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Exploring Heterogeneity in Autism Neuroendophenotypes: Effects of Genetic Risk, Gender, and Behavioral Symptomatology

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Exploring Heterogeneity in Autism Neuroendophenotypes: Effects of Genetic Risk, Gender, and Behavioral Symptomatology

A dissertation submitted in partial satisfaction of the

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by

Leanna M. Hernandez

2018

ABSTRACT OF THE DISSERTATION

Exploring Heterogeneity in Autism Neuroendophenotypes:

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Leanna M. Hernandez

Doctor of Philosophy in Neuroscience

University of California, Los Angeles, 2018

Professor Mirella Dapretto, Chair

Autism Spectrum Disorder (ASD) is a heritable neurodevelopmental disorder in which manifestations of behavioral symptomology vary widely. Core behavioral deficits associated with the disorder include impairments in social communication and social interactions, along with the presence of repetitive patterns of behavior, restricted interests, and/or altered sensory responsivity to external stimuli. The last decade has seen the rate of ASD diagnosis rise to an estimated 1 in every 59 children, making early diagnosis and effective treatment a critical public health concern. However, the neurobiological and phenotypic heterogeneity present in individuals with ASD makes discerning ASD etiologies and developing effective treatments very challenging. This dissertation seeks to examine the neurobiological underpinnings of ASD from a multidimensional perspective – investigating how brain function and connectivity are altered in ASD and how they vary among affected individuals, which may ultimately contribute to the development of targeted,

individualized treatment. Chapter 1 provides an Introduction to the research conducted in the following chapters and gives a review of the neurobiological basis of ASD, describing neuroimaging findings related to altered brain structure, function, and connectivity. Chapter 2 describes a study which investigated how functional connectivity of the brain's reward network varies as a function of genetic heterogeneity in a predominantly male cohort of youth with and without ASD. Using a seed in the subcortical hub of the reward network, the nucleus accumbens, this study showed that genetic variability in the oxytocin receptor gene (*OXTR*) is linked to distinct patterns of reward network connectivity in neurotypical children and in children with ASD. In ASD youth, increased genetic risk in the *OXTR* was associated with reduced within network connectivity, whereas neurotypical youth showed compensatory upregulation of connectivity between the nucleus accumbens and frontal cortex. These findings elucidate the neural mechanisms of risk and resilience in youth with and without autism. Chapter 2 describes a related study which used an imaging-genetics approach to examine sex-differences in ASD by investigating gender-specific effects of *OXTR* variants on reward network connectivity. Here, the results showed that under the same *OXTR* genetic risk load, females with ASD and neurotypical males display similar neuroplastic upregulation of functional connectivity between the reward network and frontal brain regions, with identical positive effects on social behavior. In addition, variability in the *OXTR* had distinct effects in male and female ASD youth, underscoring the importance of including females in studies of gene-brain interactions in ASD. Finally, Chapter 3 presents data from a study which explored how behavioral variability in auditory sensory responsivity affects discourse processing and social attention in youth with and without ASD. Study findings showed that in neurotypical youth, listening to conversations shrouded in distracting environmental noises was associated with increased activity in canonical left

hemisphere language regions, likely reflecting the automatic engagement of selective attention mechanisms due to the social salience of speech. Conversely, in ASD youth with high levels of auditory sensory over-responsivity, listening to such conversations was associated with recruitment of contralateral right hemisphere language homologues, reflecting the increased difficulty of processing the speech signal in the context of competing auditory input. These data indicate that youth with and without ASD, particularly ASD youth with high auditory sensitivity, use different neural mechanisms to "hone-in" on socially relevant information in the presence of distracting stimuli, suggesting that the intrinsic salience of speech is disrupted in individuals with ASD and high sensory-over responsivity.

The dissertation of Leanna M. Hernandez is approved.

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DEDICATION

This dissertation is dedicated to my family. To my husband, whose steady encouragement and support made it possible for me to complete this work and whose quick wit keeps me smiling. And to my daughters, whose unbounded joy, spirit and curiosity are my inspiration.

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CHAPTER 1: Introduction to the dissertation

ABSTRACT

Neuroimaging investigations of Autism Spectrum Disorders (ASDs) have advanced our understanding of atypical brain function and structure and have recently converged on a model of altered network-level connectivity. Traditional task-based functional magnetic resonance imaging (MRI) and volume-based structural MRI studies have identified widespread atypicalities in brain regions involved in social behavior and other core ASD-related behavioral deficits. More recent advances in MR-neuroimaging methods allow for quantification of brain connectivity using diffusion tensor imaging, functional connectivity, and graph theoretic methods. These newer techniques have moved the field toward a systems-level understanding of ASD etiology, integrating functional and structural measures across distal brain regions. Neuroimaging findings in ASD as a whole have been mixed and at times contradictory, likely due to the vast genetic and phenotypic heterogeneity characteristic of the disorder. Future longitudinal studies of brain development will be crucial to yield insights into mechanisms of disease etiology in ASD subpopulations. Advances in neuroimaging methods and large-scale collaborations will also allow for an integrated approach linking neuroimaging, genetics, and phenotypic data.

INTRODUCTION

Autism Spectrum Disorders (ASDs) are heritable neurodevelopmental disorders (e.g., Bailey *et al,* 1995; Nordenbæk *et al*, 2014) in which manifestations of behavioral symptomology vary widely in severity. Core behavioral deficits associated with the disorder include impairments in social communication and social interactions, along with the presence of repetitive patterns of behavior, restricted interests, and/or altered sensory responsivity to external stimuli (American Psychiatric Association [DSM-5], 2013). While these deficits serve as defining criteria on which diagnoses are currently based, not all individuals on the ASD spectrum display each symptom, and there is a broad spectrum of social, emotional, and cognitive impairment amongst diagnosed individuals.

The last decade has seen the rate of ASD diagnosis rise to an estimated 1 in every 68 children (Center for Disease Control, 2014), making early diagnosis and effective treatment a critical public health concern. However, the heterogeneity present in individuals with ASD makes discerning a singular neuropathological cause and developing effective treatments very challenging. Thus, a major goal of ASD research is to understand the neurobiological underpinnings of ASD from a multidimensional perspective – investigating how brain anatomy, function, and connectivity are altered in ASD and how they vary among affected individuals.

Over the past two decades, major advances in magnetic resonance (MR) based structural and functional neuroimaging have greatly enhanced our understanding of brain differences in ASD. In structural brain imaging, newer analysis approaches including cortical thickness mapping, voxel based morphometry, diffusion tensor imaging, and the application of these methods in younger subject populations or in longitudinal studies have led to more specific and dynamic models of abnormal brain development in autism. In functional neuroimaging, the field has moved

beyond task-based functional activation studies, which were typically limited to high-functioning children and adults. New acquisition methods, such as resting state functional magnetic resonance imaging (fMRI), and novel data- analytic approaches now allow for the study of large-scale functional brain networks and connectivity in much younger populations including toddlers and infants at high risk for developing autism. Finally, the integration of both structural and functional imaging with genetic risk and behavioral data has driven the field towards a better understanding of gene-brain-behavior pathways in autism. Together, the corpus of imaging studies in autism have led to an emerging model of abnormal developmental connectivity, a dynamic model that accounts for both genetic liability and environmental influences that shape early brain development. In this review we will present data from these major new domains of MR imaging work- structural MRI, functional MRI, imaging-genetics, and connectivity studies, focusing on developmental trajectories and ultimately converging on a model of abnormal brain development in autism.

Structural neuroimaging (sMRI) studies

Structural volume assessment (volumetry)

Historically, some of the first efforts to characterize the neural correlates of ASD investigated differences in large-scale brain structures between affected individuals and neurotypical controls. Initial evidence for altered brain structure and enlarged brain volume in ASD came from postmortem investigations (e.g., Bailey *et al*, 1998). The development of non-invasive magnetic resonance imaging (MRI) techniques subsequently allowed for more detailed study of brain volumes in specific brain regions across the lifespan in otherwise healthy individuals. The primary underlying hypothesis driving such studies has been that abnormalities

in specific brain structures early in development, often assumed to be decreased grey matter volume, may pinpoint a causal neuroanatomical basis for behavioral features in autism.

Traditional methods for assessing volume rely on manual tracing of regions of interest (ROIs), often defined by macro-structural features (i.e., hemispheres, lobes, subcortical areas, major gyri and sulci) easily visible on high-resolution structural MRI (sMRI) images. In toddlers with ASD, one of the most consistently reported global-level findings in sMRI is an increase in total brain volume of 5%-10% compared to locally recruited neurotypical children (e.g., Carper *et al*, 2002; Courchesne *et al*, 2001; Hazlett *et al*, 2005; Sparks *et al*, 2002; for a review see Amaral *et al*, 2008 and Stanfield *et al*, 2008). Analyses of brain development suggest that brain volume is normal at birth, diverging from typical trajectories during early childhood when children with ASD experience a period of brain overgrowth (reflected by enlarged total brain volume); this period is then followed by a plateau in volumetric changes during adolescence, ultimately leading to adult brain volumes that fall within range of typically developing controls (Courchesne *et al*, 2001, 2007). However, there is also some evidence for enlarged total brain volume in adolescence and adulthood (Freitag *et al*, 2009; Hazlett *et al*, 2006).

In addition to enlarged total cerebral volume, ASD is characterized by atypical grey and white matter volume in discrete brain structures (see recent reviews by Amaral *et al*, 2008; Chen *et al*, 2011; Stanfield *et al*, 2008; Steigler *et al*, 2011). The nature of these differences, unfortunately, has varied substantially across studies. An early finding in children, adolescents and adults with autism reported marked decrease in volume of the cerebellar vermis (Courchesne *et al* 1988, 1994); however, many of these subjects were low functioning. Several studies subsequently found that after controlling for or matching IQ cerebellar differences were no longer apparent (Levitt *et al*, 1999; Manes *et al,* 1999; Piven *et al*, 1992), suggesting that reduced cerebellar vermis

may relate to intellectual dysfunction but not necessarily core features of autism. In other brain areas, both increases and decreases in volumes have been reported in many regions including the frontal cortex (e.g., Hyde *et al*, 2010; McAlonan *et al*, 2005), superior temporal sulcus (e.g., Boddeart *et al*, 2004), inferior parietal lobule (e.g., Hadjikhani *et al*, 2006), cingulate, and fusiform gyrus (e.g., Bonilha *et al*, 2008) – many areas know to play a role in social cognition.

Voxel-based morphometry (VBM)

Recent advances in post-imaging processing methods allow for comparisons of grey and white matter density and volume on a voxel-by-voxel basis, referred to as voxel-based morphometry (VBM). Unlike traditional ROI-based volume analyses, VBM allows for whole-brain comparisons of grey and white matter volume between two groups. Results of these studies comparing individuals with ASD to neurotypical individuals have been extremely mixed, often reporting heterogeneous and at times contradictory results (see Chen *et al*, 2011 for a review). In a recent meta-analysis of VBM studies covering a wide age range of individuals with ASD, Nickl-Jockschat *et al* (2012) found converging evidence for alterations in the lateral occipital lobe, postcentral gyrus, medial temporal lobe, basal ganglia (right caudate and left putamen), and a region in the right hemisphere near the parietal operculum. The authors also suggested that puberty had an impact on the findings reported in VBM studies, namely that brain areas reported as having reduced grey matter in childhood were likely to be reported as having increased grey matter in adulthood, and likewise, brain areas reported as showing increased in grey matter in childhood were likely to be reported as having decreased grey matter in adulthood. Thus, discrepancies in VBM findings in ASD may also be related to differences in sample characteristics across studies,

particularly age, which impacts developmental trajectories of grey and white matter growth (Lenroot *et al*, 2007).

Importantly, in some cases the nature of the reported volumetric differences (increases, decreases, or no change from neurotypical individuals) seems to be impacted by the age of the cohort under investigation. For example in the amygdala, Schumann *et al* (2004) investigated regional volume in a cross sectional cohort of males with ASD, finding enlarged amygdala volumes in the 8-12 year old group, but no differences from control subjects in the 13-18 year old group. Degree of amygdala enlargement has also been associated with more severe social and communication impairment in children ages 3-4 years (Munson *et al*, 2006) and with anxiety symptoms in children 7-14 years (Juranek *et al*, 2006). Furthermore, in a recent meta-analysis, Stanfield *et al* (2008) found that amygdala volumes were increased in young children with ASD, but decreased with age, eventually falling to neurotypical volume levels. Even reports of head circumference changes across development, which have been consistently reported in ASD, have tended to suggest an age-specific pattern of initial overgrowth followed by reduced development in ASD (Courchesne *et al*, 2001).

White matter structure and diffusion tensor imaging (DTI)

Structural brain imaging studies in autism have increasingly moved from an early focus on grey matter volumes to the structure and organization of white matter (WM). White matter can be measured using MRI in several ways: by measuring the WM volume globally or regionally, the integrity of WM by diffusion tensor imaging (DTI; See Box 1), or the density of specific tracks using tractography, a DTI analytic technique. Atypical WM volume and other measures of WM integrity indicate a structural cause of disruptions in how different brain regions communicate with

each other. This research has contributed to an emerging understanding of autism as a disorder of developmental connectivity.

Early studies focused on WM volume in key regions associated with ASD. For instance, regional differences in white matter volume have been reported in individuals ranging from toddlerhood to middle age in the cerebellum and in multiple regions of the corpus callosum (e.g., Egaas *et al*, 1995; Hardan *et al*, 2009; Keary *et al*, 2009; Piven *et al*, 1997), a white matter tract connecting the left and right hemispheres that is critical for integration of neural information across distant brain regions. Indeed, there is evidence that global brain enlargement in many individuals with autism is due primarily to increased white matter but decreased grey matter volume. In one seminal study, Herbert *et al* (2003) divided the white matter in local "radiate" compartments composed largely of short, locally connecting circuits, and long tracts. They found that in children with ASD there was increased WM volume in more surface cortical regions while the deep, long pathway structures including the corpus callosum either exhibited lower or unchanged WM volume. The WM differences showing overgrowth in cortical regions are later to myelinate, and suggest a very specific developmental pattern of abnormal WM overgrowth. In contrast, Hardan *et al* (2009) found that children with ASD had reduced corpus callosum volumes, and that regional reductions in corpus callosum subdivisions were associated with greater reciprocal social interaction impairments, atypical speech, and sensory abnormalities. Together, these brain-based volume differences suggest altered development of brain structures implicated in high-level socio-cognitive processes in individuals with ASD.

Diffusion MRI (dMRI) provides a measure of structural connectivity by measuring the diffusion of water molecules in the brain to reconstruct white matter tracts. The extent to which water molecules diffuse in a consistent direction along the WM tract provides a proxy measure of

WM integrity, the most common of which is fractional anisotropy (FA). Diffusion studies in children, adolescents, and adults with ASD have documented multiple structural connectivity differences, mostly suggesting reduced white matter integrity in long-range anterior-posterior and interhemispheric fiber tracts (Alexander *et al*, 2007; Barnea-Goraly *et al*, 2004; Barnea-Goraly *et al*, 2010; Cheng *et al*, 2010; Shukla *et al*, 2011; Sundaram *et al*, 2008), with few studies reporting heightened white matter integrity (Bode *et al*, 2011; Cheng *et al*, 2010; Cheung *et al*, 2009). Notably, many of the specific fiber tracts reported as altered in ASD serve as structural connections between brain regions subserving social cognition. For example, Cheon *et al*, (2011) found reduced connectivity in the right anterior thalamic radiation, corpus callosum, and uncinate fasciculus in children with ASD, suggesting disruption of thalamic-to-frontal structural connectivity. In addition, reduced structural integrity of the corpus callosum has been consistently observed from childhood through adulthood (e.g., Alexander *et al*, 2007; Keller *et al*, 2007) and may be associated with lower nonverbal IQ in ASD subjects (Alexander *et al*, 2007). However, observed differences in white matter structure may not be causative in ASD, as unaffected siblings also show widespread reductions in structural integrity in the medial prefrontal white matter regions, anterior forceps, in substructures of the corpus callosum (body and splenium), cingulate, superior longitudinal fasciculous, internal and external capsules, superior temporal gyrus, and temporoparietal junction (Barnea-Goraly *et al*, 2010). Thus, it is possible that dMRI measures of structural integrity may be indicative of increased risk for ASD diagnosis only.

Despite these findings, a few critical questions remain unanswered in structural MRI research among children with ASD. First, how do these structural abnormalities relate to specific autism symptomatology? Few studies have related variations in brain structural abnormalities to specific phenotypes in autism, other than overall severity. In the language domain, Bigler *et al*

(2007) found that superior temporal gyrus volume related to receptive language ability among typically developing children and adolescents but not those with autism, suggesting a breakdown in the structure-function relationship. In an early study, Pierce and Courchese (2001) reported that cerebellar hypoplasia in children with ASD was correlated with the severity of repetitive behaviors. Together, these reports lend further support to the notion that heterogeneity in neuroimaging findings may be related to differences in subject characteristics (age, severity of behavioral symptomatology, etc.) across samples, and highlight the need for longitudinal studies of cortical volume development in ASD. However, without comprehensive, large scale studies that examine multiple regions and multiple phenotypes in the same samples, we cannot yet conclude that specific regional brain volume differences relate to autism phenotypes.

A second major issue concerns whether structural brain abnormalities are causal for ASD features, or whether early behavioral changes and abnormal interactions with the environment shape the development of brain structure to reveal these patterns of group differences. Longitudinal studies can help address this issue. The recent DTI findings of white matter abnormalities found in infants at-risk for autism, in an age range that typically precedes diagnosis, suggests that such differences precede and thus may be causal for ASD (Elison *et al*, 2013; Wolff *et al*, 2012), though this is not conclusive evidence. Ongoing studies that combine imaging with intervention may also shed light on causality, however there are as of yet no published findings of brain changes associated with early intervention.

Functional neuroimaging (fMRI) studies of core ASD deficits

In contrast to structural MRI, fMRI measures neural activity in the brain that relates directly to ongoing cognitive functions in vivo. Numerous fMRI studies in ASD have examined multiple

functions including motor, sensory, and language performance, but many studies have converged on the brain's social networks, reflecting an appreciation that autism is primarily a disorder of social cognition (other recent ASD neuroimaging reviews include Dichter *et al*, 2012; Pelphrey *et al*, 2011; Verhoeven *et al*, 2010). It is increasingly accepted that the primate brain has developed a series of brain regions constructed into networks collectively called the "social brain", evolutionarily developed to recognize and infer the intentions of others (Brothers, 1990). Among the fundamental behaviors associated with social cognition are face recognition, perceiving emotions in others, appreciating the meaning of eye gaze, discriminating biological motion, the ability to infer the mental states of others, and responding to social rewards such as smiling. The biological ability of neurons to respond specifically to social stimuli has been demonstrated in studies of non-human primates, where single cell recordings have detected neurons that fire selectively to faces in the superior temporal sulcus (Bruce *et al*, 1981; Desimone *et al*, 1984; Perrett *et al*, 1982), in response to a moving biological entity (e.g., a person walking; Bruce *et al*, 1981), head orientation, or direction of eye gaze in the temporal cortex (Perrett *et al*, 1985), and when animals perform or watch others perform an action, providing a means of encoding the goals and intentions of others, in the premotor cortex (e.g., di Pellegrino *et al*, 1992; Ferrari *et al*, 2005; Fogassi *et al*, 2005). Further, primate studies indicate that social stimuli in themselves are rewarding (e.g., Andrews and Rosenblum, 1993, 1994), and that different types of social stimuli provide differential reward value (e.g., viewing faces of high-status versus low-status conspecifics; Deaner *et al*, 2005). Together, these studies suggest that social stimuli and social interactions have an evolutionarily conserved neurobiological underpinning and provide socially relevant stimuli and brain loci with which to test and generate hypothesis about atypical neural function underlying social behavior in ASD. Current diagnostic criteria for ASD include deficits in social

communication (e.g., non verbal communication, reciprocity, etc.) and the presence of restricted or repetitive patterns of behavior (e.g., restricted interests, atypical sensitivity or interest in sensory stimuli; American Psychiatric Association [DSM-5], 2013), thus we focus our review on task-based fMRI studies tapping into core behavioral deficits observed in individuals with ASD – responding to biological motion, looking at faces, understanding and interpreting the intentions of others (theory of mind), language and reward processing, and sensory processing differences.

Face processing

Faces convey critical information not only about people's identity, but also about internal states and intentions, as well as about perceptions of the environment (e.g., a fearful face in response to a threatening situation). Research in neurotypical infants has shown a preference to attend to the human face over face-like stimuli from very early life (Fantz 1963; Goren *et al* 1975; Legerstee *et al*, 1998; Mondloch *et al*, 1999). Importantly, children with ASD do not show the same attention to faces as neurotypical children. A retrospective study of home videotapes revealed that infants later diagnosed with ASD could be distinguished from neurotypical children based on a lack of attention to other people and faces (Osterling *et al*, 2002; Osterling and Dawson, 1994), and further, toddlers with ASD appear to be less engaged by faces as they are faster to disengage from faces to attend to a peripheral target (Chawarska *et al*, 2003, 2010). Reduced attention to social stimuli very early in life may predispose children with ASD to atypical social cognition resulting in altered neuro-developmental trajectories and ultimately to atypical social behavior (Pelphrey *et al*, 2011).

