Brain and Genetic Mechanisms of Socio-Emotional Functioning in Autism Spectrum Disorders

by

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Dedication

To Andrew.

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Dissertation Abstract

The development of socio-emotional functioning is a complex process that occurs over a protracted time period and requires coordinating affective, cognitive, and social faculties. At many points in development, the trajectory of socio-emotional development can be deleteriously altered due to a combination of environmental insults and individual vulnerabilities. The result can be psychopathology. However, researchers are just beginning to understand the neural and genetic mechanisms involved in the development of healthy and disordered socio-emotional functioning. In this dissertation, I propose a translational developmental neuroscience framework to understand socio-emotional functioning in both healthy and disordered populations. I then apply this framework to healthy socio-emotional development and autism spectrum disorders, selectively reviewing current literature in light of the framework. Next, three pieces of original research serve as examples of research on socio-emotional functioning in autism spectrum disorders guided by the framework: The first study examines the influence of a genetic variant (5-HTTLPR) on habituation of a socio-emotionally relevant brain structure, the amygdala, in autism spectrum disorders. The second study compares interactions of the amygdala with other areas in the brain in the context of a socio-emotional task and in the absence of a task in autism spectrum disorders. The third study examines the influence of the same genetic variant on another socially-relevant brain network, the default network. Lastly, I examine ways that the framework can help to identify future directions of research on socio-emotional development.

CHAPTER 1 [*]

General Introduction

Understanding the processes underlying healthy socio-emotional functioning as well as altered socio-emotional functioning in developmental psychopathology requires integration across domains (Cicchetti & Blender, 2004). Moreover, this integration must include multiple levels of analysis (e.g., genetics, molecular neurobiology, brain function, cognitive-affective performance, symptoms, and disorders) in order to tease apart the many pathways to disorder versus health (Cicchetti & Blender, 2004; Cicchetti & Dawson, 2002; Masten, 2007). This dissertation builds on the perspectives set forth in prior work, which emphasized a developmental, multi-level approach to the study of psychopathology (e.g., Cicchetti, 2007; Cicchetti & Blender, 2004; Monk, 2008). Additionally, our framework incorporates the concept of transactional models and acknowledges the bidirectional effects between levels of analysis (Sameroff, 2010). In this dissertation, I propose a translational developmental neuroscience framework to understand socio-emotional functioning in both healthy and disordered populations. I then apply this framework to healthy socio-emotional development and autism spectrum disorders, selectively reviewing current literature in light of the framework in this General Introduction. The next three chapters consist of studies that are examples of research on socio-emotional functioning in autism spectrum disorders guided by the framework: The first study examines the influence of a genetic variant (5-HTTLPR) on habituation of a socio-emotionally relevant brain structure, the amygdala, in autism spectrum disorders. The second study compares interactions of the amygdala with other areas in the brain in the context of a socio-emotional task and in the absence of a task in autism spectrum disorders. The third study examines the influence of the same genetic variant on another socially-relevant brain network,

[*] Chapter 1 corresponds to a portion of the publication Wiggins and Monk (in preparation-a).

the default network. In the General Conclusion, I examine ways that the framework can help to identify future directions of research on socio-emotional development.

Translational Developmental Neuroscience Framework

Levels of Analysis

The translational developmental neuroscience framework represents a cascade of events across multiple levels of analysis (Figure 1.1). First, genetic material likely has varying levels of influence on developmental psychopathology outcomes. Traditionally, most gene-based studies of developmental psychopathology have considered only two levels of analysis, such as prevalence of a particular disorder in individuals with a specific polymorphism. However, genes do not directly cause disorders. Instead, genes exert their effects during development by coding for the proteins that in turn affect the maturation of neurons and circuits related to socio-emotional functioning. Thus, to understand the functional impact of genes, it is also important to track and understand the cascade of events that follows genotype: DNA transcription to RNA, translation to protein, proteins influencing the development of brain systems, brain mechanisms of sensations, perceptions, and cognitions that can lead to symptoms.

Further up in the levels of analysis in this framework, the brain mediates the link between genetic activity and sensations, perceptions, cognitions and behaviors. Situated at the heart of this transactional developmental neuroscience framework, the brain represents the "cross-roads" that affects or is affected by changes in the other levels of analysis. Thus, integrating functional and structural neuroimaging as another level of analysis into studies on socio-emotional functioning can help to explain equifinality and multifinality. Specifically, it can disambiguate how individuals can be homogenous in terms of genotype or environment yet heterogeneous in behavior or disorder outcome or the converse, the same disorder outcome yet with different starting points in terms of genotype or environment (Curtis & Cicchetti, 2003).

Next in the framework, affective-cognitive mechanisms lead to alterations in behavior that, in the case of maladaptive behaviors, may be classified as symptoms. Understanding the

influences on the lower level affective-cognitive mechanisms that give rise to symptoms is important, as affective-cognitive mechanisms can be a useful target for treatment. For example, attention bias modification treatment targets one probable affective-cognitive mechanism of anxiety symptoms, a tendency to attend to anxiety-provoking stimuli, by training individuals to change this attention bias (Bar-Haim, 2010). Self-reported (but not clinician-reported) anxiety symptoms decreased after youth were trained to attend toward happy faces (Britton, et al., 2012). Future research could examine whether variations in efficacy of treatments targeting affective-cognitive mechanisms are due to individual differences in other levels of the framework, such as genotype.

Currently, developmental psychopathology is diagnosed behaviorally and is based on number, intensity, and duration of symptoms. However, two people diagnosed with the same disorder may present different symptoms from each other; they may have different combinations of the symptoms that make up the criteria for a disorder, and/or one person may have more severe symptoms than the other. These different presentations may represent different etiologies and prognoses although they are still classified as the same disorder. Within our framework, genetic or brain activity linked to variations in symptom severity can elucidate different etiological mechanisms that might be obscured by using discrete diagnostic categories.

Environment

The environment cuts across levels (Figure 1.2), influencing and/or being influenced by each of the levels. For example, genetic activity (specifically, the efficacy of DNA transcription to RNA and translation to protein) can be affected by environmental influences through such epigenetic mechanisms as DNA methylation. In one study, women who were exposed to childhood sex abuse exhibited increased serotonin transporter gene methylation which in turn was associated with decreased gene expression (Vijayendran, et al., 2012). Future research could link methylation status of the serotonin transporter gene to brain function in circuits relevant to socio-emotional functioning, such as the amygdala-prefrontal circuit or the default network.

Transactional

This framework also recognizes that influences among the levels can be transactional, such that the direction of influence flows both ways (Figure 1.3). For example, the arrows linking environment and behavior/cognition are bidirectional because not only does the environment affect behaviors and cognitions, behaviors and cognitions can also change one's environment. In the case of a child who has social impairment, peers may approach and interact with the child less often. Therefore, the child has fewer opportunities to develop social skills. The result is that social impairments continue and even worsen. The present framework builds on this idea of transactional models whereby the individual is a product of continuous interactions between the individual and experience (Fiese & Sameroff, 1989; Sameroff & Mackenzie, 2003), but also recognizes the transactional nature of the relationships between brain function and behavior as well as brain function and genetic activity. Contrary to the popular notion that the brain causes behavior, behavior can affect brain function as well. Moreover, not only does genetic activity affect brain function, brain function (in response to environmental conditions) can affect the efficacy of genetic expression via mechanisms such as methylation.

Developmental

All of the levels are interacting with each other within the context of development. The relationships among levels changes depending on the developmental period (Figure 1.4). For example, serotonin transporter's effects on depression-like behaviors hinges on when in development serotonin transporter levels are altered: When serotonin transporter is decreased in early life, rodents have depression-like behaviors that emerge in adolescence. In contrast, when serotonin transporter is reduced in adulthood, depression-like behaviors do not appear (Ansorge, et al., 2008).

Translational

This framework is translational as well, because it incorporates the interface of basic and clinical science and facilitates the application of basic science to medical and behavioral treatments. The framework provides a way to conceptualize and study how different levels work together to result in psychopathology. Through this framework, studies that examine any

level, from genes to brain to behavior, can be understood in the larger context of normal and abnormal socio-emotional functioning. Moreover, the framework also gives us a way to delineate the boundaries among typical, at-risk, and abnormal functioning at any level of analysis and throughout development. Having a larger conceptualization of how all these levels of analysis work together to produce healthy or impaired socio-emotional functioning may be instrumental in creating hypothesis-driven treatments.

Concepts Within Scope of this Literature Review

In this review, I will examine what is known about links in this framework in terms of socio-emotional functioning. This framework is, by nature, broad, so for the purposes of this review I will narrow the application of this framework to a few key areas. First, I will focus on typical development to establish a normative base for the links in this framework. Next, I will examine what is known about the links in the framework in individuals diagnosed with autism spectrum disorders.

Additionally, I will focus on mid-childhood through adolescence. This is a key developmental period as multiple transitions relevant to socio-emotional function take place in the shift from pre-adolescence through adolescence: peers grow in importance; puberty onsets and hormone levels change; romantic relationships are initiated; classroom structures change from elementary, to middle, to high school; and importantly, affective disorders often onset in this period (Casey, et al., 2010; Eccles, et al., 1993).

In our discussion of brain function, I will focus on functional MRI and the two types of data it provides. The first is the measure of activation in specific brain regions. The second is functional connectivity, which measures the degree to which the changes in blood flow are synchronized between two areas in the brain. As the crossroads of this framework, the brain is subject to multiple influences, and functional MRI is a sensitive tool to examine these influences on the brain in the context of socio-emotional development in children and adolescents. Functional MRI allows researchers to visualize how brain structures respond and interact, respectively, to particular socio-emotional stimuli and situations. Through the tasks utilized in functional MRI and functional connectivity, researchers can presumably isolate socio-

emotional functions and the brain systems involved in those functions. Additionally, functional MRI may also be used to determine brain function when participants are not performing a particular task (e.g., in the absence of a task or at "rest"). Other methods (structural neuroimaging, EEG) of measuring brain phenotypes are complementary to functional MRI, but will not be discussed in this review in the interest of space. Of note, positron emission tomography (PET) scans are generally not performed for research purposes in children because of ethical issues surrounding the risks associated with injecting radioactive tracer into a developing child and thus are not a part of this review.

Although many brain structures contribute to socio-emotional functioning, I will focus on functional MRI studies on several key regions that have been most consistently implicated in this domain. First, I will include studies examining amygdala activation. The amygdala is thought to be involved in salience detection and may also index distress (Davis, 1999; Davis & Whalen, 2001; LeDoux, 1996; LeDoux, 2000). The amygdala is reliably activated in response to emotional faces and other socio-emotional stimuli (Sabatinelli, et al., 2011). Second, I will include studies examining functional connectivity of the amygdala with the prefrontal cortex. The prefrontal cortex and amygdala form a circuit via reciprocal connections found in adult humans and animal models (Carmichael & Price, 1995; Ongur & Price, 2000; Sarter & Markowitsch, 1984). The ventral, not dorsal, prefrontal cortex is likely the main area through which regulation of the amygdala occurs (Ray & Zald, 2012). Within the ventral prefrontal cortex, the ventromedial regions may be more involved in automatic regulation of the amygdala, whereas the ventrolateral prefrontal cortex is implicated in voluntary regulation of responses (Phillips, et al., 2008; Ray & Zald, 2012). In MRI studies, stronger functional connectivity suggests greater coordination of amygdala and prefrontal activation. Third, I included studies examining posterior-anterior connectivity of the default network in the context of rest, or absence of a task. These studies serve as a complement to studies on the amygdala and prefrontal cortex; the vast majority of which rely on tasks using socio-emotional stimuli. Functional connectivity of the default network increases in the absence of task and decreases during engagement in a cognitively demanding task (Buckner & Carroll, 2007; Fox, et al., 2005a; Raichle & Snyder, 2007). The default network is linked to social function, particularly projecting oneself into

others' situations or theory of mind (Buckner & Carroll, 2007; Flavell, 1999; Frith & Frith, 2003) and consolidating a narrative of the self (Gusnard, et al., 2001), although the primary purpose of the default network is a subject of debate. Default network structures include posterior medial areas such as the posterior cingulate and precuneus as well as medial prefrontal areas (Buckner, et al., 2008). Posterior-anterior connectivity of the default network is the focus of this review because posterior-anterior default network connectivity undergoes the most protracted developmental time course (Fair, et al., 2008) and is implicated in a number of disorders (e.g., Monk, et al., 2009).

Next, in choosing an emphasis for the genetics portion of the review, it is worth noting that the numbers of genetics studies on anxiety, depression, and autism spectrum disorders are vast: A PubMed search for "genetics anxiety" yields 6920 studies, "genetics depression" yields 14,637 studies, and "genetics autism" yields 4229 studies. However, there are relatively few studies that quantitatively relate genetic information with brain function. Thus, I will focus in this review on a polymorphism, the serotonin transporter-linked promoter region (5-HTTLPR), that relates to amygdala activation, amygdala-prefrontal connectivity, and posterior-anterior default connectivity and can shed light on individual differences in brain function in these areas.

The environment is often defined as any non-genetic influence. Because it is such a broad concept, I will utilize a few studies that illustrate how the environment impacts brain function in the circuits of interest in youth. Specifically, I will examine adverse environmental influences, such as child maltreatment; however, it is important to note that treatment can be considered an environmental influence as well. Treatment studies can be a natural experiment to examine brain plasticity in response to a beneficial environmental event (Maslowsky, et al., 2010). This can be accomplished by examining brain function before and after treatment. Moreover, when treatment is done in the context of a randomized control trial, in which participants are randomly assigned to receive active treatment or placebo, changes in brain function can be more precisely attributed to the treatment.

Typical Development

Functional Brain Development

Amygdala

From the time that functional MRI was first used to understand brain development, the amygdala has been the subject of intense investigation. Consistent with findings in studies of adults, healthy youth exhibit amygdala activation to fearful faces (Baird, et al., 1999). However, when adults and children were directly compared on amygdala activation, children exhibit greater amygdala activation to fearful and neutral faces than adults (Guyer, et al., 2008b; Thomas, et al., 2001) as well as greater activation to fearful versus neutral faces compared to adults (Monk, et al., 2003). Taken together, these studies suggest that amygdala reactivity decreases from childhood into adulthood. Consistent with that view, adolescents in later stages of puberty exhibit less amygdala activation to neutral faces than in earlier stages of puberty (Forbes, et al., 2011).

Amygdala-Prefrontal Connectivity

Amygdala-prefrontal functional connectivity in youth is a subject of investigation in several studies. One study demonstrated that 7-9 year old children show weaker connectivity between the amygdala and ventromedial prefrontal cortex than 19-22 year old adults (Qin, et al., 2012). Directional influence of the ventral prefrontal cortex on the amygdala also increases with age (Perlman & Pelphrey, 2011). This pattern of increased coupling between the amygdala and prefrontal cortex across adolescence has been attributed to more efficient regulation of the amygdala with age (Casey, et al., 2008). This interpretation is consistent with the protracted developmental timeline for amygdala-prefrontal development proposed by others (Casey, et al., 2008). Others, however, have challenged the notion that decreased connectivity necessarily means less emotion regulation and thus increased risk for poor socio-emotional functioning (Pfeifer & Allen, 2012). Of note, most methods of calculating functional connectivity are based on correlation between time courses from two brain areas (e.g., amygdala and prefrontal cortex). This limits the interpretation of direction of influence and does not rule out the possibility of a third variable influencing both brain regions.

Default Network

Several studies on youth populations have shown that functional connections within the default network, particularly posterior to anterior long-range connections, increase with maturation from childhood through adolescence. Using a variety of methods for calculating connectivity, children relative to adults have weaker posterior-anterior functional connectivity of the default network (Fair, et al., 2008; Stevens, et al., 2009; Supekar, et al., 2010a). Additionally, posterior-anterior default network connectivity is positively correlated with age in children and adolescents (Wiggins, et al., 2011).

Linking the Brain to Typical Variations in Socio-Emotional Functioning

Some work has been done to quantitatively link the amygdala and posterior-anterior connectivity within the default network to socio-emotional behaviors in youth. In adolescents, amygdala activation in response to fearful faces positively correlates with scores on social anxiety subscales: peer rejection, humiliation, performing in public, and being separated from loved ones (Killgore & Yurgelun-Todd, 2005). However, amygdala activation is not correlated with the non-social aspects of anxiety (Killgore & Yurgelun-Todd, 2005). Greater amygdala activation in adolescents when viewing fearful faces is also related to lower emotional intelligence (ability to effectively utilize social and emotional capacities) (Killgore & Yurgelun-Todd, 2007). For the default network, increased connectivity in the anterior portion of the default network (right middle frontal gyrus) is related to decreased anxiety scores in healthy youth, but not in adults (Dennis, et al., 2011). These studies indicate that incremental differences in brain function are linked to incremental differences in socio-emotional functioning.

Genetic Influences on the Brain and Behavior

5-HTTLPR

One genetic variant that has received considerable interest is the serotonin transporter-linked polymorphic region (5-HTTLPR). This genetic variant affects the production of serotonin transporter and consists of “short” and “long” alleles, which have a variable number of tandem repeats (Lesch, et al., 1996). Within the long allele there is a single nucleotide polymorphism where adenine is substituted for a guanine nucleotide (rs25531); a long allele with the adenine

substitution results in greater serotonin transporter expression ("high expressing") than either a long allele with adenine or the short allele ("low expressing") (Hu, et al., 2006).

Low expressing 5-HTTLPR genotypes are associated with multiple socio-emotional problems and traits in children and adolescents. Low expressing 5-HTTLPR alleles are related to increased aggressive behavior (Beitchman, et al., 2003), fear and anxiety traits (Hayden, et al., 2007), behavioral inhibition when social support is low (Fox, et al., 2005b), and affective problem scores when children are living in a one-parent family (Nobile, et al., 2009). Additionally, the low expressing alleles have been found to be associated with shyness/social anxiety in two studies (Battaglia, et al., 2005; Battaglia, et al., 2004), although another study found that the high expressing genotype is associated with shyness (Arbelle, et al., 2003). The low expressing 5-HTTLPR variants are also related to greater externalizing and internalizing behavior, but only when another genotype, the "long" allele of a dopamine receptor genetic variant (DRD4 VNTR), is present (Becker, et al., 2007; Schmidt, et al., 2007).

There have been a number of studies examining 5-HTTLPR in relation to brain activation in adults (e.g., Hariri, et al., 2005), but fewer have focused on linking 5-HTTLPR to brain function in healthy children and adolescents. In healthy adults, 5-HTTLPR low expressing genotypes have been linked to increased amygdala activation (Hariri, et al., 2005). In a study examining the contribution of 5-HTTLPR genotype during child and adolescent development, amygdala activation positively correlates with age in children and adolescents with low expressing genotypes but not high expressing genotypes (Wiggins, et al., 2012a). Moreover, the pattern of greater amygdala activation with the low expressing genotypes established in healthy adults is not evident until later adolescence (Wiggins, et al., 2012a). In the default network, typically developing youth with low expressing 5-HTTLPR genotypes show reduced posterior-anterior connectivity compared to youth with the high expressing genotypes (Wiggins, et al., 2012b). Additionally, healthy youth with the low expressing genotypes showed attenuated increases in posterior-anterior default network connectivity with age compared to high expressing genotypes (Wiggins, et al., 2012b). To summarize, low expressing 5-HTTLPR alleles are associated with brain development profiles (increased amygdala activation, decreased default

network connectivity) that are related to socio-emotional problems (e.g., Dennis, et al., 2011; Killgore & Yurgelun-Todd, 2005).

Adverse Environmental Influences on the Brain

Studies of youth that experienced adverse environments earlier in development suggest that these experiences can have lasting effects on brain function in key socio-emotional circuits. Two studies on children and early adolescents who were previously institutionalized found that these youth who experienced early adverse rearing environments exhibit increased amygdala activation to faces (Maheu, et al., 2010; Tottenham, et al., 2011). Additionally, a prospective study on a community sample found that, for girls, life stress during infancy predicts increased cortisol (a hormone related to stress) in childhood as well as decreased connectivity between the amygdala and ventromedial prefrontal cortex during adolescence, fourteen years after the stressors were measured and controlling for recent life stress (Burghy, et al., 2012). In this community sample, greater amygdala-ventromedial prefrontal cortex connectivity was related to worse depression symptoms but ameliorated anxiety symptoms in girls (Burghy, et al., 2012). These studies illustrate how early environmental influences initiate a cascade of events throughout development that includes alterations in brain function years following environmental stressors. No studies, however, have yet examined negative early environments on default network connectivity in youth.

Autism Spectrum Disorders

Functional Brain Development

Amygdala

Autism spectrum disorders are neurodevelopmental conditions characterized by impaired social functioning, as well as communication impairment and rigid, repetitive behaviors (APA, 1994). A number of studies have focused on examining alterations in brain circuitry related to socio-emotional functioning, including the amygdala, to understand the etiology and maintenance of social symptoms in autism spectrum disorders. One view regarding social symptoms is that individuals with autism spectrum disorders fail to develop social skills because they are disinterested in social stimuli, such as faces. Consistent with this

view, many studies have shown that individuals with autism spectrum disorders show less amygdala activation to faces relative to controls (e.g., Ashwin, et al., 2007; Critchley, et al., 2000; Dapretto, et al., 2006; Grelotti, et al., 2005; Hadjikhani, et al., 2007; Pelphrey, et al., 2007; Pinkham, et al., 2008). Because the amygdala is fundamentally involved in processing salient information in the environment, such as social cues, reduced amygdala activation may indicate that people with autism spectrum disorders are less interested in social information.

An alternative view is that individuals with autism spectrum disorders are not disinterested in social stimuli; rather they are distressed by social stimuli and find social stimuli aversive. This "distress" view is in line with observations that individuals with ASD avoid direct eye contact (Kliemann, et al., 2010). Moreover, children with ASD show greater autonomic arousal to faces (Joseph, et al., 2008). Evidence from functional MRI studies supports the "distress" view as well: The studies discussed above that found reduced amygdala activation in autism spectrum disorders presented faces for relatively long periods of time (two or more seconds) and did not monitor attention to the faces (e.g., Ashwin, et al., 2007; Critchley, et al., 2000; Dapretto, et al., 2006; Grelotti, et al., 2005; Hadjikhani, et al., 2007; Pelphrey, et al., 2007; Pinkham, et al., 2008). However, when attention to the faces is constrained, adolescents with ASD (Dalton, et al., 2005; Kliemann, et al., 2012; Weng, et al., 2011) as well as adults with ASD (Kleinhans, et al., 2009; Monk, et al., 2010) exhibit greater amygdala activation to faces compared to controls. Moreover, attention to the eyes of a face correlates with amygdala activation in youth with autism spectrum disorders (Dalton, et al., 2005; Kliemann, et al., 2012). Taken together, these functional MRI studies suggest that the reduced amygdala activation found in previous studies (e.g., Ashwin, et al., 2007; Critchley, et al., 2000; Dapretto, et al., 2006; Grelotti, et al., 2005; Hadjikhani, et al., 2007; Pelphrey, et al., 2007; Pinkham, et al., 2008) may be because individuals with autism spectrum disorders attended away from the faces.

Recently, in order to more fully characterize amygdala activation in autism spectrum disorders, I examined amygdala habituation to faces in youth with autism spectrum disorders as well as controls. Habituation is the initial strong neural response and reduction in response over time. In contrast to typically developing youth who consistently habituate to repeatedly presented faces, youth with autism spectrum disorders not only fail to habituate but increase

their amygdala response over time to sad and neutral faces (Swartz, et al., 2013). As increased amygdala activation may index distress (Davis, 1999; Davis & Whalen, 2001; LeDoux, 1996; LeDoux, 2000), a failure to decrease amygdala habituation or an increase in habituation over time may indicate that individuals with ASD experience social stimuli as distressing.

Amygdala-Prefrontal and Default Network Connectivity

Initial studies of connectivity in autism spectrum disorders, largely with adults, reported decreased connectivity compared to controls ("under-connectivity") in several brain systems (Hughes, 2007). Whereas under-connectivity does appear to occur often in autism spectrum disorders, recent studies on adolescents and children suggest that abnormal connectivity in autism spectrum disorders can include both under-connectivity and over-connectivity, depending on the context and the brain regions. Adolescents and children with autism spectrum disorders have weaker connectivity between the amygdala and prefrontal cortex when viewing sad faces (Swartz, et al., 2013). Additionally, in another task with emotional faces, during interference trials, children with autism spectrum disorders show decreased left amygdala connectivity with the subgenual anterior cingulate cortex (a structure within the prefrontal cortex) but increased right amygdala connectivity with the pregenual anterior cingulate cortex (Murphy, et al., 2012a). In contrast, examining the default network in the context of rest reveals that children and adolescents with autism spectrum disorders have weaker posterior-anterior connectivity (Weng, et al., 2010; Wiggins, et al., 2011). Moreover, in a cross-sectional study, children and adolescents with autism spectrum disorder fail to develop posterior-anterior default network connectivity as strong as healthy children and adolescents (Wiggins, et al., 2011).

Linking the Brain to Social Symptoms

Relatively few studies have quantitatively linked brain function to variation in social symptom severity in autism spectrum disorders. One study found that decreased amygdala habituation (i.e., persistent activation over the course of the task) to neutral faces is related to worse social symptoms in children and adolescents with autism spectrum disorders (Swartz, et al., 2013). Another found that worse social impairment symptoms are associated with weaker posterior-anterior default network connectivity in youth with autism spectrum disorders

(Weng, et al., 2010). Future research that relates brain function to dimensional symptom domains in autism spectrum disorders, as opposed to only seeking group differences between people diagnosed with autism spectrum disorders and controls, may prove to be beneficial. This is because the triad of symptom domains in autism spectrum disorders appears not to represent a single "autism spectrum disorder" concept, but rather separate subdomains with potentially different genetic and brain etiologies that co-occur in autism spectrum disorders (Kuenssberg, et al., 2011). Considering the symptom domains separately and quantitatively linking them to potential etiological mechanisms could reduce noise and heterogeneity in these types of studies.

Genetic Influences on the Brain and Behavior

5-HTTLPR

Evidence indicates that the genetic variant 5-HTTLPR plays a role in socio-emotionally relevant brain activation and symptoms in autism spectrum disorders. Low expressing 5-HTTLPR genotypes are associated with greater social symptoms, but not to an overall diagnosis of autism spectrum disorders (Brune, et al., 2006; Tordjman, et al., 2001). 5-HTTLPR genotype influences amygdala habituation to sad faces differently for individuals with ASD versus controls. Specifically, whereas controls of any genotype demonstrate amygdala habituation to the faces, youth with autism spectrum disorders fail to display amygdala habituation; moreover, youth with autism spectrum disorders and the low expressing genotypes exhibit increased amygdala responses to the faces over time, a process known as sensitization (Wiggins, et al., in press). In the default network, low expressing 5-HTTLPR genotypes are associated with increased posterior-anterior connectivity for youth with autism spectrum disorders, but the converse is true for controls (Wiggins, et al., 2013). Moreover, youth with autism spectrum disorders and low expressing genotypes have greater age-related increases in posterior-anterior default network connectivity compared to youth with autism spectrum disorders and high expressing genotypes as well as controls with either genotype classification (Wiggins, et al., 2013).

Adverse Environmental Influences on the Brain

To date, there have been no studies of adverse environmental influences on amygdala-prefrontal or default network function in youth with autism spectrum disorders. Including environmental influences as a level of analysis in studies on brain function in autism spectrum disorders can help to trace the multiple pathways to impaired functioning.

The Translational Developmental Neuroscience Framework in Action

To summarize, the translational developmental neuroscience framework provides a model to understand socio-emotional functioning in both healthy and disordered populations. This framework emphasizes that the development of socio-emotional functioning is a complex process that occurs over a protracted time period and requires coordinating affective, cognitive, and social faculties. At many points in development, the trajectory of socio-emotional development can be deleteriously altered due to a combination of environmental insults and individual vulnerabilities. The result can be psychopathology, such as autism spectrum disorders. The upcoming three chapters are examples of studies on socio-emotional functioning in autism spectrum disorders guided by the framework: The first study examines the influence of the 5-HTTLPR genetic variant on amygdala habituation in autism spectrum disorders (Wiggins, et al., in press). The second study compares amygdala-prefrontal connectivity in the context of a socio-emotional task and in the absence of a task in autism spectrum disorders (Wiggins, et al., in preparation). The third study investigates the influence of 5-HTTLPR on the default network (Wiggins, et al., 2013).

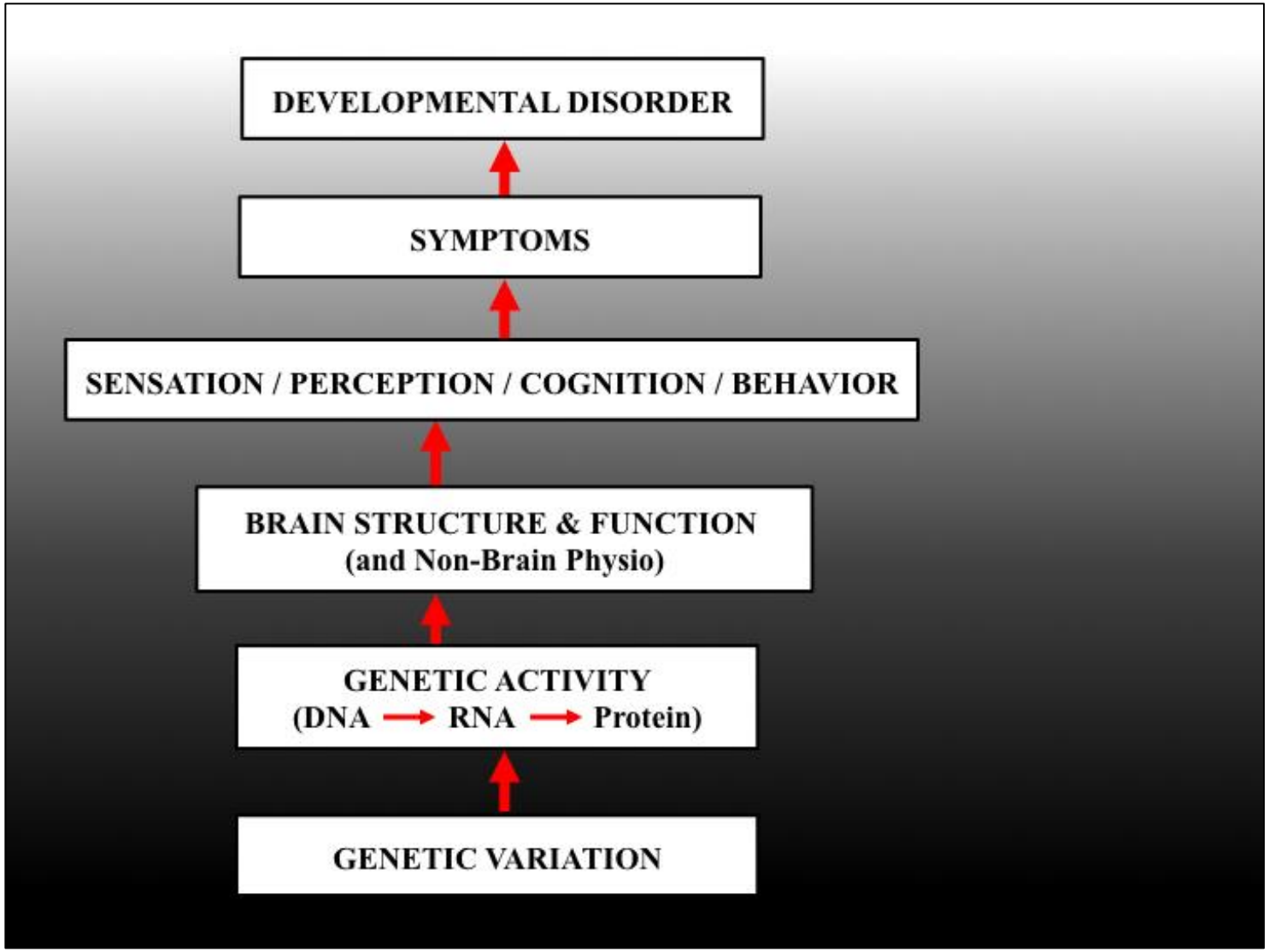


Figure 1.1. Levels of analysis in framework.

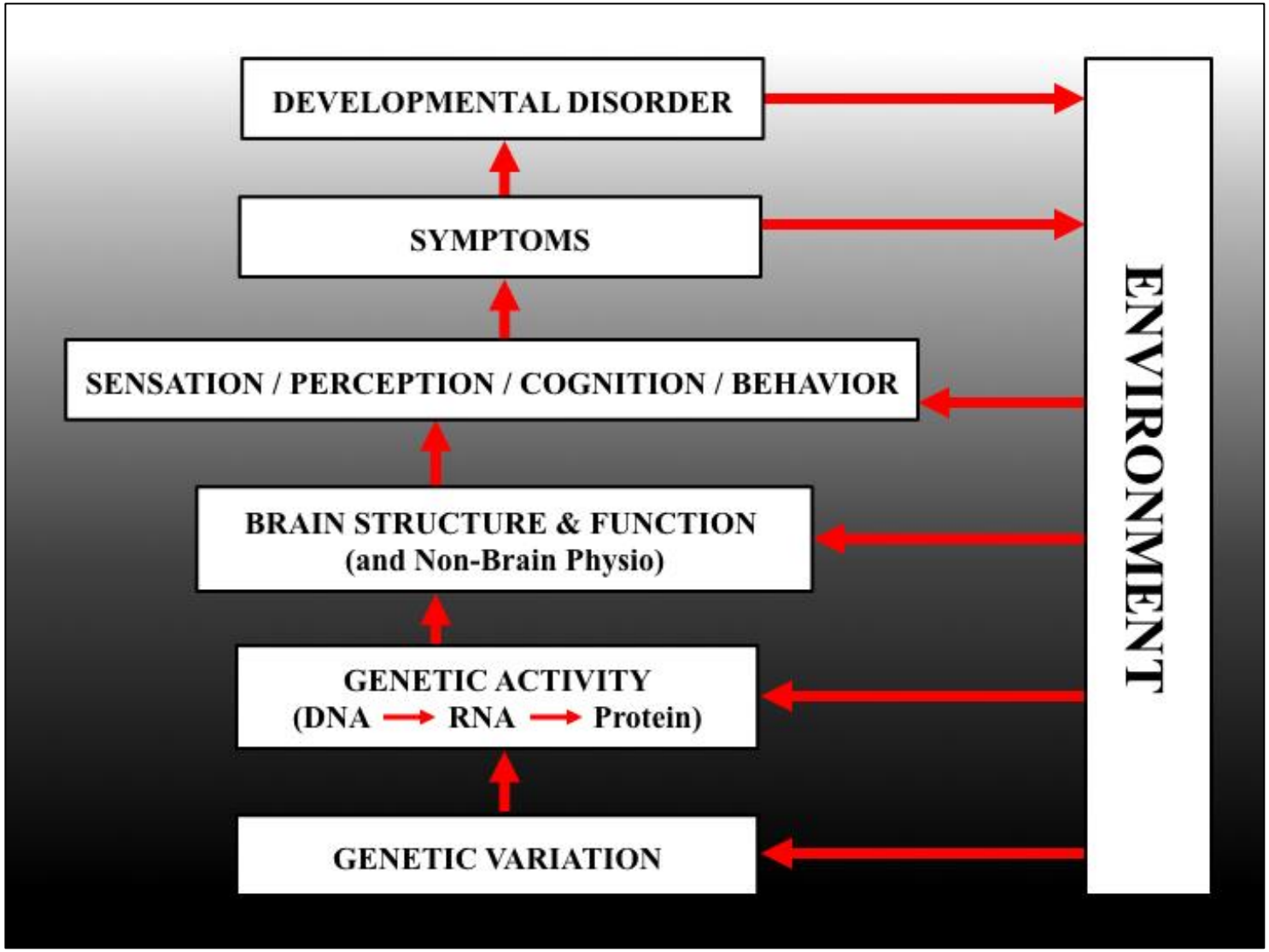


Figure 1.2. Environmental influences cut across all levels of analysis.

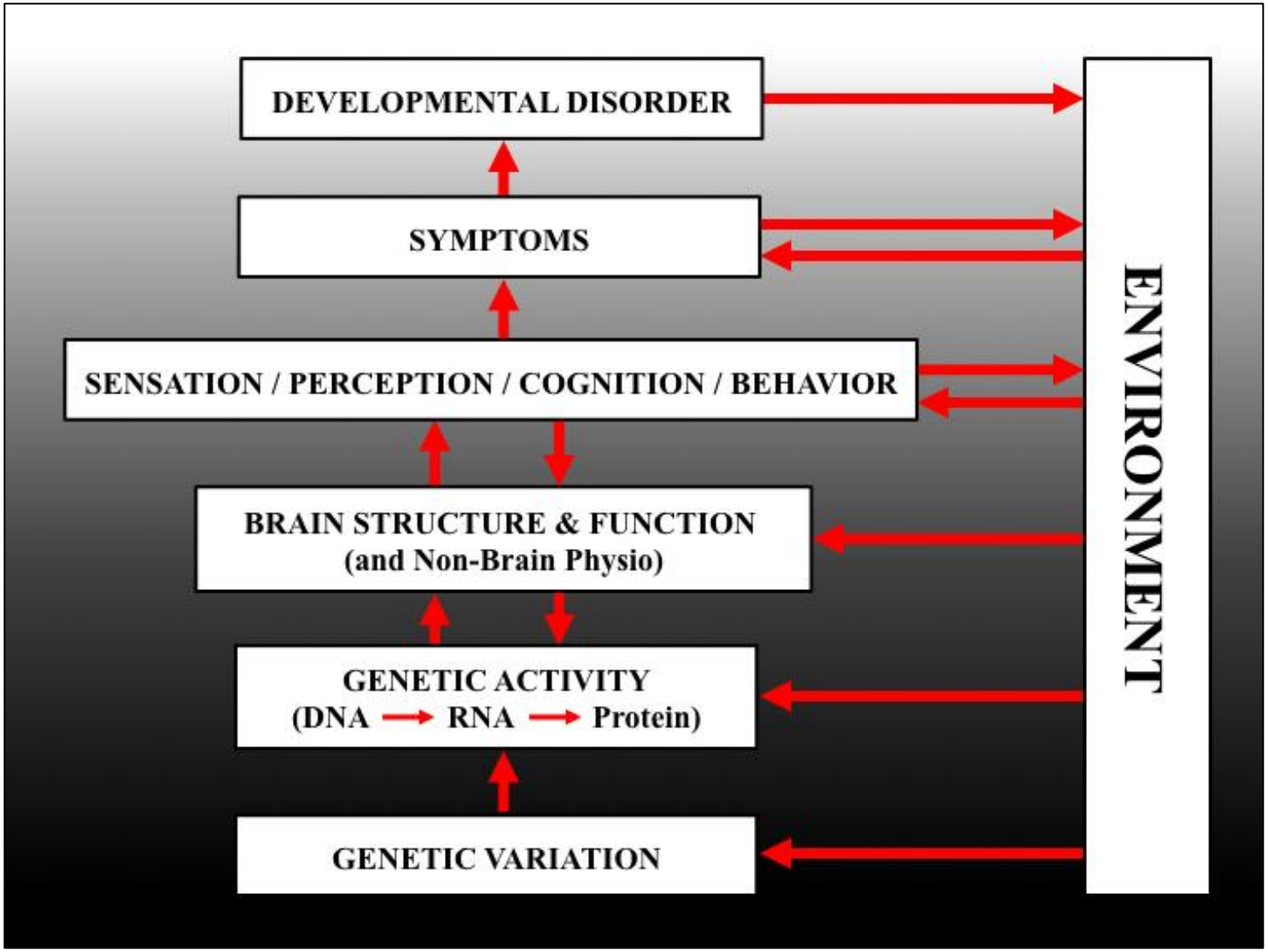


Figure 1.3. Influences are transactional, the product of continuous interactions among levels and environment.

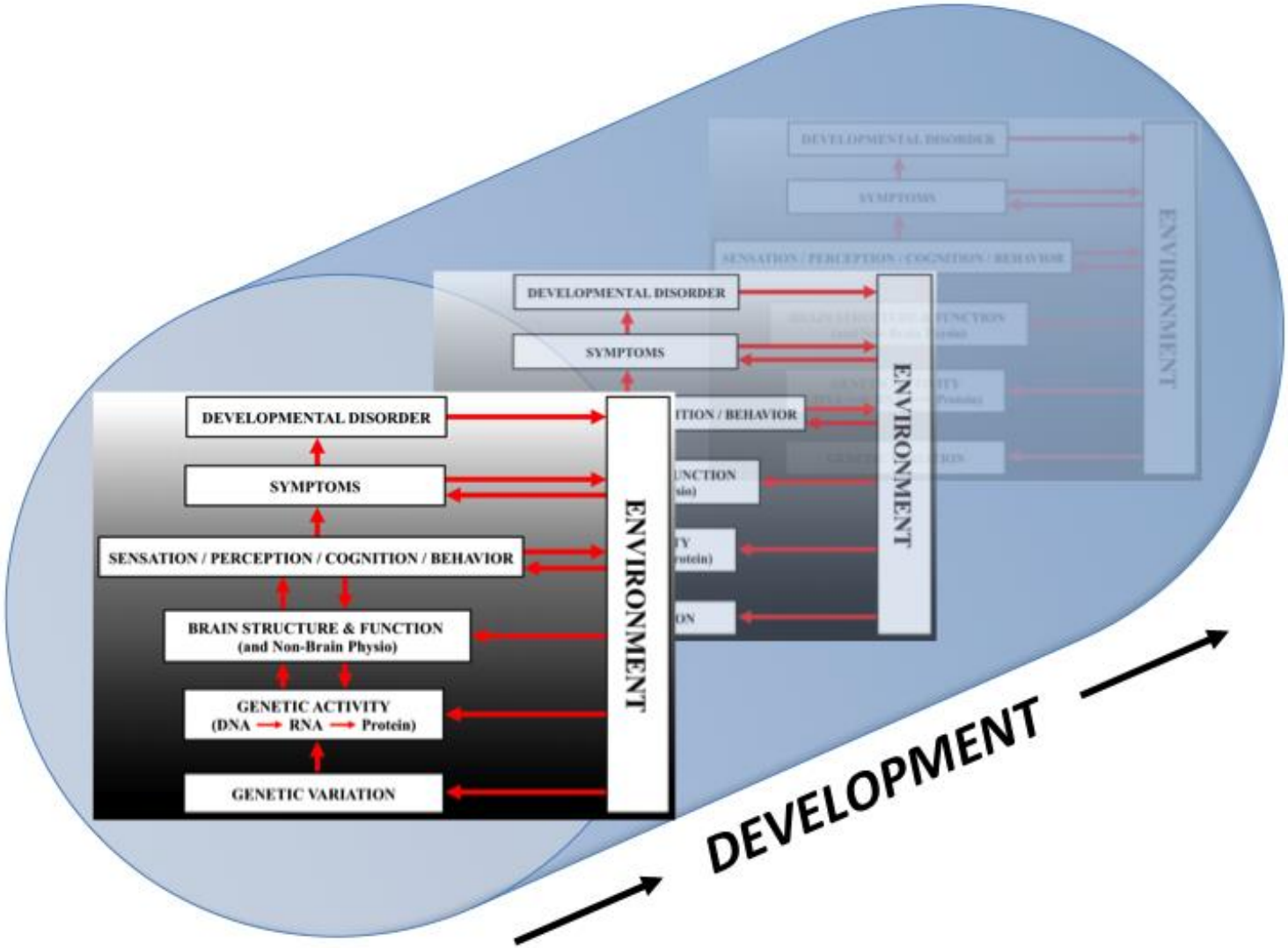


Figure 1.4. Relationships among levels of analysis change over development.

CHAPTER 2 [†]

Serotonin Transporter Genotype Impacts Amygdala Habituation in Youth with Autism Spectrum Disorders

Summary

Failure of the amygdala to habituate, or decrease response intensity, to repeatedly presented faces may be one mechanism by which individuals with autism spectrum disorders (ASD) develop and maintain social symptoms. However, genetic influences on habituation in ASD have not been examined. I hypothesized that serotonin transporter-linked promoter region (5-HTTLPR) genotype affects change in amygdala response to repeated sad faces differently in individuals with ASD versus healthy controls. Forty-four youth with ASD and 65 controls aged 8-19 years were genotyped and underwent an event-related fMRI scan where they identified the gender of emotional faces presented for 250 ms. The first half of the run was compared to the second half to assess habituation. 5-HTTLPR genotype influences amygdala habituation to sad faces differently for individuals with ASD versus controls. The genotype-by-diagnosis-by-run half interaction was driven by individuals with ASD and low expressing genotypes (S/S, S/L_G, and L_G/L_G), who trended toward sensitization (increase in amygdala activation) and whose habituation scores significantly differed from individuals with ASD and higher expressing genotypes (L_A/L_A, S/L_A, and L_A/L_G) as well as controls with low expressing genotypes. Our results show that amygdala response to social stimuli in ASD, which may contribute to social symptoms, is genetically influenced.

[†] Chapter 2 corresponds to the publication Wiggins and colleagues (in press).

Introduction

The social impairment symptoms characteristic of autism spectrum disorders (ASD) may have their roots in altered processing of social stimuli, such as emotional faces. Altered activation of the amygdala, a brain structure that responds to faces and likely indexes emotional salience of stimuli (Adolphs, 2010) may contribute to social deficits in ASD.

There are two main views regarding the development of social symptoms in ASD and amygdala activation. The first view is that individuals with ASD may be disinterested in social stimuli, such as faces. If youth with ASD are disinterested in social stimuli, they may not seek out social stimuli and miss opportunities to develop social skills (Sasson, 2006). Supporting this view, a number of studies have found reduced amygdala activation in ASD in response to emotional faces (e.g., Kleinhans, et al., 2011; see reviews Pelphrey, et al., 2011; Volkmar, 2011). However, in these studies, the emotional face stimuli were presented for relatively long periods of time (several seconds) and attention to the faces was not monitored or controlled. However, when group differences in attention to faces are limited (Dalton, et al., 2005; Monk, et al., 2010; Weng, et al., 2011) and when individuals with ASD initially fixate on the eyes (Kliemann, et al., 2012), individuals with ASD have increased amygdala activation to faces. These studies support an alternative view, that individuals with ASD are not disinterested in faces, but rather find social stimuli to be aversive. Thus, individuals with ASD may actively avoid faces, and thus miss developmental opportunities to hone social skills. Indeed, children with ASD exhibit greater skin conductance responses to faces than healthy controls (Joseph, et al., 2008) and actively avoid looking at the eyes of a face (Kliemann, et al., 2012; Kliemann, et al., 2010). Additionally, the more time spent looking at the eyes, the greater the amygdala activation in individuals with ASD (Dalton, et al., 2005).

Increased amygdala activation in ASD (e.g., Weng, et al., 2011) may be due to a failure to habituate to faces. Habituation refers to the decrease in neural response to repeated presentation of a stimulus (Rankin, et al., 2009). Amygdala habituation may represent a learning process by which adaptive levels of arousal are maintained to social stimuli (Herry, et al., 2007). In healthy controls, the amygdala quickly habituates to faces, decreasing responses as faces are repeatedly presented (e.g., Fischer, et al., 2003). However, adults with ASD fail to

habituate to faces (Kleinhans, et al., 2009) and youth with ASD exhibit sensitization, or increase in response to faces (Swartz, et al., 2013). Sustained activation to faces for the duration of the scan may be one reason previous studies found amygdala overactivation in ASD.

Genetic factors, particularly the serotonin transporter-linked polymorphic region variant (5-HTTLPR), may play a role in amygdala habituation. The S and L_G alleles of 5-HTTLPR are associated with decreased serotonin transporter expression relative to the L_A allele (A to G SNP in L allele, rs25531; Hu, et al., 2006). A meta-analysis showed that individuals with the low expressing genotypes of 5-HTTLPR show greater amygdala activation (Murphy, et al., 2012b). This may be caused by a failure to habituate to socio-emotional stimuli, as healthy controls with low expressing genotypes, relative to high expressing genotypes, fail to habituate to faces (Lonsdorf, et al., 2011). Since individuals with ASD, as a group, show reduced habituation to faces (Kleinhans, et al., 2009; Swartz, et al., 2013), this genetic effect of 5-HTTLPR on habituation may be heightened in the ASD group. There is evidence that the low expressing genotype may represent a subtype in ASD in terms of symptoms: Individuals with ASD and low expressing 5-HTTLPR genotypes exhibit worse social symptoms (e.g., Brune, et al., 2006). However, the role of 5-HTTLPR in amygdala habituation in individuals with ASD has not yet been examined.