Imaging studies suggest that viewing faces is associated with activity in the bilateral fusiform face area (a region of the fusiform gyrus), lateral occipital cortex, posterior superior

temporal sulcus (e.g., Grill-Spector *et al*, 2004), and amygdala (especially during fearful face processing; e.g., Guyer *et al*, 2008). It was originally proposed that children, adolescents and adults with ASD display reduced activation to faces based on fMRI studies finding reduced activity in the fusiform gyrus (Corbett *et al*, 2009; Critchley *et al*, 2000; Dalton *et al*, 2005; Hubl *et al*, 2003; Kleinhans *et al*, 2011; Pierce *et al*, 2001; Piggot *et al*, 2004; Schultz *et al*, 2000; Wang *et al*, 2004), and amygdala (Corbett *et al*, 2009; Kleinhans *et al*, 2011) during face and emotion processing. However, brain activity in these areas may be mediated by extrinsic factors that increase attention. For example, presenting ASD individuals with a cue to direct visual attention to the eye region (i.e., a cross hair at eye level prior to face stimuli presentation) (Bookheimer *et al*, 2008; Davies *et al*, 2011; Hadjikhani *et al*, 2004) is associated with increased activity in the fusiform gyrus. Furthermore, in a combined eye-tracking and neuroimaging study, Dalton (2005) found that the amount of time spent looking at the eyes was associated with greater activity in the fusiform gyrus and amygdala in individuals with ASD. Additionally, Pierce and colleagues (2004, 2008) found that when participants with ASD viewed faces of personally significant individuals (i.e., mother, friend), activity in the fusiform gyrus was comparable to that of controls. Together, these studies suggest that the original findings of hypoactive responses to faces in ASD is due to either avoidance of or lack of attention to the eye area. It appears that "normative" levels of activity in the fusiform gyrus may be elicited from ASD subjects by explicitly cueing attention to face stimuli, but doing so may also increase anxiety responses; indeed increased anxiety with direct gaze may underlie eye contact avoidance in ASD (Kleinhans *et al*, 2010).

Biological motion

The ability to detect salient social stimuli depends critically on the ability to detect biological motion, a behavior present in infancy (Hoehl *et al*, 2009; Simion *et al*, 2009). Examples of biological motion include following eye gaze or discerning the patterns of how people walk and move, as opposed to mechanical motion. This skill develops quickly in early infancy, as neurotypical children can detect eye gaze deviations during social interactions at five months of age (Symons *et al*, 1998). Early attention to biological motion likely heightens the amount of exposure infants receive to social stimuli, setting the stage for ongoing cognitive development in neural substrates involved in social and emotional cognition. Importantly, two-year old children with autism show an early behavioral deficit in attention to human biological motion visualized in point-light animations (Klin *et al*, 2009), an impairment that persists into late childhood (Blake *et al*, 2003).

Neuroimaging investigations of biological motion have employed a variety of stimuli including animated human characters moving their mouth, hand, or eyes (Morris *et al*, 2008; Pelphrey *et al*, 2005; Pelphrey and Morris, 2006), moving elements designed to display a range of human-like qualities (e.g., a human, a robot, a grandfather clock, and a mechanical assembly; Carter and Pelphrey, 2006), and point-light displays of both non-biological (e.g., a spinning wheel) and biological (e.g., walking) motion (Herrington *et al*, 2011). These studies suggest that in neurotypical individuals, processing biological motion relies on neural activity in the superior temporal sulcus, (Carter and Pelphrey, 2006; Morris *et al*, 2008; Pelphrey *et al*, 2005; Pelphrey and Morris, 2006), inferior frontal gyrus, amygdala, and visual areas including the fusiform gyrus (e.g., Herrington *et al*, 2011). In individuals with ASD, perception of biological motion is consistently associated with reduced activity in the superior temporal sulcus compared to controls

when viewing point-light displays (Freitag *et al*, 2008; Herrington *et al*, 2007; Koldewyn *et al*, 2011). Individuals with ASD also show hypoactivation of the ventrolateral prefrontal cortex (Davies *et al*, 2011), temporo-parietal junction and superior temporal sulcus (von dem Hagen *et al*, 2013) when viewing directs versus averted gaze. Reduced activity in neurotypical correlates of biological motion in individuals with ASD suggests atypical processing of biological motion cues present in everyday social contexts. In neurotypical individuals, detection of biological motion may serve to direct attention to socially relevant stimuli, a behavior that appears to be impaired in ASD.

Theory of mind and pragmatic language

"Theory of mind" (TOM) is described as the ability to understand and infer the actions and intentions of others (Baron-Cohen, 1991; Frith and Frith, 1999). It is well documented that individuals with ASD show deficits in higher-level social information processing, such as recognizing social faux pas (Baron-Cohen *et al*, 1999), and extrapolating the mental state of others based on cues expressed through the eyes and by voice (Baron-Cohen *et al*, 1997, 2001; Baron-Cohen and Hammer, 1997). In neurotypical individuals, TOM skills such as reasoning about the beliefs of others or their state of mind (e.g., Saxe *et al*, 2009; Saxe and Kanwisher 2003) elicit neural activity in the superior temporal sulcus, temporo-parietal junction, medial prefrontal cortex, and temporal poles (Gallagher *et al*, 2000; Saxe *et al*, 2009; Saxe and Kanwisher, 2003). In ASD, neuroimaging studies of TOM have shown reduced activity in the left inferior frontal gyrus (Harris *et al*, 2006; Just *et al*, 2004), medial prefrontal cortex (Castelli *et al*, 2002; Kana *et al*, 2009, 2014; Nieminen-von Wendt *et al*, 2003; Wang *et al*, 2007), and temporo-parietal junction (Kana *et al*,

2014). Notably, many of these regions overlap with those activated in biological motion, suggesting a potential common network underlying a range of social deficits in ASD.

Paralleling the deficits seen in theory of mind abilities, individuals with ASD also show deficits in language processing, particularly high-level language processing such as the proper use and comprehension of pragmatics, or appropriate use of language in a social context (Boucher, 2003; see Groen *et al*, 2008 for review). Behavioral studies have reported atypical production and comprehension of prosody (the rhythm, stress or intonation of speech) in children with high-functioning ASD (e.g., McCann *et al*, 2007). Deficits in prosodic comprehension have been documented in both children and adults with ASD, who have difficulty inferring the mental state of others when relying on vocal intonation cues (e.g., Golan *et al*, 2007; Peppé *et al*, 2007; Rutherford *et al*, 2002). In line with the behavioral deficits observed in ASD, functional neuroimaging studies also report atypical activation of language relevant brain areas. For example, a growing number of fMRI studies have reported more bilateral or right-lateralized activity in adults (Kleinhans, *et al*, 2008a; Mason *et al*, 2008; Tesink *et al*, 2009) and children (Knaus, *et al*, 2008; Redcay and Courchesne, 2008) with ASD. While some studies have shown hyperactivation (Wang *et al*, 2006), others have reported hypoactivation (Gaffrey *et al*, 2007; Wang *et al*, 2007), and a few others have reported simultaneous decreased activity in frontal brain regions and increased activity in posterior temporal areas (Harris *et al*, 2006; Just *et al*, 2004) during basic language processing.

A handful of studies have focused on higher-level language abilities in ASD by assessing the neural correlates of pragmatic or prosodic cues in language comprehension. In one study of pragmatic language, Tesink *et al* (2009) examined neural activity during a task in which adults with ASD made inferences about a speaker's characteristics (i.e., gender, age, social background)

using voice-based cues. While both ASD and neurotypical controls showed equivalent behavioral performance, the ASD groups showed greater activity in the right inferior frontal gyrus, suggesting an atypical and potentially compensatory neural mechanism in individuals with ASD. A second study of pragmatic language processing found that adults with ASD showed increased activity in the left supramarignal gyrus compared to controls (Hesling *et al*, 2010). Several other investigations have focused on the neural correlates of prosodic cues in ASD by examining brain activity during processing of ironic versus sincere speech. Wang *et al* (2006) employed a task in which children and adolescents with ASD listened to short scenarios and made a choice to indicate whether the speaker was sincere or sarcastic. While both the ASD and neurotypical control groups performed well on the task, the children with ASD showed increased activity in the right inferior frontal gyrus and bilateral temporal pole, suggesting more effortful processing in the ASD group during high-level prosodic language processing. A second study by Wang *et al* (2007) tested whether explicit instructions to attend to facial or tone of voice cues could regulate neural activity in children with ASD during ironic speech processing. Indeed, the authors demonstrated that while the ASD group showed reduced activity in the medial prefrontal cortex during ironic versus sincere speech (a region associated with theory of mind), this activity was modulated by specific instructions to attend to face or voice cues, and that activity in this region was negatively correlated with social impairment in ASD (i.e., less activity in the medial prefrontal cortex was associated with greater social impairment). Finally, a recent study by Colich *et al*, (2012) showed that when processing ironic versus sincere speech, children with ASD show a more bilateral pattern of activation in right hemisphere language homologues, and in brain areas implicated in theory of mind processing (such as the medial prefrontal cortex) compared to neurotypical children. Together, these studies suggest that compensatory activity in distributed brain regions in

individuals with ASD may allow for normal behavioral performance during high-level language processing, and that atypical neural activity may be modulated by attention to secondary social (i.e., face, voice) cues. Further, while language deficits are a common feature of ASD, it is no longer required for an ASD diagnosis (DSM V); thus heterogeneity in the ASD phenotype may help explain the range of findings in language regions among children with ASD.

Reward processing

In humans, social cues such as smiles are processed early in infancy and appear to be highly rewarding, biasing attention towards these cues. Early attentional biases to social stimuli may be modulated by increased neural mediated reward-value for these stimuli. Evolutionarily, successful social interaction in infancy is critical for survival as it impacts mother-offspring bonding (Lévy *et al*, 1995). One general theory of autism posits that reduced social motivation underlies the development of autism (Chevallier *et al*, 2012); reduced reward responsiveness could provide a potential neural basis of reduced social motivation. Animal studies suggest that reward processing involves activity in the anterior cingulate cortex, orbitofrontal cortex, and ventral striatum (Febo *et al*, 2005; Young *et al*, 2005). Importantly, recent neuroimaging studies have found that the same areas underlie reward processing in humans, including food reward (O'Doherty *et al*, 2002), monetary rewards (O'Doherty *et al*, 2001; Thut *et al*, 1997), and social rewards such as viewing happy faces (Phillips *et al*, 1998).

Given that children with ASD display reduced attentional biases to social stimuli, recent neuroimaging work has begun to investigate whether decreased activity in social-reward brain regions may play a role in atypical ASD social behavioral and neural phenotypes. These studies have primarily contrasted social reward stimuli (i.e., faces) to monetary reward in individuals with

ASD and neurotypical controls. Compared to controls, children, adolescents and adults with ASD show aberrant activity during monetary reward conditions in the anterior cingulate, frontal cortex and ventral striatum (Dichter *et al*, 2012; Kohls *et al*, 2013; Schmitz *et al*, 2008; Scott Van-Zeeland *et al*, 2010). During positive social reward conditions (i.e., smiling faces), ASD participants show reduced striatal activity in comparison to controls (Delmonte *et al*, 2012; Scott Van-Zeeland *et al*, 2010). Recently, Cascio *et al*, 2014 investigated brain activity in children with ASD during exposure to images of participant-specific restricted interest objects, aiming to disentangle whether the observed difference in neural activity during reward processing in ASD may be modulated by the salience of the reward, or instead represent broad functional atypicalities. While both controls and individuals with ASD showed activity in reward-related circuitry when viewing pictures of their own interests, only the neurotypical group showed similar activity when seeing pictures of another child's interest. Conversely, the ASD group showed reduced activity in reward-related areas including the insula and anterior cingulate cortex when seeing pictures of another child's interest. Thus, similar to results in neuroimaging studies of face processing, extrinsic factors that increase attention personally relevant rewarding stimuli may be associated with more neurotypical activity in individuals with ASD. Since narrow, selective interests are a core feature of children with ASD, reduced neural responses in reward related brain regions to non-preferred stimuli has face validity; however these data do not provide a mechanistic causative explanation for these atypical fMRI findings.

Sensory over-responsivity

Many children with ASD display over-responsivity to sensory stimuli (e.g., scratchy clothing, vacuum cleaners), but others are under-responsive and many are both over- and under-

responsive depending on the situation (e.g., Liss *et al*, 2006). Sensory processing issues in individuals with ASD have been challenging to study. Physiological studies often fail to find group (ASD vs. control) differences, likely because they fail to take into account within-ASD heterogeneity (Rogers *et al*, 2005). A recent neuroimaging study of sensory over-responsivity (SOR) found that children with ASD display hyper-reactivity in limbic areas (amygdala and hippocampus) and primary sensory cortices to mildly aversive visual and auditory sensory stimuli (Green *et al*, 2013). Notably, this study took into account individual levels of SOR, and found that activation in these areas was related to parent-reported SOR in both groups of children. Cascio *et al* (2012) compared neural response to pleasant, neutral, and unpleasant tactile stimuli in adults with and without ASD. They found that in general, the ASD group was under-responsive to the stimuli compared to the TD group, with the TD group having greater activation in multiple brain areas including the primary somatosensory cortex, middle frontal gyrus, superior temporal gyrus, and cingulate cortex. However, the ASD group had greater activation in the thalamus (pulvinar) in response to both the pleasant and unpleasant stimuli, and in the posterior cingulate and insula in response to the unpleasant stimulus. Greater insula response was correlated with severity of ASD symptoms. The authors hypothesized that individuals with ASD are hyporesponsive to pleasant tactile stimuli in primary and association somatosensory areas, but are hyperresponsive to unpleasant stimuli in areas associated with emotional processing of sensory stimuli. Voos *et al* (2013) also found that in neurotypical adults, higher levels of autism traits were associated with diminished response to pleasant touch in the superior temporal sulcus and orbital frontal cortex. While neither the Cascio *et al* (2012) or Voos *et al* (2013) study examined individual differences in sensory processing, these studies suggest that social touch is processed differently from unpleasant sensory stimuli in individuals with ASD.

In summary, a broad range of fMRI studies tapping into several aspects of social cognition and other core deficits show abnormal brain activity in ASD. A recent meta-analysis of 39 task-based fMRI studies in children and adults with ASD found that during social neuroimaging experiments, individuals with ASD had reduced probability of activation in the anterior cingulate cortex, right amygdala, and left frontal gyrus, as well as greater probability of activation in somatosensory brain regions (Di Martino *et al*, 2009). In particular, research shows reduced engagement throughout brain networks involved in processing social stimuli and responding to both positive and negative affect, as well as brain systems involved in understanding the emotional states of others (e.g., Dapretto *et al*, 2006) and regulating sensory and emotional experience. Interestingly, in some cases there is increased activity in regions not typically associated with task performance that may suggest engagement of compensatory neural systems. Of note, many regions showing reduced activity under certain conditions can show more normative responses when attention is explicitly directed to the task at hand, when the salience of the stimuli is increased, or when stimuli or tasks are more personally relevant to the individual. This argues in favor of the social motivation hypothesis of ASD, whereby a lack of intrinsic motivation to attend to social stimuli early in life leads to an altered developmental trajectory of neuro-cognitive development in ASD.

Studies of brain connectivity in ASD

While functional neuroimaging studies have informed our understanding of the regional neurobiological underpinnings of ASD during social-emotional cognition, recent work has focused on elucidating differences at the network/systems-level. Another way to conceptualize functional brain abnormalities in autism is to look at activity not within individual brain regions, but at the

way in which regions within social brain networks connect with each other, working in concert to perform complex tasks. It has been hypothesized that very early disruption in both the structural architecture and functional connectivity of local circuits in individuals with ASD may impact maturation of large-scale brain networks required for complex cognitive processing (Belmonte *et al*, 2004; Courchesne and Pierce 2005; Geschwind and Levitt 2007; Just *et al*, 2004; Mundy *et al*, 2009). These structural and functional abnormalities may thus prevent typical experience-dependent reorganization of neural circuitry into fully integrated networks, which are critical for understanding and initiating complex social behavior.

Connectivity in task-related brain networks

In ASD, task-based functional connectivity (fcMRI; See Box 2) studies have shown altered connectivity in multiple brain networks underlying complex social and emotional information processing. In an early study, Just *et al* (2004) found that during a sentence comprehension task, individuals with ASD displayed reduced connectivity between multiple high-level association cortical areas. These findings led the authors to suggest that cognitive deficits in ASD may be due to a general underconnectivity of brain regions important for information integration. In support of this hypothesis, many other task-based connectivity studies have reported underconnectivity in individuals with ASD in task-related brain areas including fronto-parietal connections during tasks involving working memory (Koshino *et al*, 2005), visuomotor coordination (Villalobos *et al*, 2005), visual imagery (Kana *et al*, 2006), executive functioning (Just *et al*, 2007), response inhibition (Kana *et al*, 2007), facial processing (Kleinhans *et al*, 2008), theory of mind (Kana *et al*, 2009), and during rest (Kennedy and Courshesne 2008). However, others have reported

overconnectivity of neural networks in ASD, (e.g., Mizuno *et al*, 2006; Noonan *et al*, 2009; Shih *et al*, 2010; Turner *et al*, 2006).

Importantly, recent functional and structural neuroimaging studies have demonstrated that altered brain connectivity is related to behavioral phenotypes in ASD. In a recent study, Abrams *et al* (2013) investigated resting state functional connectivity of the bilateral posterior superior temporal sulcus in children with ASD. The posterior superior temporal sulcus is involved in human voice processing in neurotypical individuals (Belin *et al*, 2000), but fails to activate in individuals with ASD (Gervais *et al*, 2004). Children with ASD showed reduced connectivity between the posterior superior temporal sulcus and many reward-related brain regions including the nucleus accumbens, insula, orbitofrontal cortex, and ventromedial prefrontal cortex. Importantly, the authors found that reduced connectivity between the posterior superior temporal sulcus and reward circuitry was associated with greater communication deficits. These findings suggest that the human voice may be less intrinsically rewarding for children with ASD and as a consequence negatively impact language outcomes.

Resting state connectivity fMRI

One major limitation of task-based functional MRI studies is that they require subject participation and thus are typically limited to older, higher functioning children with autism. Brain changes in later years may be a consequence rather than a cause of abnormal social development, and findings may not generalize to lower-functioning or non-verbal children or those with more severe social deficits. Furthermore, traditional fMRI studies comparing neural functioning between ASD and neurotypical populations must control for baseline differences in task

performance, and ensure that the task is well designed to address specific questions relating to ASD neurobiology. A relatively new approach that alleviates many of these concerns aims to understand functional brain connectivity by examining the interactions between brain regions not during task performance but while subjects are at rest. Resting state-functional connectivity MRI (rs-fcMRI) is a method in which fMRI is performed in the absence of an overt task in order to detect low frequency (<0.1 Hz) fluctuations in neural activity and identify co-activating brain regions (i.e., intrinsic functional brain networks) (Biswal *et al*, 1995; see Fox and Raichle 2007, for review). Findings of synchronized activity across brain regions, both at rest and during task conditions, suggest that functional brain organization consists of multiple interacting large-scale neural networks (e.g., Calhoun *et al*, 2008; Smith *et al*, 2009). Neuroimaging studies of ASD have recently begun to characterize functional connections within and between brain networks.

In neurotypical individuals, rs-fcMRI studies have identified multiple, widely replicated brain networks (see Hoff *et al*, 2013 for a review of resting state networks present in early development, and Van den Heuval and Hulshoff Pol, 2010 for a review of findings in adults). Here we will focus on networks implicated in ASD etiology. Perhaps the most widely studied functional connectivity network important for social cognition is the so-called "default mode network" (DMN), which is comprised of the posterior cingulate cortex, medial prefrontal cortex, lateral temporal cortex, inferior parietal lobule, and hippocampal formation (Buckner *et al*, 2008). The DMN has been shown to be involved in internally-directed cognition as it is deactivated during goal-directed behaviors and shows an anticorrelated relationship with the "attentional control network" (Stevens *et al*, 2009). In children, adolescents and adults with ASD, reports consistently suggest that connectivity between nodes of the DMN is diminished (Assaf *et al*, 2010; Cherkassky *et al*, 2006; Kennedy and Courchesne, 2008; Monk *et al*, 2009; Rudie *et al*, 2012; Weng *et al*,

2010). This is consistent with the known role of specific DMN nodes in tasks of social cognition (e.g., watching social interactions; Iacoboni *et al*, 2004) and the observed behavioral deficits characteristic of ASD (i.e., atypical theory of mind processing and social interactions). However, the DMN interacts in a dynamic fashion with other brain systems and is unlikely to be the only functional network affected in ASD.

Another network that has recently received a substantial amount of attention in the ASD literature is the salience network (Seeley *et al*, 2007), which is involved in identification of the most relevant information in one's environment, including social stimuli. Primary nodes of the salience network are the anterior cingulate cortex and anterior insula, which neuroimaging studies suggest play a role in perception of social exclusion (Bolling *et al*, 2011; Masten *et al*, 2011) and cognitive control (Agam *et al*, 2010). A recent study (Uddin *et al*, 2013) compared connectivity in large-scale brain networks in children with ASD and matched controls. Hyperconnectivity was observed in a number of brain networks, including the DMN and the salience network. Importantly, salience network connectivity values showed the highest classification accuracy in parsing neurotypical from ASD individuals, and correlated with severity scores for restricted interests and repetitive behaviors. Another study investigating connectivity of the salience network in adolescents and adults found reduced connections with the medial temporal lobe network, including the amygdala (von dem Hagen *et al*, 2013). Reduced functional connectivity between networks may suggest altered integration of social-emotional information across distributed brain regions in individuals with ASD.

Further evidence for network-level dysfunction in ASD comes from two recent studies by Keown *et al* (2013) and Supekar *et al* (2013), which found increased functional connectivity across multiple brain networks in children with ASD. Both studies also found that increased connectivity

was associated with greater severity of ASD impairments. As both under- and over-connectivity have been reported in ASD, a major challenge facing the autism neuroimaging field is to reconcile these seemingly discrepant findings. Findings of hyper- vs hypo- connectivity in ASD may depend on the nature of the task-related demands, the specific brain networks under investigation, study-specific methodological choices, as well the age of the cohort under investigation (Rudie and Dapretto, 2013). For example, Uddin *et al*'s (2013) review of the functional connectivity literature in ASD suggests that studies of adults and adolescents with ASD tend to report hypo connectivity compared to neurotypical controls, whereas studies of younger children often report hyper connectivity - indicating that differences in sample characteristics can lead to opposite findings. Taken together, these studies highlight the complexity of brain network organization in ASD and the need for longitudinal investigations in order to elucidate the entire developmental trajectory of altered connectivity in ASD.