The goal of the present study is to address this gap in the literature by examining whether 5-HTTLPR impacts amygdala habituation to sad faces differently in ASD. I specifically focused on sad faces for several reasons: First, compared to controls, individuals with ASD consistently show greater amygdala activation to sad faces (Monk, et al., 2010; Weng, et al., 2011). Moreover, individuals with ASD require more intense sad facial expressions to accurately identify the face as sad, and diminished sensitivity to sad faces is related to worse social impairment (Wallace, et al., 2011). Next, evidence from controls indicates that 5-HTTLPR genotype affects amygdala activation to sad faces, but not happy or neutral faces (Dannlowski, et al., 2010). Last, the amygdala in healthy controls may not reliably habituate to fearful faces, as one study found habituation with fearful faces (Fisher, et al., 2009), another did not (Swartz, et al., 2013), and a third found habituation in a single voxel in the amygdala (Fischer, et al., 2003). Thus, to maximize potential group differences, sad faces presented early in the scan

were compared to sad faces presented late in the scan. I hypothesized that 5-HTTLPR affects change in amygdala response to repeated sad faces differently in individuals with ASD versus healthy controls.

Methods

Participants

Forty-four children and adolescents with ASD and 65 healthy controls, aged 8 to 19 years, were included (Table 2.1). Of 103 participants with ASD and 86 controls, all data from 56 participants with ASD and 21 controls were excluded because of head movement exceeding 2.25 mm translation or degrees rotation in any frame compared to the first, inability to complete the MRI scan, scoring less than 80% accuracy in identifying gender in the face task, failure to return a saliva sample for genotyping, or technical problems with the MRI. Three participants with ASD were excluded as outliers, with amygdala responses more than 2.5 standard deviations from the mean. Individuals were excluded if they had braces, medical conditions contraindicated for MRI, or history of seizures or neurological disorders.

Controls were recruited through flyers posted at community organizations. Clinicians at the University of Michigan Autism and Communication Disorders Center diagnosed participants with an ASD (Autistic Disorder, Asperger's syndrome, or Pervasive Developmental Disorder – Not Otherwise Specified) using the Autism Diagnostic Observation Schedule (ADOS; Lord, et al., 2000), the Autism Diagnostic Interview-Revised (ADI-R; Lord, et al., 1994), and clinical consensus (Lord, et al., 2006). The University of Michigan Institutional Review Board approved procedures. Participants over age 18 and parents of minors gave written informed consent; participants under 18 gave written assent.

Participants were given a battery of self- and parent report symptom and behavioral measures (Table 2.2). All control participants scored below clinical cutoffs for affected status. Individuals with the low and higher expressing genotypes did not differ in any of the symptom measures or cognitive functioning in either the ASD or control group. There was a significant diagnosis-by-genotype interaction predicting age and puberty; specifically, participants with ASD and the low expressing genotypes were younger and less advanced in pubertal

development. Therefore, I conducted additional analyses controlling for age and pubertal status. Prior studies utilized portions of this dataset (Swartz, et al., 2013; Weng, et al., 2011; Weng, et al., 2010; Wiggins, et al., 2012b; Wiggins, et al., 2011).

Genetic Analyses

5-HTTLPR genotype was assessed using established procedures (Wiggins, et al., 2012b). The Oragene DNA kit (DNA Genotek; Kanata, Canada) was used to collect saliva samples from each participant. PCR and agarose gel genotyping were utilized to discriminate between the S and L alleles. Subsequently, Sanger sequencing was used to determine the A to G SNP in the L allele (rs25531; Hu et al., 2006) and to confirm PCR genotyping.

Participants were grouped by expression level of genotype: low expressing genotypes (S/S, S/L_G, L_G/L_G) versus medium plus high expressing genotypes (L_A/L_A, S/L_A, L_A/L_G, referred to as "higher expressing" genotypes). As the L_G allele results in serotonin transporter expression equivalent to the S allele (Hu, et al., 2006), the S and L_G alleles were grouped together for the purpose of analysis. This genotype grouping is consistent with a number of non-autism spectrum disorder studies that found recessive effects of the low expressing 5-HTTLPR alleles, often in adolescent populations (e.g., Benjet, et al., 2010; Cicchetti, et al., 2007; Surguladze, et al., 2008). Within the ASD group, there were 15 individuals with low and 29 with higher expressing genotypes. There were 22 controls with low and 43 with higher expressing genotypes. Hardy-Weinberg equilibrium was tested for low versus medium versus high expressing genotypes. Genotype frequencies were in Hardy-Weinberg equilibrium for the ASD group (χ^2 = 1.49, df = 1, p = 0.222) and the control group (χ^2 = 2.60, df = 1, p = 0.107).

Emotional Faces Task (In Scanner)

We utilized a faces task known to reliably activate the amygdala (Weng et al., 2011). During image acquisition, participants were instructed to identify the gender of emotional and neutral faces from NimStim (model numbers: 1, 7, 10, 12, 15, 16, 17, 20, 23, 25, 30, 34, 38, 40, and 42; Tottenham, et al., 2009). Each model was pictured four times, showing sad, happy, fearful, and neutral expressions. Half of the models were male, and half were female. Eight of

the models were European American, 4 were African American, and 3 were Asian American. Prior to the MRI scan, participants practiced the task with different faces in a mock scanner.

Each trial consisted of a fixation cross displayed for 500 ms, followed by a face for 250 ms, and a blank screen for 1500 ms. Any time after the face appeared, participants pressed a button with their right hand to indicate whether the face was male or female. I minimized group differences in attention to the faces by presenting the face very briefly (250 ms) and having participants do a task (identify gender) immediately following the face presentation. Inter-trial intervals were jittered between 0 ms and 6000 ms at intervals of 2000 ms. The blank screen displayed between trials served as baseline. E-prime (Psychological Software Tools, Pittsburgh, PA) presented stimuli and recorded responses. Sixty trials (15 trials of each emotion) were presented in a different randomized order for each participant. No picture (model displaying a particular emotion) was presented more than once.

fMRI Data Acquisition

Details on MRI acquisition have been previously published (Weng, et al., 2011). High resolution spoiled gradient images and T_2*-weighted blood oxygen level dependent (BOLD) images, using a reverse spiral sequence (Glover & Law, 2001) to ensure maximum coverage of the amygdala, were acquired.

FMRI Data Analysis

Data Preprocessing

The University of Michigan fMRI Center's standard pre-processing procedure was applied to the functional images, which includes removing outliers from the raw k-space data, reconstructing the k-space data to image space, applying a field map correction to reduce artifacts from susceptibility regions, and correcting for slice timing. To address head motion, functional images were realigned to the 10^{th} image (see Monk, et al., 2010 for details).

Using SPM8 (Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk), anatomical images were co-registered to the functional images.

Functional images were smoothed using an isotropic 8 mm full width at half maximum Gaussian kernel and normalized to the Montreal Neurological Image space.

Contrast Images for Habituation

We examined habituation by comparing activation to early faces (in the first half of the run) to activation to late faces (the second half of the run). This approach allowed us to control for all other activity related to the viewing of faces or specific face models, yielding differences in activation between early faces and late faces due to the timing of the faces. Habituation occurs if activation to late faces is less than activation to early faces; the converse is sensitization.

Face conditions were modeled with SPM8's canonical hemodynamic response function. The individual-level model included separate regressors for each of the face emotions. Additionally, trials in which gender was incorrectly identified were modeled as a separate regressor and excluded from further analyses. Two contrast images were generated for each participant, early sad faces versus baseline and late sad faces versus baseline, by estimating the contrast value at every voxel. These images, which convey how much activation differed between the two conditions (seeing either early or late sad faces versus a blank baseline screen) at every voxel in the brain for that individual, were then used in group-level analyses.

Group-Level Analyses

The images for early sad faces versus baseline and late sad faces versus baseline were then entered into second-level analyses in SPM8. To address our hypothesis, a voxel-wise model was created to examine the three-way interaction of genotype (low (S/S, S/L_G, L_G/L_G) versus higher expressing (L_A/L_A, S/L_A, L_A/L_G)) by diagnosis (ASD versus control) by run half (early sad faces versus late sad faces). Genotype and diagnosis were between subjects factors, and run half was a within subjects factor. All lower order terms, as well as the three-way interaction, were included in the model. A *t* contrast was used in the group level model to assess the beta of the three-way interaction. A small volume correction using the bilateral amygdala from the AAL atlas in the Wake Forest Pickatlas (Maldjian, et al., 2002) was applied to test the three-way interaction. This small volume correction restricted the search for voxels

with a significant interaction to the bilateral amygdala and applied a family-wise error correction based on the size of the bilateral amygdala (Worsley, et al., 1996).

Post-hoc analyses were conducted to characterize the interaction in SPSS. Values from a 4mm sphere around the peak voxel (xyz = -30, -6, -14) were extracted and averaged for the image representing the first half of the run and the image representing the second half of the run, then exported to SPSS. The low and higher expressing genotypes were compared on habituation scores within the ASD and control groups, and individuals with ASD and controls were compared within the low and higher expressing groups. Significance testing was corrected with the Holm-Bonferroni method with an initial α of 0.05/4 = 0.0125 (Holm, 1979). Post-hoc tests were also performed to compare the activation change from the early faces to late faces for the four groups.

Emotion Recognition Task (Outside Scanner)

After the scan, participants also performed a computer task to assess accuracy in identifying emotional facial expressions. The same face stimuli were used in the fMRI task, as well as an additional 15 faces from NimStim (Tottenham, et al., 2009). Each trial consisted of a 500 ms fixation cross, then a face for 250 ms, followed by an instruction screen asking participants to indicate the emotion of the face by pressing a button corresponding to fearful, happy, sad, or neutral. There were 120 trials, 30 of each emotion.

Results

The four groups (individuals with ASD and low expressing genotypes, individuals with ASD and higher expressing genotypes, controls with low expressing genotypes, and controls with higher expressing genotypes) did not differ in their accuracy ($F_{1,103} = 1.261$, $p = 0.264$, controlling for age and gender) nor in their reaction time ($F_{1,101} = 2.512$, $p = 0.116$, controlling for age and gender) to identify face gender during the faces task in the scanner. In the emotion recognition task outside the scanner, the four groups did not differ in accuracy to identify sad faces ($F_{1,99} = 0.009$, $p = 0.923$, controlling for age and gender). Neither did they significantly differ in accuracy to identify other emotions (fearful: $F_{1,99} = 0.001$, $p = 0.970$; happy: $F_{1,99} = 1.155$, $p = 0.285$; neutral: $F_{1,99} = 1.504$, $p = 0.223$; each analysis controlling for age and gender).

The number of faces shown in the first half versus second half of the run did not differ across the four groups for sad ($F_{1,105}$ = 0.448, p = 0.505), fearful ($F_{1,105}$ = 0.732, p = 0.394), happy ($F_{1,105}$ = 0.395, p = 0.531), and neutral ($F_{1,105}$ = 1.234, p = 0.269) faces. Cognitive functioning did not differ across the four groups, and individuals with the low and higher expressing genotypes did not differ on symptom measures within both the control group and the ASD group (Table 2.2).

Our hypothesis, that the relationship between 5-HTTLPR genotype and amygdala habituation to sad faces differs in the ASD group versus controls, was confirmed. There was a significant genotype-by-diagnosis-by-run half interaction predicting left amygdala activation to sad faces (xyz = -30, -6, -14, cluster size = 27, t_{210} = 3.31, p = 0.023, corrected for multiple comparisons within bilateral amygdala; Figure 2.1). Specifically, the impact of 5-HTTLPR genotype on amygdala habituation was different for individuals with ASD versus controls. Post-hoc analyses indicated that individuals with ASD and low expressing genotypes failed to habituate and displayed a trend toward sensitization (i.e., greater activation to late faces compared to early faces, p = .065). Additionally, individuals with ASD and low expressing genotypes had greater increases in amygdala activation from the early to late sad faces compared to individuals with ASD and higher expressing genotypes (p = 0.012) as well as controls with low expressing genotypes (p = 0.013).

Other Emotion Contrasts

To determine whether the hypothesized effect was specific to sad faces, additional analyses to examine potential genotype-by-diagnosis-by-run half interactions were conducted with the other emotional face types. I reran the model using early and late faces for fearful versus baseline, happy versus baseline, and neutral versus baseline images. None of these models yielded significant voxels within the bilateral amygdala for the genotype-by-diagnosis-by-run half interaction (fearful: xyz = -28, 4, -18, t_{210} = 2.29, p = 0.215; happy: xyz = -26, 4, -22, t_{210} = 2.29, p = 0.249; neutral: xyz = -26, -8, -12, t_{210} = 1.40, p = 0.701, all corrected for multiple comparisons within the bilateral amygdala).

Additional Analyses

In imaging and genetic studies with disordered populations, head motion, developmental differences, population stratification, psychotropic medication status, and allele grouping are potential factors influencing associations. Thus, additional analyses were performed to determine whether these factors account for our main result, a significant genotype-by-diagnosis-by-run half interaction for sad faces. To summarize, when taking into account each of these factors, the results still stood. Details on the analyses are in Supplemental Results (p. 38).

Discussion

This is the first study, to our knowledge, to examine genetic influences on amygdala function in ASD. I found that 5-HTTLPR impacted changes in amygdala response to repeated sad face presentation differently in individuals with ASD compared to controls. Specifically, whereas our previous work found that, overall, individuals with ASD fail to habituate to sad faces (Swartz, et al., 2013), the present study found that the degree to which individuals with ASD fail to habituate to sad faces depends on genotype. Individuals with ASD and low expressing genotypes failed to habituate to the sad faces and in fact displayed a statistical trend toward sensitization, or increase in activation over time; these individuals sensitized more than individuals who also have ASD but with higher expressing genotypes.

Our finding of lack of habituation and a trend toward increased sensitization to sad faces in the individuals with ASD and low expressing genotypes provides support for the theory that individuals with ASD experience faces as aversive (Weng, et al., 2011). In avoiding faces, individuals with ASD may miss opportunities to develop social skills and maintain deficits in social communication. Our results suggest that this mechanism by which social impairment develops and is maintained is genetically influenced. Specifically, previous findings of sensitization (Swartz, et al., 2013) and lack of habituation (Kleinhans, et al., 2009) in the ASD group may be driven by individuals with ASD and the low expressing genotype.

The failure to habituate in individuals with ASD and the low expressing genotype was specific to sad faces; this effect was not found in fearful, happy, or neutral faces. It is possible

that amygdala response increases to sad faces because they are more ambiguous for those individuals to interpret. The amygdala is known to activate in ambiguous situations (Hsu, et al., 2005). However, in our study, groups did not differ on accuracy to identify the emotion in sad faces, providing evidence against the idea that individuals with ASD and low expressing genotypes experienced sad faces as more ambiguous. Of note, the face task inside the scanner involved implicit processing of the emotion (instructions were to identify the gender of the face), whereas the face task outside the scanner required explicit processing (instructions were to identify the emotion on the face). It is possible that individuals with ASD can correctly identify sad emotion faces when explicitly instructed to do so, but have difficulties implicitly processing the same stimuli. Another explanation for the failure to habituate is that individuals with ASD and low expressing genotypes find sad faces either anxiety provoking or distressing. However, self-report (MASC, OCI-R, CDI) and parent-report (CBCL-Internalizing) anxiety and depression symptom measures were not significantly correlated with amygdala habituation within the ASD group (MASC: r = -0.195, p = 0.234; OCI-R: r = -0.159, p = 0.327; CDI: r = -0.174, p = 0.259; CBCL-Internalizing: r = -0.206, p = 0.201) or the control group (MASC: r = 0.062, p = 0.663; OCI-R: r = 0.116, p = 0.358; CDI: r = -0.068, p = 0.589; CBCL-Internalizing: r = -0.078, p = 0.543). These findings are consistent with Swartz et al (2013; overlapping sample). Although our study was not designed to investigate why sad faces in particular might be an effective probe for group differences, I offer the following possibility regarding the internal experiences of the emotional faces, which could be evaluated in future research. When confronted with happy or fearful faces, the social protocol is clearer: happy faces are an invitation to socially interact with the other person, and fearful faces are a sign to scan the environment for threat. However, the social protocol for sad faces is less clear. When confronted with a sad person, should one comfort them or give them space? Not knowing exactly what the social protocol is may be distressing or anxiety provoking, particularly for individuals with ASD. Perhaps because dealing with sad faces can be difficult even for typically developing individuals, this is the probe that revealed group differences. Future research should explore these possibilities to better understand the role of sad faces in genetically-influenced lack of habituation and sensitization in ASD.

Of note, individuals with ASD and the low versus higher expressing genotypes did not differ on any of the symptom measures nor on accuracy or reaction times in the fMRI task, although it is important to note that as a forced-choice task (e.g., identify male or female gender), performance may be inflated. Moreover, the genotype groups within ASD did not differ on DSM-IV-TR diagnoses (Fisher's Exact Test, $p = 0.242$). Participants did differ on brain activation patterns however, such that individuals with ASD and higher expressing genotypes failed to habituate but did not sensitize as much as individuals with ASD and the low expressing genotypes. When groups are equivalent on the behavioral measures, it suggests that genotype is not simply acting as a proxy for symptoms. The brain differences I found in the absence of statistically significant differences on the symptom measures speak to the possibility that the brain measures may have been more sensitive to genetic effects than current parent or self-report measures. Our study, which examined individuals homogenous in terms of parent or self-report symptom measures but heterogeneous in terms of brain and genetic profiles, represents a step toward identifying subtypes based on brain and genetic profiles within ASD. Moreover, the development of more finely tuned behavioral measures and tasks, used in combination with brain and genetic information, may aid identification of subtypes. Identifying subtypes is important in heterogeneous disorders like ASD to tease apart multiple pathways to developing the disorder, as subtypes may represent different etiologies for the same disorder. Additionally, different subtypes may be associated with different prognoses and treatment responses. Longitudinal analyses will be necessary to determine what the outcomes are for individuals with ASD and low compared to high expressing genotypes. If individuals who display sensitization to sad faces are more suited toward some medical and behavioral treatments, early identification based on genotype could increase the efficacy of treatment plans.

This study has several limitations. First, our sample size is modest. Within the ASD group, I had 15 individuals with low expressing and 29 with higher expressing genotypes, and 22 controls with low and 43 with higher expressing genotypes. This sample size is comparable to other 5-HTTLPR and neuroimaging studies with controls (e.g., 15 low and 15 high expressing adults, 31 lower and 20 high expressing children, 13 lower and 6 high expressing children in Battaglia, et al., 2011, respectively; Roiser, et al., 2009; Thomason, et al., 2010) and with an ASD

sample (two cohorts from different sites: 6 low and 23 higher expressing children, 3 low and 12 higher expressing children in Wassink, et al., 2007). However, replication with a larger sample is necessary to make our results more generalizable.

Second, our groups differed in age and pubertal status. Because of this, I covaried age as well as pubertal status to determine whether development accounted for the genotype-by-diagnosis-by-run half interaction predicting amygdala response. I found that even after controlling for these developmental measures, our results still stood, which makes it unlikely that age and puberty are driving our results (Supplemental Results, p. 38).

Third, our groups differed in mean head motion as calculated according to Van Dijk et al (2012). However, when removing variance associated with head motion, our hypothesis was still confirmed (Supplemental Results, p. 38).

The present study lays a foundation for future studies to better understand the brain and genetic mechanisms involved in the etiology and maintenance of ASD symptoms. I found that individuals with ASD and low expressing genotypes did not display habituation to repeated sad faces; conversely, they exhibited a trend toward sensitization, unlike individuals with ASD and higher expressing genotypes and controls of any 5-HTTLPR genotype. Future research could expand on these findings by designing studies to understand amygdala habituation and sensitization within the context of a network, using functional connectivity and diffusion tensor imaging tools. Additionally, future researchers may wish to include measures of stress and the individuals' environments, as 5-HTTLPR may act in conjunction with environmental input (Belsky, et al., 2009). Such studies could be used to examine potential gene-by-environment interactions in predicting amygdala habituation and sensitization in ASD. To conclude, the findings from our study open a path to better understand genetic influences on brain function in ASD.

Table 2.1. Participant characteristics.

	Autism Spectrum Disorders Group						Control Group					
	Low Expressing Genotypes			Higher expressing Genotypes			Low Expressing Genotypes			Higher expressing Genotypes		
	S/S	S/L_G	L_G/L_G	L_A/L_A	S/L_A	L_A/L_G	S/S	S/L_G	L_G/L_G	L_A/L_A	S/L_A	L_A/L_G
Number of participants	10	4	1	8	20	1	19	2	1	20	22	1
Total N	15			29			22			43		
Gender (F:M)	1 : 14			4 : 25			5 : 17			11 : 32		
Handedness* (L:R)	3 : 11			4 : 20			4 : 18			4 : 36		
fMRI task accuracy	95.2% (4.17%)			94.9% (5.00%)			95.9% (4.53%)			96.6% (3.56%)		
fMRI task RT (ms)	799 (159)			771 (125)			687 (131)			797 (148)		
DSM-IV-TR Diagnosis	10 AD; 5 AS			23 AD; 4 AS			N/A			N/A		
Age	12.9 (2.37)			14.1 (2.24)			15.3 (1.77)			14.1 (3.28)		
Verbal CF	115 (25.3)			111 (18.5)			114 (13.2)			114 (14.1)		
Nonverbal CF	109 (18.7)			104 (20.9)			105 (10.6)			100 (14.0)		
SRS	73.9 (11.9)			77.1 (11.3)			44.5 (7.51)			42.5 (6.95)		
SCQ	18.8 (7.20)			20.8 (7.02)			3.0 (2.55)			3.2 (4.15)		

CDI	7.67 (5.18)	8.62 (6.07)	5.41 (3.67)	4.44 (5.37)
CBCL-Internal	63.4 (8.71)	63.4 (9.01)	46.3 (9.17)	46.4 (8.82)
OCI-R	20.0 (16.4)	17.0 (11.1)	10.6 (8.02)	10.1 (8.88)
MASC	42.5 (21.6)	45.6 (16.2)	32.1 (13.3)	31.2 (15.3)
Caucasian	93%	93%	64%	77%

*Nine individuals missing handedness data, 4 missing non-verbal cognitive functioning, 1 missing SRS, 8 missing SCQ, 2 missing RT. 2 missing clinical consensus diagnostic category for DSM-IV-TR due to data failure; however, all participants received an ASD diagnosis via clinical consensus and met cutoffs for autism spectrum on both the ADI-R and ADOS.

Means and standard deviations (in parentheses) reported. fMRI task accuracy = accuracy in identifying gender of all emotional or neutral faces, fMRI task RT = reaction time in milliseconds to identify gender of all emotional or neutral faces, AD = Autistic Disorder, AS = Asperger Syndrome, CF = cognitive functioning, SRS = Social Responsiveness Scale, SCQ = Social Communication Questionnaire – Lifetime, CDI = Children's Depression Inventory, CBCL-Internal = Internalizing subscale of the Child Behavior Checklist, OCI-R = Obsessive-Compulsive Inventory-Revised, MASC = Multidimensional Anxiety Scale for Children. Table 2.2 contains more details on subject characteristics.