Graph theoretical methods

As descried above, many neuroimaging studies report differences in regional network connectivity in ASD; however, how these findings might be reflective of more complex systems-level dysfunctions across the brain in individuals with ASD remains unclear. Recently, researchers have begun using graph theory methods to address this question by modeling the brain as a network of integrated and segregated systems composed of hundreds of brain regions or "nodes". Graph theoretical approaches depict the brain as a hierarchically organized network comprised of large-scale functional systems or modules (see Wang *et al*, 2010, for review). Each module is made up of discrete brain regions (nodes) and the connections between these nodes (edges). In the brain, functional and structural networks are "small-world" in nature, meaning that they are efficient at

both a local-systems and global-systems level (Watts and Strogatz, 1998). Graph theory measures such as the number of nodes, edges, and small-world characteristics such as modularity can be quantified (Bullmore *et al*, 2009; Rubinov and Sporns, 2010) and compared across populations during development (e.g., Fair *et al*, 2009; Hagmann *et al*, 2010), and in diseases such as schizophrenia (e.g., Bassett *et al*, 2008) and Alzheimer's disease (e.g., Supekar *et al*, 2008). Graph theoretical metrics are useful in that they go beyond simple connectivity analyses to describe large-scale properties of how brain networks are organized and how they interact.

To date, there have been relatively few graph theory studies of ASD, and reported findings have been mixed. In a whole brain investigation of network properties across four functional brain networks, Redcay *et al* (2013) found few differences between adolescents with ASD and neurotypical controls, except for greater "betweenness centrality" (a measure of how often the shortest path goes through a given node, or its centrality to the network) in a parietal region of the DMN in individuals with ASD. In another study of adolescents with ASD, Keown *et al* (2013) found increased local functional connectivity in temporo-occipital regions, which was associated with greater scores of ASD symptom severity. In a third study of children and adolescents with ASD, Rudie *et al* (2012) investigated both functional and structural connectivity using graph theory methods, finding alterations in local and global network measures including modularity and local efficiency of brain networks in children with ASD. Overall, graph theory studies of ASD suggest wide-scale disruptions in how brain networks communicate, suggesting that in autism, critical networks are less modular and less segregated from one another, with abnormalities both within and between networks. Although graph theoretical approaches are in their infancy, further research may elucidate more complex interactions between large-scale brain networks in ASD.

Integrating imaging and genetics

The identification of genetic contributions to ASD has progressed rapidly in the last decade (see Huguet *et al*, 2013, and Persico and Napolioni, 2013 for a review). Mirroring the heterogeneity observed in behavioral phenotypes, hundreds of genes have been implicated in conferring increased risk for ASD. Importantly, the biological functions of many ASD-associated genes impact the formation of neural circuits in the developing brain (Won *et al*, 2013), including prenatal transcription regulation and synapse development, and are enriched in outer cortical layers of the brain (Parikshak *et al*, 2013). However little is known about how autism risk genes relate to brain structure, function, and behavior. For over a decade, research on a range of common genetic variants related to neurobehavioral disorders has demonstrated differences in brain structure and function in risk gene carriers despite having no overt behavioral symptomatology (for a review see Hariri and Weinberger 2003). The field of imaging-genetics examines the relationship between risk genes and brain structure and function, conceptualizing neuroimaging metrics as potential endophenotypes. As MRI metrics of brain functional and structural connectivity are both heritable (Chiang *et al*, 2011; Fornito *et al*, 2011; Glahn *et al*, 2010; Kochunov *et al*, 2010; Koten *et al*, 2009) and altered in individuals with ASD, neuroimaging endophenotypes are well suited to inform our understanding of how genetic risk impacts brain circuitry. A key goal of imaging-genetics research is to elucidate neural mechanisms by which genetic heterogeneity may give rise to phenotypic heterogeneity in ASD. As genetics research suggests that many common single nucleotide polymorphisms (SNPs) are related to increased risk for autism diagnosis (see Klei *et al*, 2012 for a review), imaging studies have investigated whether stratifying neuroimaging data by common genetic risk factors can inform understanding of ASD neurobiology.

To date, neuroimaging-genetics studies have taken two forms: studies of the effects of ASD-associated risk alleles on brain measures in neurotypical children, adolescents and adults (e.g., Clemm von Hohenberg *et al*, 2013; Dennis *et al*, 2011; Hedrick *et al*, 2012; Raznahan *et al*, 2012; Sauer *et al*, 2012; Tan *et al*, 2010; Voineskos *et al*, 2011; Whalley *et al*, 2011) and studies comparing the effects of ASD-associated risk alleles on children and adolescents with ASD compared to neurotypical controls (e.g., Rudie *et al*, 2012a; Scott-Van Zeeland *et al*, 2010). Scott-Van Zeeland *et al* (2010) investigated the impact of the contactin-associated protein-like 2 (CNTNAP2) rs2710102, C risk allele on functional connectivity in children and adolescent with ASD. Results suggested that while nonrisk allele carriers (in both neurotypical and ASD groups) displayed connectivity between frontal cortex and language regions in the left hemisphere, risk allele carriers showed a pattern of diffusely increased functional connectivity with frontal cortex and temporal regions. A second study by Rudie *et al* (2012) found that children and adolescents carrying the met receptor tyrosine kinase (MET) rs1858830, C risk allele had decreased functional connectivity between the posterior cingulate cortex (PCC) and medial prefrontal cortex (MPFC) and reduced white matter integrity in the splenium of the corpus callosum, cortical spinal tract, and inferior longitudinal fasciculus. Interestingly, the authors also identified a significant interaction whereby the presence of one or two risk alleles in ASD children had a significantly larger impact on functional connectivity values than in neurotypical children.

Together, these results suggest that these and other ASD liability genes may confer risk through their effects of brain function and structure in regions involved in social and emotional cognition. While not causal, autism risk genes may bias the brain towards patterns of neural activity and connectivity that are atypical, and in combination with a range of additional genetic and environmental factors may contribute to abnormal brain development that ultimately underlies

ASD symptoms. Future neuroimaging studies should continue to use relevant genetics data to help explain variance observed in both behavioral and brain-based phenotypes of ASD, as well as to improve diagnostic tools and treatment strategies for individuals falling throughout the autism spectrum (Fox and Greicius 2010).

Machine learning

Prediction and classification of diagnostic status based on neuroimaging data represents a powerful tool for improved clinical care in ASD. Machine learning algorithms extract highly relevant components from neuroimaging data to classify an individuals' diagnostic status (see Box 3). Machine learning algorithms have been applied to neuroimaging data to identify ASD from neurotypical subjects using MRI measures of grey and white matter volume (classification accuracy (CA) 81% and 68% respectively, Ecker *et al*, 2010), regional cortical thickness (CA 70%-87%; Jiao *et al*, 2010; Zhou *et al*, 2014), voxel-based morphometry (CA 79-92%; Uddin *et al*, 2011), diffusion tensor imaging (CA 80%; Ingalhalikar *et al*, 2011), and resting state functional connectivity (CA 71-89%; Anderson *et al*, 2011; Uddin *et al*, 2013). Variations in classification accuracy may be attributed to the types of classification systems employed, which MRI-based metrics are utilized, and the number of features on which classification is determined (e.g., number of brain areas included in measures of regional cortical thickness). In a recent study, Nielsen *et al* (2013) used machine learning to evaluate whole brain resting state fMRI data collected from 16 sites in 964 subjects ranging in age from 7-64 years from the Autism Brain Imaging Data Exchange (ABIDE). The maximum CA achieved by this study was just 60%, much lower than CA's reported for single site studies. However, the authors note that higher CAs were calculated for sites where

longer resting state scans were collected, providing support for longer fMRI imaging times in future machine learning paradigms.

Very recently, machine learning algorithms have been applied to address questions of heterogeneity in ASD - aiming to distinguish between ASD subpopulations. Sato *et al* (2013) used inter-regional whole brain cortical thickness correlations and machine learning to predict scores on the Autism Diagnostic Observation Scale (ADOS; Lord *et al*, 1989) in children, adolescents, and adults with ASD, yielding a significant correlation ($r = 0.362$) between predicted and actual values. In a another study, Uddin *et al* (2013) classified children with and without ASD using large-scale brain network connectivity measures. Connectivity of the salience network was best able to classify subjects, achieving a CA of 83%, with BOLD signal in this network also predicting restricted and repetitive behaviors in the sample of children with ASD. While the machine learning literature in ASD is just beginning to emerge, these studies suggest that neuroimaging data evaluated with machine learning may help to identify brain-based biomarkers that correlate with severity of ASD symptomatology. Continued methodological development of this technique will undoubtedly further our understanding of neural signatures of ASD subpopulations.

Methodological considerations

Despite the growing corpus of brain imaging studies in ASD, many discrepant findings make it difficult to draw broad and definitive conclusions about brain abnormalities in ASD. As described in detail by Rudie and Dapretto (2013), there are a few major methodological considerations that may help to explain the seemingly discrepant findings between many prior studies of neural systems in ASD and more recent studies. First, these recent studies employed rigorous motion-correction methods. Recently it has been reported that even very small amounts

of motion in resting state functional connectivity data (e.g., Power *et al*, 2012; Van Dijk *et al*, 2012) and in structural diffusion MRI data (Yendiki *et al*, 2013) can bias findings of group differences in connectivity metrics. These findings are crucial to the interpretation of reports of altered brain connectivity in ASD, as ASD cohorts may display increased amounts of motion related artifacts in their MRI data compared to neurotypical controls, and similarly, the degree of motion present in the data is likely to be correlated with subject age (Satterthwaite *et al*, 2013), producing a possible age and diagnostic status confound. A multitude of studies have recently been published speaking to how best to account for motion related artifacts and aiming to identify the optimal series of processing steps for connectivity data analysis (e.g., Carp *et al*, 2013; Hallquist *et al*, 2013; Power *et al*, 2012, 2013, 2014; Satterthwaite *et al*, 2013). On the heels of these reports, scientists have begun to revisit published results of connectivity-related findings in ASD, and in more recently published studies to analyze their data using a variety of processing streams. Other methods-related choices such as use of low-pass filtering, task regression, seed selection, and whether connectivity results are obtained for the whole brain (versus within a priori ROIs) may also impact findings of over- or under connectivity in ASD (see Müller *et al*, 2013 for a review). Importantly, when analyzing data using a variety of suggested processing streams, findings of altered connectivity in children and adolescents with ASD appear to hold (e.g., Maximo *et al*, 2014; Nair *et al*, 2014; Starck *et al*, 2013; Uddin *et al*, 2013).

A second cause of discrepant findings is the differences in age and severity among different studies. The trajectory of age-related development in both brain structure and function may be complex, not holding to a simple linear increase or decrease with increasing age. For example, Schumann *et al*'s (2004) work showing that the pattern of amygdala brain overgrowth seen early in life in ASD reverses later in development indicates that differences in sampling characteristics

can lead to opposite findings. Ultimately, large-scale studies examining developmental trajectories will be essential if we are to understand how brain structure and function differ in ASD.

A third problem in brain imaging studies relates to the heterogeneity of the autism phenotype. The vast heterogeneity present in behavioral symptomatology and genetic liability to ASD likely reflects variation in disease etiology between diagnosed individuals (Geschwind 2009). Dissimilar disease etiology would additionally imply varied neuro-developmental trajectories in ASD affected individuals. Discrepant findings in the neuroimaging literature may reflect variance in the severity of ASD symptomatology, the constellation of ASD behavioral symptoms represented, or the presence or absence of associated features such as mental retardation and severe language impairment. Furthermore, as described above, evidence suggests that the atypical functional brain responses to task stimuli in ASD may not simply be a result of a fundamental deficit in neural functioning, but rather, that incorporating extrinsic factors that increase attention or interest into neuroimaging task designs are associated with more normal activity (Hadjikhani *et al*, 2004; Perlman *et al*, 2011; Pierce *et al*, 2004; Pierce and Redcay 2008; Wang *et al*, 2007; Zürcher *et al*, 2013). Thus, discrepant findings in the neuroimaging literature may also be due to variation in sample characteristics or task demands. To address these issues replication of previous findings will be essential.

Future directions and clinical implications

While neuroimaging techniques allow us to relate behavioral traits and genetic risks to structural and functional brain development, an important question in autism neuroimaging research is whether findings of altered brain activity and connectivity during childhood, adolescence, and adulthood are the *cause of*, or the *result of* ASD behavioral pathology. Experience

shapes brain structure, function, and connectivity; reduced social attention will affect a child's social experience, and the brain changes observed in older children may simply reflect this socially-deprived experience. Recent advances in neuroimaging methods will allow researchers to begin to address some of these complex interactions by studying very young infants who are at high genetic risk for developing ASD, and by following individuals with ASD over time to map long-term neurodevelopmental trajectories. Our understanding of causality will be further guided by animal models, which can manipulate both genetic risk and experience to understand brain development in ASD, as well as large-scale neuroimaging data sharing projects designed to achieve sufficient power to detect true brain-gene-behavior relationships. Such work will be greatly enhanced by large-scale collaborations such as ABIDE (the Autism Brain Imaging Data Exchange; Di Martino *et al*, 2014) and NDAR (the National Database for Autism Research), combining imaging data from multiple sites and integrating these data with both genetic and behavioral data. Given the correlational nature of imaging and genetic methods, causality can only begin to be addressed in the context of longitudinal studies of both affected and unaffected individuals with different genetic vulnerability for ASD. Recently, neuroimaging studies have begun to assess brain structure and function in unaffected family members of individuals with ASD. By mapping developmental trajectories in unaffected siblings, we may also gain insight into possible genetic and neurodevelopmental protective factors and critical periods during which such factors come on line.

Understanding developmental trajectories will be essential, both for understanding brain development in heterogeneous subgroups of individuals with ASD, as well as for determining the impact of treatment on brain structure and function in these populations. Because the infant brain is very plastic and adaptable, early interventions should help to shape the emerging activity and

connectivity patterns that experience helps to create. As our technologies improve, brain imaging may allow us to measure the impact of interventions on brain development, as well as to inform the choice of optimal interventions. With the increasing move towards imaging infants and toddlers at risk for autism, we should be able to identify abnormal neurodevelopmental trajectories and guide intervention strategies that will be most effective at this stage of greatest brain plasticity.

Any application of neuroimaging data to aid in diagnosis and treatment will ultimately rely on the ability to accurately identify children at risk for developing ASD. A major question is whether differences in neuroimaging measures (which are typically interpreted at the group level) are robust at the individual subject level, a prerequisite for identifying and developing treatment options for *individuals* at high risk for ASD. Recent advances in data analytic methods, such as machine learning classification techniques that allow for integration of information across multiple modalities (e.g., Ingalhalikar *et al*, 2012) and identification of subpopulations within a heterogeneous population (e.g., Filipovych *et al*, 2012), may be able to identify the most relevant signatures of ASD from neuroimaging, genetic, and behavioral data in order to accurately predict diagnostic status, treatment response, and/or developmental outcome at the individual level.

BOXES

1.

DTI: Diffusion tensor imaging (DTI) allows for mapping of white matter tracts by measuring the diffusion of water molecules in the brain (e.g., Basser & Pieraoli, 1996). When unrestricted by physical boundaries, water diffuses in an isotropic manner (equally in all directions). In the brain, the diffusion of water is restricted by the physical boundaries of axons and myelin. Thus, in the brain the diffusion of water occurs preferentially along the long axis of the axon. DTI measures the principle direction of water diffusion in each brain voxel and models this information in the shape of an ellipsoid whose long axis points in the direction of the mean axonal direction for the voxel – this information can be reconstructed to identify gross trajectories of white matter fiber tracts in the whole brain. The most reported measure in DTI neuroimaging studies is fractional anisotropy (FA), which provides a metric of the degree of diffusion for each voxel and is thought to be a reflection of white matter integrity. In DTI, the direction in which fiber tracts travel is typically expressed with different colors that correspond to the primary direction at each point. In addition, various mathematic algorithms can approximate the pathway a particular fiber tract takes as it curves and turns to reach its target, thus isolating fiber bundles through the brain. This approach is called tractography. While DTI has greatly improved our understanding of structural brain connectivity at the macroscale, confounds including the inability to distinguish crossing fibers due to spatial resolution limitations imposed by imaging at the voxel level, the limited ability to quantify connection strengths, and the probabilistic nature of DTI tractography (potentially leading to inaccurate models), which remain active areas of methodological development (Craddock *et al*, 2013).

2.

Resting State Connectivity: Resting state-functional connectivity MRI (rs-fcMRI) refers to a growing body of literature in which imaging is performed in the absence of an overt task to detect brain regions where the BOLD fMRI signal is co-fluctuating; these methods provide a map of intrinsic functional brain networks. There are two main methodological approaches used to identify resting state brain networks. 1) Seed-based methods, in which the average BOLD time course is extracted from a seed region and correlated with every other voxel in the brain, thereby identifying voxels with BOLD signal time series co-fluctuating with that of the seed. 2) Independent component analysis (ICA), in which statistically independent components of the resting state BOLD scan are identified in a data-driven manner, providing the user with all statistically independent time-varying signals within the data. While a main advantage of ICA is that it provides an unbiased metric of all components in the data (i.e., no a priori hypotheses related to a seed region of interest are needed), a critical disadvantage is that individual users must decide which components are biologically meaningful (as opposed to noise components, for example) and merit reporting and further investigation. Some of the most common resting state brain networks include, sensory networks such as visual and auditory systems, attentional networks that include regions such as dorsal prefrontal cortex, the default mode network which is negatively correlated with task performance, and the salience network, which relates to identifying relevant information in one's environment. Critically, these networks persist both during task-based activity and when individuals are at rest, as well as during sleep and sedation. Connectivity-based methods are uniquely suited to inform our understanding of network-level brain activation, providing insight into the history of co-activation between brain regions, and

atypicalities indicative of clinical diagnoses. However, interpretation of fcMRI results can be difficult since connectivity measures can be impacted by differences in underlying brain structure, cognitive state, and subject motion during data acquisition (Buckner *et al*, 2013).

3.

Machine Learning. Traditional machine learning methods (also see Klöppel *et al,* 2012) begin by feeding neuroimaging data from labeled cases and controls (the training data set) into a mathematical classifier and identifying relevant components (or features) from the neuroimaging data that contribute to group discrimination. The classifier then uses the training data set to establish a set of rules that allow for optimal discrimination of patient and control groups, converging on an optimal classification algorithm. Finally, this algorithm is applied to a new set of neuroimaging data consisting of non-labeled cases and controls and classification accuracy (CA) is assessed by calculating the number of correct positive (ASD) and negative (neurotypical) classifications for the new data. A major advantage of this approach is that it allows *individual* subjects to be evaluated for likelihood of ASD diagnosis. An important limitation to the application of this method to the study of autism is the inherent heterogeneity of the ASD subject population, which can decrease overall CA. There is great potential to overcome this limitation by studying circumscribed ASD sub-groups (including restrictions based on age, comorbid symptoms, etc.) and collection of larger data sets.

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CHAPTER 2: Additive effects of oxytocin receptor gene polymorphisms on reward circuitry in youth with autism

ABSTRACT

Several common alleles in the oxytocin receptor gene (*OXTR*) are associated with altered brain function in reward circuitry in neurotypical adults and may increase risk for autism spectrum disorders (ASD). Yet, it is currently unknown how variation in the *OXTR* relates to brain functioning *in individuals with ASD*, and, critically, whether neural endophenotypes vary as a function of aggregate genetic risk. Here, for the first time, we use a multi-locus approach to examine how genetic variation across several *OXTR* single nucleotide polymorphisms (SNPs) affect functional connectivity of the brain's reward network. Using data from 41 children with ASD and 41 neurotypical children, we examined functional connectivity of the nucleus accumbens (NAcc) – a hub of the reward network – focusing on how connectivity varies with *OXTR* risk-allele dosage. Youth with ASD showed reduced NAcc connectivity with other areas in the reward circuit as a function of increased *OXTR* risk-allele dosage, as well as a positive association between risk-allele dosage and symptom severity, whereas neurotypical youth showed increased NAcc connectivity with frontal brain regions involved in mentalizing. Additionally, we found that increased NAcc-frontal cortex connectivity in TD youth was related to better scores on a standardized measure of social functioning. Our results indicate that cumulative genetic variation on the *OXTR* impacts reward system connectivity in both youth with ASD and neurotypical controls. By showing differential genetic effects on neuroendophenotypes, these pathways elucidate mechanisms of vulnerability versus resilience in carriers of disease-associated risk alleles.

INTRODUCTION

Autism spectrum disorders (ASD) are genetically complex neurodevelopmental disorders. While hundreds of genes have been implicated in the etiology of ASD, recent evidence suggests that most genetic liability is conferred by inherited variations in single nucleotide polymorphisms (SNPs) that are commonly distributed throughout the population.[1,2] Findings of risk variants clustered within particular genes may suggest biological pathways likely to be altered in ASD, supporting an additive, polygenic model of autism risk whereby individuals who carry greater numbers of risk alleles are at increased risk for ASD and may present more severe symptomatology.[3] However, thus far, studies relating common genetic variants to neurobiological functioning in ASD have focused on single SNPs. This approach fails to take advantage of genetic variability across multiple loci, which can be leveraged to parse the considerable neurobiological heterogeneity observed in ASD.

As social deficits are core features of the ASD phenotype, genes linked to variations in social behavior are logical targets for investigation. Allelic variations on the oxytocin receptor gene (*OXTR*) have been associated with increased rates of ASD,[4] lower social responsiveness,[5] and increased severity of social deficits in individuals with ASD.[5-7] Further evidence for the critical role of *OXTR* in social functioning comes from animal models demonstrating that dense expression of the oxytocin receptor in the nucleus accumbens (NAcc) is associated with social affiliative behaviors,[8] reduces susceptibility to early adverse experiences (e.g., neglect),[9] and critically, that allelic variation in the *OXTR* is associated with variability in receptor expression specifically in brain areas important for social attachment, including the NAcc.[10] Further, disruption of oxytocin receptor signaling in the NAcc inhibits social attachment[11,12] and the formation of positive associations with social rewards.[13] Together, these studies suggest that oxytocin receptor function

in the NAcc is required to form associations with social rewards through its effects on neural excitability in reward-related brain regions.

Notably, a large body of work indicates that reward processing is altered at the neural level in individuals with ASD. Compared to neurotypical controls, individuals with ASD show aberrant brain activity to monetary rewards in several nodes of reward circuitry including the ventral striatum, anterior cingulate, and prefrontal cortex,[14-17] as well as reduced striatal activity to positive social rewards (i.e., smiling faces).[15,18] According to a leading theory of ASD, reduced ability to represent the reward-value of social stimuli results in poor motivation to engage in social interactions, decreasing opportunities for social learning and thus leading to social impairments.[19,20] Importantly, imaging-genetics studies in neurotypical adults suggest that inheritance of certain *OXTR* alleles linked to poor social skills are related to altered functional activity, connectivity, and volume of brain regions implicated in reward and social-emotional processing.[21-23] However, no studies have examined how any *OXTR* SNPs relate to brain functioning in individuals with ASD and, importantly, whether neural endophenotypes vary as a function of carrying multiple *OXTR* risk alleles.

We addressed these questions in 41 youth with ASD and 41 typically developing (TD) matched controls (age range 9-17 years; Table 1) using resting-state functional magnetic resonance imaging (rs-fMRI) to examine the relationship between four *OXTR* SNPs previously associated with ASD[4,24-26] (rs53576, rs237887, rs1042778, rs2254298) and functional connectivity in reward circuitry. Based on the animal and human work presented above, we hypothesized that greater *OXTR* genetic risk would be associated with reduced NAcc functional connectivity with other nodes of the reward system, particularly so in youth with ASD.

MATERIALS AND METHODS

Participants

High-functioning children and adolescents with ASD (N=56) and typically-developing (TD) controls (N=45) were recruited through the UCLA's Center for Autism Research and Treatment (CART) or by flyers posted throughout the Los Angeles area. Participants with a history of claustrophobia, diagnosed neurological disorders, genetic conditions, structural brain abnormalities, or metal implants were excluded from study participation. Study protocols were approved by the UCLA Institutional Review Board (IRB). Before assessment, informed consent and assent to participate in research were obtained from legal guardians and study participants. For ASD participants, inclusionary criteria included a prior diagnosis of autism confirmed at the UCLA Autism Evaluation Clinic with the Autism Diagnostic Observation Schedule – 2nd Edition (ADOS-2),[27] the Autism Diagnostic Interview – Revised (ADI-R),[28] and best clinical judgment. Table 1 displays mean scores on social and communication subscales of the ADOS and ADI, as well as mean verbal, performance, and full scale IQ for ASD and TD participants, as assessed with the Wechsler Intelligence Scale for Children – 3rd Edition (WISC)[29] or the Wechsler Abbreviated Scale of Intelligence (WASI).[30] Twenty-seven of the ASD children reported current use of psychotropic medications; 13 children reported use of a single medication, 14 children were on a combination of one or more medications (see Supplementary Information).

Behavioral Measures

Behavioral measures included the Social Responsiveness Scale (SRS)[31] and the Autism Diagnostic Observation Schedule (ADOS-2).[27] The SRS is a parent-report questionnaire intended for use in children 4–18 years of age that provides a quantitative measure of social impairment. Autism severity scores were calculated for each of the ASD participants by converting ADOS raw

total scores into calibrated severity scores, which take into account chronological age and language level in assessing the severity of core autism features.[32]

Genotyping

DNA was extracted from saliva samples using standard protocols from the OraGene Collection Kit (DNA GenoTek). The SNPs rs53576 and rs2254298 were genotyped at the UCLA Genotyping and Sequencing Core (GenoSeq Core; http://genoseq.ucla.edu) using a 5' nuclease assay to discriminate between the two alleles (Taqman SNP Genotyping Assay, Applied Biosystems Inc.). Polymerase chain reactions were performed using 5-µL reaction volumes in 384-well plates with 25 ng of DNA and Taqman genotyping master mix from Applied Biosystems Inc. The standard protocol provided with the kit was followed. End point reads of fluorescence levels were obtained with an ABI 7900HT Sequence Detection System. Genotyping for rs237887 and rs1042778 was performed at the UCLA Neuroscience Genomics Core (UNGC; https://www.semel.ucla.edu/ungc) according to standard manufacturer protocols using the HumanOmni2.5-8 BeadChip microarray (Illumina Inc.). Genome-wide SNP data, including rs237887 and rs1042778, were generated by the UCLA Neuroscience Genomics Core (UNGC; https://www.semel.ucla.edu/ungc) using the Illumina Omni-1 or Omni-2.5-exome platforms according to standard manufacturer protocols. After quality filtering (<5% missing per person/per SNP, >1% minor allele frequency, Hardy-Weinberg equilibrium $p>10^{-7}$), multi-dimensional scaling was performed in PLINK (http://pngu.mgh.harvard.edu/purcell/plink/)[33] using the default settings with the HapMap 3 reference panel (http://hapmap.ncbi.nlm.nih.gov/).[34]

MRI Data Acquisition

Resting-state functional connectivity MRI (rs-fcMRI) data were collected on a Siemens 3T Trio whole-body scanner using a 16-channel phased-array head coil. For each subject, a scout localizing scan was first acquired for graphic prescription followed by a T2-weighted echo planar imaging (EPI) volume (TR=5000 ms, TE=34 ms, 128x128 matrix size, 19.2 cm FoV, 36 4 mm axial slices, in plane voxel dimension=1.50x1.50 mm), which was acquired co-planar to the functional volumes to ensure identical distortion characteristics. For the rs-fcMRI data, a 6 min T2*-weighted functional scan was acquired while the subjects were asked to fixate on a black crosshair presented on a white screen (TR=3000 ms, TE=28 ms, 64x64 matrix size, 19.2 cm FoV, 34 4 mm slices, in plane voxel dimension=3.0x3.0 mm). In order to ensure that subjects were at ease during the scanning session, all participants participated in a mock scan prior to the date of their MRI.

Resting-State Data Processing

Neuroimaging data were analyzed using FSL (FMRIB's Software Library, www.fmri.ox.ac.uk/fsl)[35] and AFNI (Analysis of Functional NeuroImages).[36] Briefly, functional and structural volumes were skull stripped and functional data were motion corrected to the average functional volume using FSL's MCFLIRT (Motion Correction Linear Registration Tool);[37] translations in the x, y, and z dimensions were calculated from volume to volume, then averaged to create a measure of mean displacement. Subjects with greater than 3 mm of motion from one volume to the next were excluded from further analyses (N=7 ASD, N=1 TD). Functional data were linearly registered to the high-resolution T2-weighted EPI volume (6 degrees of freedom), then to the MNI152 2 mm standard brain (12 degrees of freedom) in light of empirical evidence indicating that the use of an adult brain template is appropriate for children aged 7 and

above.[38,39] Next, FSL's Automatic Segmentation Tool (FAST) was used to segment the high-resolution scans, creating subject-specific masks of grey matter (GM), white matter (WM), and cerebral spinal fluid (CSF). The data were then band-pass filtered (0.1 Hz > t > 0.01 Hz), smoothed (FWHM 5 mm), and FSL's FEAT was used to regress out nuisance variables including WM, CSF, and global time-series. Motion scrubbing was performed; volumes for which framewise displacement (FD) exceeded 0.5 mm and BOLD percent signal change from the prior volume (DVARS) exceeded 0.5% were removed ("scrubbed") from the data; one volume immediately proceeding and two volumes following the scrubbed volume were also removed.[40] Volumes were removed for 13 ASD youth (mean volumes removed = 8.6, range = 4-19) and 13 TD youth (mean volumes removed = 9.1, range = 4-18); there was no difference between diagnostic groups in the number of removed volumes (p=0.89). After scrubbing, participants with less than 5 min of rs-fcMRI data were excluded from further analyses (N=8 ASD, N=3 TD), leaving a final sample of 41 youth with ASD and 41 TD youth. All volume removal was completed prior to subsequent statistical analysis of the data. Finally, using an affine transformation, residuals from the motion scrubbed data were aligned to the T2-weighted high-resolution volume (6 degrees of freedom), then to the MNI 152 2 mm standard brain (12 degrees of freedom) with FLIRT (FMRIB's Linear Image Registration Tool).

Resting-State Data Analysis

To examine functional connectivity of the nucleus accumbens (NAcc), time series were extracted from the bilateral NAcc defined using the Harvard-Oxford Atlas at a threshold of 25% probability. Averaged time series from this region of interest (ROI) were correlated with time series from every other voxel in the brain to generate maps of NAcc functional connectivity for each participant. Correlation maps were converted to z-statistic maps (Fisher's r to z transform).

Single-subject maps were then combined at the group level and compared between diagnostic groups. Statistical analyses were preformed using FEAT version 5.98. Whole-brain connectivity maps were compared between ASD and TD participants (Figure 1, Supplementary Table 1). For imaging-genetics analysis, a risk score was calculated for each subject by summing the number of risk alleles inherited across the 4 SNPs of interest (Supplementary Figure 1A). The group mean was then calculated across ASD and TD participants and used to de-mean single subject risk scores. Demeaned values were used as covariates in higher-level FEAT analyses using FLAME, an FSL mixed effects model. To identify brain areas with different *OXTR* modulation effects in the TD and ASD groups, the interaction effect was tested focusing on areas showing significant *OXTR* modulation in either group. All imaging results are presented at $z>2.3$, $p<0.01$, cluster corrected for multiple comparisons at $p<0.05$.

RESULTS

Additive *OXTR* Risk

The average number of risk alleles for all participants was 3.11 (*SD*=1.02, range 1-6); neurotypical participants had an average of 2.95 risk alleles (*SD*=1.05); participants with ASD had an average of 3.26 risk alleles (*SD*=0.98; Supplementary Figure 1B,D). An independent samples t-test showed there were no significant differences in the aggregate number of risk alleles between TD and ASD groups. Risk allele frequencies were equally distributed with skewness of 0.278 (*SE*=0.266) and kurtosis of -0.337 (*SE*=0.526). To ensure that each SNP was inherited independently of the others, pairwise linkage disequilibrium was calculated in Haploview version 4.2;[41] pairwise r^2 in the data was less than 0.32 (Supplementary Figure 1C,E).

NAcc Functional Connectivity

As expected, the NAcc showed positive connectivity with other regions of the reward network including the bilateral caudate, putamen, anterior cingulate and frontal cortex, as well as negative connectivity with the thalamus, superior parietal, and occipital brain areas (Figure 1A,B, Supplementary Table 1). While overall similar patterns of positive and negative connectivity were found in both groups, direct between-group comparisons showed that youth with ASD had less connectivity between NAcc and superior frontal gyrus, as well as posterior cingulate/precuneus (a hub of the default mode network; Figure 1C, Supplementary Table 1).

Modulation by Aggregate OXTR Risk

In youth with ASD, carrying more *OXTR* risk alleles was associated with reduced NAcc connectivity with other nodes of the reward circuitry, specifically bilateral caudate, putamen, and anterior cingulate gyrus (Figure 2A, Supplementary Table 2); no decreased connectivity was observed in TD youth as a function of higher aggregate risk score. In contrast, TD youth carrying more *OXTR* risk alleles showed increased NAcc connectivity with regions in the middle prefrontal cortex (Figure 2B, Supplementary Table 2); no increased connectivity was seen in ASD youth as a function of higher aggregate risk score. Next, to identify brain areas with significantly different *OXTR* modulation effects in the TD and ASD groups, the interaction effect was tested. Results confirmed that, as compared to their TD counterparts, ASD youth showed significantly reduced connectivity with striatal and mesolimbic regions, whereas TD participants showed significantly stronger NAcc connectivity with frontal cortex (Supplementary Figure 2).[42]

To evaluate whether imaging-genetics findings were influenced by ancestry, the first two components from multi-dimensional scaling of genome-wide data were controlled for in a correlation between *OXTR* aggregate risk score and connectivity values in regions modulated by

risk-allele dosage. The observed correlations between aggregate *OXTR*-risk and NAcc-connectivity in both ASD and TD groups remained significant ($p<0.001$).

Correlations with Social Responsiveness

Average connectivity values between the NAcc and regions modulated by the *OXTR* in TD and ASD groups were extracted and correlated with t-scores from subscales of the Social Responsiveness Scale (SRS).[31] While there were no significant findings in the ASD group, greater connectivity between the NAcc and frontal cortex in the TD group was correlated with better scores on the social cognition subscale of the SRS (r=-0.35, $p<0.05$; Figure 3). To confirm these findings, we conducted a separate, independent analysis using SRS social cognition t-scores as a regressor of interest in a bottom-up analysis. Verifying our original finding, a cluster in the frontal pole reached statistical significance (peak coordinate -20, 46, 28, cluster size 63 voxels), indicating that more NAcc-frontal pole connectivity in the TD group is associated with better scores of social functioning. As each of the four *OXTR* SNPs used to create the aggregate risk score has independently been associated with ASD, we further hypothesized that inheritance of more risk-alleles would be related to greater ASD symptom severity. Indeed, in ASD participants, the correlation between *OXTR* risk-allele dosage and Autism Diagnostic Observation Schedule (ADOS)[27] severity score was significant ($r(40)=0.28$, $p<0.05$, one-tailed), such that individuals with greater numbers of *OXTR* risk alleles displayed more severe ASD symptomatology.

DISCUSSION

Here we show, for the first time, that common ASD-associated genetic variants in the *OXTR* act additively to impact functional connectivity in the human brain. Specifically, we found that *OXTR* risk alleles coalesce to decrease connectivity between brain areas critical for reward

processing in youth with ASD. These additive effects on brain circuits in individuals with ASD are consistent with evidence suggesting that ASDs follow a polygenic pattern of inheritance, with genetic variants at many loci contributing to expression of the ASD phenotype.[1] In our ASD sample, greater *OXTR* risk-allele dosage was associated with more severe ASD symptomatology as indexed by the ADOS, demonstrating that cumulative risk on this gene does exert significant negative effects on social functioning in individuals with ASD. The oxytocin receptor is a g-protein-coupled receptor that, through a cascade of events, affects intracellular calcium levels and neural excitability.[43] Thus, alterations in neural excitability in the nucleus accumbens (NAcc) – the hub of the reward circuit – may explain our findings of reduced functional connectivity, as well as previous reports of hypoactivity during reward processing in ASD.[15,16] Furthermore, we found that in both youth with ASD and neurotypical controls, greater genetic risk in the *OXTR* modulates NAcc functional connectivity, although in different ways. Specifically, greater *OXTR* risk in ASD children was associated with *decreased* connectivity in the striatum, a set of brain regions critical for reward processing and implicit learning; conversely, greater *OXTR* risk in neurotypical children was associated with *increased* connectivity with the frontal pole, an area implicated in the ability to understand the mental states of the self and others.

Remarkably, our observations of reduced connectivity as a function of increased genetic risk were limited to individuals with ASD; significant interaction effects confirmed that, compared to their TD counterparts, ASD youth showed significantly reduced NAcc connectivity with subcortical brain regions implicated in reward processing, whereas TD youth showed stronger NAcc connectivity with frontal regions implicated in mentalizing processes.[42] This finding of increased connectivity in TD children could reflect a compensatory mechanism in the face of increased genetic risk. Consistent with this interpretation, we found that greater NAcc-frontal pole

connectivity in TD youth was related to better social cognition measured with the SRS.[31] These findings are in agreement with prior imaging-genetics work showing that neurotypical individuals who are carriers of ASD-risk variants show alterations in brain function and structure.[44,45] Our results in neurotypical youth highlight possible compensatory mechanisms, which may lead to improved social cognition despite increased *OXTR* risk-allele dosage. Importantly, there is also evidence that genetic variants may have enhanced effects (i.e., increased penetrance) on disease-related brain circuits in individuals who have a diagnosis of ASD[46] – an effect likely due to the presence of other genetic (i.e., epistatic) and environmental susceptibilities. Our data indicate that additive genetic effects in the *OXTR* have more pronounced effects on connectivity with reward-related brain circuits in individuals who express the ASD phenotype. More generally, these findings indicate that additive genetic vulnerability in biological pathways underlying reward processing in the human brain may be one mechanism by which neural vulnerability leads to atypical social behavior in ASD.

While the present study focused on the effects of multiple SNPs in a single gene, it will be important for future research to examine risk factors across multiple genes implicated in autism, which may modulate brain activity and connectivity in other neural circuits known to be compromised in ASD. Our study focused on a small set of ASD-associated *OXTR* SNPs that demonstrated a lack of linkage disequilibrium (LD) with one another in order to assure statistical independence of each SNP's inheritance. Additional variance in neural endophenotypes may be explained by using more complex statistical models to account for the degree of LD between loci and thus examine the additive effects of ASD-associated SNPs in close proximity to one another. While there is some evidence that several *OXTR* SNPs affect expression of the oxytocin receptor in the human brain (i.e., they are expressed quantitative trait loci; http://www.braineac.org), these

effects are not genome-wide significant, thus limiting our ability to relate our results to variation in NAcc oxytocin receptor expression. Nevertheless, our findings show that functional connectivity in the reward network varies significantly with *OXTR* risk-allele dosage and provide a model for integrating genetic risk across multiple loci with neuroimaging data to further elucidate mechanisms of vulnerability and resilience to neurocognitive disorders.

Given recent interest in the use of intranasal oxytocin as a treatment option for individuals with ASD,[47] our results also have broad clinical relevance. Several studies have shown that intranasal oxytocin administration affects brain activity during social-emotional processing in neurotypical individuals[48] and in individuals with ASD;[49-52] further, oxytocin administration has been shown to rescue social behaviors in a mouse model of ASD.[53] Importantly, the effects of intranasal administration on brain activity in humans may be moderated by genotypic variation in the *OXTR*[54] and other genes in the same pathway. For instance, during a task designed to elicit social cooperation, neurotypical male and female carriers of the *OXTR* rs53576 GG genotype show different patterns of ventral striatal activity in response to oxytocin administration, a sex-specific effect not seen in carriers of other rs53576 genotypes.[55] Similarly, when exposed to intranasal oxytocin, individuals homozygous for the ASD-associated allele of rs3796863 in the *CD38* gene (involved in endogenous oxytocin secretion) show increased brain activity in the fusiform gyrus during a face processing task, an effect again not seen in individuals carrying non-risk alleles.[56] Together, these studies suggest that aggregate genetic variability may be an important biomarker to consider when determining which individuals would optimally benefit from oxytocin treatment. The identification of subgroups, within the heterogeneous ASD population, is a prerequisite step to implement targeted interventions; our findings are relevant to this goal as they indicate that

stratification by risk-allele dosage may reveal distinct neural endophenotypes and thus help parse the considerable heterogeneity observed in ASD.

Overall, our results expand upon other lines of evidence, from both animal models and human studies, indicating that genetic variation in the *OXTR* is related to reward system functioning. Importantly, as the observed additive genetic effects differed in youth with and without ASD, this work underscores the benefits of examining cumulative genetic risk to elucidate neural pathways conferring increased vulnerability, as well as resilience, in carriers of disease-associated risk alleles. Our findings of additive genetic effects on neural endophenotypes indicate that future research should embrace complex genetic variability by examining aggregate risk both within disease-associated genes and across genes in disease-associated biological pathways.

SUPPLEMENTARY INFORMATION

Aggregate *OXTR* Risk

In order to investigate whether any of the SNPs was contributing significantly more to the observed effects than the others, correlations were run between average connectivity values in subcortical and frontal regions modulated by the *OXTR* in ASD and TD groups (i.e., areas shown in Figure 2A,B) and aggregate risk on 3 of the 4 *OXTR* SNPs in an iterative fashion, leaving out one SNP at a time. While the strength of the correlation decreased in the analyses built on the 3-SNP aggregate scores, they remained significant in both ASD and TD groups ($p<0.05$). Further suggesting that no single risk allele disproportionately contributed to the reported effects, the strength of the observed correlations between aggregate risk allele dosage and connectivity did not differ significantly across any of these three SNP models.

Connectivity values were extracted from areas showing *OXTR*-dose-dependent modulation (i.e., areas shown in Figure 2) and correlated with age in the ASD and TD groups separately, as well as in the total sample, in order to determine whether finding were influenced by age. There were no significant associations ($p>0.05$). Furthermore, Leven's test for equality of variances was not significant when comparing ages in the TD and ASD sample, nor was there a difference in means between TD (M=13.11, SD=1.85) and ASD (M=13.52, SD=2.20) participants ($t(80)=0.91$, $p>0.05$, $d=0.20$).

NAcc-Frontal Connectivity in TD Youth

To further test whether increased NAcc-frontal connectivity in TD youth might reflect a compensatory neural mechanism in response to increased genetic risk, we divided our TD sample into high-risk (3-6 risk alleles) and low-risk (0-2 risk alleles) groups and tested the correlation between NAcc-frontal cortex connectivity and SRS scores in each group separately. In the high-risk TD group, the significant correlation with SRS social cognition scores held ($r(25)=-0.34$, $p<0.05$, one-tailed), while in the low-risk group the correlation did not reach significance ($r(15)=-0.29$, $p=0.15$, one-tailed). Consistent with the notion that the observed relationship between increased NAcc-frontal connectivity and SRS scores may reflect a compensatory mechanism, we also found that connectivity between the NAcc and frontal cortex was significantly stronger in the TD high-risk group than in the TD low-risk group (M=0.19, 0.04, SD=0.14, 0.12, respectively; $t(39)=-3.38$, $p<0.01$, $d=1.10$).