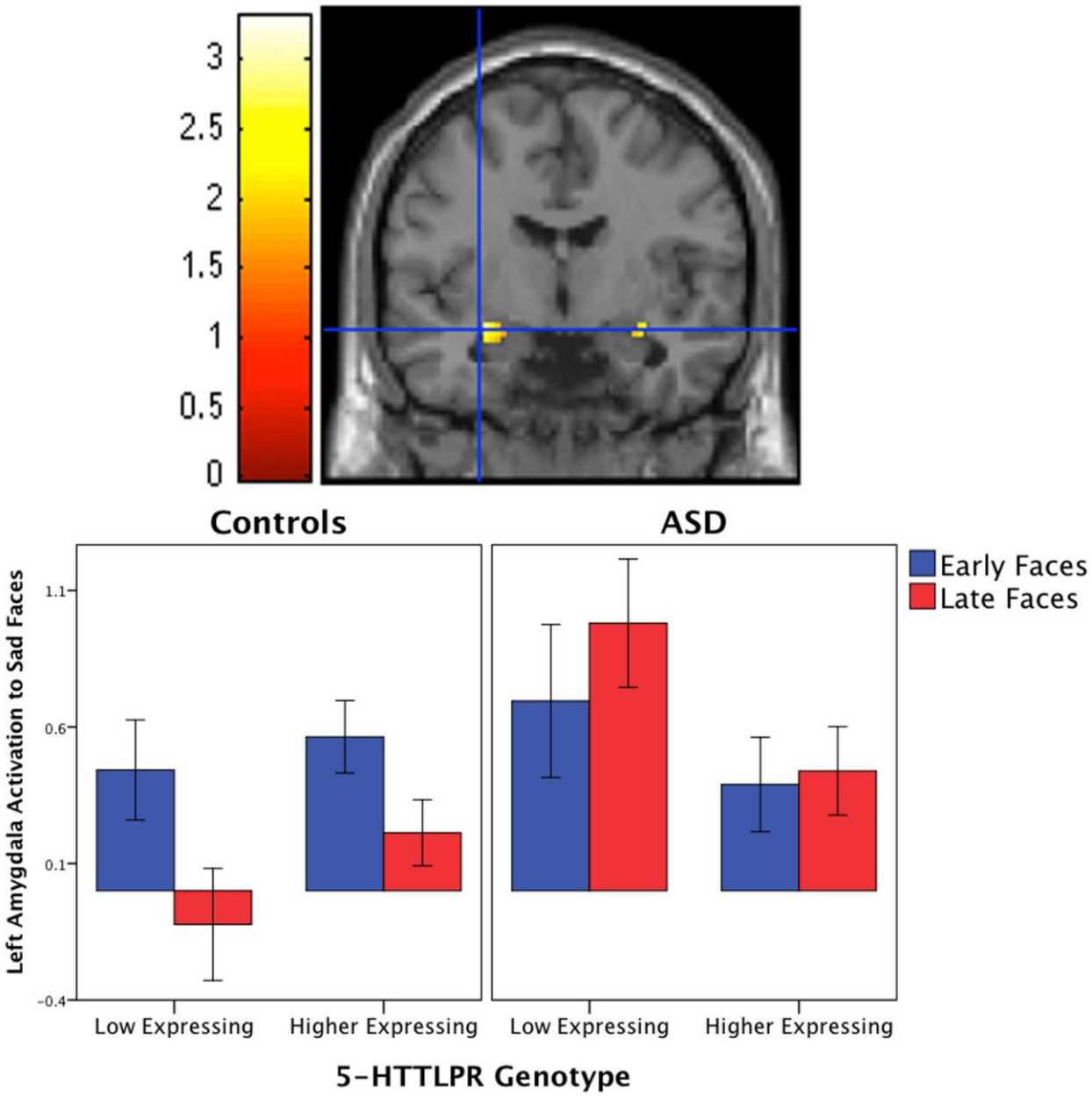


Figure 2.1. Impact of 5-HTTLPR genotypes on amygdala habituation is different in youth with autism spectrum disorders versus controls.

A significant genotype-by-diagnosis-by-run half interaction in the amygdala is depicted in the coronal section of the brain (upper). Color on brain image indicates places where change in response to face presentation early in the task versus later in the task is differentially influenced by 5-HTTLPR in the ASD group compared to controls. For illustration purposes, the threshold was set at $p < 0.05$ and the image masked for the bilateral amygdala. Crosshairs are at the peak voxel (xyz = -30, -6, -14). Contrast values from the whole left amygdala were extracted and plotted (lower). Higher expressing 5-HTTLPR genotypes consisted of L_A/L_A, S/L_A, and L_A/L_G; low expressing 5-HTTLPR genotypes consisted of S/S, S/L_G, and L_G/L_G. Error bars indicate standard error of the mean.

Table 2.2. Detailed participant characteristics.

	Autism Spectrum Disorders Group																	
	Low Expressing Genotypes			Higher Expressing Genotypes						Low Expressing Genotypes			Higher Expressing Genotypes					
	S/S	S/L_G	L_G/L_G	L_A/L_A	S/L_A	L_A/L_G				S/S	S/L_G	L_G/L_G	L_A/L_A	S/L_A	L_A/L_G			
Number of participants	10	4	1	8	20	1				19	2	1	20	22	1			
Total N	15			29						22			43					
Gender (F:M)	1:14			4:25						5:17			11:32					
Handedness* (L:R)	3:11			4:20						4:18			4:36			F	df	p
fMRI task accuracy	95.2% (4.17%)			94.9% (5.00%)						95.9% (4.53%)			96.6% (3.56%)			0.311	1,103	0.264
fMRI task RT	799 (159)			771 (125)						687 (131)			797 (148)			2.51	1, 101	0.116
Age	12.9 (2.37)			14.1 (2.24)						15.3 (1.77)			14.1 (3.28)			4.71	1,105	0.032
Puberty	1.99 (0.93)			2.58 (1.00)						3.09 (0.60)			2.41 (1.01)			11.1	1,104	0.001
Verbal CF	115 (25.3)			111 (18.5)						114 (13.2)			114 (14.1)			0.39	1,105	0.534
Nonverbal CF	109 (18.7)			104 (20.9)						105 (10.6)			100 (14.0)			0.004	1,101	0.949
							t	df	p							t	df	p
CDI	7.67 (5.18)			8.62 (6.07)			0.518	42	0.607	5.41 (3.67)			4.44 (5.37)			0.497	43	0.622
OCI-R	20.0 (16.4)			17.0 (11.1)			0.690	38	0.495	10.6 (8.02)			10.1 (8.88)			0.796	39	0.431
MASC	42.5 (21.6)			45.6 (16.2)			0.502	37	0.618	32.1 (13.3)			31.2 (15.3)			0.333	38	0.741

CBCL - Internal	63.4 (8.71)	63.4 (9.01)	0.000	38	1.000	46.3 (9.17)	46.4 (8.82)	0.292	39	0.772
CBCL - External	52.4 (8.92)	56.7 (12.0)	1.196	38	0.239	46.3 (6.65)	43.0 (8.31)	1.28	39	0.207
CBCL Total	61.4 (8.09)	64.7 (8.90)	1.167	38	0.251	46.9 (8.53)	43.6 (8.50)	1.09	39	0.283
SRS	73.9 (11.9)	77.1 (11.3)	0.857	42	0.396	44.5 (7.51)	42.5 (6.95)	0.833	43	0.409
SCQ	18.8 (7.20)	20.8 (7.02)	0.842	36	0.405	3.0 (2.55)	3.2 (4.15)	0.847	37	0.403
Caucasian	93%	93%				64%	77%			

*Nine individuals missing handedness data, 5 missing accuracy scores, 1 missing puberty, 4 missing non-verbal cognitive functioning, 1 missing CDI, 4 missing OCI-R, 8 missing MASC, 6 missing CBCL, 1 missing SRS, 8 missing SCQ, 2 missing RT.

Means and standard deviations (in parentheses) reported. fMRI task accuracy = accuracy in identifying gender of all emotional or neutral faces, fMRI task RT = reaction time in milliseconds to identify gender of all emotional or neutral faces, Puberty = Pubertal Development Scale (Petersen, et al., 1988), CF = cognitive functioning (see below), CDI = Children's Depression Inventory (Kovacs, 1992), OCI-R = Obsessive Compulsive Inventory – Revised (Foa, et al., 2010), MASC = Multidimensional Anxiety Scale for Children (March, et al., 1997), CBCL = Child Behavior Checklist (Achenbach & Edelbrock, 1981), CBCL Internal = Child Behavior Checklist – Internalizing Subscale, CBCL External = Child Behavior Checklist – Externalizing Subscale, SRS = Social Responsiveness Scale (Constantino, et al., 2003), SCQ = Social Communication Questionnaire – Lifetime (Rutter, et al., 2003), Caucasian = self-reported Caucasian descent.

Cognitive Functioning: The Peabody Picture Vocabulary Test (Dunn & Dunn, 1997) and the Ravens Progressive Matrices (Raven, 1960) were utilized to assess cognitive functioning in controls; participants with ASD were given these measures or the Differential Ability Scales II – School Age (Elliott, 2005), the Stanford-Binet Intelligence Scales (Roid, 2003), the Wechsler Intelligence Scale for Children IV (Wechsler, 2003), or the Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999).

fMRI Task Accuracy: Before calculating accuracy, trials in which reaction time was less than 100 ms or greater than 5000 ms were excluded.

Supplemental Results

In imaging and genetic studies with individuals who have clinical or developmental disorders, head motion, developmental differences, population stratification, psychotropic medication status, and allele grouping are potential factors influencing associations. Because of this, additional analyses were performed to determine whether these factors account for our main result, which is a significant genotype-by-diagnosis-by-run half interaction for sad faces. Values from a 4 mm sphere surrounding the peak voxel from our main finding (xyz = -30, -6, -14) were extracted and averaged. The change in activation from early to late faces was calculated for each person, then exported to SPSS for additional analyses (examining a genotype-by-diagnosis interaction, as run half is accounted for with the difference score). This approach allowed us to constrain our additional analyses to the locus of effects within the amygdala in our main finding, examining whether these potential confounds account for our main result.

First, because mean head motion, calculated as in Van Dijk (2012), differed among the four groups ($F_{1,105} = 10.9$, $p = 0.001$), I re-ran the model covarying mean head motion. When removing variance associated with head motion, our hypothesis was still confirmed: the genotype-by-diagnosis interaction significantly predicts habituation to sad faces ($t_{104} = 2.712$, $p = 0.025$).

Second, development is a potential confounding factor in our sample as the four groups differed in age and pubertal development (age: $F_{1,105} = 4.71$, $p = 0.032$; puberty: $F_{1,104} = 11.1$, $p = 0.001$; see Table 2.2). However, when rerunning the models with age and pubertal status as covariates, the hypothesis was still confirmed (genotype-by-diagnosis interaction covarying age: $t_{104} = 3.111$, $p = 0.002$; and covarying pubertal development: $t_{103} = 2.793$, $p = 0.006$).

Third, to determine whether our findings were primarily driven by non-Caucasian individuals in our sample, I excluded 3 of 44 participants with ASD and 18 of 65 controls who self-reported as non-Caucasian and re-ran the analyses. When including only 41 Caucasian individuals with ASD and 47 controls, our hypothesized genotype-by-diagnosis interaction predicting habituation to sad faces was still significant ($t_{84} = 2.285$, $p = 0.025$).

Fourth, medication usage among individuals with ASD is very high (Oswald & Sonenklar, 2007) and could potentially influence brain activation findings. Accordingly, 19 individuals with ASD taking psychotropic medications and one control taking levothyroxine were excluded and analyses were repeated. Even with 25 non-medicated individuals with ASD and 64 non-medicated controls, our hypothesized genotype-by-diagnosis interaction remained significant (t_{85} = 2.512, p = 0.014).

Last, additional analyses were conducted with alternate genotype groupings – low (S/S, S/L_G, L_G/L_G) versus medium (S/L_A, L_A/L_G) vs high expressing (L_A/L_A) genotypes as well as S/S versus intermediate genotypes (S/L_A, S/L_G, L_G/L_G, L_A/L_G) versus L_A/L_A – to assess whether the findings persisted when participants were split into different genotype groups. The genotype-by-diagnosis interaction predicting habituation to sad faces persisted even when genotypes were grouped different ways in the statistical analyses (interaction of diagnosis with low vs. medium vs. high expressing genotypes: $F_{2,103}$ = 4.479, p = 0.014; and with S/S vs. intermediate genotypes vs. L_A/L_A: $F_{2,103}$ = 5.070, p = 0.008).

CHAPTER 3 ‡

Context-Dependent Amygdala-Prefrontal Connectivity in Youth with Autism Spectrum Disorders

Summary

Objective: The amygdala and prefrontal cortex are involved in processing responses to socio-emotional cues and may thus mediate social impairment symptoms in autism spectrum disorders (ASD). However, it is unknown if amygdala-prefrontal connectivity is altered in ASD only in the presence of stimuli requiring overt socio-emotional processing (such as faces) or also altered in the absence of a task. I tested whether alterations in amygdala-ventral prefrontal connectivity in youth with ASD compared to controls would differ or be the same by context (faces task versus rest). **Method:** Forty-five youth with ASD and 65 healthy controls, aged 8-19 years, performed an emotional faces task and underwent a resting acquisition in an MRI scanner. Amygdala connectivity was calculated for each individual during both contexts. **Results:** Alterations in amygdala-ventrolateral prefrontal connectivity in ASD differ in the faces task versus rest (context-by-diagnosis interaction, xyz = -42, 28, -8, k = 85, $t_{206} = 3.56$, $p = 0.043$ corrected). Relative to controls, the ASD group has weaker amygdala-ventrolateral prefrontal connectivity during the faces task ($p = .026$) but greater connectivity during rest ($p = .039$). Moreover, controls show decreased ($p = .013$) connectivity during rest compared to during the faces task, but youth with ASD show increased ($p = .010$) connectivity during rest versus the faces task. **Conclusions:** Findings suggest that ASD in youth is characterized by inappropriate modulation of amygdala-ventrolateral prefrontal connectivity across different contexts. Understanding context-dependent brain alterations in ASD may help to disambiguate the brain mechanisms subserving social impairment and provide targets for treatment.

‡ Chapter 3 corresponds to the publication Wiggins and colleagues (in preparation-b).

Introduction

Together, the amygdala and prefrontal cortex are involved in evaluating and processing responses to socio-emotional cues in the environment. A structure integral to salience detection (Davis, 1999; Davis & Whalen, 2001; LeDoux, 1996; LeDoux, 2000), the amygdala robustly activates in response to emotional faces and other socio-emotional stimuli (Sabatinelli, et al., 2011). Structural studies in adult humans and animal models indicate that the prefrontal cortex and amygdala form a circuit via reciprocal connections (Carmichael & Price, 1995; Ongur & Price, 2000; Sarter & Markowitsch, 1984). Regulation of the amygdala is thought to occur primarily through the ventral portion of the prefrontal cortex, which includes the anterior cingulate and orbitofrontal cortices (Ray & Zald, 2012). Amygdala-prefrontal circuitry may thus mediate social impairment symptoms in autism spectrum disorders (ASD; APA, 1994).

Individuals with ASD exhibit altered functional connectivity between the amygdala and ventromedial prefrontal cortex when performing tasks with emotional faces. First, adults with ASD show greater positive connectivity of the amygdala with the ventromedial prefrontal cortex compared to healthy controls when viewing happy faces versus neutral faces in an attention cuing task (Monk, et al., 2010). Second, adolescents with ASD have reduced amygdala-ventromedial prefrontal connectivity when viewing sad faces (Swartz, et al., 2013). Moreover, whereas greater amygdala-ventromedial prefrontal connectivity when viewing sad faces relates to more amygdala habituation to sad faces for controls, youth with ASD fail to show this relationship between amygdala-prefrontal connectivity and amygdala function (Swartz, et al., 2013). Third, in a Stroop-like task with emotional faces, youth with ASD demonstrate altered connectivity between the amygdala and portions of the prefrontal cortex depending on trial type: during trials in which the Stroop cues are congruent, amygdala-dorsal anterior cingulate cortex connectivity is increased in ASD, but for trials where the Stroop cues are incongruent, amygdala-subgenual anterior cingulate cortex connectivity decreases in the ASD group (Murphy, et al., 2012a). These mixed findings of reduced and increased amygdala-ventromedial prefrontal connectivity in ASD may be due to the variety of face tasks utilized.

In addition to the ventromedial prefrontal cortex, the lateral portion of the ventral prefrontal cortex is also implicated in ASD and social dysfunction cutting across developmental

disorders. In contrast to the ventromedial prefrontal cortex, which is thought to relate to automatic processes involving the amygdala, the ventrolateral prefrontal cortex may relate to voluntary regulation of amygdala responses (Phillips, et al., 2008; Ray & Zald, 2012). Adults with ASD show reduced activation in the ventrolateral prefrontal cortex and amygdala when performing a social judgment task (rating trustworthiness of faces; Pinkham, et al., 2008). Moreover, children with ASD display reduced ventrolateral prefrontal activation compared to controls when looking at faces with direct versus averted gazes (Davies, et al., 2011) and after a social exclusion task (Masten, et al., 2011). The ventrolateral prefrontal cortex also demonstrates altered connectivity with the amygdala in other pediatric disorders that feature social dysfunction: First, adolescents at high risk for schizophrenia, a disorder that includes socio-emotional deficits, show decreased amygdala-ventrolateral prefrontal cortex connectivity when labeling emotional expressions on faces (Gee, et al., 2012). Next, adolescents with social anxiety exhibit greater positive connectivity between the right amygdala and left ventrolateral prefrontal cortex compared to controls when assessing how they would be socially evaluated by peers they had previously rated as low desirability (Guyer, et al., 2008a). However, when opportunities for elaborative, strategic, or regulatory processing in response to angry faces are limited via extremely short (17 millisecond), masked presentation, children with generalized anxiety disorder (which includes social anxiety features) have weaker amygdala-ventrolateral prefrontal cortex connectivity (Monk, et al., 2008). Taken together, the studies reviewed here indicate that amygdala-ventral prefrontal cortex connectivity may be relate to social impairment and is altered in ASD, although whether connectivity is increased or decreased may depend on the particular socio-emotional task.

To date, all studies on amygdala-prefrontal connectivity on children and adolescents with ASD have examined connectivity in the context of a social task with emotional face stimuli. However, amygdala-prefrontal connectivity continues even in the absence of a task (i.e., "rest"; Roy, et al., 2009). Alterations in amygdala-prefrontal connectivity when individuals with ASD are undirected and not required to perform a task to isolate socio-emotional function may be indicative of pervasive deficits (Prater, et al., 2012). One study on adults with ASD found reduced ventromedial prefrontal connectivity with the amygdala during rest (von dem Hagen,

et al., 2012). Studies on amygdala-ventral prefrontal connectivity during rest in social anxiety in adults were mixed: one found increased connectivity (Liao, et al., 2010), two found decreased connectivity (Hahn, et al., 2011; Prater, et al., 2012), and one failed to find significant differences in connectivity (Ding, et al., 2011).

The amygdala is commonly thought to confer liabilities and the prefrontal cortex to provide suppression of those liabilities; thus, decreased amygdala-prefrontal connectivity would be detrimental. However, this conceptualization of the amygdala and prefrontal cortex has recently faced challenges (Pfeifer & Allen, 2012). Instead, whether decreased amygdala-prefrontal connectivity is adaptive or maladaptive may depend on context (Pfeifer & Allen, 2012). Only one study directly compared connectivity across contexts; this study found that adults with social anxiety show decreased amygdala-rostral anterior cingulate connectivity during both a faces task as well as during rest (Prater, et al., 2012).

No studies, however, have examined amygdala-prefrontal resting connectivity in youth. Moreover, it is unknown if amygdala-prefrontal connectivity is altered only in the presence of stimuli requiring overt socio-emotional processing (such as faces) or altered both in a socio-emotional task and in the absence of a task. Understanding the circumstances under which brain connectivity is altered in ASD would have an impact on how connectivity findings are interpreted and future studies are designed. If amygdala-prefrontal connectivity alterations in ASD differ in the socio-emotional task versus rest, this would provide evidence that connectivity alterations are an evoked phenomenon that is neither inherently maladaptive or adaptive but rather context-dependent. Conversely, if the same alterations in connectivity are found across contexts (e.g., either increased or decreased in both a socio-emotional task and in the absence of a task), this would provide evidence for a pervasive, spontaneous, task-independent brain disturbance in ASD. I tested these two competing hypotheses – that alterations in amygdala-ventral prefrontal connectivity in youth with ASD compared to controls would either differ or be the same depending on the context.

Methods

Participants

Forty-five children and adolescents with ASD and 65 healthy controls, aged 8 to 19 years, were included (Table 3.1). Of 103 participants with ASD and 86 controls, 58 participants with ASD and 21 controls were excluded from all analyses because of head movement exceeding 2.5 mm translation or degrees rotation in any frame compared to the first, inability to complete either the emotional face task or the resting acquisition in the MRI, scoring less than 80% accuracy in identifying gender in the face task, or technical problems with the MRI. Individuals were excluded if they had braces, medical conditions contraindicated for MRI, or history of seizures or neurological disorders.

Controls were recruited through flyers posted at local organizations. Participants with an ASD (Autistic Disorder, Asperger's syndrome, or Pervasive Developmental Disorder – Not Otherwise Specified) were referred to our fMRI study by clinicians at the University of Michigan Autism and Communication Disorders Center, where diagnoses were made using the Autism Diagnostic Observation Schedule (ADOS; Lord, et al., 2000), the Autism Diagnostic Interview-Revised (ADI-R; Lord, et al., 1994), and clinical consensus (Lord, et al., 2006). The University of Michigan Institutional Review Board approved procedures. Parents gave written informed consent; juvenile participants gave written assent.

Participants completed self- and parent report symptom and behavioral measures (Table 3.1, Table 3.2). All control participants scored below clinical cutoffs for affected status. Prior studies utilized portions of this dataset (Swartz, et al., 2013; Weng, et al., 2011; Weng, et al., 2010; Wiggins, et al., 2012b; Wiggins, et al., 2011; Wiggins, et al., 2013).

Tasks in Scanner

Emotional Faces Task

Participants performed a faces task known to reliably activate the amygdala (Weng, et al., 2011) in the scanner. During image acquisition, participants were instructed to identify the gender of sad, happy, fearful, and neutral faces from NimStim (Tottenham, et al., 2009).

Each trial consisted of a fixation cross displayed for 500 milliseconds (ms), followed by a face for 250 ms, and a blank screen for 1500 ms. Any time after the face appeared, participants

pressed a button with their right hand to indicate whether the face was male or female. Group differences in attention to the faces were minimized by presenting the face very briefly (250 ms) and having participants do a task (identify gender) immediately following the face presentation. Inter-trial intervals were jittered between 0 ms and 6000 ms at intervals of 2000 ms. The blank screen displayed between trials served as baseline. E-prime (Psychological Software Tools, Pittsburgh, PA) presented stimuli and recorded responses. Sixty trials (15 trials of each emotion) were presented in a different randomized order for each participant. Prior to the MRI scan, participants practiced the task with different faces in a mock scanner.

Resting State Instructions

In addition to the faces task, participants underwent a resting state acquisition. During the 10-minute scan, participants were instructed to let their minds wander and not to think of anything in particular while looking at a fixation cross.

fMRI Data Acquisition

Details on MRI acquisition have been previously published for both the emotional face task (Weng, et al., 2011) and resting state (Wiggins, et al., 2012b). High resolution spoiled gradient images and T2*-weighted blood oxygen level dependent (BOLD) images, using a reverse spiral sequence (Glover & Law, 2001) to ensure maximum coverage of the amygdala, were acquired.

Physiological Noise Correction

Physiological data were collected during the resting state acquisition for subsequent noise correction. An abdominal pressure belt recorded respiratory rhythms, and a pulse oximeter on the participant's left middle finger recorded oxygenation. The physiological data were synchronized to the fMRI data.

fMRI Data Analysis

Data Preprocessing

The functional images underwent University of Michigan fMRI Center's standard pre-processing procedure (Monk, et al., 2010), including eliminating outliers from the raw k-space

data, reconstructing the k-space data to image space, utilizing a field map correction to reduce artifacts from susceptibility regions, and performing slice timing correction. To address head motion, functional images were realigned to the 10th image. Noise from cardiac and respiratory rhythms was removed using RETROICOR (Glover, et al., 2000).

Using SPM8 (Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk), anatomical images were co-registered to the functional images. Functional images were smoothed using an isotropic 8 mm full width at half maximum Gaussian kernel. To eliminate higher frequency sources of noise and to isolate the frequency band in which resting-state connectivity has previously been observed in functional MRI data, a low-pass filter with a 0.08 Hz cutoff was applied to the time courses from each voxel (Biswal, et al., 1995; Cordes, et al., 2000; Wiggins, et al., 2011).

Addressing Head Motion

Excessive head motion can introduce spurious correlations in connectivity analyses (Power, et al., 2012; Satterthwaite, et al., 2012; Van Dijk, et al., 2012). Thus, several steps were taken to address head motion in our sample beyond standard realignment of the functional images. First, any participant whose head motion exceeded 2.5 mm in the x, y, or z directions or 2.5 degrees in the roll, pitch, or yaw directions was excluded from all analyses. Second, I removed variance associated with head motion at the individual level by creating nuisance regressors from motion estimated in the x, y, z, roll, pitch, yaw directions and retaining the residuals for further processing. Third, I calculated mean and maximum motion for each person (mean absolute displacement of each volume as compared to the previous volume, as done previously; Van Dijk, et al., 2012) to test whether mean motion differed between the ASD and control groups. Moreover, at the group level, I repeated our analyses covarying mean and maximum head motion (Van Dijk, et al., 2012) to examine whether group differences in motion were primarily driving our results (Additional Analyses, p. 49).

Connectivity Images

In order for the images to be comparable, I calculated connectivity of the amygdala with the rest of the brain using the same method for both the faces task and the resting acquisition. Low-pass filtered BOLD time courses from voxels within the whole right amygdala were

extracted and averaged. The right amygdala was chosen as the seed because the right amygdala has been identified as the locus of functional alterations multiple times in individuals with autism spectrum disorders, (e.g., Dalton, et al., 2005; Hadjikhani, et al., 2007; Monk, et al., 2010). For each subject, this reference time course was then used as a seed to correlate with every other voxel in the brain. The resulting connectivity maps were normalized to Montreal Neurological Image (MNI) space by estimating the transformation matrix for the high resolution SPGR image to the MNI template image in SPM8, then applying this transformation to the connectivity maps. Pearson r values at every voxel in the connectivity image were converted to z values using Fisher's r to z transformation. The end product was two connectivity images for each subject, one image depicting connectivity with the right amygdala during the faces task and the other during rest.

Group-Level Analyses

The connectivity images were then entered into second-level analyses in SPM8. A voxel-wise mixed-design ANOVA was utilized to examine interaction of diagnosis (ASD versus control) by context (face task versus rest) as well as the main effect of diagnosis (regardless of context). Diagnosis was a between subjects factor, and context was a within subjects factor. To determine whether alterations in amygdala-prefrontal connectivity in ASD relative to controls differ by context, a *t* contrast tested the beta of the interaction term. To examine the alternative, that alterations in amygdala-prefrontal connectivity persist despite context, another *t* contrast tested the main effect of diagnosis, regardless of context. A small volume correction using the bilateral ventral prefrontal cortex was applied for both contrasts. Using the Wake Forest PickAtlas (Maldjian, et al., 2002), the ventral prefrontal cortex mask was created by adding the intersection between the inferior orbitofrontal gyrus and Brodmann's Area (BA) 47 to the intersection of the anterior cingulate cortex and BA 25. This mask represents the ventrolateral and ventromedial prefrontal areas that have previously been found to be sensitive to group differences in adolescents with social dysfunction (Gee, et al., 2012; Guyer, et al., 2008a; Monk, et al., 2008) and ASD in particular (Davies, et al., 2011; Masten, et al., 2011; Monk, et al., 2010; Murphy, et al., 2012a; Pinkham, et al., 2008; Swartz, et al., 2013). This small volume correction restricted the search for voxels with a significant context-by-diagnosis

interaction or main effect of diagnosis to the ventral prefrontal cortex and applied a family-wise error correction based on the size of the bilateral ventral prefrontal cortex (Worsley, et al., 1996).

Post-hoc analyses were conducted to characterize the interaction in SPSS using the protected Fisher's Least Significant Difference test, which allows post-hoc tests only when the overall ANOVA is significant. Values from a 4 mm sphere around the peak voxel (xyz = -42, 28, -8) were extracted and averaged from the image representing connectivity during the face task and from the image representing connectivity during rest, then exported to SPSS. The post-hoc analyses were performed using these values in SPSS and compared the control and ASD groups on connectivity differences during the faces task and during rest. The post-hoc contrasts also compared the contexts (faces task versus rest) within the ASD and control groups.

Results

We confirmed our hypothesis that alterations in amygdala-ventral prefrontal connectivity in the ASD group compared to controls differ by context (faces task versus rest). The context-by-diagnosis interaction significantly predicts connectivity with the right amygdala in the ventrolateral prefrontal cortex (xyz = -42, 28, -8, cluster size = 85, $t_{206} = 3.56$, $p = 0.043$, corrected for multiple comparisons within bilateral ventral prefrontal cortex; Figure 3.1). Specifically, relative to controls, the ASD group has weaker amygdala-ventrolateral prefrontal connectivity during the faces task ($p = .026$) but greater connectivity during rest ($p = .039$). Moreover, controls show decreased ($p = .013$) but youth with ASD show increased ($p = .010$) connectivity during rest compared to during the faces task (Figure 3.1). The alternative hypothesis, that alterations in connectivity in the ASD group persist regardless of context, was not supported. There were no significant clusters within the ventral prefrontal cortex for the contrast comparing the ASD group to the control on amygdala connectivity across both contexts.

Additional Analyses

The ASD group did not differ from the control group in mean or in maximum head displacement during the faces task versus during rest (mean: $F_{1, 103} = 0.600$, $p = 0.440$, maximum: $F_{1, 103} = 1.356$, $p = 0.247$). Moreover, the ASD and control groups did not differ in reaction time ($t_{102} = 0.683$, $p = 0.496$) or in accuracy ($t_{102} = 1.761$, $p = 0.081$) to identify face gender during the faces task in the scanner. Cognitive functioning (verbal: $t_{102} = 0.617$, $p = 0.538$; non-verbal: $t_{99} = 1.518$, $p = 0.132$), age ($t_{103} = 0.310$, $p = 0.757$), and pubertal status ($t_{103} = 0.048$, $p = 0.962$) did not differ between the ASD and control groups. More information on subject characteristics, including symptom presentation, is available in Table 3.1 and Table 3.2.

Neuroimaging studies with disordered and/or youth populations engender several potential confounds, including head motion, developmental differences, and psychotropic medication usage. Thus, additional analyses were performed to determine whether these factors are driving our finding of differing alterations in connectivity in the ASD group depending on context. Connectivity values from a 4 mm sphere surrounding the peak voxel from our main finding (xyz = -42, 28, -8) were extracted and averaged for the connectivity map from the faces task as well as from rest. These values were then exported to SPSS to examine the context-by-diagnosis interaction, via a mixed-design ANOVA consistent with our main analysis, taking into account each of the potential confounds. This approach allowed us to limit our additional analyses to the locus of effects within the ventrolateral prefrontal cortex, as documented in this study, to assess whether these other factors are driving the main finding.

First, mean and maximum head displacement, calculated as recommended in a seminal paper on head motion (Van Dijk, et al., 2012), did not differ between the ASD and control groups during the face task versus during rest (mean: $F_{1, 103} = 0.600$, $p = 0.440$, maximum: $F_{1, 103} = 1.356$, $p = 0.247$). Nevertheless, I re-ran the model twice, covarying mean head displacement (averaged for faces task and rest) and then covarying maximum head displacement (also averaged for faces task and rest). After variance associated with head displacement was removed, our hypothesis was still confirmed: the context-by-diagnosis interaction significantly predicts amygdala-ventrolateral prefrontal connectivity (covarying mean head displacement: $F_{1, 102} = 12.757$, $p = 0.00054$; covarying maximum head displacement: $F_{1, 102} = 13.123$, $p = 0.00046$).

Second, development is a potential confounding factor in our sample as previous research has shown that developmentally related changes in connectivity may differ in disordered populations (Wiggins, et al., 2012a; Wiggins, et al., 2013). However, when rerunning the models with age and pubertal status as covariates, the hypothesis was still confirmed (context-by-diagnosis interaction significant when covarying age: $F_{1, 102} = 13.235$, $p = 0.00043$; and covarying pubertal status: $F_{1, 102} = 13.069$, $p = 0.00047$).

Third, psychotropic medication usage among individuals with ASD is very common (Oswald & Sonenklar, 2007) and may influence the brain structures of interest. To address this, the analysis was repeated excluding 20 youth with ASD currently prescribed psychotropic medications (whether the medication was taken the day of the scan or not) and one control currently prescribed levothyroxine. Even with a reduced sample consisting of 20 non-medicated individuals with ASD and 64 non-medicated controls, the context-by-diagnosis interaction was still significant ($F_{1, 82} = 8.655$, $p = 0.0042$), confirming our hypothesis. To summarize, when taking into account head motion, developmental differences, and psychotropic medication usage, the results pattern is the same.

Discussion

The present study is novel in several ways: This is the first study, to our knowledge, to compare amygdala-prefrontal connectivity in the absence of a task with task-based connectivity in typically developing children and adolescents. I found that typically developing youth modulate amygdala-ventrolateral prefrontal connectivity such that connectivity is stronger during the faces task but weaker in the absence of a task. This is also the first study to examine amygdala-prefrontal connectivity during rest in children and adolescents with ASD as well as to compare ASD and control participants on task-based and resting connectivity. Youth with ASD show the opposite pattern to controls: youth with ASD have less connectivity during the faces task compared to during rest. Moreover, relative to controls, youth with ASD have weaker connectivity during the faces task but stronger connectivity during rest compared to controls.

Our results suggest that ASD in youth is characterized by inappropriate modulation of amygdala-ventrolateral prefrontal connectivity across different contexts. The increased

connectivity controls exhibit during the faces task may represent increased coordination of the amygdala and ventrolateral prefrontal cortex required for emotion processing and regulation during the faces task; conversely, the decreased connectivity controls exhibit during rest may reflect the reduced need for emotion processing and regulation in the absence of overtly social or emotional stimuli like faces. However, the opposite pattern of connectivity found in youth with ASD may signify that youth with ASD are not able to adapt amygdala-prefrontal connectivity to social task demands, which could contribute to symptoms. Our findings are consistent with recent work that has challenged the notion that reduced amygdala-prefrontal connectivity indexes risk for poorer socio-emotional functioning (Pfeifer & Allen, 2012). In line with Pfeifer and Allen's (2012) argument, decreased connectivity alone is not associated with the disordered group in our study; rather, both decreased and increased connectivity confer risk, depending on context.

This study has at least two limitations. First, recent papers have documented head motion's potential to produce spurious correlations in connectivity analyses, particularly in resting acquisitions and with pediatric disordered populations (Power, et al., 2012; Satterthwaite, et al., 2012; Van Dijk, et al., 2012). Thus, it is possible that head motion may have influenced our findings. However, several pieces of information make this possibility less likely: Our participants with ASD and controls did not differ in mean or maximum head displacement (Additional Analyses, p. 29). Nevertheless, I took several steps to remove potential motion-related artifacts in the data (Addressing Head Motion, p. 46). Also, I covaried mean and maximum head displacement (Additional Analyses, p. 29), and demonstrated that the main finding was still significant even after removing variance associated with head displacement. After implementing all of these steps to mitigate head motion, if head motion was still the driving force behind our results, one would expect to see weaker connectivity between amygdala and prefrontal cortex in the ASD group regardless of context, as head motion decreases longer-range correlations (such as amygdala to prefrontal cortex) and increases correlations for nearby regions (Van Dijk, et al., 2012). However, I found that the decreased connectivity in the ASD group was specific to the faces task and connectivity during rest was increased in the ASD group (the group more likely to be affected by head motion; Van Dijk, et

al., 2012) compared to controls. Thus, our findings are not consistent with the explanation that head motion drove our results.

Second, our sample size is modest (40 youth with ASD, 65 controls) compared with large datasets like the Autism Brain Imaging Data Exchange (ABIDE) that aggregated resting connectivity images from multiple sites. However, the advantage of our sample is that each individual performed both the faces task and the resting acquisition. Thus, I can directly link connectivity during the faces task and during resting acquisition within participants. This allows us to avoid systematic group differences between those who did one task versus the other. Nevertheless, our findings will need to be replicated with larger samples.

The present research lays the groundwork for a program of research on context-driven connectivity in ASD. Our study looked at only two contexts, a commonly used social task with face stimuli (Swartz, et al., 2013; Weng, et al., 2011; Wiggins, et al., 2012a) and the absence of a task (rest). However, there are many variations on contexts that would be relevant to studying how brain function and connectivity of structures related to social impairment may differ. For example, future research could examine how connectivity differs when individuals with ASD are viewing dynamic, not static faces; perceiving different types of social interactions; and other contexts that capture the large variation in real-life social and non-social situations. Knowledge of how individuals with ASD may have differing levels of success compared to controls in modulating connectivity across particular contexts may help to disambiguate the brain mechanisms subserving social symptoms and provide medical and behavioral targets for treatment. Future research could examine whether the degree to which individuals with ASD inappropriately modulate brain connectivity is predictive of responsiveness to types of treatments and prognoses.

Table 3.1. Participant characteristics.

	Autism Spectrum Disorders Group	**Typically Developing Controls**	χ^2 ***df = 1***	***p***
Number of participants	40	65		
Gender (F:M)	6 : 34	16 : 49	1.382	.240
Handedness* (L:R)	7 : 28	7 : 55	1.347	.241
			t ***df = 103****	***p***
fMRI task accuracy	95.0% (4.65%)	96.5% (3.85%)	1.761 (df = 102)	.081
fMRI task RT (ms)	751 (145)	772 (158)	0.683 (df = 101)	.496
Age	14.1 (2.3)	14.2 (3.0)	0.310	.757
Puberty	2.54 (0.94)	2.55 (0.98)	0.048	.962
Mean head displacement (faces task)	0.00099 (0.00090)	0.00091 (0.00087)	0.453	.651
Max head displacement (faces task)	0.0068 (0.0055)	0.0081 (0.013)	0.592	.555
Mean head displacement (rest)	0.0010 (0.0015)	0.00077 (0.00068)	1.170	.245
Max head displacement (rest)	0.0097 (0.013)	0.0082 (0.012)	0.591	.556
Verbal CF	113 (21)	115 (13)	0.617 (df = 102)	.538

Nonverbal CF	106 (20)	101 (13)	1.518 (df = 99)	.132
SRS	75.6 (11.9)	42.7 (6.7)	18.000 (df = 102)	< .001
SCQ	19.9 (8.1)	3.06 (3.7)	14.001 (df = 95)	< .001

*Degrees of freedom = 103 unless otherwise indicated. Eight individuals missing handedness data, 4 missing non-verbal CF, 1 missing verbal CF, 1 missing SRS, 8 missing SCQ, 2 missing RT. Accuracy score for one person was lost due to computer failure; examiner visually monitored responses during the task and reported very high accuracy for that individual. fMRI task accuracy = accuracy in identifying gender of all emotional or neutral faces, fMRI task RT = reaction time in milliseconds to identify gender of all emotional or neutral faces, Puberty = Pubertal Development Scale, head displacement = frame-wise displacement in millimeters, CF = cognitive functioning, SRS = Social Responsiveness Scale, SCQ = Social Communication Questionnaire – Lifetime.

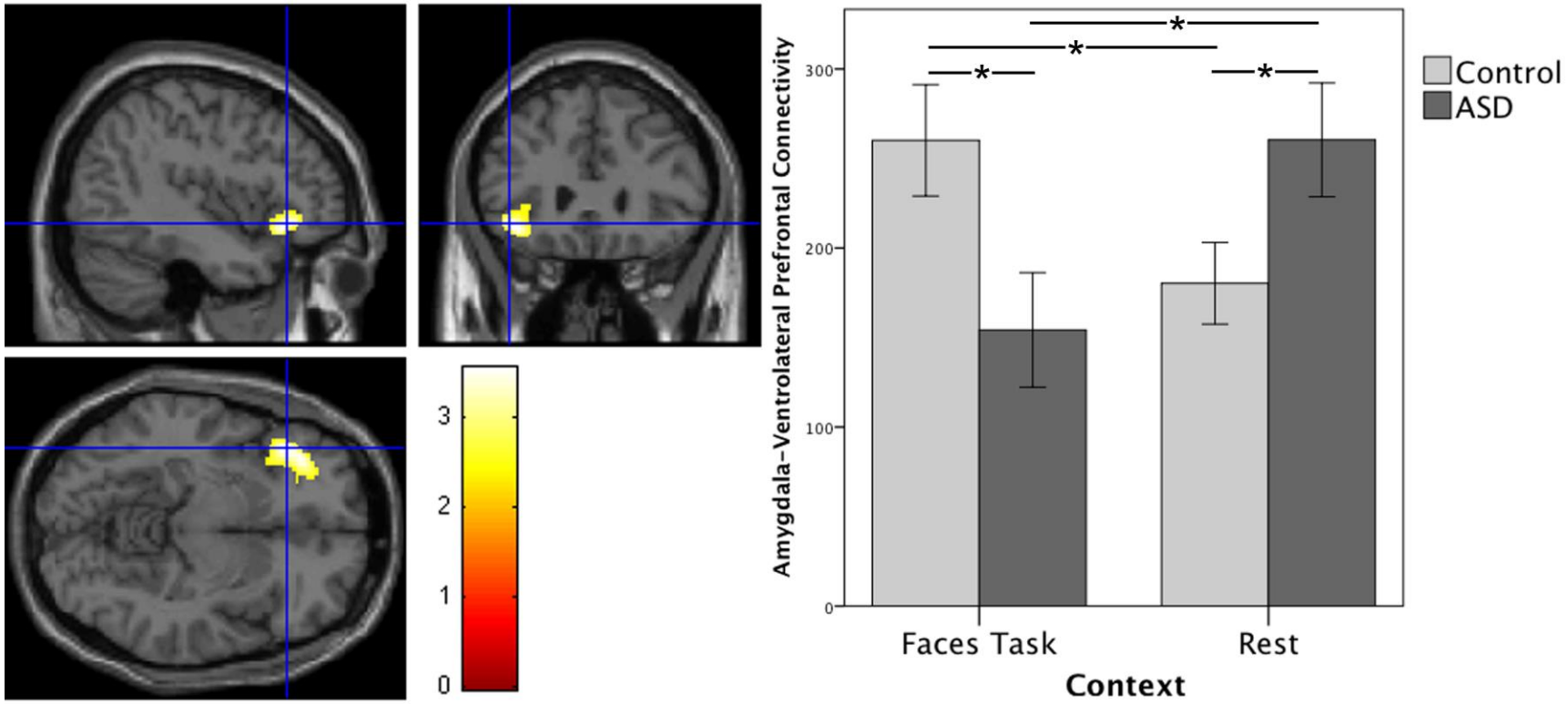


Figure 3.1. Alterations in amygdala-ventrolateral prefrontal connectivity in ASD differ by context (faces task versus rest). Brain images show ventrolateral prefrontal cluster where context-by-diagnosis interaction significantly predicts connectivity with amygdala. Brain shown in sagittal (upper left), coronal (upper right), and transverse (lower left) planes. For illustration purposes, threshold on brain images set to $p < 0.01$, k > 50 voxels. Crosshairs on brain images show peak voxel (xyz = -42, 28, -8) of cluster. Values from a 4 mm sphere around peak voxel were extracted, averaged, and plotted in bar graph on far right to illustrate interaction. Asterisks indicate significant differences in post-hoc tests at $p < 0.05$.

Table 3.2. Comorbid symptom presentation.

	Autism Spectrum Disorders Group	Typically Developing Controls	*t* *df* = 103*	*p*
CDI	7.93 (5.6)	4.88 (4.8)	2.964	.004
OCI-R	17.5 (12)	10.4 (8.4)	3.474 (df = 100)	.001
MASC	41.3 (16)	32.0 (15)	2.825 (df = 92)	.006
CBCL-Internal	62.4 (9.3)	46.8 (8.6)	8.540 (df = 100)	< .001
CBCL-External	54.3 (12)	44.1 (8.8)	5.015 (df = 100)	< .001

*Degrees of freedom = 103 unless otherwise indicated. 3 missing OCI-R, 11 missing MASC, 3 missing CBCL, 1 missing accuracy, 1 missing RT. Means and standard deviations (in parentheses) reported. CDI = Children's Depression Inventory, OCI-R = Obsessive-Compulsive Inventory-Revised, MASC = Multidimensional Anxiety Scale for Children, CBCL = Child Behavior Checklist, Internalizing and Externalizing subscales.

CHAPTER 4 [§]

The Impact of Serotonin Transporter Genotype on Default Network Connectivity in Children and Adolescents with Autism Spectrum Disorders

Summary

Compared to healthy controls, individuals with autism spectrum disorders (ASD) have weaker posterior-anterior connectivity that strengthens less with age within the default network, a set of brain structures connected in the absence of a task and likely involved in social function. The serotonin transporter-linked polymorphic region (5-HTTLPR) genotypes that result in lowered serotonin transporter expression are associated with social impairment in ASD. Additionally, in healthy controls, low expressing 5-HTTLPR genotypes are associated with weaker default network connectivity. However, in ASD, the effect of 5-HTTLPR on the default network is unknown. I hypothesized that 5-HTTLPR's influence on posterior-anterior default network connectivity strength as well as on age-related changes in connectivity differs in the ASD group versus controls. Youth with ASD and healthy controls, ages 8-19, underwent a resting fMRI acquisition. Connectivity was calculated by correlating the posterior hub of the default network with all voxels. Triallelic genotype was assessed via PCR and Sanger sequencing. A genotype-by-diagnosis interaction significantly predicted posterior-anterior connectivity, such that low expressing genotypes (S/S, S/L_G, L_G/L_G) were associated with stronger connectivity than higher expressing genotypes (L_A/L_A, S/L_A, L_A/L_G) in the ASD group, but the converse was true for controls. Also, youth with ASD and low expressing genotypes had greater age-related increases in connectivity values compared to those with higher expressing genotypes and controls in either genotype group. Our findings suggest that the cascade of

[§] Chapter 4 corresponds to the publication Wiggins and colleagues (2013).

events from genetic variation to brain function differs in ASD. Also, low expressing genotypes may represent a subtype within ASD.

Introduction

Autism spectrum disorders (ASD) are neurodevelopmental conditions characterized by social and communicative impairments and rigid repetitive behaviors. The prevalence of ASD has sharply increased in recent years and is currently 1 in 88 (CDC, 2012). Deciphering the complex etiology of ASD is thus a priority, and progress will likely involve examining the condition using multiple methodologies, including neuroimaging and molecular genetics.

As alterations in brain connectivity have been repeatedly implicated in ASD (Hughes, 2007), attention has been focused on identifying perturbations in fundamental, large-scale networks, such as the default network, that may contribute to ASD symptoms. In healthy adults, the default network (including the posterior cingulate, angular gyri, superior frontal gyri/Brodmann's area (BA) 10, and anterior cingulate/BA 10) is active and functionally connected in the absence of a demanding task (Raichle & Snyder, 2007). Functional connectivity reflects structural connectivity of the default network in healthy adults (Greicius, et al., 2009). The default network contains posterior and anterior hubs (Buckner, et al., 2008) that typically display strong long-range connectivity but are distinct from one another (Horovitz, et al., 2009).

The primary purpose of the default network is a subject of debate. The default network may relate to basic central nervous system functions such as maintaining the balance of excitatory and inhibitory inputs or interpreting information from the environment (Raichle & Snyder, 2007). Alternatively, the primary purpose of the default network may be related to social cognition, including self-referential processes (Gusnard, et al., 2001) and mentally projecting oneself into hypothetical situations (Buckner & Carroll, 2007).

Studies on adults with ASD (Cherkassky, et al., 2006; Kennedy & Courchesne, 2008; Monk, et al., 2009) as well as adolescents (Anderson, et al., 2011; Weng, et al., 2010; Wiggins, et al., 2011) found weaker connectivity between the posterior and anterior default network compared to controls. Moreover, the weaker the posterior-anterior default network

connectivity, the worse the social impairment in individuals with ASD (Monk, et al., 2009; Weng, et al., 2010).

A few studies have investigated the development of the default network. For healthy individuals, posterior-anterior connectivity is weaker during childhood and adolescence than adulthood both functionally (Fair, et al., 2008; Stevens, et al., 2009; Wiggins, et al., 2011) and structurally (Supekar, et al., 2010b). These studies indicate that connectivity of this network increases in strength over childhood and adolescence in healthy individuals. In contrast, youth with ASD have attenuated increases in posterior-anterior connectivity with age compared to controls (Wiggins, et al., 2011).