Medication Usage

Twenty-seven of the ASD children reported current use of psychotropic medications: 7 were taking psychostimulants, 3 were taking atypical antipsychotics, 3 were taking a selective serotonin reuptake inhibitor (SSRI), 1 was taking an alpha 2 adrenergic agonist, 2 were taking both

a psychostimulant and SSRI, 2 were taking both an atypical antipsychotic and SSRI, 1 was taking both a psychostimulant and a norepinephrine reuptake inhibitor, 1 was taking both a psychostimulant and an atypical antipsychotic, 6 of the remaining participants were taking a combination of three different classes of psychotropic medications and 1 was taking a combination of four different classes of medications. To assess whether medication status affected our findings in the ASD group, we directly compared parameter estimates from regions showing greater NAcc functional connectivity as a function of increased *OXTR* aggregate risk between medicated and non-medicated participants; there was no difference in connectivity strength between the two groups.

Characteristic	TD Subjects	ASD Subjects	p-value
N	41	41	
Gender	34 male	37 male	
Handedness	38 right-handed	36 right-handed	
Age (years)	13.11 (1.8)	13.52 (2.2)	0.36
Full Scale IQ	106.12 (10.2)	104.54 (14.4)	0.57
Verbal IQ	106.59 (11.0)	103.24 (13.3)	0.22
Performance IQ	105.51 (11.1)	105.12 (14.4)	0.89
ADOS (Comm+ Soc)		11.85 (4.63)	
ADI (Comm) Total		16.29 (4.41)	
Mean absolute motion	0.25 (0.16)	0.26 (0.14)	0.72
Max absolute motion	0.75 (0.60)	0.78 (0.45)	0.84
Mean framewise displacement	0.06 (0.03)	0.07 (0.03)	0.22
Max framewise displacement	0.56 (0.62)	0.54 (0.42)	0.83
Self-reported ethnicity/race			
Asian	1	2	
Black or African American	2	5	
Hispanic/White	13	12	
Non-Hispanic/White	18	16	
Other/Mixed	7	6	
Total *OXTR* Risk Alleles	2.95 (1.05)	3.26 (0.98)	0.16
Subjects (N) with ≥ 1 Risk Allele			
OXTR rs1042778	28	33	
OXTR rs2254298	10	8	
OXTR rs53576	15	23	
OXTR rs237887	34	32	

Table 1. Mean (standard deviation). Subjects' characteristics were matched across TD and ASD diagnostic groups. Comm, communication; Soc, social; Max, maximum; OXTR, oxytocin receptor gene; N/A, not applicable.

Supplementary Table 1. Peak coordinates of NAcc connectivity.

		TD Positive Connectivity				ASD Positive Connectivity				TD Negative Connectivity				ASD Negative Connectivity				TD > ASD Connectivity			
		MNI Peak (mm)			Max Z	MNI Peak (mm)			Max Z	MNI Peak (mm)			Max Z	MNI Peak (mm)			Max Z	MNI Peak (mm)			Max Z
	R/L	x	y	z		x	y	z		x	y	z		x	y	z		x	y	z	
Accumbens	R	8	12	-4	13.2	10	10	-8	13.0												
Accumbens	L	-8	12	-4	13.1	-8	14	-4	13.1												
Angular Gyrus	R	62	-60	16	3.3																
Angular Gyrus	L	-58	-58	42	2.6																
Amygdala	R													24	-12	-12	3.0				
Amygdala	L													-30	-4	-24	4.7				
Caudate	R	12	14	14	5.6	18	26	2	7.9												
Caudate	L	-14	14	14	4.9	-14	22	0	10.3												
Cingulate Gyrus, Anterior	R	6	38	-4	8.7	14	44	6	7.4	4	4	32	3.7	10	-8	38	3.9				
Cingulate Gyrus, Anterior	L	-6	36	-4	9.0	-4	36	-2	9.0					-6	0	38	2.8				
Cingulate Gyrus, Posterior	R	6	-50	18	5.2													4	-48	30	3.6
Cingulate Gyrus, Posterior	L	-6	-52	18	4.8									-12	-16	36	3.6	-6	-50	26	3.0
Cuneal Cortex	R									24	-68	24	4.5								
Cuneal Cortex	L									-20	-76	22	4.6	-20	-76	26	2.7				
Frontal Medial Cortex	R	12	40	-14	5.9	12	46	-8	7.9												
Frontal Medial Cortex	L	-4	38	-14	7.0	-8	36	-20	5.9												
Frontal Operculum Cortex	L	-34	26	8	2.6	-40	22	8	3.1												
Frontal Orbital Cortex	R	22	30	-10	6.1	32	30	-4	4.5												

Frontal Orbital Cortex	L	-26	38	-10	5.0	-26	26	-16	6.3											
Frontal Pole	R	18	56	0	6.4	12	56	-4	7.7	52	38	16	4.0							
Frontal Pole	L	0	64	4	6.7	-4	66	10	5.7	-48	42	2	4.0					-12	42	54	3.7
Hippocampus	R									34	-12	-24	3.5	24	-34	-6	2.6			
Hippocampus	L									-22	-10	-20	3.2	-32	-16	-22	4.0			
Inferior Frontal Gyrus, Pars Opercularis	R									44	12	16	4.1							
Inferior Frontal Gyrus, Pars Opercularis	L									-46	14	22	4.2							
Inferior Frontal Gyrus, Pars Triangularis	R									42	30	12	3.6							
Inferior Frontal Gyrus, Pars Triangularis	L									-42	36	10	3.4							
Inferior Temporal Gyrus, Anterior	L	-48	-8	-26	2.9															
Inferior Temporal Gyrus, Posterior	R	50	-18	-24	2.6					56	-34	-28	3.6							
Inferior Temporal Gyrus, Posterior	L													-44	-24	-30	4.8			
Inferior Temporal Gyrus, Temporooccipital	R									46	-52	-10	4.9	58	-54	-16	3.3			
Inferior Temporal Gyrus, Temporooccipital	L									-46	-54	-22	3.6							
Insular Cortex	R	36	14	-12	3.2	34	14	-12	3.2	38	0	-6	3.8	38	-8	-2	4.1			
Insular Cortex	L	-36	10	-8	2.6	-40	16	-12	3.6	-42	0	-8	3.4	-36	-12	-2	3.5			
Intracalcarine Cortex	R									20	-64	6	4.9	20	-72	2	5.1			
Intracalcarine Cortex	L									-14	-72	10	4.9	-20	-76	2	3.9			

Lateral Occipital Cortex, Inferior	R									36	-84	-4	3.8	36	-60	12	3.7
Lateral Occipital Cortex, Inferior	L									-50	-72	-10	4.1	-38	-64	10	3.9
Lateral Occipital Cortex, Superior	R	44	-70	50	4.8					26	-60	46	5.8	26	-64	60	3.6
Lateral Occipital Cortex, Superior	L	-46	-78	36	5.1					-30	-82	20	5.7	-32	-82	20	4.1
Lingual Gyrus	R									14	-70	-2	4.9	22	-54	-8	4.2
Lingual Gyrus	L									-16	-52	-8	4.6	-8	-74	2	4.0
Middle Frontal Gyrus	R									40	4	54	4.8	36	16	60	3.4
Middle Frontal Gyrus	L					-26	24	44	3.5	-54	18	30	4.4	-30	-2	52	3.3
Middle Temporal Gyrus, Anterior	R	56	2	-32	3.2												
Middle Temporal Gyrus, Anterior	L	-60	-4	-26	3.2												
Middle Temporal Gyrus, Posterior	R	58	-16	-18	4.3												
Middle Temporal Gyrus, Posterior	L	-62	-16	-20	4.5												
Middle Temporal Gyrus, Temporooccipital	R									52	-54	0	4.0	48	-54	6	3.7
Middle Temporal Gyrus, Temporooccipital	L									-56	-58	-2	2.5	-50	-62	2	3.5
Occipital Fusiform Gyrus	R									24	-74	-12	4.0	28	-70	-6	3.8
Occipital Fusiform Gyrus	L									-30	-70	-10	4.5	-34	-72	-6	3.2
Occipital Pole	R									12	-90	18	3.4				
Occipital Pole	L									-18	-92	18	3.9				
Pallidum	R									22	-4	0	4.4	20	-2	0	6.1

Pallidum	L									-20	-4	0	3.9	-20	-6	0	4.7						
Paracingulate Gyrus	R	2	50	-2	7.9	4	44	-2	7.5														
Paracingulate Gyrus	L	-10	50	2	8.4	-8	48	-4	7.9														
Parrahippocampal Gyrus, Anterior	R	16	0	-30	2.8									24	-18	-30	4.6						
Parrahippocampal Gyrus, Anterior	L									-20	-22	-26	2.6	-32	-14	-32	4.3						
Parrahippocampal Gyrus, Posterior	R													22	-28	-26	2.7						
Plamun Polare	R													44	-6	-16	2.8						
Postcentral Gyrus	R									46	-38	58	4.2	14	-38	48	3.6						
Postcentral Gyrus	L									-64	-22	40	4.0										
Precentral Gyrus	R									46	-8	54	4.4	50	0	54	4.0						
Precentral Gyrus	L									-46	-2	30	3.8	-44	-2	60	4.9						
Precuneus Cortex	R									12	-42	50	4.2	38	-2	56	4.8	10	-58	36	2.5		
Precuneus Cortex	L	-8	-54	34	5.1					-8	-66	58	3.5	-4	-52	66	3.1	-12	-54	34	3.7		
Putamen	R													34	0	4	4.7						
Putamen	L													-30	-4	6	3.8						
Superior Frontal Gyrus	R	20	32	40	4.6	6	44	38	2.9	18	2	66	4.0	18	-4	68	4.0	2	48	48	4.5		
Superior Frontal Gyrus	L	-6	54	32	5.0	-20	28	36	5.0	-22	0	64	4.8	-24	-2	62	3.6	-12	30	58	3.2		
Superior Parietal Lobule	R									36	-46	54	5.0	30	-38	44	4.0						
Superior Parietal Lobule	L									-32	-58	56	4.8	-22	-46	70	3.2						
Superior Temporal Gyrus, Anterior	L	-54	-4	-14	3.1																		
Supramarginal Gyrus, Anterior	R									60	-34	44	4.8										
Supramarginal Gyrus, Anterior	L									-42	-40	42	5.5										

Supramarginal Gyrus, Posterior	R									38	-44	40	5.1					
Supramarginal Gyrus, Posterior	L									-48	-42	54	4.8					
Subcallosal Cortex	R	6	28	-6	7.7	6	30	-8	8.9									
Subcallosal Cortex	L	-6	30	-8	8.8	-4	32	-8	8.1									
Supplementary Motor Cortex	R									12	-2	58	4.1	2	-12	62	4.5	
Supplementary Motor Cortex	L									-4	4	48	3.5	-12	-14	48	3.7	
Temporal Fusiform Cortex, Posterior	R									40	-20	-28	3.4	30	-34	-24	3.7	
Temporal Fusiform Cortex, Posterior	L													-36	-28	-20	3.4	
Temporal Occipital Fusiform Cortex	R									30	-46	-12	4.5	40	-18	-30	3.5	
Temporal Occipital Fusiform Cortex	L									-30	-60	-8	4.5	-28	-60	-12	4.4	
Temporal Pole	R	42	22	-34	4.3	40	26	-24	4.7									
Temporal Pole	L	-36	24	-30	4.7	-40	22	-26	4.2									
Thalamus	R									8	-16	-4	5.0	8	-16	-4	5.5	
Thalamus	L	0	-8	8	4.9	0	-10	10	5.3	-10	-24	2	4.0	-4	-18	-4	5.3	

Coordinates are in Montreal Neurological Institute space. Results are presented at z>2.3, p<0.01 (corrected for multiple comparisons at p<0.05).

Supplementary Table 2. Areas modulated by number of *OXTR* risk alleles.

		TD OXTR+ Modulation				ASD OXTR- Modulation			
		MNI Peak (mm)			Max Z	MNI Peak (mm)			Max Z
	R/L	x	y	z		x	y	z	
Frontal Pole	L	-10	56	36	4.3				
Paracingulate Gyrus	L	-12	40	28	3.7	-8	36	24	3.2
Paracingulate Gyrus	R					12	44	14	4.6
Superior Frontal Gyrus	L					-24	54	22	2.8
Frontal Orbital Cortex	R					38	16	-22	4.0
Frontal Orbital Cortex	L					-34	16	-22	3.9
Insular Cortex	L					-34	16	-14	3.6
Insular Cortex	R					26	16	-12	3.8
Putamen	L					-24	12	-6	3.2
Putamen	R					16	12	-6	3.2
Pallidum	R					16	-2	2	3.5
Cingulate Gyrus, Anterior	L					-8	38	4	3.3
Caudate	L					-8	6	10	4.8
Caudate	R					12	4	14	4.0
Cingulate Gyrus, Anterior	R					4	26	16	4.4

Coordinates are in Montreal Neurological Institute space. Results are presented at $z>2.3$, $p<0.01$ (corrected for multiple comparisons at $p<0.05$).

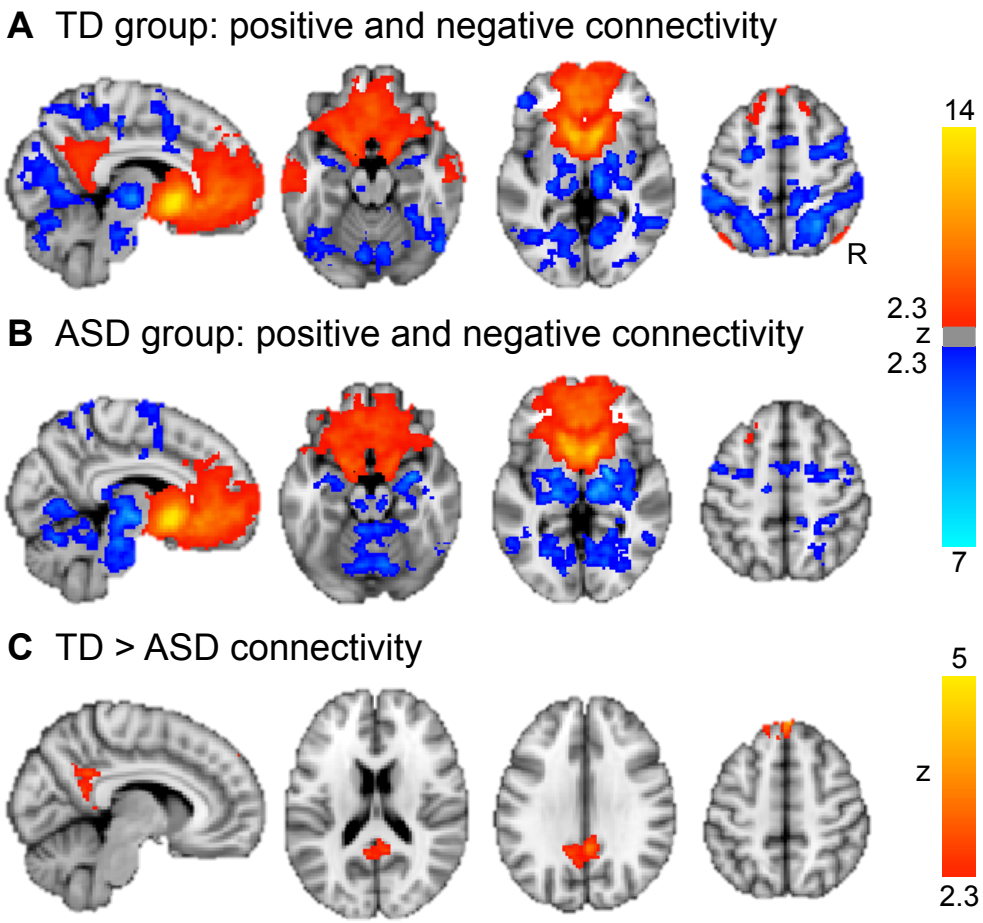


Figure 1. NAcc whole-brain connectivity. Warm colors indicate positive connectivity with the seed region; cool colors represent negative connectivity. (**A**) Connectivity in the TD group. (**B**) Connectivity in the ASD group. Results for **A, B** presented at $z>2.3$, $p<0.01$, cluster corrected for multiple comparisons at $p<0.05$. (**C**) Areas for which NAcc showed differential connectivity between diagnostic groups, $z>2.3$, $p<0.01$, cluster corrected for multiple comparisons at $p<0.05$. See also Supplementary Table 1.

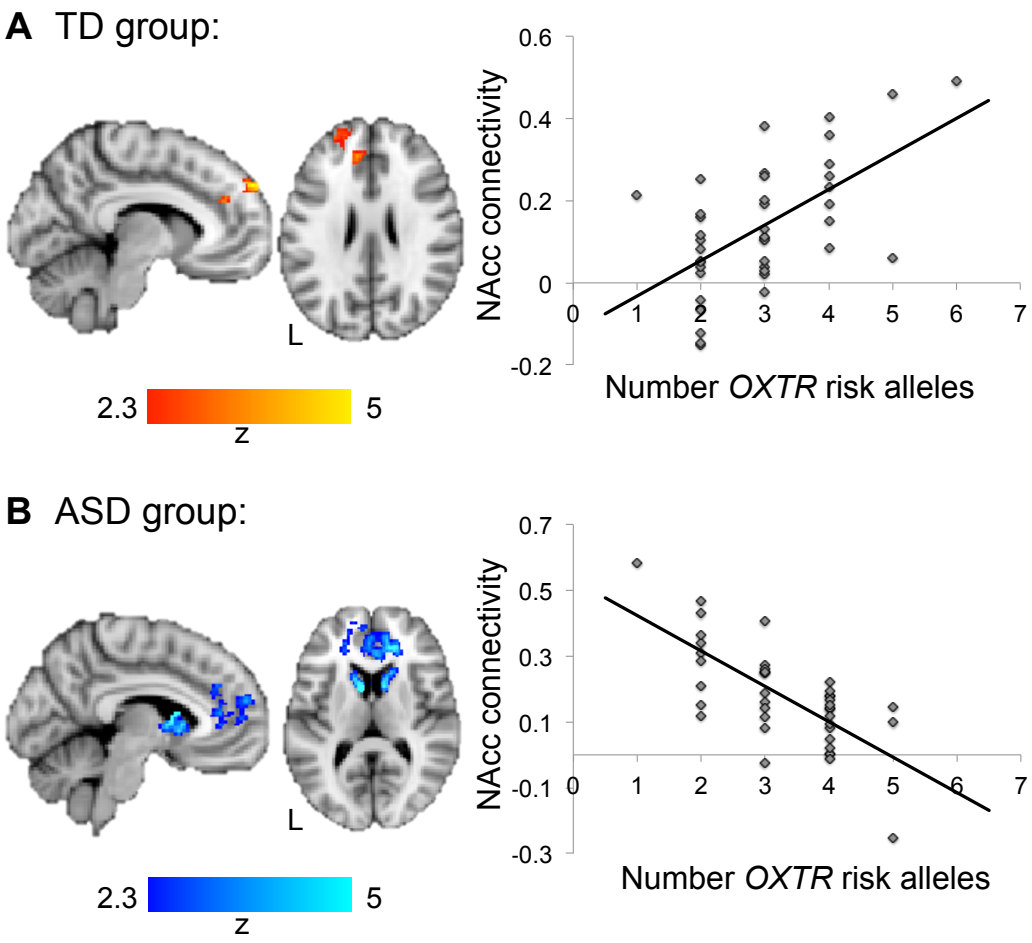


Figure 2. Effects of aggregate *OXTR* risk on functional connectivity ($z>2.3$, $p<0.01$, cluster corrected for multiple comparisons at $p<0.05$). (**A**) TD Group: Areas showing greater connectivity with the NAcc as a function of greater numbers of *OXTR* risk alleles. No increased functional connectivity with the NAcc as a function of *OXTR* risk alleles was observed in the ASD group (not shown). (**B**) ASD Group: Areas showing less connectivity with the NAcc as a function of greater *OXTR* aggregate risk. No decreased functional connectivity with the NAcc as a function of *OXTR* risk alleles was observed in the TD group (not shown). Scatterplots are shown for illustrative purposes and represent connectivity values extracted from the regions displayed at left as a function of number of *OXTR* risk alleles. See also Supplementary Table 2.