Identifying the genetic factors that influence the default network in ASD is important to further elucidate the complex etiology of ASD. The serotonin transporter-linked polymorphic region variant (5-HTTLPR; Lesch, et al., 1996) in the promoter region of the serotonin transporter gene (*SLC6A4*) is relevant to the default network in ASD. The S and L_G alleles of 5-HTTLPR are associated with decreased serotonin transporter expression relative to the L_A allele (A to G SNP in L allele, rs25531; Hu, et al., 2006). The low expressing alleles of 5-HTTLPR have been associated with worse social symptoms in ASD (Brune, et al., 2006; Tordjman, et al., 2001). In healthy adolescents, 5-HTTLPR is known to influence the default network: those with low expressing genotypes exhibit weaker posterior-anterior connectivity than adolescents with higher expressing genotypes (Wiggins, et al., 2012b). Moreover, in healthy children and adolescents, 5-HTTLPR also impacts the development of default network connectivity such that youth with higher expressing genotypes have greater age-related increases in posterior-anterior connectivity than those with low expressing genotypes (Wiggins, et al., 2012b). A previous study found that serotonin transporter binding in the anterior default network is decreased in individuals with autism (Nakamura, et al., 2010). However, no study has yet examined how 5-HTTLPR affects default network connectivity or its development in individuals with ASD.

The present study addresses these two gaps in the literature on ASD: the role of 5-HTTLPR in default network connectivity and in the development of default network connectivity. This is accomplished by directly examining the influence of 5-HTTLPR variants on

posterior-anterior default network connectivity as well on age-related changes in connectivity in a sample of children and adolescents with ASD and controls. I hypothesized that the relationship between 5-HTTLPR genotype and posterior-anterior default network connectivity strength differs in the ASD group versus controls. Additionally, I hypothesized that the relationship between 5-HTTLPR and changes in connectivity across childhood and adolescence differs in the ASD group compared to controls.

Methods

Participants

Fifty-four children and adolescents with ASD and 66 healthy controls, aged 8.3 to 19.6 years, were included in this study (see Table 4.1 for participant characteristics). Of a total 105 participants with ASD and 82 controls recruited, 51 participants with ASD and 16 controls were excluded because of head movement exceeding 2.5 mm translation or 2.5 degrees rotation, declining to complete the MRI scan due to discomfort, failure to return a saliva sample for genotyping, or technical problems with the MRI.

Controls were recruited through flyers posted at community organizations in the Ann Arbor, Michigan area. The University of Michigan Autism and Communication Disorders Center (UMACC) referred potential participants to our study and diagnosed participants with an ASD (Autistic Disorder, Asperger's syndrome, or Pervasive Developmental Disorder – Not Otherwise Specified) using the Autism Diagnostic Observation Schedule (ADOS; Lord, et al., 2000), the Autism Diagnostic Interview-Revised (ADI-R; Lord, et al., 1994), and clinical consensus (Lord, et al., 2006). The University of Michigan Institutional Review Board approved the procedures. Participants over age 18 gave written informed consent; participants under age 18 gave written assent and their parents gave written informed consent. Cognitive functioning was evaluated for controls with the Peabody Picture Vocabulary Test (PPVT; Dunn & Dunn, 1997) and the Ravens Progressive Matrices (Raven, 1960); participants with ASD were given these measures or the Differential Ability Scales II – School Age (Elliott, 2005), the Stanford-Binet Intelligence Scales (Roid, 2003), the Wechsler Intelligence Scale for Children IV (Wechsler, 2003), or the Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999). Participants with orthodontic

braces, medical conditions contraindicated for MRI, or history of seizures or neurological disorders were excluded. Control participants were screened for psychological disorders with the Child Behavior Checklist (Achenbach & Edelbrock, 1981), Social Responsiveness Scale (Constantino, et al., 2003), Social Communication Questionnaire (Rutter, et al., 2003), Obsessive Compulsive Inventory – Revised (Foa, et al., 2010), Child Depression Inventory (Kovacs, 1992), and Multidimensional Anxiety Scale for Children (March, et al., 1997). All control participants scored below clinical cutoffs for affected status. Individuals with the low and higher expressing genotypes did not differ in any of the symptom measures or cognitive functioning in either the ASD or control group (Table 4.2). Prior studies utilized portions of this dataset (Weng, et al., 2011; Weng, et al., 2010; Wiggins, et al., 2012b; Wiggins, et al., 2011).

Genetic Analyses

5-HTTLPR genotype was ascertained using previously published procedures (Wiggins, et al., 2012b). Participants donated saliva samples using the Oragene DNA kit (DNA Genotek; Kanata, Canada). PCR and agarose genotyping were used to determine S versus L allele. Sanger sequencing was utilized to determine the A to G single nucleotide polymorphism (SNP) in the L allele (rs25531; Hu, et al., 2006) and to confirm PCR genotyping.

In autism, individuals with the low expressing genotype (S/S) have been shown to differ in neurochemical metabolism compared to L allele carriers in the anterior portion of the default network (Endo, et al., 2010). As such, participants were put into two genotype groups: low expressing genotypes (S/S, S/L_G, L_G/L_G) versus higher expressing genotypes (L_A/L_A, S/L_A, L_A/L_G). (The L_G allele is equivalent to the S allele in serotonin transporter expression level (Hu, et al., 2006), so for the purposes of the analyses, the two alleles were grouped together.) This genotype grouping is consistent with a number of non-ASD studies that found recessive effects of the low expressing 5-HTTLPR alleles, often in adolescent populations (e.g., Benjet, et al., 2010; Cicchetti, et al., 2007; Surguladze, et al., 2008). Nevertheless, I conducted additional analyses to examine whether our results still stood when the alleles were grouped differently (see Alternative Genotype Groupings, p. 68).

Hardy-Weinberg equilibrium was tested based on the insertion/deletion polymorphism. Genotype frequencies were in Hardy-Weinberg equilibrium for the ASD group (χ^2 = 0.742, df = 1, p = 0.389), but there was a trend toward disequilibrium for the control group (χ^2 = 3.74, df = 1, p = 0.053). When including only Caucasians for the control group, the trend disappeared (χ^2 = 0.654, df = 1, p = 0.419). Because of this, additional analyses were performed to address potential effects of multiple ancestries within the sample.

fMRI Data Acquisition

T_2*-weighted blood oxygen level dependent (BOLD) images were acquired during a resting state scan, in which participants were instructed not to think of anything in particular and to let their minds wander while looking at a fixation cross. Over the 10-minute resting state scan, 300 images were acquired (Glover and Law, 2001; TR=2000 ms, TE=30 ms, flip angle=90°, FOV=22 cm, 64×64 matrix, 40 contiguous axial 3mm slices). Slices were acquired parallel to the intercommissural (AC-PC) line. High-resolution 3D T1 axial overlay (TR=8.9, TE=1.8, flip angle= 15°, FOV=26 cm, slice thickness=1.4 mm, 124 slices; matrix= 256×160) and spoiled gradient (SPGR; flip angle=15°, FOV=26 cm, 1.4mm slice thickness, 110 slices) anatomical images were also collected. Participants wore a pulse oximeter and abdominal pressure belt to record cardiac and respiratory rhythms, synchronized to the fMRI data, for subsequent physiological artifact correction. Further details on the acquisition parameters have been previously published (Weng, et al., 2010; Wiggins, et al., 2012b). Prior to the MRI scan, participants practiced in a mock scanner to acclimate to the scanning environment.

fMRI Data Analysis

Data Preprocessing

The standard pre-processing procedure from the University of Michigan Functional MRI Center was applied to the fMRI data. This procedure includes removing outliers from the raw k-space data, reconstructing the k-space data to image space, applying a field map correction to reduce artifacts from susceptibility regions, and correcting for slice timing. RETROICOR was utilized to remove noise associated with cardiac and respiratory rhythms (Glover, et al., 2000).

To address potential effects of head motion, functional images were realigned to the 10th image. Details on these steps are available in multiple papers utilizing this pre-processing stream (e.g., Weng, et al., 2010; Wiggins, et al., 2012b). The high-resolution T1 anatomical images were then co-registered to the functional images using the SPM5 Matlab toolbox (Wellcome Department of Neurology, London, UK; http://www.fil.ion.ucl.ac.uk). After removing variance associated with head motion (see Addressing Head Motion, p. 63), functional images were smoothed with an isotropic 8 mm full width at half maximum (FWHM) Gaussian kernel using SPM5. A low-pass filter of .08 Hz was applied as well to isolate the frequency band where default network activation has been found.

Addressing Head Motion

Recent papers have emphasized the importance of addressing head motion, which can introduce spurious correlations in connectivity analyses (Power, et al., 2012; Satterthwaite, et al., 2012; Van Dijk, et al., 2012). In addition to the standard realignment of images to correct for head motion, I took several steps to address potential effects of head motion on our results. First, I excluded participants whose head motion exceeded 2.5 mm in the x, y, or z direction or 2.5 degrees in the roll, pitch, or yaw directions.

Second, I removed variance associated with head motion by creating nuisance regressors from motion estimated in the x, y, z, roll, pitch, and yaw directions and retaining the residuals for processing.

Third, I calculated mean motion for each person (i.e., mean absolute displacement of each volume as compared to the previous volume, calculated as square root($(x_{i+1} - x_i)^2 + (y_{i+1} - y_i)^2 + (z_{i+1} - z_i)^2$) for i = 1, …, 300 images), as in Van Dijk et al (2012). I then conducted a 2-way ANOVA with post-hoc contrasts to examine whether mean motion differed between the ASD and control groups and by genotype. I also performed a 3-way interaction analysis to examine whether the interaction of genotype-by-diagnosis-by-age significantly predicted mean motion.

Fourth, even though groups may not differ on overall mean motion, it is possible that the different motion distributions within groups could influence findings. Because of this, I repeated the analyses for our main hypotheses with a subsample of our participants matched on mean motion to examine whether the results persisted when the groups' motion

distributions were equivalent (see Matched Head Motion Distributions, p. 67). Several other studies have utilized matching to reduce the likelihood that the connectivity patterns they observed were an artifact of head motion (Dosenbach, et al., 2010; Fair, et al., 2008; Fair, et al., 2007). Our matching procedure was as follows: first, I split participants into four groups: individuals with the low expressing genotypes (ASD and control) and individuals with the higher expressing genotypes (ASD and control). Within each group, I binned participants by mean motion into .001 mm bins. Participants were removed randomly until the number of participants in each corresponding bin for the ASD and control groups were the same within both the low and higher expressing genotypes.

Connectivity Images

A self-organizing map algorithm was applied to the images to derive a data-driven reference from the posterior hub of the default network to calculate connectivity for each individual, as described in previous publications (Peltier, et al., 2003; Wiggins, et al., 2012b; Wiggins, et al., 2011). An example of a posterior hub is shown in Figure 4.3. The advantage of using this data-driven method is that, unlike traditional *a priori* seed analyses, seed placement is not based on data from adult control brains. When using the self-organizing map algorithm, the default network reference, which is correlated with every other voxel in the brain to calculate connectivity, is not biased toward the control group but rather tailored for each individual.

The connectivity images generated by this method were normalized to Montreal Neurological Image (MNI) space by estimating the transformation matrix for the SPGR image to SPM's template MNI image, and applying that transformation to the connectivity images. The end product is a normalized image for each subject that indicates, with a Z value at each voxel, how highly functionally connected (correlated) that voxel is to the posterior hub of the default network identified by the self-organizing map algorithm.

Group-Level Analyses

The connectivity images were then entered into second-level analyses in SPM8 to test hypotheses at a group level. As a preliminary step, I first examined whether the ASD group and the control group exhibited default network connectivity by applying small volume corrections

using masks covering the default network (posterior cingulate, precuneus, angular gyri, inferior parietal lobules, parahippocampal gyri, superior frontal gyri, anterior cingulate, BA 32, BA 10), from the Wake Forest Pickatlas (Maldjian, et al., 2002). Also as a preliminary step, I compared the ASD and control groups on long-range default network connectivity, using the anterior masks in small volume corrections (Table 4.2).

To address our first hypothesis, a voxel-wise multiple regression was created to examine the interaction of genotype (low expressing (S/S, S/L_G, L_G/L_G) versus higher expressing (L_A/L_A, S/L_A, L_A/L_G) by diagnosis (ASD versus control group). For this model, three regressors were entered – genotype, diagnosis, and the interaction of genotype-by-diagnosis – predicting connectivity with the posterior hub. To determine whether the beta for the interaction was significant in the anterior default network, a small volume correction was performed on the image mapping the betas of the interaction using a mask of the bilateral BA 10, where alterations in long-range default network connectivity have consistently been found in ASD samples (e.g., Monk, et al., 2009; Weng, et al., 2010; Wiggins, et al., 2011), as well as where effects of 5-HTTLPR have been found (Wiggins, et al., 2012b). The small volume correction takes into account the geometric qualities of the mask when doing a correction for multiple comparisons based on the number of resels (a measure related to the number of independent observations) within the mask (Worsley, et al., 1996). Significance thresholds within BA 10 were corrected for multiple comparisons using family wise error (FWE) correction (Worsley, et al., 1996). Post-hoc comparisons were also performed in SPSS, comparing each subgroup pair on connectivity values extracted and averaged from a 4 mm sphere around the peak of the interaction, with a Bonferroni-corrected α level of 0.05/6 = 0.0083.

To address our second hypothesis, I created a model to examine the three-way interaction among genotype (low versus higher expressing), diagnosis (ASD versus control group), and age. In this model, the three-way interaction term was entered, as well as all lower order terms. A small volume correction applied the same mask as in the first hypothesis, bilateral BA 10, to the image mapping the betas for the 3-way interaction to examine whether the three-way interaction significantly predicted connectivity with the posterior hub in the anterior default network. Post-hoc analyses were also performed to further characterize the

interaction. Connectivity values from a 4 mm sphere around the peak of the 3-way interaction were extracted and averaged, then exported to SPSS. In SPSS, the simple slopes (changes in connectivity for every unit increase in age) for four subgroups (controls with low expressing genotypes, controls with higher expressing genotypes, individuals with ASD and low expressing genotypes, individuals with ASD and higher expressing genotypes) were tested against zero.

Results

Individuals with the low and higher expressing genotypes within the control and the ASD groups did not differ on any of the symptom measures (Table 4.2). Both the ASD group and the control group exhibited default network connectivity, and previous findings of weaker posterior-anterior default network connectivity in the ASD group were replicated (see Table 4.3) (Cherkassky, et al., 2006; Kennedy & Courchesne, 2008; Monk, et al., 2009; Weng, et al., 2010; Wiggins, et al., 2011).

The four groups (individuals with ASD and low expressing genotypes, individuals with ASD and higher expressing genotypes, controls with low expressing genotypes, controls with higher expressing genotypes) did not differ in mean head motion (genotype-by-diagnosis: $F_{1,116}$ = 0.040, p = .841). Additionally, age did not relate to head motion differently across the four groups (genotype-by-diagnosis-by-age: β = .058, t_{112} = 0.256, p = 0.799).

The first hypothesis, that the relationship between 5-HTTLPR genotype and posterior-anterior default network connectivity strength differs in the ASD group versus controls, was confirmed. There was a significant genotype-by-diagnosis interaction predicting degree of connectivity between the posterior hub and the anterior default network in the left hemisphere (xyz = -34, 62, 0, t_{116} = 4.24, p = 0.021, corrected for multiple comparisons within bilateral BA 10; Figure 1). Specifically, 5-HTTLPR genotype influences posterior-anterior connectivity strength differently for individuals with ASD versus controls. Two pair-wise comparisons survived a Bonferroni correction, indicating that individuals with low expressing genotypes within the ASD group had significantly stronger connectivity than individuals with ASD and higher expressing genotypes (p = 0.001) as well as controls with low expressing genotypes (p = 0.003). The genotype-by-diagnosis interaction was also significant in the right anterior default

network (xyz = 44, 56, -6, t_{116} = 4.17, p = 0.027, corrected for multiple comparisons within bilateral BA 10; Figure 4.4).

Our second hypothesis, that the relationship between 5-HTTLPR and changes in posterior-anterior connectivity across childhood and adolescence differs in ASD compared to controls, was also confirmed. I found a significant genotype-by-diagnosis-by-age interaction predicting degree of connectivity between the posterior and anterior default network (xyz = -6, 40, -6, t_{112} = 4.09, p = 0.037, corrected for multiple comparisons within bilateral BA 10; Figure 4.2). 5-HTTLPR genotype differentially influences age-related changes in posterior-anterior connectivity strength in individuals with ASD compared to controls. Post-hoc analyses to further characterize the interaction indicated that only individuals with ASD with low expressing genotypes had significant increases in connectivity values with age (simple slope = 0.708, p = 0.002), whereas the other subgroups' relationships between connectivity and age did not significantly differ from zero (ASD group, high expressing genotype, simple slope = -.268, p = 0.104; controls, low expressing, simple slope = 0.216, p = 0.335; controls, higher expressing, simple slope = -.185, p = 0.229).

Additional Analyses

In imaging and genetic studies with disordered populations, head motion, population stratification, psychotropic medication status, allele grouping, and degree of smoothing are potential factors influencing associations. Because of this, additional analyses were performed to determine whether these factors account for our results. As these additional analyses required a reduced sample size and/or more complex models, thereby diminishing the power to detect effects, I utilized a threshold of $p < 0.05$ without family-wise error correction. To summarize, the hypotheses were still confirmed even when taking into account each of these factors.

Matched Head Motion Distributions

Our matching procedure is described in Addressing Head Motion (p. 63). In total, 24 participants (20%) were removed in order to match the groups' motion distributions. (See Figure 4.5 for a visual representation of the participants removed from each bin). After

removing participants until the group distributions were matched on mean head motion, our first hypothesis was still confirmed. The interaction of genotype-by-diagnosis significantly predicted connectivity in bilateral BA 10 (left: xyz = -34, 62, 0, t_{92} = 4.22, p = 0.000029; right: xyz = 38, 60, 2, t_{92} = 3.84, p = 0.00011) with a subsample of participants matched on head motion. Our second hypothesis was confirmed as well with the matched subsample, as a genotype-by-diagnosis-by-age interaction significantly predicted connectivity in BA 10 (xyz = -10, 36, -6, t_{88} = 4.22, p = 0.000029).

Population Stratification

To determine whether the findings were primarily driven by differing ancestries within our sample, 5 non-Caucasian individuals with ASD and sixteen non-Caucasian controls were excluded and the group-level analyses addressing our hypotheses were repeated. In line with our first hypothesis, including Caucasian participants only, the genotype-by-diagnosis interaction predicting connectivity strength was significant in both left (xyz = -34, 62, -2, t_{95} = 3.95, p = 0.000075) and right (xyz = 38, 58, 4, t_{95} = 3.14, p = 0.0011) BA 10. Supporting our second hypothesis, the genotype-by-diagnosis-by-age interaction predicting connectivity was significant in BA 10 when including only Caucasian participants (xyz = -20, 50, 2, t_{91} = 3.51, p = 0.00035).

Medication Effects

Next, twenty-four individuals with ASD taking psychotropic medication and 1 control taking levothyroxine (a thyroid medication) were excluded before repeating the analyses. Like the analyses only including Caucasians, findings including only non-medicated participants also mirrored the original findings with the entire dataset. Supporting the first hypothesis, with only non-medicated participants, the genotype-by-diagnosis interaction was significant (left BA 10: xyz = -34, 62, 0, t_{91} = 3.71, p = 0 .00018; right BA 10: xyz = 32, 60, 6, t_{91} = 4.55, p = 0.0000084). The second hypothesis, genotype-by-diagnosis-by-age interaction predicting connectivity, also held with non-medicated participants only in BA 10 (xyz = -6, 40, -6, t_{87} = 2.97, p = 0 .0019).

Alternative Genotype Groupings

Moreover, additional analyses were conducted with alternate genotype groupings to examine whether the findings persisted when participants split into different genotype groups.

First, I ran the analyses with the genotype in three groups based on expressing level: low expressing (S/S, S/L_G, L_G/L_G) versus medium expressing (S/L_A, L_A/L_G) vs high expressing (L_A/L_A) genotypes. Consistent with the first hypothesis, there was a significant genotype-by-diagnosis interaction in both the left (xyz = -34, 62, 0, $F_{2,114}$ = 9.22, p = 0.00019) and right (xyz = 44, 56, -6, $F_{2,114}$ = 9.19, p = 0.00020) anterior default network. The three-way genotype-by-diagnosis-by-age interaction was also significant with this genotype grouping in the anterior default network (xyz = -6, 40, -8, $F_{2,108}$ = 11.23, p = 0.000037), consistent with the second hypothesis.

Second, I examined the hypotheses with the genotype grouping S/S versus heterozygotes (S/L_A and S/L_G) versus L_A/L_A. With this alternative genotype grouping, there was a significant genotype-by-diagnosis in the left (xyz = -34, 62, -2, $F_{2,114}$ = 7.68, p = 0.00074) and right (xyz = 44, 56, -6, $F_{2,114}$ = 10.45, p = 0.000068) anterior default network. Moreover, consistent with the second hypothesis, the three-way interaction was significant in the anterior default network (xyz = -6, 40, -8, $F_{2,108}$ = 8.11, p = 0.00052) with this alternative grouping. To summarize, the original results pattern persisted even when genotypes were grouped in two alternate ways in the statistical analyses.

5 mm Smoothing Kernel

The degree of smoothing can also affect results. I re-did the analyses with a 5 mm (instead of 8 mm) FWHM Gaussian kernel for spatial smoothing of the functional images. Consistent with the first hypothesis, the genotype-by-diagnosis was significant in the left (xyz = -48, 54, 6, t_{116} = 2.25, p = 0.013) and right (xyz = 42, 58, 10, t_{116} = 3.96, p = 0.00065) BA 10. Moreover, consistent with the second hypothesis, the three-way interaction was significant in BA 10 (xyz = 42, 48, 24, t_{112} = 3.03, p = 0.002) with the 5 mm smoothing kernel, albeit in a more lateral location within BA 10.

Discussion

In results confirming both the first hypothesis (genotype-by-diagnosis) and the second hypothesis (genotype-by-diagnosis-by-age), individuals with ASD and low expressing genotypes stood out among the other subgroups, exhibiting the greatest connectivity as well as the sharpest increase in connectivity values with age. This overall pattern suggests that individuals

with low expressing genotypes may represent a subtype of ASD, which is consistent with previous research linking 5-HTTLPR to symptom subtypes rather than a global ASD diagnosis in a larger sample (Brune, et al., 2006; Tordjman, et al., 2001). Linking genotype to brain phenotypes may be a more sensitive way to identify subtypes of ASD than linking genotype to behavior, as individuals with ASD and the low and higher expressing genotypes did not differ on any of the symptom measures (Table 4.2). Future research could examine other aspects of this potential subtype of ASD, including responsiveness to specific interventions and long-term prognosis.

There are two main possibilities to explain why 5-HTTLPR influences the ASD group and control group differently. First, a gene-by-gene interaction may account for our results. The specifics of the complex genetic etiology of ASD are a subject of intense inquiry. Nonetheless, as ASD is highly heritable (Miles, 2011), individuals with ASD may carry a systematically different overall genetic profile than controls. Causative gene products may interact with 5-HTTLPR, leading to alterations in expression levels that then produce a different brain phenotype. Future research probing this possibility may include examining other autism genes and their involvement in serotonin metabolism.

Alternatively, a gene-by-environment interaction may explain our findings. As 5-HTTLPR is sensitive to environmental input (Belsky, et al., 2009), it may be that 5-HTTLPR affects brain function in individuals with ASD differently than controls because individuals with ASD experience an altered social environment brought about by the reactions of others to their symptoms. Particularly during adolescence, an important social development period in which relationships with peers become more important (Youniss & Haynie, 1992), individuals with ASD may miss out on social opportunities with peers and thus find themselves in an environment with reduced social stimuli. This environment could affect epigenetically-sensitive serotonin transporter expression and subsequently, brain function. Future studies incorporating comprehensive environmental measures and focusing on molecular mechanisms of altered serotonin transporter expression, such as methylation, will be necessary to probe this possibility.

This study has several limitations, as some confounds make imaging and genetics research, particularly with pediatric clinical populations, more challenging. First, in our study, mean head motion did not differ among the groups I compared: individuals with ASD and low expressing genotypes, individuals with ASD and higher expressing genotypes, controls with low expressing genotypes, and controls with higher expressing genotypes. Neither did age relate to head motion differently across the four groups. Nevertheless, motion remains a concern in all functional connectivity studies, so I took several steps to address motion: first, only participants with movement under 2.5 mm or degrees in all translation and rotation directions were included; second, I realigned the functional images; third, I removed variance associated with movement in the x, y, z, roll, pitch, and yaw directions; fourth, I repeated the analyses with a subsample matched on head motion and found that our hypotheses were still confirmed even when motion distributions were the same across individuals with ASD and controls in the low and higher expressing genotype groups.

Another limitation is that the cross-sectional design utilized in this study precludes inferences about developmental trajectories within individuals. Future studies may use a longitudinal design to rule out birth cohort effects. Additionally, longitudinal studies will be useful to examine whether brain differences earlier in development predict later symptom presentation and responsiveness to particular treatments.

Third, I did not exclude any racial or ethnic group when recruiting participants, which can contribute to spurious associations in genetic studies. Although it should be acknowledged that Caucasians in our sample may not all be of the same ancestry, I repeated the analyses with non-Caucasian participants removed to determine whether results were primarily due to several different ancestries within the sample. The genotype-by-diagnosis and genotype-by-diagnosis-by-age interactions predicting posterior-anterior connectivity were found even with non-Caucasians excluded from the analyses, suggesting that the results were not primarily driven by population stratification. Nevertheless, the lack of understanding of genetic effects in different racial/ethnic groups is a widespread problem in the field that must be addressed in future work.

Conclusions

This is the first study, to our knowledge, to examine the influence of 5-HTTLPR genotype on the default network in individuals with ASD. I found that the relationship between 5-HTTLPR genotype and posterior-anterior default network connectivity is different in individuals with ASD compared to controls. Specifically, consistent with previous research in controls (Wiggins, et al., 2012b), higher expressing genotypes were associated with stronger connectivity than low expressing genotypes. However, the pattern was reversed for the ASD group: individuals with ASD and low expressing genotypes had stronger connectivity than individuals with ASD and higher expressing genotypes. Also, I found that youth with ASD and low expressing genotypes had greater age-related increases in connectivity values compared to others in the ASD group with higher expressing genotypes and to controls with either low or higher expressing genotypes. The present findings provide evidence that the cascade of events from genetic variation to brain function is markedly different in ASD versus typically developing, healthy individuals. Moreover, the findings suggest that the impact of genotype on brain function is not static but rather develops and changes with age. Thus, understanding how 5-HTTLPR affects brain function in ASD is dependent on the developmental timeframe.

Although replication of our findings with a larger sample is necessary, the present study lays the groundwork to better understand the genetic and brain mechanisms that are involved in ASD. The present study documented a different impact of 5-HTTLPR on both default network connectivity and the development of default network connectivity in ASD compared to controls. Future studies may expand on these findings by examining the structural connections within the default network in vivo using diffusion tensor imaging. Moreover, the resting connectivity approach used in this study will be useful to examine the brain activation patterns of lower functioning individuals with ASD or very young children. These individuals are underrepresented in functional MRI studies because they are often unable to comply with the demands of a task requiring responses in the scanner. The relatively low demand of a resting fMRI acquisition, on the other hand, may allow lower functioning and younger participants to be successfully scanned. Obtaining brain data from individuals with a greater range of cognitive abilities and ages will allow researchers to gain a broader, more representative picture of ASD

and the developmental trajectory of ASD earlier than mid-childhood. To conclude, the findings from our study open a path for a research program to better understand genetic influences on brain function in ASD.

Table 4.1. Participant characteristics.

	Autism Spectrum Disorders Group						Control Group					
	Low Expressing Genotypes			Higher Expressing Genotypes			Low Expressing Genotypes			Higher Expressing Genotypes		
	S/S	S/L_G	L_G/L_G	L_A/L_A	S/L_A	L_A/L_G	S/S	S/L_G	L_G/L_G	L_A/L_A	S/L_A	L_A/L_G
Number of participants	10	5	1	12	25	1	18	3	1	21	22	1
Total N	16			38			22			44		
Gender (F:M)	1 : 15			7 : 31			6 : 16			11 : 33		
Handedness* (L:R:ambidextrous)	4 : 11 : 0			5 : 28 : 1			4 : 18 : 0			3 : 38 : 0		
Age	13.5 (2.78)			13.9 (2.72)			14.8 (2.35)			14.1 (3.26)		
Verbal CF	115 (25.6)			111 (18.8)			113 (13.1)			115 (13.7)		
Nonverbal CF	113 (17.6)			101 (21.9)			103 (11.6)			101 (13.0)		
SRS	74.5 (12.2)			78.0 (11.1)			43.6 (7.68)			42.5 (6.37)		
SCQ	19.4 (7.67)			21.73 (6.85)			2.95 (2.84)			3.36 (4.23)		
Caucasian	94%			90%			64%			82%		

Means and standard deviations (in parentheses) reported. *Note: 8 individuals were missing handedness data. CF = cognitive functioning, SRS = Social Responsiveness Scale, SCQ = Social Communication Questionnaire – Lifetime, Caucasian = self-reported Caucasian descent. Table 4.2 contains a more detailed version of the subject characteristics presented here.

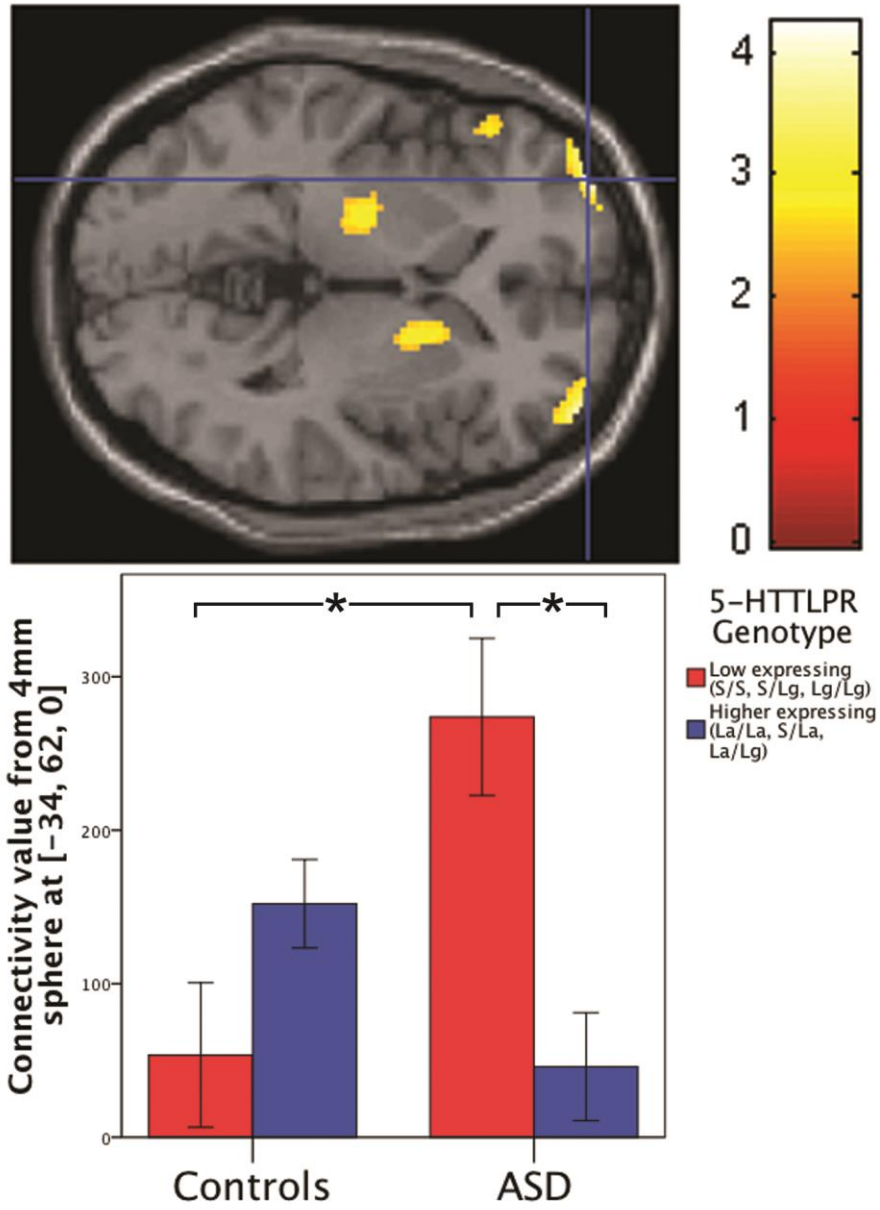


Figure 4.1. Impact of 5-HTTLPR genotypes on posterior-anterior default network connectivity is different in youth with ASD compared to controls.

Voxels in color indicate places where connectivity between that area and the posterior default network is differentially influenced by 5-HTTLPR in the ASD group versus controls. A significant genotype-by-diagnosis interaction in the anterior default network (xyz = -34, 62, 0, $t_{116} = 4.24$, $p = 0.021$, corrected for multiple comparisons within bilateral BA 10) is depicted in the transverse section of the brain (upper). For this and the subsequent brain image, the threshold was set at $p < 0.01$ for illustration purposes. To show the interaction, contrast values from a 4 mm sphere around the peak voxel (xyz = -34, 62, 0) were extracted and plotted (lower). In the bar graph, controls show the pattern found in previous research (Wiggins et al., 2012), but youth with ASD show a different pattern. Brackets with asterisks indicate significant differences at a Bonferroni-corrected α-level of 0.05/6 = 0.0083.

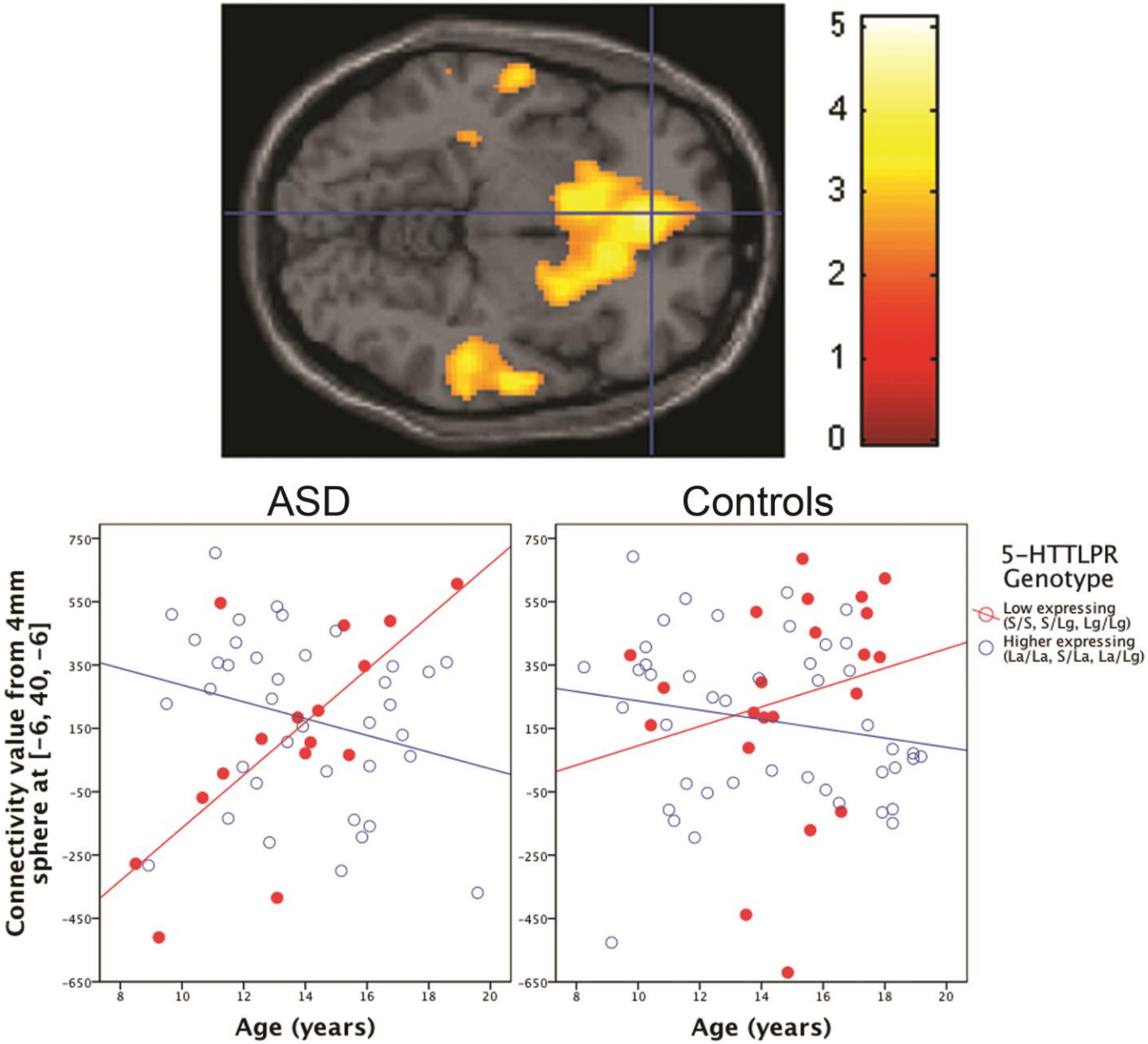

5
4
3
2
1
0
ASD
Controls
Connectivity value from 4mm sphere at [-6, 40, -6]
750
550
350
150
-50
-250
-450
-650
8
10
12
14
16
18
20
Age (years)
5-HTTLPR Genotype
Low expressing (S/S, S/Lg, Lg/Lg)
Higher expressing (La/La, S/La, La/Lg)

Figure 4.2. 5-HTTLPR influences age-related changes in posterior-anterior default network connectivity differently in youth with ASD compared to controls.

Voxels in color indicate places where connectivity between that area and the posterior hub changes across age differently for the ASD group and the control group. A significant genotype-by-diagnosis-by-age interaction in the anterior default network (xyz = -6, 40, -6, t_{112} = 4.09, p = 0.037, corrected for multiple comparisons within bilateral BA 10) is depicted in the transverse section of the brain (upper). To illustrate connectivity levels in each individual, contrast values from a 4 mm sphere around the peak voxel (xyz = -6, 40, -6) were extracted and plotted (lower).

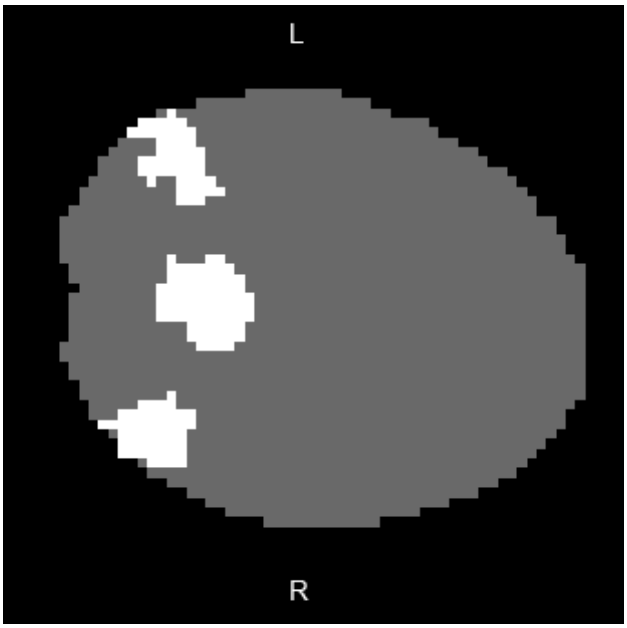


Figure 4.3. An example of the default network posterior hub identified through the self-organizing map algorithm.
Following procedures described in Wiggins et al (Wiggins, et al., 2011) the self-organizing map algorithm, a data-driven method, was applied to the data to organize voxels into networks. An experienced investigator blind to condition identified the network that contained the posterior hub (posterior cingulate and angular gyri/inferior parietal lobules) of the default network for each individual. The posterior hub was then used as an individualized reference to calculate default network connectivity for each participant. An example of the posterior hub from one individual is shown here. Data are from a single 64x64 slice in the transverse plane. White indicates that the voxel is a member of the posterior hub; gray indicates that the voxel does not belong in the posterior hub. The brain is masked for illustration purposes to highlight the posterior hub. At this point in the data-processing stream, brains are not yet normalized.

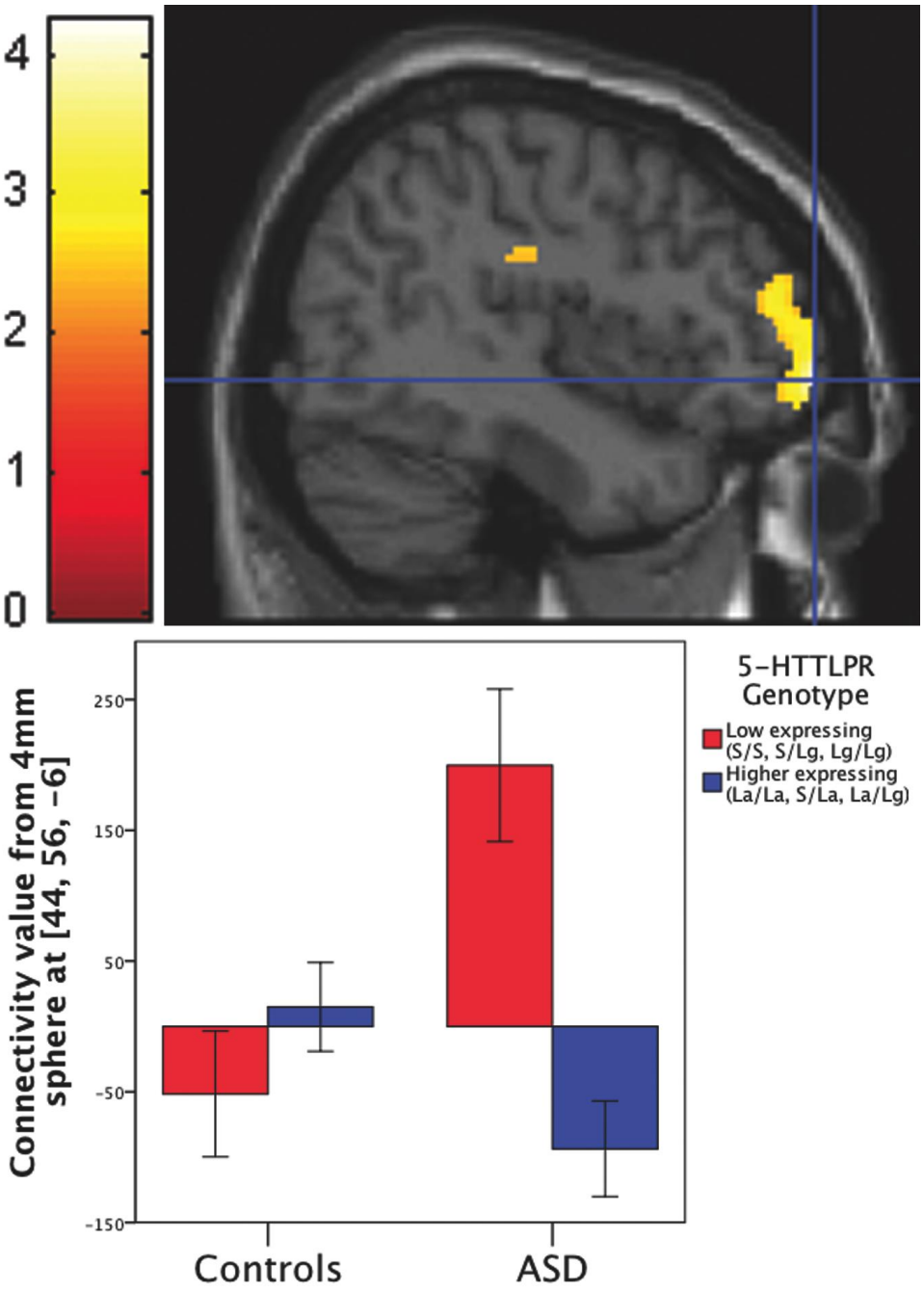
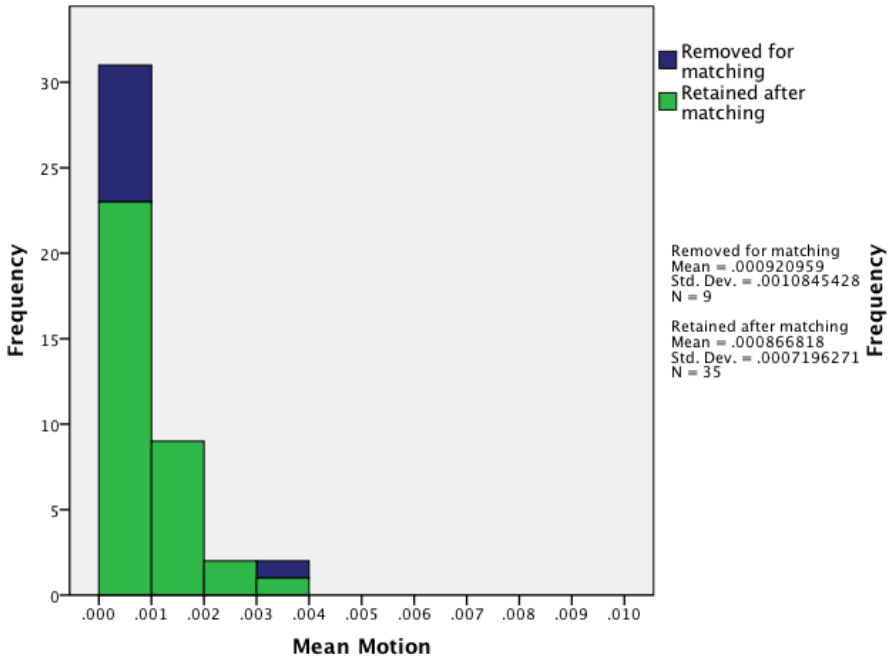


Figure 4.4. Right hemisphere – Impact of 5-HTTLPR on posterior-anterior default network connectivity differs in youth with ASD compared to controls.

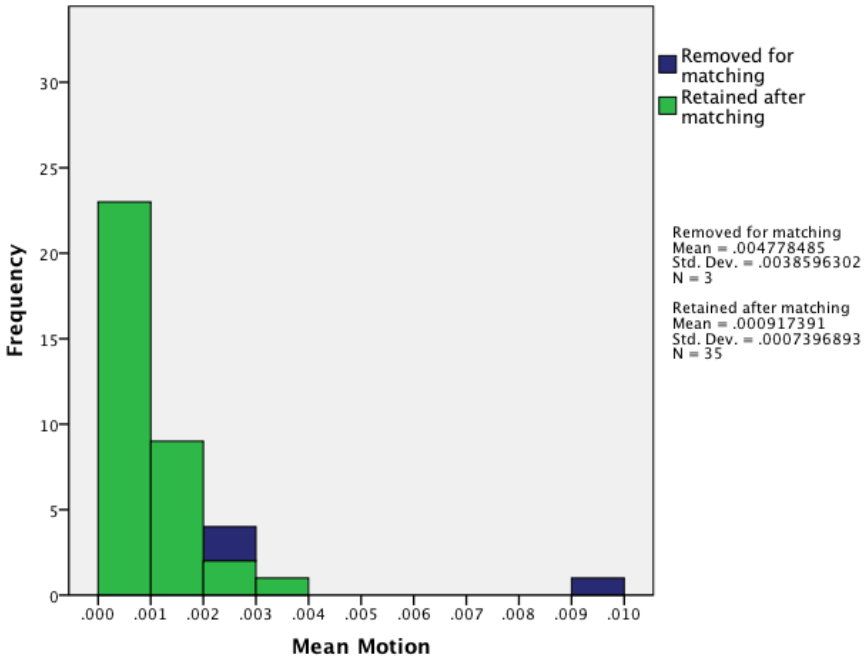
Voxels in color indicate places where genotype influenced connectivity between that area and the posterior hub differently for the ASD group and the control group. A significant genotype-by-diagnosis interaction in the anterior default network (xyz = 44, 56, -6, $t_{116} = 4.17$, $p = 0.027$, corrected for multiple comparisons within bilateral BA 10) is depicted in the sagittal section of the brain (upper), with the threshold set at $p < 0.01$ for illustration purposes. To depict the interaction, contrast values from a 4 mm sphere around the peak voxel (xyz = 44, 56, -6) were extracted and plotted (lower).