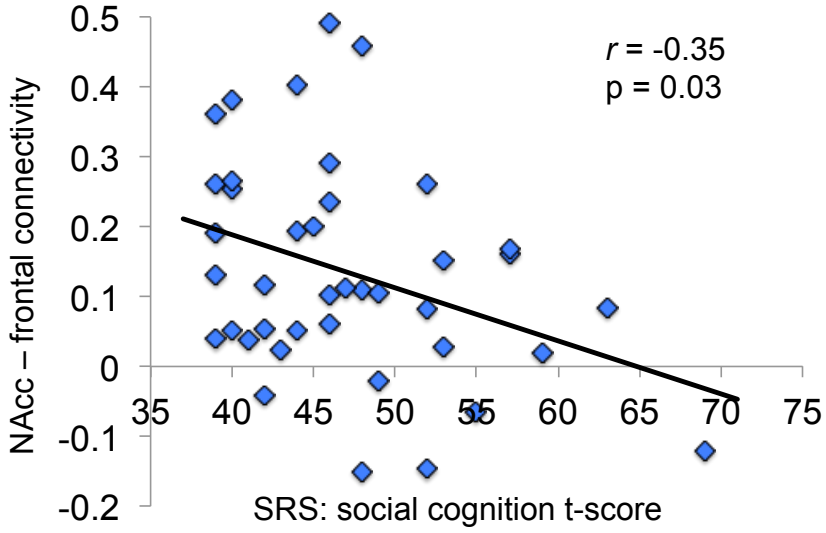
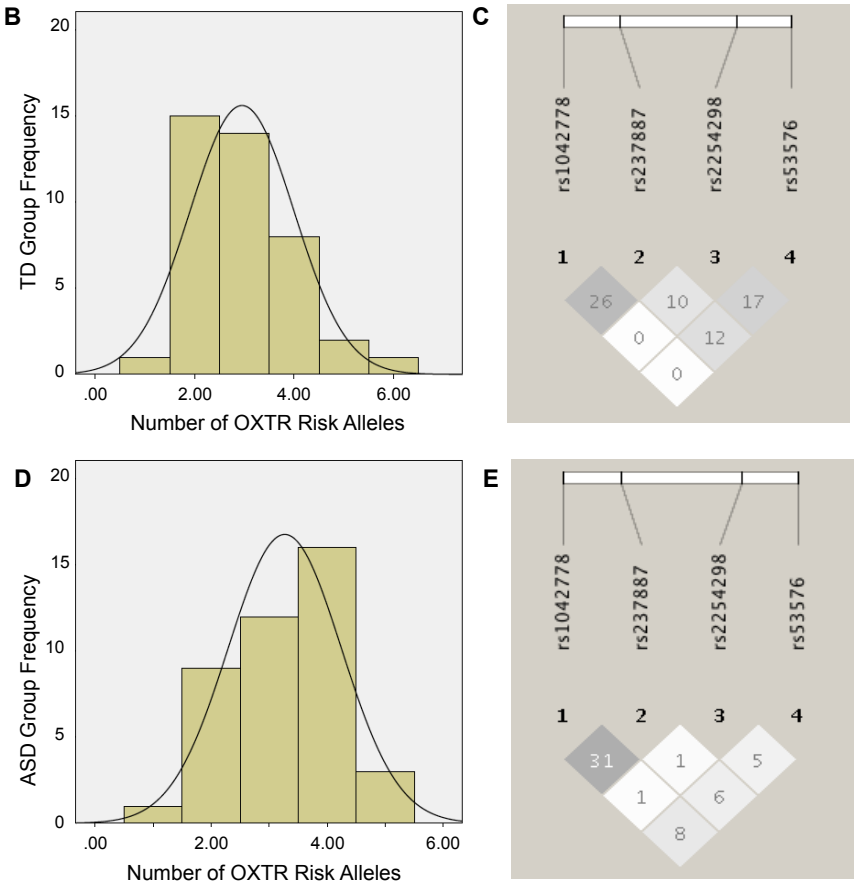


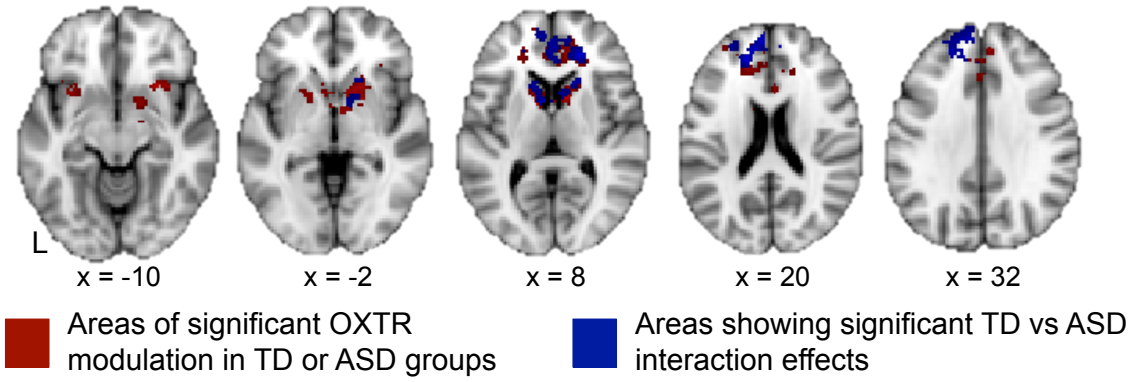
Figure 3. Behavioral correlation. Relationship between NAcc-frontal cortex connectivity in the TD group and SRS social cognition t-scores.

A

Polymorphism	Risk Allele	Genetic Score = 0	Genetic Score = 1	Genetic Score = 2
OXTR rs53576	A	GG	AG	AA
OXTR rs237887	A	GG	AG	AA
OXTR rs1042778	G	TT	TG	GG
OXTR rs2254298	A	GG	AG	AA



Supplementary Figure 1. *OXTR* risk allele descriptives. (**A**) Additive risk scores were calculated by summing the number of ASD-associated risk alleles across four *OXTR* SNPs. (**B**) Distribution of the number of inherited ASD-associated risk alleles for the TD group (N=41). (**C**) Pair-wise linkage disequilibrium (LD) plot in 41 typically developing youth; LD values are r^2 x 100. (**D**) Distribution of the number of inherited ASD-associated risk alleles for the ASD group (N=41). (**E**) Pair-wise linkage disequilibrium (LD) plot in 41 youth with ASD; LD values are r^2 x 100.



Supplementary Figure 2. Significant interactions by diagnostic group and number of risk allele. Regions in red represent the overlap of areas modulated by aggregate *OXTR* risk in TD and ASD groups (i.e., areas displayed in Figure 2). Areas showing significant interactions when comparing TD and ASD *OXTR* effects are displayed in blue.

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CHAPTER 3: Imaging-genetics of sex differences in ASD: Distinct effects of *OXTR* variants on brain connectivity

ABSTRACT

Autism spectrum disorder (ASD) is more prevalent in males than females but the neurobiological mechanisms that give rise to this sex-bias are poorly understood. The female protective hypothesis suggests that the manifestation of ASD in females requires higher cumulative genetic and environmental risk relative to males. Here, we assessed the additive impact of several *OXTR* risk-variants on brain connectivity in males and females with and without ASD, focusing our analysis on brain regions underlying social reward processing, and exploring how interactions between genotype, sex, and diagnosis relate to heterogeneity in neuroendophenotypes. We found that females with ASD who carry higher genetic risk in the *OXTR* showed upregulation of functional connectivity between the reward network and subcortical brain areas important for motor learning, which, was related to higher restricted and repetitive behavior symptoms. Further, we demonstrated that female youth with ASD and higher *OXTR* risk showed increased connectivity between reward regions and frontal brain areas involved in mentalizing. Increased frontal cortex connectivity in ASD females was related to better social functioning, a finding that, remarkably, mirrored the gene-brain-behavior pattern observed in neurotypical males. These findings suggest that, under the same *OXTR* genetic risk load, females with ASD and neurotypical males display similar neuroplastic upregulation of frontal cortex connectivity, with identical effects on behavior. These results indicate that the neurobiological correlates of genetic liability for ASD vary as a function of both sex and diagnostic status, highlighting the importance of including females in studies investigating gene-brain mechanisms in ASD.

INTRODUCTION

Across species, the neuropeptide oxytocin plays a critical role in a wide range of reproductive and complex social processes including initiation of uterine contractions during childbirth, lactation, pair bonding, and social reward processing.[1] The effects of oxytocin are dependent on expression of its receptor throughout the brain and body, and several variants in the oxytocin receptor gene (*OXTR*) have been linked to increased rates of autism spectrum disorder (ASD).[2–5] As social deficits are a core feature of ASD, the oxytocin system has attracted considerable attention as a potential target for pharmacological manipulation in individuals with autism.[6,7] Indeed, in males with ASD, treatment with intranasal oxytocin has been shown to improve social responsivity, seemingly through effects on reward-related subcortical brain areas and frontal brain regions important for social cognition.[8–13]

Given the known male bias in rates of ASD diagnoses,[14] it is unsurprising that much of the research conducted to date on the neurobiological correlates of oxytocin functioning in individuals with ASD has been in males. However, critically, sex-specific regulation of the oxytocin system has been reported in both animals[15,16] and humans,[17] suggesting that findings in males may not be generalizable to females. Theories on the neurogenetic underpinnings of sex-differences in ASD have hypothesized a female protective effect (FPE) whereby females require a higher burden of genetic and environmental risk factors to develop ASD.[18] Evidence for a FPE comes from genetics research showing that females with ASD carry more deleterious copy number and single nucleotide variants relative to males with ASD,[19,20] and further that genes highly expressed in the male brain also tend to be upregulated in the brains of individuals with ASD.[21] Together, these studies suggest that the male-bias in ASD may be due to inherent differences in sex-specific neurobiology, which in turn affect the impact that risk variants have on neuroendophenotypes.[21,22]

At the neural systems level, differences in the capacity of the brain to adapt to genetic and environmental risk factors may mediate the effects of genetic risk for ASD on the brain and ultimately behavior;[23] indeed, genes involved in the regulation of synaptic plasticity have been associated with increased ASD risk.[24]

Recent genetics work suggests that ASD follows a polygenic mode of inheritance whereby inheriting higher numbers of ASD-associated single nucleotide polymorphisms (SNPs) confers greater risk for developing the disorder.[25–27] This dose-dependent genetic effect on diagnostic outcome suggests that ASD-risk variants may also have additive effects on brain function, which ultimately manifest in atypical behavior. As multiple SNPs on the *OXTR* have been linked to higher rates of ASD, the oxytocin system serves as an ideal model in which to test whether increased genetic risk for ASD affects neural functioning in a dose-dependent manner. In a predominantly male sample, we have previously reported that in the presence of increased genetic risk for ASD on the *OXTR*, youth with ASD show reduced connectivity between the nucleus accumbens (NAcc; a hub of the reward-network) and other subcortical brain regions critical for implicit learning and reward processing.[28] Conversely, typically developing youth with increased *OXTR* genetic risk display compensatory functional connectivity between the NAcc and frontal cortex, which buffers them from expressing social cognitive deficits indicative of ASD.[28]

Notably, there is evidence for sexually dimorphic effects of intranasal oxytocin on brain function in subcortical and frontal brain regions important in reward and salience processing,[29] as well as interactions between sex and *OXTR* genotype which mediate neural responses to intranasal oxytocin in neurotypical adults.[30,31] These findings are in agreement with gene expression data which has shown that in men and women, the oxytocin receptor is expressed in subcortical brain regions, with enhanced expression in reward-related brain regions including the NAcc,

hypothalamus, and substantia niagra.[32] As intranasal oxytocin is a leading candidate in the search for a pharmacological treatment for ASD, elucidating potential sex-specific differences in the effects of *OXTR* variants on brain circuitry is of critical importance. Here, we address this issue by relating cumulative genetic risk for ASD across four *OXTR* SNPs to brain connectivity in females with and without ASD, exploring the moderating effects of diagnosis and gender on neuroendophenotypes and associations with behavioral measures of social functioning.

Using data from a multisite study focused on girls with ASD, we examined how ASD risk-allele-dosage on the *OXTR* relates to heterogeneity in NAcc network connectivity in females and critically, investigate sex differences in the effects of genetic risk on the brain by comparing cohorts of males and females with ASD. Results of these analyses elucidate how *OXTR* genotypes modulate reward network connectivity in male and female youth with and without ASD, providing a framework upon which to understand sex-differences which have previously been reported in studies of intranasal oxytocin use in neurotypical adults, and motivating future studies investigating sex-differences in the effects of intranasal oxytocin on reward network function in individuals with ASD.

METHODS

Subjects

Participants were high functioning females with ASD (N=50) and neurotypical (NT) females (N=52) who were recruited from four different sites (Harvard, Seattle Children's Hospital, University of California, Los Angeles (UCLA), and Yale) as part of the Gender Exploration of Neurogenetics and Development to Advance Autism Research (GENDAAR) multisite consortium. Participants with a history of claustrophobia, diagnosed neurological disorders,

genetic conditions, structural brain abnormalities, or metal implants were excluded from study participation. Study protocols were approved by the Institutional Review Board at each participating site and informed consent and assent to participate in research were obtained from legal guardians and study participants.

Male subjects were the same whose results were previously published in Hernandez et al (2017). Note that the Hernandez et al. 2017 sample included several female participants. For the purposes of the current study, females were removed, and analyses were re-run on male only ASD (N=37) and NT (N=34) groups. All previously reported results held in this male-only sample.

All participants with ASD had a prior diagnosis for the disorder based on the Diagnostic and Statistical Manual of Mental Disorders[33], which was confirmed using the Autism Diagnostic Observation Schedule – 2nd Edition[34] (ADOS-2), Autism Diagnostic Interview-Revised[35] (ADI-R), and best clinical judgement by licensed clinicians at each participating site. Criteria for study inclusion was an IQ above 70 as assessed by the Differential Ability Scales II[36], Wechsler Intelligence Scale for Children-IV,[37] or the Wechsler Abbreviated Scale of Intelligence.[38] Additional behavioral measures included the Social Responsiveness Scale[39] (SRS), a measure that provides a quantitative index of autistic traits. The SRS is designed to assess reciprocal social behavior in both NT and ASD individuals ages 4-18 years; questionnaires were completed by parents of study participants. A summary of demographic information is presented in Table 1.

Genotyping

Genomic DNA from whole blood was obtained from the Rutgers University Cell and Data Repository (RUCDR) or extracted using standard protocols (Gentra Puregene Blood DNA extraction kit; Qiagen). Genotyping was performed at the UCLA Neuroscience Genomics Core

(UNGC; https://www.semel.ucla.edu/ungc) according to standard manufacturer protocols using the HumanOmni2.5-8 BeadChip microarray (Illumina Inc.) After quality filtering (<5% missing per person/per SNP, >1% minor allele frequency, Hardy-Weinberg equilibrium p>10-7), multi-dimensional scaling was performed in PLINK (http://pngu.mgh.harvard.edu/purcell/plink/) using the default settings with the HapMap 3 reference panel (http://hapmap.ncbi.nlm.nih.gov/). Commercially available TaqMan assays were used to genotype SNPs rs53576 (C_3290335_20) and rs2254298 (C_15981334_10) at the UCLA Genotyping and Sequencing Core (GenoSeq Core; http://genoseq.ucla.edu) using a 5' nuclease assay to discriminate between the two alleles (Taqman SNP Genotyping Assay, Applied Biosystems Inc.). Polymerase chain reactions were performed using 5-μL reaction volumes in 384-well plates with 25 ng of DNA and Taqman genotyping master mix from Applied Biosystems Inc. End point reads of fluorescence levels were obtained with a Roche 480 lightcycler following manufacturer's protocol.

MRI Data Acquisition

Resting-state functional magnetic resonance imaging (rs-fcMRI) data were collected at each of the four GENDAAR participating sites on either a Siemens 3T Trio (12-channel head coil) or a Prisma 3T whole-body scanner (20-channel head coil). For each subject, a matched-bandwidth echo-planar image was acquired (Siemens Trio: TR=5000ms, TE=34ms, FOV=192mm, 34 slices, slice thickness 4mm, in-plane voxel size 1.5x1.5mm; Siemens Prisma: identical parameters except for TE=35ms) followed by a T2*-weighted resting-state functional MRI sequence (TR=2000ms, TE=30ms, FOV=192mm, 34 slices, slice thickness 4mm, in-plane voxel size 3x3mm on both platforms) during which participants were asked to look at a white crosshair presented on black screen.

fMRI Data Analysis

The rs-fMRI data were analyzed using FSL[40] (FMRIB's Software Library, www.fmri.ox.ac.uk/fsl) and AFNI[41] (Analysis of Functional NeuroImages). Data were processed using the same pipeline described in Hernandez et al. (2017) to ensure valid comparisons of results obtained for the male and female groups. Images were skull-stripped using AFNI. Next, FSL's Motion Correction Linear Registration Tool[42] (MCFLIRT) was used to align the functional images with the mean functional volume. Translations in the x, y, and z dimensions were calculated from volume to volume, then averaged to create a measure of mean displacement. Functional data were linearly registered to the matched-bandwidth EPI volume (6 degrees of freedom), followed by registration to the MNI 152 2mm standard brain (12 degrees of freedom). FSL's Automatic Segmentation Tool (FAST) was applied to high-resolution scans to create masks for grey matter, white matter, and cerebral spinal fluid for each subject; these variables, as well as the global signal were regressed from the data using FSL's FEAT. Images were smoothed using a 5mm FWHM Gaussian kernel. Bandpass filtering was applied to the data (0.1 Hz > t > 0.01 Hz).

Motion scrubbing was performed,[43] including removal of volumes for which frame-wise displacement exceeded 0.5mm and DVARS exceeded 0.5%; two volumes preceding and one volume following a scrubbed image were also removed. Participants with less than 5 minutes of data after scrubbing were excluded from final analyses (18 ASD subjects, 19 NT subjects), resulting in a final sample of 32 ASD females and 33 NT females. For subjects in the final analyses, the mean number of volumes scrubbed was 15 for ASD females and 9 for NT females; an independent samples t-test showed that there was not a significant difference in the number of volumes removed between diagnostic groups (p=0.21). FSL's FLIRT was used to register residuals

from the motion scrubbed data to the EPI volume (6 degrees of freedom), followed by registration to the MNI 152 2mm standard brain (12 degrees of freedom).

Statistical analyses were preformed using the general linear model in FSL's FEAT. The NAcc was defined bilaterally using the Harvard Oxford Atlas at a probability threshold of 25%. Average timeseries were extracted from the bilateral NAcc and correlated with every other voxel in the brain to generate functional connectivity maps for each participant. Individual subject-level correlation maps were transformed to *z*-statistic maps using Fisher's *r* to *z* transform. Single-subject maps were combined in higher-level group analyses in FSL's FEAT using FLAME 1+2, a mixed effects model.

Results of NAcc-whole brain connectivity analyses in females are presented at $z>3.1$ ($p<0.001$), corrected for multiple comparisons at $p<0.05$. Within-group maps of NAcc positive and negative whole-brain connectivity for NT and ASD females, as well as comparisons between diagnostic groups, included covariates for MRI data collection site and IQ. Analyses investigating diagnostic group differences in NAcc connectivity focused on regions showing significant positive or negative connectivity ($z>3.1$, cluster-corrected at $p<0.05$) in either NT or ASD females.

Group-level analyses testing the interaction between sex and diagnostic status on NAcc connectivity were also covaried MRI data collection site, IQ, and number of functional volumes remaining after motion scrubbing. Male subjects completed a shorter rs-fcMRI protocol, resulting in fewer overall volumes remaining after scrubbing. The mean number of volumes remaining after scrubbing were 179, 117, 176, and 117, for NT females, NT males, and ASD females, and ASD males, respectively. Examination of sex-differences in positive and negative NAcc connectivity focused on regions showing significant connectivity ($z>3.1$, cluster-corrected at $p<0.05$) in any group (NT females, NT males, ASD females, ASD males).

To assess the aggregate effect of *OXTR* genetic risk on NAcc connectivity, cumulative genetic risk scores were computed for each participant as the sum of the number of ASD-associated risk alleles across 4 *OXTR* SNPs: rs53576 (A risk, G non-risk), rs227887 (A risk, G non-risk), rs1042778 (G risk, T non-risk), rs2254298 (A risk, G non-risk). Mean genetic risk was calculated across NT and ASD groups; subjects' demeaned genetic risk score was entered as a regressor in higher-level FEAT analyses (FLAME 1+2). Modulation of NAcc connectivity by aggregate *OXTR* risk was assessed at $z>3.1$, corrected for multiple comparisons at $p<0.05$.

RESULTS

OXTR Genetic Variation in Females

In females, the average number of risk alleles on the *OXTR* was 3.45 (*SD*=1.03, range 2-6); NT females had on average 3.45 *OXTR* risk alleles (*SD*=1.03), ASD females had on average of 3.44 *OXTR* risk alleles (*SD*=1.05; Table 1). An independent samples t-test indicated that, as expected, that NT and ASD females had the same average number of *OXTR* risk alleles. As previously reported in ASD males,[28] inheriting greater numbers of *OXTR* risk alleles was associated with higher calibrated severity scores on the ADOS in females with ASD (*r*=0.29, one-tailed p=0.05). Risk allele frequencies were normally distributed in NT and ASD females; skewness 0.21 (*SE*=0.29), kurtosis -0.50 (*SE*=0.56).

NAcc Whole-Brain Connectivity

The NAcc showed significant connectivity with other key reward-related brain regions in both NT females and females with ASD (Figure 1). Both groups showed positive connectivity between the NAcc and frontal pole, superior frontal gyrus, frontal medial and orbital cortex,

paracingulate and cingulate cortex, caudate, and putamen; connectivity between NAcc and amygdala was observed in NT females only. Negative (anticorrelated) connectivity was observed between the NAcc and occipital cortex (lingual and fusiform gyri). Females with ASD displayed additional negative connectivity between the NAcc and sensorimotor brain regions (bilateral middle frontal gyrus and thalamus, left precentral gyrus, right pallidum and insula) and areas involved in auditory/language processing (i.e., middle temporal gyrus, angular gyrus, parietal operculum). These results lend support to findings previously reported in NT and ASD males, who showed positive connectivity between the NAcc and frontal cortex, anterior cingulate, bilateral caudate and putamen (with NT males having additional NAcc-precuneus positive connectivity), and negative connectivity to thalamus, occipital, and parietal cortex.[28] In the present female cohort, there were no significant diagnostic group differences in NAcc whole-brain positive or negative connectivity. Relative to ASD males, ASD females showed stronger negative connectivity between the NAcc and right visual and auditory regions including Heschl's gyrus, middle temporal gyrus, and lingual gyrus (Supplementary Table 1); no sex-differences were observed when comparing NT males and females.

Cumulative Genetic Risk: Effects on NAcc Connectivity in Females

In females with ASD, increased *OXTR* genetic risk was associated with stronger connectivity between the NAcc and subcortical brain regions involved in reward and sensory processing including the caudate and thalamus, as well as weaker connectivity with visual cortex (Figure 2A; Table 2). Extracting parameter estimates from these brain regions showed that increased NAcc-subcortical connectivity in ASD females was related to higher RRB scores on the ADOS (r=0.44, p=0.01; Figure 2B). In contrast, in NT females, increased genetic risk for ASD on

the *OXTR* was associated with increased connectivity between the NAcc and frontal brain areas implicated in social cognition/mentalizing (i.e., frontal medial cortex) and sensory brain regions (precentral gyrus, occipital pole). As *OXTR* risk-allele dosage increased, NT females also showed weaker functional connectivity between the NAcc and anterior cingulate gyrus (Figure 2A; Table 2). A significant interaction revealed that aggregate *OXTR* risk differentially modulated NAcc connectivity in NT and ASD females in sensory brain regions (bilateral lateral occipital cortex, postcentral gyrus), as well as subcortical and frontal brain regions (Figure 2C; Table 2). To confirm that results were not biased by ancestry, the first two components from multidimensional scaling of genome-wide data were controlled for in a correlation analysis between cumulative risk-allele dosage and parameter estimates extracted from brain regions modulated by *OXTR* risk at the whole brain level; results in both NT and ASD females remained significant.