A. Higher Expressing Genotypes

Control Group



ASD Group



B. Low Expressing Genotypes

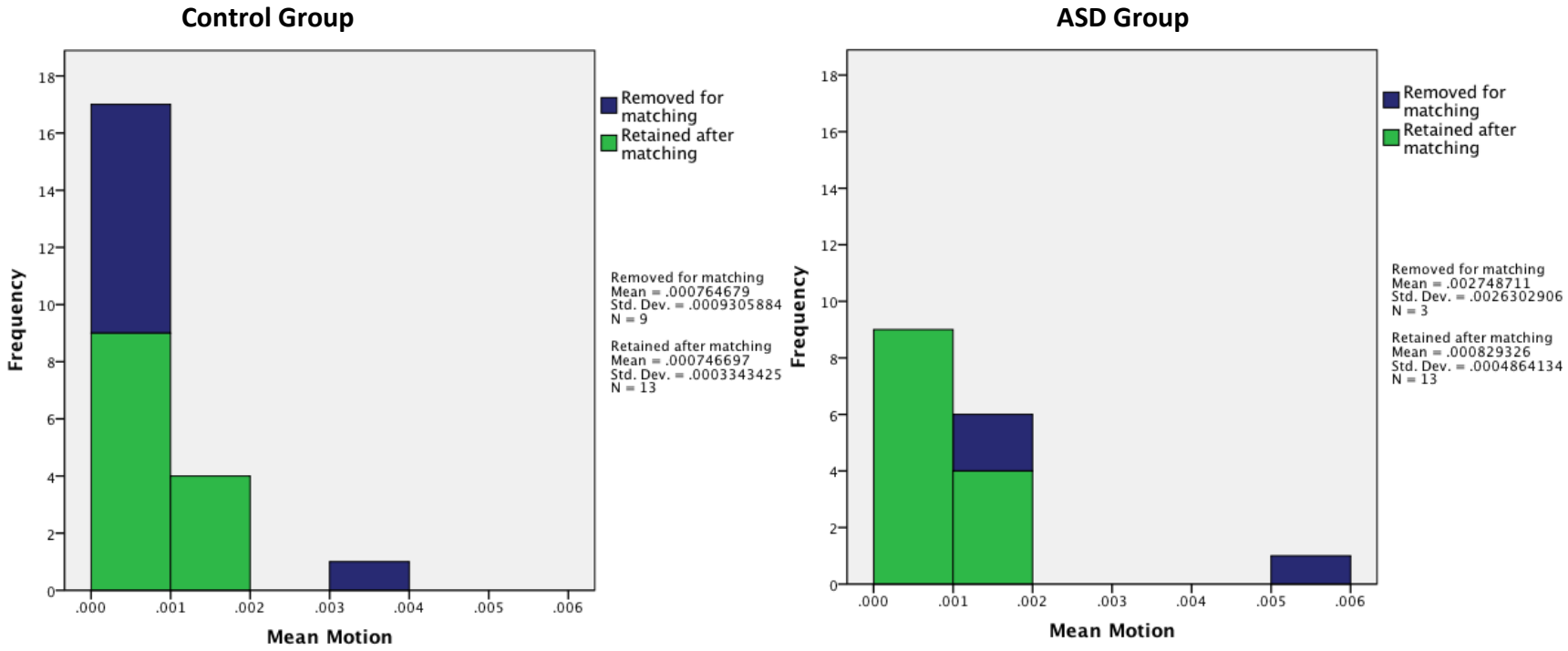


Figure 4.5. Matching participants on mean motion.
Participants were randomly removed from .001 mm bins until the same number of participants remained in corresponding bins across the ASD and control groups for both (A) high expressing and (B) low expressing genotypes. Bar graph shows proportion removed in each bin.

Table 4.2. Detailed participant characteristics.

	Autism Spectrum Disorders Group						Control Group							
	Low Expressing Genotypes			Higher Expressing Genotypes			Low Expressing Genotypes			Higher Expressing Genotypes				
	S/S	S/L_G	L_G/L_G	L_A/L_A	S/L_A	L_A/L_G	S/S	S/L_G	L_G/L_G	L_A/L_A	S/L_A	L_A/L_G		
Number of participants	10	5	1	12	25	1	18	3	1	21	22	1		
Total N	16			38			22			44				
													χ^2	**p**
Gender (F:M)	1:15			7:31			6:16			11:33			3.76 (df=3)	0.288
Handedness (L:R:ambidextrous)	4:11:00			5:28:01			4:18:00			3:38:00			6.17 (df=6)	0.405
Caucasian	94%			90%			64%			82%				
													F(1,116)*	**p**
Age	13.5 (2.78)			13.9 (2.72)			14.8 (2.35)			14.1 (3.26)			1.107	0.295
Verbal CF	115 (25.6)			111 (18.8)			113 (13.1)			115 (13.7)			0.811	0.370
Nonverbal CF	113 (17.6)			101 (21.9)			103 (11.6)			101 (13.0)			2.756 (df=1,112)	0.100
					t(52)**	**p**							**t(64)*****	**p**
SRS	74.5 (12.2)			78.0 (11.1)	1.01 (df=51)	0.318	43.6 (7.68)			42.5 (6.37)			.590 (df=63)	0.557
SCQ	19.4 (7.67)			21.73 (6.85)	1.05 (df=46)	0.299	2.95 (2.84)			3.36 (4.23)			.400 (df=59)	0.691

MASC	42.3 (21.5)	45.2 (16.8)	.509 (df=47)	0.613	34.8 (14.1)	31.4 (15.2)	.847 (df=60)	0.400
CDI	7.13 (4.26)	8.47 (6.38)	.748 (df=51)	0.458	5.86 (4.00)	4.47 (5.27)	1.094 (df=63)	0.278
CBCL Internal	60.0 (18.0)	56.4 (21.3)	0.537	0.593	45.0 (9.43)	45.2 (10.7)	0.093	0.926
CBCL External	49.0 (15.6)	51.7 (20.9)	0.457	0.650	44.4 (7.33)	41.7 (10.4)	1.092	0.279
CBCL Total	57.4 (17.1)	58.1 (22.0)	0.110	0.913	44.2 (8.81)	42.4 (10.48)	0.673	0.504
OCI-R	18.0 (13.8)	18.9 (13.3)	.225 (df=47)	0.823	10.1 (6.81)	10.1 (9.10)	0.004 (df=59)	0.997

Note: Some participants were missing data. This is noted with altered df.

*df=1,116 unless otherwise specified

**df=52 unless otherwise specified

***df=64 unless otherwise specified

CF= cognitive functioning

SRS = Social Responsiveness Scale.

SCQ = Social Communication Questionnaire – Lifetime

MASC = Multidimensional Anxiety Scale for Children

CDI = Children's Depression Inventory

CBCL = Child Behavior Checklist

OCI-R = Obsessive Compulsive Inventory – Revised;

Likelihood ratio test used for chi-square analyses

Table 4.3. Default network connectivity for ASD and control groups.
Functional connectivity in the (A) Control group, (B) ASD group, (C) ASD > Control group, (D) Controls > ASD group. The threshold was set at $p < 0.05$ uncorrected with the number of contiguous voxels set at $k \geq 10$. L = left, R = right. A full list of the default network structures used can be found in Methods (p. 60).

(A). Control group

Region	Brodmann's Area	Cluster size	*t* *df = 65*	MNI Coordinates x	y	z
L posterior cingulate	23	808	14.75	-6	-52	24
R posterior cingulate	10	853	15.22	4	-52	24
L precuneus	31	2062	15.55	-6	-50	30
	39	229	10.89	-44	-74	38
R precuneus	31	2291	16.42	4	-52	32
	39	206	11.11	42	-68	34
L angular gyrus	39	390	12.20	-50	-64	34
R angular gyrus	39	396	13.89	50	-62	30
L inferior parietal lobule	39	735	11.88	-46	-68	38
	40	74	6.42	-48	-50	24
R inferior parietal lobule	40	587	12.66	50	-62	38
	13	82	8.15	46	-50	24
L parahippocampal gyrus	--	901	5.03	-28	-34	-10
R parahippocampal gyrus	30	776	4.26	10	-46	2
L superior frontal gyrus	10	3432	9.70	-8	58	-8
R superior frontal gyrus	8	3159	10.82	18	30	48
L anterior cingulate	10	810	9.09	-2	58	2
R anterior cingulate	10	981	9.75	4	58	2
L prefrontal cortex	10	1006	10.07	-4	56	-8

	32	311	7.80	-2	50	0
R prefrontal cortex	10	905	10.80	8	66	8
	32	307	8.11	6	40	-10

(B). ASD group

Region	Brodmann's Area	Cluster size	*t* *df = 53*	MNI Coordinates x	y	z
L posterior cingulate	23	817	11.14	-2	-44	24
R posterior cingulate	23	818	12.10	4	-44	24
L precuneus	31	1978	11.34	-2	-50	30
	19	203	7.89	-44	-74	40
R precuneus	31	2335	13.74	12	-48	30
	39	233	7.00	46	-76	34
L angular gyrus	39	381	9.13	-46	-68	30
R angular gyrus	39	399	9.15	48	-74	34
L inferior parietal lobule	39	1090	8.62	-42	-64	38
R inferior parietal lobule	39	649	7.61	44	-72	38
	39	294	6.43	46	-50	22
L parahippocampal gyrus	39	54	3.27	-10	-48	2
	--	12	2.44	-24	-12	-14
	30	34	2.02	-14	-34	-6
R parahippocampal gyrus	35	379	3.73	22	-28	-14
L superior frontal gyrus	10	3307	7.50	-12	66	18
R superior frontal gyrus	10	2576	6.90	8	64	24
L anterior cingulate	11	682	6.07	-2	42	-10
R anterior cingulate	11	719	6.44	2	42	-10
L prefrontal cortex	10	1044	7.56	-10	66	18
	32	260	5.79	-2	40	-10
R prefrontal cortex	10	849	6.90	8	64	24
	32	248	6.08	2	46	-4

(C). Controls > ASD group

Region	Brodmann's Area	Cluster size	*t* *df = 118*	MNI Coordinates x	y	z
L posterior cingulate	31	331	2.94	-2	-62	24
R posterior cingulate	31	408	3.77	8	-58	24
L precuneus	31	903	3.75	-2	-70	28
	19	42	2.09	-44	-74	38
R precuneus	31	1009	4.02	12	-56	30
	39	33	3.11	42	-68	34
L angular gyrus	39	72	2.29	-50	-72	34
R angular gyrus	39	261	3.95	44	-66	30
L inferior parietal lobule	39	51	2.17	-46	-72	38
R inferior parietal lobule	39	186	3.04	46	-70	42
L parahippocampal gyrus	35	715	3.75	-24	-22	-20
R parahippocampal gyrus	20	287	2.94	34	-22	-28
L superior frontal gyrus	11	334	3.75	-6	58	-24
	6	72	2.63	-16	24	56
	10	30	2.46	-2	62	2
	9	92	2.43	-4	52	28
R superior frontal gyrus	11	505	4.21	6	58	-24
	8	1040	3.36	22	36	50
L anterior cingulate	25	63	2.88	-2	12	-10
R anterior cingulate	25	76	2.98	4	8	-12
	10	97	2.60	4	58	2
L prefrontal cortex	10	132	2.56	-8	60	-8
R prefrontal cortex	10	375	3.66	12	66	-4

32	13	1.98	14	46	-4
32	16	1.97	8	46	4

(D). ASD > Control group

Region	Brodmann's Area	Cluster size	*t* *df = 118*	MNI Coordinates x	y	z
L posterior cingulate	30	44	2.73	-24	-70	6
L precuneus	7	11	1.98	-28	-56	54
R precuneus	7	331	3.50	14	-58	60
L inferior parietal lobule	40	1258	3.41	-60	-38	28
R inferior parietal lobule	40	1412	3.81	64	-46	22
L superior frontal gyrus	6	520	3.58	-4	10	54
	9	94	2.74	-38	44	36
	10	32	2.64	-38	58	18
R superior frontal gyrus	6	217	3.15	2	10	56
	6	164	2.62	20	-4	74
	10	19	2.33	38	58	18
	6	12	2.06	24	4	58
L anterior cingulate	32	15	1.91	-10	26	28
L prefrontal cortex	10	136	3.48	-46	50	14
	10	43	3.36	-44	50	8
	32	264	3.61	-12	10	40
R prefrontal cortex	10	53	2.96	48	50	4
	10	26	2.51	38	58	16
	32	199	2.90	12	6	42

CHAPTER 5 [**]

General Conclusion

Summary

As discussed in the General Introduction (CHAPTER 1, p. 1), researchers are beginning to flesh out the links on the translational developmental neuroscience framework in terms of socio-emotional functioning in both healthy and impaired development. Three studies on youth with ASD were offered as examples of research driven by the translational developmental neuroscience framework. The first study (CHAPTER 2, p. 20) examined the impact of serotonin transporter genotype on amygdala habituation, which may represent a mechanism by which adaptive levels of arousal to socio-emotional stimuli are maintained. Our previous work (Swartz, et al., 2013) found that, overall, youth with ASD fail to habituate to socio-emotional stimuli (sad faces); in CHAPTER 2, I showed that the degree to which individuals with ASD fail to habituate to sad faces depends on 5-HTTLPR genotype. Individuals with ASD and low expressing genotypes failed to habituate to the sad faces and in fact displayed a statistical trend toward sensitization, an increase in activation over time; these individuals sensitized more than individuals who also have ASD but with higher expressing genotypes. Our results suggest that the brain mechanisms by which social impairment develops and is maintained is genetically influenced.

The second study (CHAPTER 3, p. 40) tested whether alterations in amygdala-ventral prefrontal connectivity in youth with ASD would differ or be the same by context (socio-emotional task with faces versus rest). Supporting the hypothesis that alterations would differ by context, this study found that relative to controls, the ASD group has weaker amygdala-ventrolateral prefrontal connectivity during the faces task but greater connectivity during rest. Moreover, controls show decreased but youth with ASD show increased connectivity during

[**] Chapter 5 corresponds to a portion of the publication Wiggins and Monk (in preparation-a).

rest versus during the faces task. This suggests that ASD may be characterized by inappropriate modulation of amygdala-ventrolateral prefrontal connectivity across different contexts.

The last study (CHAPTER 4, p. 57) investigated the influence of serotonin transporter genotype on another set of socially-relevant brain structures, the default network, in the absence of a task. In this study, low expressing genotypes were associated with stronger connectivity than higher expressing genotypes in the ASD group, but the converse was true for controls. Also, youth with ASD and low expressing genotypes had greater age-related increases in connectivity values compared to those with higher expressing genotypes and controls in either genotype group. These findings suggest that the cascade of events from genetic variation to brain function differs in ASD. Also, low expressing genotypes may represent a subtype within ASD. Taken together, these three studies illustrate that bringing together information from multiple levels of analysis (genetic and multiple brain measures) can help to disambiguate subtypes within a complex socio-emotional disorder, ASD.

However, much work remains to be done to fully understand the multiple etiologies and trajectories of ASD and other socio-emotional disorders as well as the multiple pathways to a healthy outcome. The translational developmental neuroscience framework is useful to guide the research questions that I pose and shapes future directions in understanding the development of socio-emotional functioning.

Future Directions

Genetics

So far, the majority of imaging genetics studies on socio-emotional functioning link a single polymorphism to brain function. However, the single-gene approach is limited in that it leaves out the larger context, which likely involves additive or interactive effects of multiple genes as well as gene-by-environment, and gene-by-development interaction effects. One response to the limitations of single-gene association studies has been to use genome-wide association studies in which brain activation or behavior can be tested against hundreds of thousands or even millions of single nucleotide polymorphisms simultaneously (Pearson & Manolio, 2008). For example, this exploratory, hypothesis-free approach was used to identify a

single nucleotide polymorphism (rs2023454) in the gene DOK5 that is significantly associated with amygdala activation, and penetrance of this genotype was greater in youth with bipolar disorder than healthy youth (Liu, et al., 2010). However, the frequent failure to replicate genome wide association findings (e.g., Hart, et al., 2012; Ousdal, et al., 2012) has led to reticence to make the large expenditures for genome-wide association studies. One of several issues that may be affecting the difficulty in replicating genome-wide association studies is that multiple statistical tests (hundreds of thousands or more, one for each single nucleotide polymorphism) introduce the problem of finding a balance between alpha inflation and applying corrections for multiple comparisons that are too harsh, particularly because the tests may involve some degree of dependency (see Moskvina & Schmidt, 2008 for a discussion of multiple comparison issues). One way forward that takes into account multiple genes yet imposes some limits on the number of statistical tests is to examine only polymorphisms along a particular molecular signaling pathway that are related to a neural or behavioral phenotype of interest (Nikolova, et al., 2011). For example, building on the single-gene studies linking 5-HTTLPR to socio-emotionally relevant brain function in youth reviewed in this article, future research could expand the focus to include other polymorphisms in the serotonergic signaling pathway, such as 5-HT1A and 5-HT1B (serotonin receptor) genes, and consider the impact of all of these genes simultaneously. However, this approach, as well as genome-wide or single-gene association studies, needs to be combined with other information, such as environment, development, or gene-by-gene interactions (Musani, et al., 2007) to more fully capture the multiple and interacting influences on brain and behavior.

Epigenetics

Whereas functional polymorphisms have served as proxies for expression level of genetic products (e.g., 5-HTTLPR variants can result in high or low expression of serotonin transporter), other factors can also affect the efficacy of gene expression without changing the underlying DNA sequence. One such epigenetic mechanism is methylation, in which a methyl group added to a cytosine nucleotide linearly adjacent to a guanine nucleotide in the promoter region of a gene can alter the degree of gene expression. Methylation can occur in response to psychosocial environmental influences such as stress. For example, adults with depression who

experienced childhood stress and adversity have higher serotonin transporter promoter methylation (Kang, et al., 2013). Methylation is also related to worse clinical presentation in the adults with depression (Kang, et al., 2013). Methylation studies, however, involve challenging methodological issues (Aberg & van den Oord, 2011). Specifically, although methylation in response to environmental stress may affect some types of tissues equally (e.g., T cells and prefrontal cortex; Provencal, et al., 2012), it is possible for methylation status to differ by tissue or location in the body (Grafodatskaya, et al., 2010; Sun, et al., 2010). Methylation of specific brain structures is of greatest interest in order to link functional significance of genes to brain activation and subsequently, behavior. However, presently, there is no ethical way to measure methylation status in the central nervous system of living humans. One way to move forward in epigenetic research on socio-emotional function would be to combine neuroimaging information from living humans with methylation information obtained from postmortem brain tissue. Specifically, living subjects (e.g., a group of adolescents with major depressive disorder and controls) could be scanned and methylation status based on blood could be related to brain function (see Ursini, et al., 2011 for an example of this approach applied to study working memory). To provide further support that there are group differences in methylation status in the central nervous system, postmortem brain tissue samples from existing brain banks could be analyzed. The functional MRI findings could be used to identify the precise location to sample the tissue.

Combining Multiple Methods

Although brain activation and connectivity are the main brain measurement tools discussed, combining multiple methods of measuring the brain will be necessary to obtain converging evidence of brain alterations due to genetic and/or environmental effects in developmental socio-emotional psychopathology. In particular, functional connectivity during different contexts, such as during a socio-emotional task (in which brain structures are actively recruited in response to socio-emotional stimuli) and during rest (when structures are allowed to operate without specific task demands), can reveal the extent to which symptom-related alterations are elicited or suppressed in response to specific stimuli or, alternatively, pervasive even in the absence of a task. Another complementary mode of measuring the brain is diffusion

tensor imaging (DTI). DTI provides information about the structural connectivity of white matter tracts in vivo and, combined with functional connectivity measures, can tease apart whether functional alterations in a brain circuit are due to reduced structural integrity in a particular white matter pathway. Rudie and colleagues' (2012) study is one example that brings together these three types of evidence (connectivity during a task with socio-emotional stimuli, connectivity during rest, structural connectivity via DTI) to look at genetic effects on autism spectrum disorders. In this study, all three of these measures were applied to the same sample of children and adolescents with autism spectrum disorders and typically developing controls. Rudie and colleagues (2012) found that the risk genotype of Met Receptor Tyrosine Kinase predicts atypical amygdala activation and connectivity using all three measures, and that the degree to which the genotype affects the brain phenotype in all three measures is greater for individuals with autism spectrum disorders than controls.

Combining methodologies may also help to mitigate the problem of spurious connectivity due to head movement in the MRI scanner. In developmental studies of functional connectivity, particularly resting state functional connectivity, movement is an issue because younger children and individuals with disorders move more than healthy adults (Power, et al., 2012; Van Dijk, et al., 2012). In addition to taking steps to reduce movement, having converging evidence from multiple methods (fMRI, DTI, post-mortem, animal studies) can help to evaluate whether connectivity differences are due to movement. If an alteration in connectivity persists across pieces of evidences in a particular disorder, regardless of movement levels in any one scan, the alteration is less likely to be spurious.

Large Samples

Sample sizes in the majority of current studies linking multiple levels of analysis (e.g., brain and genetics) are relatively modest, due to the large expenditures required for both brain imaging and genetic assays. Moreover, sample sizes are often even smaller for clinical youth populations due to both the increased difficulty in recruiting these specialized populations as well as the cost of diagnosing participants. To increase sample sizes, there have been efforts to cooperate and share resting state MRI images among researchers for both autism spectrum disorders via the Autism Brain Imaging Data Exchange

(http://fcon_1000.projects.nitrc.org/indi/abide/) as well as for typical development via the 1000 Connectome (http://fcon_1000.projects.nitrc.org/) and the National Institutes of Health MRI Study of Normal Brain Development (http://pediatricmri.nih.gov/nihpd/info/index.html), which includes longitudinal data. Also, there have been efforts to share genetic information and biological samples across multiple sites, such as the Simons Simplex Collection (http://sfari.org/sfari-initiatives/simons-simplex-collection) and the Autism Genetic Resource Exchange (https://research.agre.org/) for individuals with autism spectrum disorders. Moving forward, however, databases that are the product of cooperation of many research sites and take multiple measures across several different levels (genetics, brain, behavior, etc.) delineated in the translational developmental neuroscience framework will be extremely valuable to tease apart multiple paths to health and psychopathology. Moreover, longitudinal studies will be necessary to examine individual developmental trajectories and identify the causes of the emergence of psychopathology. One example of a database that combines brain, behavior, and genetics – but to examine risk-taking behavior in a normative population, not psychopathology – is the Imagen study in Europe (http://www.imagen-info.com/). Future efforts for aggregation of data can follow a similar model for individuals with socio-emotional developmental psychopathology.

Implications for Intervention

The studies in this dissertation focused on characterizing brain activation patterns of genetic subgroups within autism as well as autism as a whole. Establishing the brain activation patterns associated with socio-emotional impairment is important, because brain activation patterns may be useful as a biomarker to measure responses to intervention. In the two genetics studies (Chapters 2 and 4), individuals with different genotypes did not differ in symptom presentation as measured by self- or parent-report measures. However, the brain measures were able to detect differences between the genotype groups. Thus, the brain may be a more sensitive measure of social impairment than self or parent reports of behavior. Future intervention studies may leverage this knowledge to test efficacy of interventions with smaller samples. If the brain activation patterns of individuals with a disorder become more

similar to typically developing participants after an intervention, this could indicate that the intervention is be effective. Moreover, the brain results could indicate that deploying more resources to subsequently do a randomized control trial with behavioral measures would be a prudent investment.

In conclusion, the translational developmental neuroscience framework can provide guidance for a research program with the goal of fully understanding the complex process of socio-emotional development. Understanding the multiple developmental pathways to health and disorder can provide targets for treatments to improve the well being of individuals with socio-emotional impairment.

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