Cumulative Genetic Risk: Sex-specific Effects on NAcc Connectivity

Gender significantly modulated the relationship between *OXTR* genetic risk and NAcc connectivity in the ASD group only. Relative to their male counterparts, as genetic risk for ASD increased, females with ASD showed significantly greater connectivity between the NAcc and regions of the mesolimbic reward system including the caudate, pallidum, and putamen, as well as bilateral thalamus, and frontal brain regions important for metalizing (Figure 3A, Table 3). These frontal brain areas that showing greater connectivity with the NAcc in ASD females with high genetic risk (compared to ASD males with high risk) are the same regions where NT males have stronger connectivity as a function of increasing *OXTR* aggregate risk.[28] Furthermore, in NT males this increased NAcc-frontal connectivity is related to better social cognition as measured by the SRS. Here too, in females with ASD, extracting parameter estimates indexing NAcc-frontal

connectivity showed that stronger reward-frontal cortex connectivity as a function of *OXTR* risk was related to better social cognition as measured by the SRS (r=0.43, p=0.02; Figure 3B).

DISCUSSION

Here, we report that common variants on the *OXTR* modulate reward network functional connectivity in both a sex and diagnosis dependent manner. Directly comparing males and females with ASD, we found sexually dimorphic effects of *OXTR* variants on brain circuitry such that in the presence of increased genetic risk, females showed upregulation of connectivity between the reward network and frontal brain regions important for social cognition, whereas males showed the opposite pattern. Further, in females with ASD, this increased NAcc-frontal connectivity represents a neurobiological compensatory mechanism as individuals with greater NAcc-frontal connectivity had better (lower) scores on the social cognition subscale of the SRS. Remarkably, these gene-brain-behavior results directly mirrors our previously reported findings in NT males, who showed exactly the same pattern whereby NAcc-frontal connectivity increased as a function of increased *OXTR* risk-allele-dosage and was related to better social cognition scores on the SRS.[28] These findings suggest that brain connectivity in females with ASD, rather than being more similar to NT females, are more akin to NT males both in terms of reward network-frontal connectivity and brain-behavior relationships. These findings are in agreement with a female protective model of ASD susceptibility in which ASD risk variants (such as those on the *OXTR*) interact with other genes that are expressed in a sexually dimorphic and regionally-specific manner throughout the brain, resulting in the manifestation of sex-specific neuroendophenotypes in individuals with ASD. Gene expression analyses in post mortem human brain samples have shown that individuals with ASD (compared to neurotypical individuals) and males (compared to

females) show downregulation of genes with neural and synaptic functions, as well as upregulation of genes involved in neuroimmune and inflammatory processes.[21,44] Thus, male-typical gene expression profiles may create a background on which ASD-risk genes are likely to have more penetrant/deleterious effects whereas female gene expression profiles may act to buffer the effects of ASD-risk variants on neural circuitry.[21] Our data support this theory, as we found that cumulative genetic risk for ASD on the *OXTR* has more penetrant effects on connectivity within the reward network in males with ASD compared to NT males[28] and, that females with ASD were in part buffered from the neurobehavioral effects of high *OXTR* genetic risk through formation of compensatory NAcc-frontal connectivity. Interestingly, behavioral research suggests that high functioning females with ASD show greater social motivation compared to their male counterparts,[45–47] a finding which has been attributed to the ability of females with higher IQ to mask their social-communicative deficits.[46,48] Our findings of compensatory connectivity between brain regions involved in reward processing and frontal brain areas important for metalizing in high functioning females with ASD may provide a neurobiological mechanism supporting this behavioral process.

A second feature of ASD females that distinguished them from both NT females and ASD males was that higher *OXTR* risk was related to greater connectivity between the NAcc and subcortical brain regions; a pattern not observed in any other group. Subcortical brain areas in the striatum are known to play a critical role in implicit learning of motor repertoires through cortico-basal ganglia feedback circuits,[49,50] and indeed this increased NAcc-subcortical connectivity was associated with higher restricted interests and repetitive behavior (RRB) scores on the ADOS in females with ASD. Notably, a sex-bias in expression of repetitive behaviors has been documented in individuals with ASD whereby males generally display more repetitive behaviors and restricted

interests relative to females.[51] Our imaging-genetics results in ASD females suggest that increased genetic risk on the *OXTR*, as well as associated up regulation of connectivity between the reward network and subcortical brain regions, puts ASD females at a greater likelihood of expressing RRBs. Although females in our study did not display more severe RRB than males with ASD, we also did not see the typical pattern whereby males with ASD show increased RRB relative to females. In sum, we found that in ASD females increased *OXTR* risk was associated with greater neuroplasticity between the reward network and both subcortical and frontal brain regions, and that increased connectivity between these areas had both positive and negative effects on behavior.

That the *OXTR* risk variants investigated in this study would affect frontal and subcortical brain regions in a sex-specific manner are in line with findings from studies using intranasal oxytocin to alter neural activity. In adult neurotypical females, administration of intranasal oxytocin increases connectivity between subcortical brain regions involved in reward and social-emotional processing, and, importantly the effects of oxytocin are greatest in women with higher autistic traits.[32] Sex-differences in the effects of intranasal oxytocin have been documented in neurotypical adults such that oxytocin increases brain activity in the caudate and putamen in men, while decreasing activity in these brain regions in women.[29] Furthermore, these sex-dependent effects of oxytocin on the brain interact with genetic variants on the *OXTR*. For instance, for the rs53576 SNP on which the A allele has been associated with increased rates of ASD diagnosis, male GG homozygotes (i.e., the non-risk group) show greater caudate activity during a game eliciting social cooperation under oxytocin vs. placebo, while female GG carriers have greater caudate activity in the placebo condition.[30] Similarly, for the rs1042778 SNP for which the G allele confers ASD-risk, male TT homozygotes (i.e., the non-risk group) display greater increases in amygdala activity in response to angry faces compared to risk-allele carriers, while no significant

difference is observed between female risk and non-risk groups.[31] How these results may translate to males and females *with ASD* and how the modulatory effects of oxytocin may be affected by cumulative genetic risk for ASD on the *OXTR* have yet to be empirically determined. Taken together with previous data, our findings suggest that in addition to investigating the effect of sex on oxytocin treatment outcomes, it is critical that future work also consider sex x diagnosis interactions, as ASD males and females are likely to show different neural responses, at baseline and in response to treatment, from their NT male and female counterparts. Notably, recent research suggests that the effects of oxytocin on neural activity may be modulated not only by sex, but also by age,[17] pointing to the need to study the effects of pharmacological treatments in the context of sex-specific neurodevelopmental trajectories.

In NT females, greater genetic risk for ASD on the *OXTR* was related to reduced functional connectivity between the NAcc and anterior cingulate (ACC), and increased connectivity with medial orbital frontal cortex (OFC). Both the ACC and OFC are components of the limbic fronto-striatal circuit, which plays a role in reward processing and goal directed planning based on distant outcomes.[50] Neuroanatomically, when a reward stimulus is given (i.e., a smiling face, money, etc.), dopamine projections from the midbrain encode reward prediction error[52] and signal to the ventral striatum and frontal cortex, which encode the subjective reward value of the stimulus.[53] Thus, the ventral striatum and frontal cortex form a feedback loop that is critical for implicit learning of the relationship between reward prediction and likely outcomes. Our findings in NT females suggest that variability in *OXTR* genotypes impact the relative balance of connectivity between nodes of the fronto-striatial loop in NT females, possibly affecting signaling from the frontal lobe back to subcortical brain regions to impact decision making based on previous reward. Notably, there are sex and age dependent differences in structural connectivity between the NAcc and OFC in

neurotypical individuals with males showing earlier peak in structural connectivity between these two regions relative to females.[54] The developmental trajectory of accumbo-frontal connectivity remains to be explored in males and females with ASD and is an important future direction.

In sum, this study examined resting-state functional connectivity of the nucleus accumbens in females with and without ASD to investigate how ASD risk-allele-dosage on the *OXTR* relates to between-subject heterogeneity in network connectivity. Critically, we also investigated sex differences in the effects of genetic risk on the brain by comparing cohorts of males and females with ASD. Taken together, our results confirm previous evidence that *OXTR* risk variants do indeed impact neuroendophenotypes in both NT and ASD individuals. Our findings show that the specific brain regions affected and the direction of the observed effect varies as a function of both sex and ASD-diagnostic status. These findings have important implications for treatment studies investigating the effects of intranasal oxytocin on brain activity as they suggest that males and females may have different responses to pharmacological treatment depending on their genetic background and diagnostic status. More broadly, these findings underscore the importance of including females in studies investigating the neural basis of ASD, which in addition to providing insights into the etiology of ASD, can inform our understanding of how variability at the genetic, neural, and behavioral level gives rise to sex differences in rates of ASD diagnosis, and ultimately inform personalized approaches to the treatment of ASD.

	ASD Females	NT Females	P-Value
Age	13.84	13.49	0.59
Full IQ	97.91	112.97	0.001*
Nonverbal IQ	99.53	110.82	0.009*
Verbal IQ	101.16	111.18	0.018*
ADOS Social	7.97	-	-
ADOS Repetitive Behavior	2.06	-	-
ADOS Severity Score	6.13	-	-
ADI Social	19.09	-	-
ADI Communication	15.77	-	-
ADI Repetitive Behavior	6.00	-	-
Mean Relative Motion	0.12	0.09	0.16
Mean Volumes Scrubbed	14.97	8.58	0.21
Mean *OXTR* risk variants	3.44	3.45	0.94
Subjects with ≥ 1 risk allele			
rs1042778	28	27	-
rs2254298	6	8	-
rs53576	21	21	-
rs237887	28	25	-
Self-Reported Ethnicity			
Asian	2	3	-
Black/African American	0	3	-
More than One Race	4	5	-
White	26	22	-

Table 1. Sample Descriptive. Mean values and p-values derived from independent samples t-tests. ASD, autism spectrum disorder; NT neurotypical; IQ, intelligence quotient; ADOS, Autism Diagnostic Observation Schedule; ADI, Autism Diagnostic Interview; *OXTR,* oxytocin receptor gene.

ASD OXTR Modulation

	MNI Peak (mm)			Max		MNI Peak (mm)			Max
OXTR+	**x**	**y**	**z**	***Z***	***OXTR-***	**x**	**y**	**z**	***Z***
Caudate	-12	0	20	4.44	Lateral Occipital Cotrex, superior division	36	-86	12	3.96
Thalamus	-12	-16	6	3.69	Lateral Occipital Cotrex, inferior division	40	-80	4	3.49
Putamen	-24	-6	14	3.27					

TD OXTR Modulation

	MNI Peak (mm)			Max		MNI Peak (mm)			Max
OXTR+	**x**	**y**	**z**	***Z***	***OXTR-***	**x**	**y**	**z**	***Z***
Precentral Gyrus	-12	-34	62	5.34	Cingulate Gyrus, anterior division	4	26	22	4.46
Lateral Occipital Cortex, superior division	26	-88	28	4.85	Paracingulate Gyrus	12	18	42	4.42
Frontal Medial Cortex	-2	50	-12	4.59	Frontal Pole	22	38	28	4.19
Postcentral Gyrus	-14	-46	62	4.15					
Occipital Pole	32	-92	20	3.98					
Frontal Pole	2	64	-10	3.98					

Interactions

	MNI Peak (mm)			Max		MNI Peak (mm)			Max
ASD connectivity increases, TD connectivity decreases with more risk	**x**	**y**	**z**	***Z***	***ASD connectivity decreases, TD connectivity increases with more risk***	**x**	**y**	**z**	***Z***
Thalamus	-10	-16	2	4.03	Lateral Occipital Cortex, inferior division	36	-86	8	4.91
Frontal Pole	26	38	26	3.90	Postcentral Gyrus	-10	-36	60	4.67
					Occipital Pole	26	-96	20	3.65

Table 2. Peak coordinates of brain regions modulated by variability in *OXTR* risk-allele dosage; z>3.1, corrected for multiple comparisons at p<0.05.

Gender Differences in *OXTR* modulation				
	MNI Peak (mm)			**Max**
ASD female connectivity increases, ASD male connectivity decreases with more risk	**x**	**y**	**z**	***Z***
Caudate	-12	10	10	5.09
Frontal Pole	26	50	34	4.95
Thalamus	-8	-8	16	4.56
Putamen	20	8	2	3.20

Table 3. Peak coordinates of sex-differences in NAcc functional connectivity as a function of *OXTR* risk in youth with ASD; $z>3.1$, corrected for multiple comparisons at $p<0.05$.

ASD female > ASD male : Nacc Negative Connectivity				
	MNI Peak (mm)			Max
	x	y	z	Z
Supramarginal Gyrus	46	-38	8	4.43
Lingual Gyrus	18	-58	-6	4.33
Heschl's Gyrus	48	-8	6	3.95
Lateral Occipital Cortex	34	-66	28	3.78
Middle Temporal Gyrus	56	-42	-4	3.70
Planum Temporale	48	-30	14	3.62
Central Opercular Cortex	58	-4	8	3.54

Supplementary Table 1. Peak coordinates of sex-differences in NAcc functional connectivity in youth with ASD; $z>3.1$, corrected for multiple comparisons at $p<0.05$.

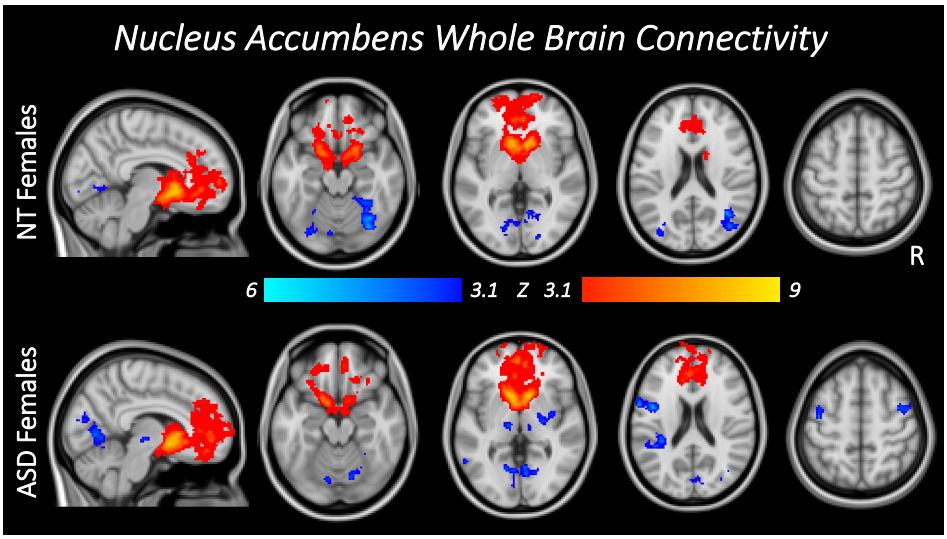

Figure 1. NAcc whole-brain connectivity in females with and without ASD. Red/yellow indicate positive connectivity with the seed; blue/cyan indicate negative connectivity with the NAcc seed. Maps are shown at $z>3.1$, corrected for multiple comparisons at $p<0.05$. NT, neurotypical; ASD, autism spectrum disorder.

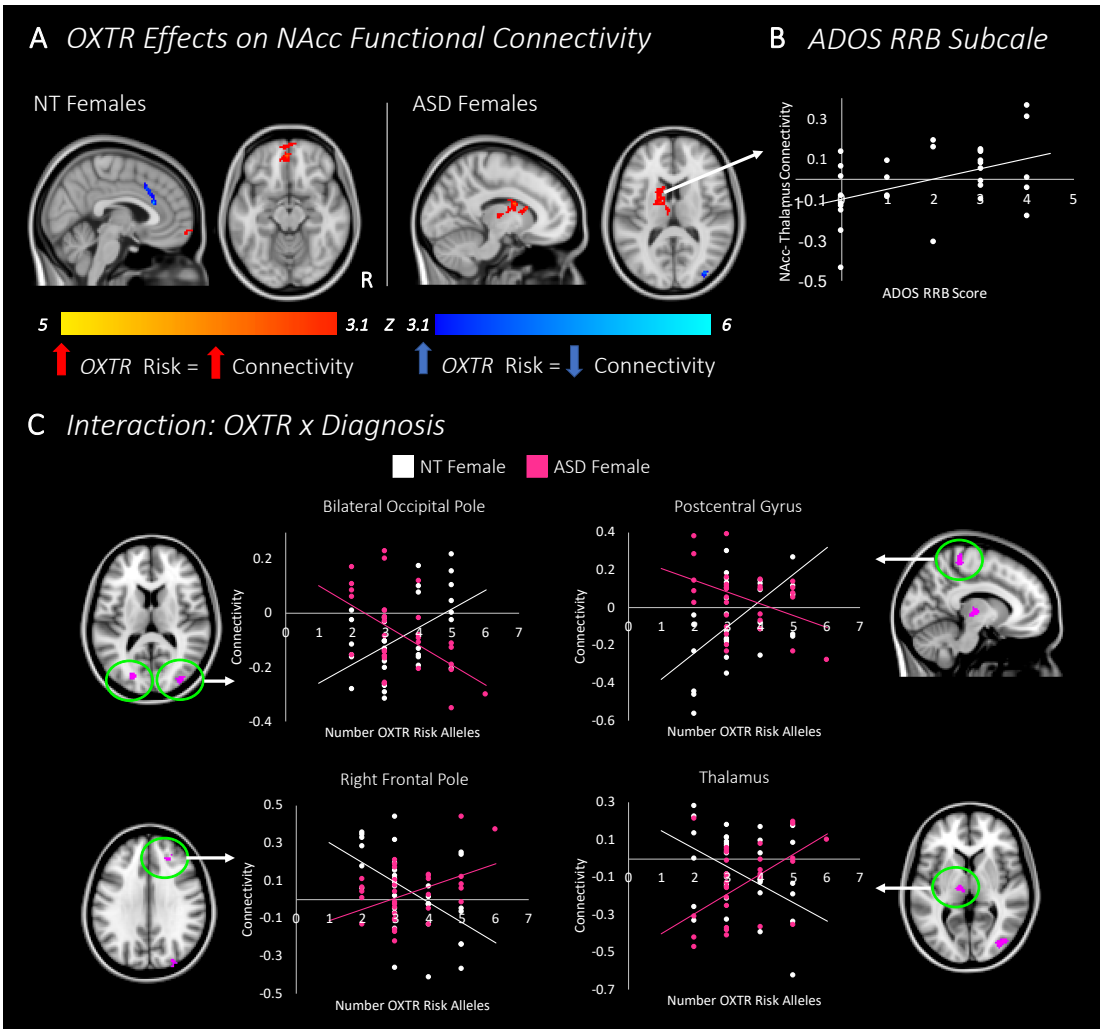

Figure 2. Effects of *OXTR* genetic risk on connectivity of the NAcc. Brain regions showing greater connectivity with the NAcc as a function of increased *OXTR* genetic risk are shown in red. Areas showing reduced functional connectivity with the NAcc as a function of increased *OXTR* genetic risk are shown in blue. Graphs are for illustrative purposes and show the relationship between NAcc connectivity and number of *OXTR* risk alleles for each participant.

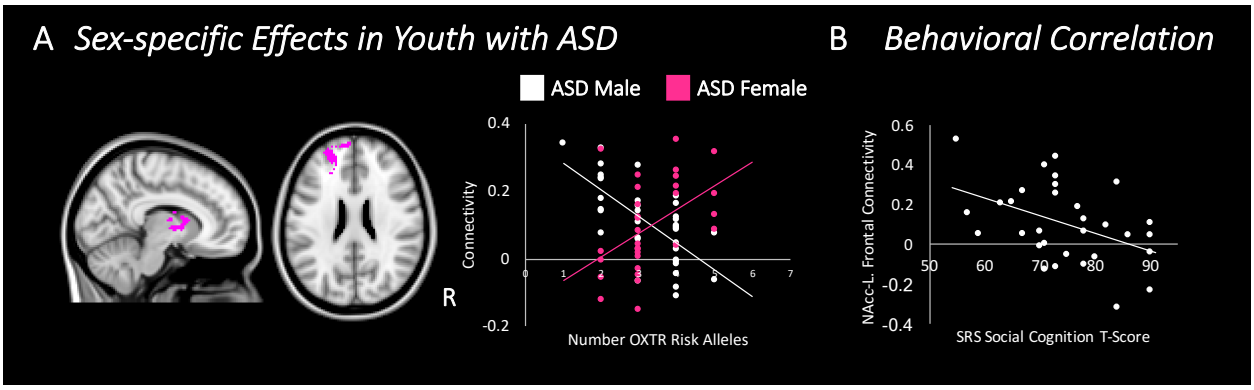

Figure 3. Distinct effects of *OXTR* variants on brain connectivity in males and females with autism. A) Brain areas showing a significant interaction as a function of sex ($z>3.1$, $p<0.05$). B) Increased NAcc – frontal pole connectivity in ASD females is associated with lower social cognition T-scores on the SRS (indicative of less severe social-cognitive symptomatology).

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CHAPTER 4: Brain responses to conversation-in-noise in youth with ASD: Effects of sensory-over responsivity and attention

ABSTRACT

Individuals with Autism Spectrum Disorder (ASD) often have difficulty attending to speech in the context of distracting non-social environmental noise; however, the effects that heightened auditory sensory sensitivity and selective attention have on speech-in-noise processing remains poorly understood. Here, we employ a novel functional magnetic resonance imaging (fMRI) paradigm where youth with ASD and typically developing (TD) controls listened passively to two-person conversation in the context of common environmental noises (e.g., police siren, helicopter), as well as two-person conversation or common environmental noises alone, to elucidate the network of brain regions underlying selective attention to social auditory stimuli. We further assessed how parent-reported auditory sensory over-responsivity (SOR) related to heterogeneity in neural responses during exposure to simultaneous conversation and noise, and how brain response when listening to speech in the context of environmental noise related to later memory for conversations heard in the MRI scanner. We found that, during exposure to conversation-in-noise, ASD youth showed greater activity than TD youth in right posterior superior temporal cortex. This difference was driven by greater activity in ASD youth with higher levels of SOR, who also showed higher activity in the auditory thalamus relative to both TD and ASD youth with lower SOR. In ASD youth with higher SOR, activity in the right posterior superior temporal cortex was related to better recall of conversations heard in the presence of distracting background noise, suggesting that increased thalamic and right temporal activity served as a compensatory mechanism in this group. Conversely, in TD youth, greater recall accuracy was

related to the ability to sustain activity in canonical language regions during conversation-in-noise listening. Furthermore, when examining task-based functional connectivity of the voice-selective cortex, ASD youth showed reduced connectivity with auditory cortex during simultaneous exposure to conversation and noise, whereas TD youth showed reduced functional connectivity with fronto-parietal attention regions. These data indicate that in neurotypical individuals the attentional mechanisms at work during conversation-in-noise processing involve upregulation of language-related brain regions, whereas individuals with ASD recruit contralateral right-hemisphere brain areas. Thus, the neurobiological salient nature of speech stimuli may be disrupted in ASD, making the task of attending to conversation-in-noise more difficult, and ultimately requiring recruitment of additional neural resources to resolve this cognitively demanding task.

INTRODUCTION

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by difficulty in social communication and the presence of repetitive behaviors and restricted interests, including hyper or hypo responsivity to sensory stimuli.[1] Delayed language acquisition is one of the earliest concerns expressed by parents[2] and multiple studies in infants who later go on to get an ASD diagnosis have shown that allocation of attention to social stimuli is disrupted early in development.[3] In addition to showing reduced attention to faces,[4] young children with ASD fail to show a preference for listening to their mothers' voice,[5] as well as to child-directed speech.[6] Disrupted attention to language stimuli in infancy may set the stage for subsequent atypical language acquisition in children with ASD,[6] and, ultimately, alter the development of neural systems responsible for language processing. Heightened auditory sensory sensitivity is observed in a significant number of children with ASD[7] and may interfere with language processing throughout development, skewing attention away from speech stimuli in favor of other noises present in the environment. Despite growing interest on the relationship between altered sensory processing and social impairments in ASD,[8] little research to date has investigated how individual variability in sensory responsivity may impact language processing. Here, we used functional magnetic resonance imaging (fMRI) to investigate how heightened auditory sensory over-responsivity may disrupt brain activity in neural networks subserving speech processing in youth with and without ASD.

Converging neuroimaging data indicate altered brain responses to language in individuals with ASD. While ASD is characterized by a great deal of heterogeneity in language outcomes, young children with ASD who go on to have poorer language skills show hypoactivity in temporal cortex during language listening,[9] as well as reduced functional connectivity between nodes of the

language network.[10] In children and adolescents with ASD, fMRI studies have found reduced functional lateralization and increased rightward asymmetry during a variety of language processing tasks compared to the leftward asymmetry observed in neurotypical individuals.[11–16] However, in most real-life situations, language is not heard in isolation, but against the background of other competing sensory distractors (e.g., a buzzing fan, a barking dog). Thus, the brain must learn to "hone-in" on social stimuli by downregulating competing non-relevant sensory inputs and promoting attention to salient social stimuli, such as the human voice. In neurotypical adults, the bilateral pSTS responds selectively to vocal stimuli, and activity in this region is reduced when voice stimuli are degraded or masked by background noise.[17,18] Individuals with ASD fail to activate voice-selective regions in the posterior superior temporal sulcus (pSTS),[11] and show increased recruitment of right hemisphere language homologues[19] during exposure to vocal stimuli. Reduced resting-state functional connectivity between voice-selective cortex and many limbic and reward-related brain regions has also been observed in children with autism,[20] suggesting that both activity and connectivity of speech-selective brain regions are disrupted in ASD. Furthermore, the ability to detect speech-in-noise appears reduced in individuals with ASD, who are poorer at identifying speech heard in the context of background noise.[21,22] Interestingly, a recent study showed that sensory processing atypicalities modulate brain activity during language processing in youth with ASD during simultaneous exposure to distracting tactile stimuli.[23] However, how individual differences in auditory sensory responsivity in individuals with ASD may impact processing of speech-in-noise at the neural level is not yet known.

In adults with ASD, heightened sensory over-responsivity (SOR), characterized by extreme behavioral response to everyday sensory stimuli, is related to higher autism traits.[24] Importantly, roughly 65% of children with ASD also show atypical sensory responsivity to non-social auditory

stimuli[25,26] including a lower tolerance for loud noises[27,28] and hypersensitivity to certain environmental noises, such as the sound of a dog barking or a vacuum cleaner.[29] Further, ASD youth with SOR display reduced neural habituation to mildly aversive sensory stimuli compared to neurotypical youth and youth with ASD who do not have SOR,[30] as well as increased functional connectivity between brain regions involved in salience and sensory processing.[31] Children with ASD also show altered connectivity between the thalamus (a subcritical brain region critical to the relay of sensory information) and cortex,[32] and notably, increased connectivity between the thalamus and somatosensory regions has been observed during exposure to mildly aversive sensory stimulation.[33] A small but growing body of neuroimaging research also suggests that children with ASD who have high levels of SOR display neural hyper-responsivity to mildly aversive visual, tactile, and auditory stimuli in primary sensory brain regions and areas important for salience detection,[30,34] suggesting that there may be an over-allocation of attentional resources to sensory stimuli in youth with ASD. Together, these data suggest that language processing within social contexts in which there are other competing sensory stimuli – such as those that occur in the natural environment – may be particularly aversive to some individuals with ASD. Over the course of development, hypersensitivity to auditory stimuli may disturb the typical allocation of attentional resources to language and set the stage for atypical neural and behavioral language outcomes.

Here, we examined how behavioral hyper-responsivity to auditory stimuli affects neural response to language listening in a paradigm where participants heard two-speaker conversation shrouded in competing environmental noise. Ecologically valid stimuli were developed to examine the effects of ASD diagnosis and auditory SOR on neural processing of commonly encountered environmental noise, conversation, and conversation-in-noise (i.e., noise and conversation

presented simultaneously). In the present study, we explicitly probed differences in brain responses to simultaneous exposure to conversation and noise *above and beyond* neural responses to conversation and noise alone. In addition, participants completed a post-scan computerized test that probed recognition of noises and topics of conversation heard during the fMRI task, thus providing a measure of attention. Lastly, functional connectivity of the voice-selective cortex during the auditory fMRI task was examined using a psychophysiological interaction (PPI) analysis to elucidate brain regions showing differential connectivity with voice-selective areas as a function of experimental conditions. In TD youth, we expected to see a normative selective attention response whereby activity increased in canonical left hemisphere language regions during conversation-in-noise listening, as well as perhaps some additional recruitment of frontal brain regions involved in social cognition. Youth with ASD were not expected to show this neurotypical pattern, instead displaying increased activity in primary auditory cortex and subcortical brain regions such as the amygdala and thalamus during conversation-in-noise listening (due to the distracting and mildly aversive nature of the noise stimuli). Alternately, we expected that youth with ASD might show upregulation of right hemisphere language homologues, reflective of more effortful processing of speech stimuli during conversation-in-noise listening.

METHODS

Participants

Participants were 26 youth with ASD and 28 age-matched typically-developing (TD) youth who were recruited through referrals from the UCLA Child and Adult Neurodevelopmental (CAN) Clinic as well as from posted advertisements throughout the greater Los Angeles area. Exclusionary criteria included diagnosed neurological disorders, genetic conditions, known

structural brain abnormalities, or metal implants. ASD participants had a prior clinical diagnosis of autism, which was confirmed using the Autism Diagnostic Observation Schedule – 2nd Edition (ADOS-2)[35] and Autism Diagnostic Interview-Revised (ADI-R)[36] by licensed clinicians at the UCLA CAN Clinic. All participants had full-scale IQ above 70 as assessed by the Wechsler Abbreviated Scale of Intelligence[37] (M=108, SD=14.9; Table 1). Data were originally acquired for 30 ASD and 30 TD youth; 4 ASD participants and 2 TD participants were excluded from the final sample due to excessive head motion during fMRI data acquisition (i.e., greater than 5mm of maximum relative motion). Study procedures were approved by the UCLA Institutional Review Board and informed consent and assent to participate in this research were obtained from legal guardians and study participants.

Behavioral Measures

Sensory responsivity was assessed in both ASD and TD youth using the Short Sensory Profile (SSP)[38] and the Sensory Over-Responsivity (SensOR) Scales.[39] The SSP is a 38-item parent report measure of how children process sensory information in a variety of every-day situations and across multiple sensory modalities. Questions probe sensory processing patterns in the context of home, school, and community-based activities. The SensOR Scales include 42 items, asking parents which sensory stimuli bother their child including questions about tactile, visual, and auditory sensations. As in prior studies,[30,33,34] an auditory sensory over-responsivity (SOR) composite score was computed by standardizing and averaging the auditory subscales from the SSP and SensOR (i.e., SSP auditory filtering and SensOR auditory score). Children scoring in the top quartile of the composite score were defined as having high auditory SOR.

Experimental Design

During the fMRI scan, auditory stimuli were presented according to a canonical block design (Figure 1A), using E-Prime 2.0 Software on a Dell Latitude E6430 laptop computer. Each block consisted of 15s of auditory stimulus presentation alternating with 7.5s of rest. A crosshair was presented at the center of a white screen throughout the duration of the scan. Blocks consisted of three types: Conversation (C), Noise (N), and Conversation-in-Noise (CIN; i.e., conversation and noise presented simultaneously). Stimuli were ecologically valid and mimicked those encountered in everyday life, in situations when one overhears two people engaged in a conversation that is shrouded by competing auditory stimuli, thus forcing the listener to "hone-in" on the socially relevant speech. Inspiration for conversation topics were taken from scripted television series focusing on childhood/adolescence (Figure 1B). Speech passages were recorded by two actors (one male, one female) using GarageBand 6.0.5 and an Apogee MiC digital microphone connected to a Macintosh computer. Noise stimuli were downloaded from Freesound.org. Selection of noise stimuli ensured that they were ecologically valid (i.e., commonly encountered in everyday life). The aversive nature of the selected noises was rated in an independent sample (N=30) using a 7-point Likert scale (1=not aversive, 7=extremely aversive); the final 12 noise stimuli used in the fMRI task were rated as mildly aversive (rating M=4.7, range 3.6-5.5) and included such sounds as a jackhammer, a police siren, and a blender. Each participant heard each conversation and noise recording only once. Stimuli were counterbalanced such that half of the participants heard a given conversation without noise, whereas the other half of participants heard the same conversation masked by noise (i.e., in the CIN condition). Likewise, for any given noise, half of participants heard the noise alone, while the other half heard the noise in the CIN condition. Each block type (C, N, CIN) was presented 6 times (with orders counter

balanced across subjects). The total run time was 7 minutes and 7.5s. Prior to the fMRI scan, participants were told that they would hear some people talking and some noises and were instructed to listen and look at the crosshair on the screen.

To assess the participants' ability to recognize stimuli presented in the three experimental conditions (and thus gain a proximal measure of in-scanner attention), a brief post-MRI scanning questionnaire was administered using E-Prime 2.0 Software on a Dell Latitude E6430 laptop computer. During this post-scanning test, participants heard and read questions about the conversations and noises they were exposed to during the fMRI data acquisition, interspersed with questions about conversations and noises they did not hear (i.e., foils). For each conversation and noise stimuli presented during the fMRI scan, participants were first asked to answer a question about whether they heard a particular noise/conversation topic. The post-scan test was programmed such that if a participant's yes/no response to this initial question was correct (Figure 1C, Part A and C), a more nuanced multiple-choice question about that conversation was then presented (Figure 1C, Part B and D). Incorrect responses to the initial yes/no questions resulted in being presented the next set of questions about a different conversation topic (i.e., skipping multiple-choice questions). Participants responses, as well as reaction time, were recorded in E-Prime. Accuracy on the post-scan test was computed for each subject by adding the number of correct responses across all post-scan questions about conversations heard in the MRI scanner during the CIN condition.

MRI Data Acquisition

MRI data were collected on a 3.0 Tesla Siemens Prisma MRI Scanner using a 64-channel head coil. For each subject, a multi-slice echo-planar (EPI) sequence was used to acquire functional

data (TR=720ms, TE=37ms, FOV=208mm, 72 slices, slice thickness 2mm, voxel size 2x2x2mm). Visual and auditory stimuli were presented via magnetic resonance compatible goggles and headphones (Optoacoustics LTD., Or Yehuda, Israel). Subjects wore earplugs and headphones to lessen scanner noise.

fMRI Data Analysis

Data were processed using FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl)[40] and AFNI (Analysis of Functional NeuroImages).[41] Functional data were motion corrected to the average functional volume with FSL's Motion Correction Linear Registration Tool (MCFLIRT)[42] using sinc interpolation and skull stripped using FSL's Brain Extraction Tool (BET).[43] Time series statistical analyses were run in FSL's FMRI Expert Analysis Tool (FEAT) version 6.0. Functional images were spatially smoothed (FWHM 5mm) and a temporal high pass filter of 67.5s was applied. Functional data were linearly registered to the Montreal Neurological Institute (MNI) 2mm standard brain with 12 degrees of freedom. Motion outliers were identified using FSL's motion outliers tool (comparing the root mean square intensity difference from the center volume to identify outliers) and were included as a confound explanatory variable in the single subject analyses; there was no difference in the mean number of volumes censored between ASD and TD participants ($p=0.31$). Condition effects were estimated by convolving a box-function for each condition with a double-gamma hemodynamic response function, along with the temporal derivative and temporal filtering. Each condition was modeled with respect to rest (C, N, CIN); single-subject models were combined into a group-level mixed effects model (FLAME1+2). Verbal IQ was entered as a covariate in all group-level analyses. Within-group and between-group maps were thresholded at $z>2.3$ ($p<0.01$), cluster-corrected for multiple comparisons at $p<0.05$.

To investigate how brain activity and connectivity during the fMRI task were related to diagnostic group and auditory sensory profiles, the following analyses were conducted: First, to assess brain activity associated with simultaneous exposure to conversation and noise (*above and beyond* either one alone), we examined the contrast of CIN>(C+N) as this allowed for optimal differentiation of neural systems supporting attention during *simultaneous* exposure to social and non-social auditory stimuli. Between-group comparisons (ASD vs. TD) were masked by regions that showed significant activity for the C, N, or CIN conditions in either ASD or TD participants. In keeping with previous literature that looked at speech-in-noise processing, we also examined the contrasts of CIN>C and C>CIN; these results are presented in Supplemental Materials. Second, as the auditory thalamus was an a priori region of interest, activity from 2mm spheres in the bilateral medial geniculate nuclei (MNI coordinates Left: -14, -25, -6; Right: 13, -25, -7)[44] were extracted for our contrast of interest and compared between diagnostic (ASD, TD) and SOR (ASD with high SOR, ASD with low SOR, and TD with low SOR) groups. Third, to examine the relationship between brain activity and our behavioral measure of attention, a bottom-up fMRI analysis was run using as a covariate of interest the total number of correct responses about the topics of the conversations heard in the CIN condition on the post-fMRI computer task. Here, again, analyses focused on the contrast of CIN>(C+N), elucidating brain regions whose activity increased in the CIN condition relative to conditions in which the noise and conversation stimuli were presented alone. Finally, functional connectivity of the left and right voice-selective cortex was examined by conducting a psychophysiological interaction analysis (PPI) in FSL[45,46] using the same seeds as in Abrams et al. (2013; 6mm spheres, MNI coordinates - Left: -63, -42, 9, Right: 57, -31, 5). This analysis allowed for investigation of brain regions showing changes in functional connectivity to the voice selective-cortex between the CIN and C+N conditions.

RESULTS

Behavioral Results

As expected, ASD youth had higher sensory symptoms compared to TD youth on the auditory subscales of both the SSP and SensOR, as well as higher composite SOR values (Table 1). Only one TD child showed elevated levels of SOR, falling within the top 25% of all participants. This subject was excluded from analyses comparing subjects by SOR group (i.e., ASD with high SOR, ASD with low SOR, TD with low SOR).

Post-scan Computerized Test

Relative to TD youth, ASD youth had lower accuracy for post-scan questions about noises heard during the fMRI task ($p=0.014$); there were no differences between ASD and TD youth in accuracy for topics of conversation heard in the CIN and C conditions, or for foils ($p>0.05$). GroupXCondition differences in accuracy for topics of conversation heard in the MRI scanner were explored using a repeated-measures ANOVA with Condition (CIN and C) as the within-groups variable, and Group (ASD and TD) as the between-groups variable. The analysis revealed a main effect of Condition ($F(52)=24.3$, $p<0.001$) in which both ASD and TD participants had lower accuracy for topics heard in CIN condition as compared to when listening to conversation (alone); the interaction was not significant. We ran a similar ANOVA breaking down the ASD group into those with high and low SOR. As expected, results again revealed a main effect of Condition ($F(50)=24.4$, $p<0.001$); the interaction approached significance ($p=0.06$). Follow-up analyses revealed that ASD youth with high SOR showed the greatest decline in accuracy for topics heard in the CIN condition compared to C.

fMRI Results

Within-Group Analyses

Figure 2 shows brain activity for each of the three conditions; corresponding peak coordinates can be found in Supplementary Table 1. Across all three conditions, the ASD and TD groups showed similar patterns of activity, with no statistically significant differences at this threshold ($z>2.3$, $p>0.01$).

When looking at our fMRI contrast of interest (i.e., brain regions more active during simultaneous exposure to conversation and noise than either alone (i.e., CIN>(C+N)), both TD and ASD youth displayed wide-spread upregulation of brain regions involved in theory-of-mind and auditory processing and (i.e., angular gyri, superior frontal gyrus, and superior temporal regions); ASD youth displayed additional activity in the precuneus while TD youth showed activity in ventral medial frontal cortex (Figure 2; Supplementary Table 2). For the opposite contrast, CIN<(C+N), TD youth showed reduced activity during exposure to conversation-in-noise (versus conversation or noise alone) in the precuneus (a hub of the default-mode network) and a number of brain regions involved in working memory (bilateral frontal cortex) and salience detection (bilateral insula, anterior cingulate); ASD youth showed no significant activity for this contrast (Figure 2; Supplementary Table 2).

Similar to previous findings in neurotypical adults showing that greater activity in the inferior frontal gyrus is associated better speech-in-noise accuracy,[47] both ASD and TD youth showed greater activity in this region for the contrast of CIN>C, as well as additional activity in the middle frontal gyrus, frontal pole, and lateral occipital cortex. ASD youth showed additional activity in the precuneus, right angular gyrus, and bilateral insula. For the opposite contrast, TD youth showed reduced activity in the posterior superior temporal sulcus (pSTS) when listening to

conversation-in-noise relative to conversation alone (CIN<C), again in-line with previous findings that temporal cortex activity becomes reduced as speech stimuli become increasingly degraded[18] (Supplementary Figure 1).

Between-Group Analyses

Significant between-group differences were observed only for the contrast of CIN>(N+C), where ASD youth showed increased activity in right hemisphere auditory regions and insular cortex relative to TD youth (Figure 3A; Supplementary Table 2). Parameter estimates extracted from these brain regions for the CIN and C+N conditions showed that the observed group differences (ASD vs. TD) were driven by higher activity during the CIN condition in right auditory cortex and insula in the ASD group with high SOR relative to TD youth; there was not a significant difference in activity when comparing ASD youth with low SOR to TD youth during CIN listening (Figure 3B). In ASD youth with high levels of SOR, greater right hemisphere activity during CIN exposure was related to greater accuracy on the post-scan test for conversations heard in the CIN condition (r= 0.72, p=0.005); correlations between activity in these regions and post-scan test accuracy were not significant in either in ASD youth with low SOR or TD youth (Figure 3C). Between-group differences in our a priori region of interest in the MGN of the thalamus were assessed by extracting and plotting BOLD signal intensity as a function of SOR; ASD youth with high SOR had significantly greater left (2-tailed t-test, p=0.012) and right (2-tailed t-test, p=0.002) MGN activity compared to TD youth with low SOR (results survive Bonferroni correction for 4 between-group comparisons; Figure 3D).

Post-Scan Accuracy

The relationship between brain activity and accuracy in the post-scan test was examined. There were no differences in response time between ASD and TD participants ($p>0.05$). The average number of post-scan correct responses (for conversations heard in the CIN condition) was entered as a regressor of interest in a whole-brain bottom-up group level analysis. TD youth showing higher accuracy had greater activity in canonical language regions including the left middle temporal gyrus, bilateral inferior frontal gyrus, and bilateral temporal pole (Figure 4, top; Supplementary Table 3). By contrast, ASD youth with higher accuracy had greater activity in right hemisphere temporal cortex, superior temporal gyrus and supramarginal gyrus, as well as bilateral planum temporale, temporal pole, and occipital cortex (Figure 4, middle; Supplementary Table 3). The direct between group comparison showed that relative to TD youth, higher accuracy in the ASD group was more strongly related to activity in right hemisphere auditory and language regions including the right supramarginal gyrus, angular gyrus, and superior temporal gyrus, as well as bilateral visual cortex (Figure 4, bottom; Supplementary Table 3). There were no regions where TD youth showed greater activity relative to ASD youth.

PPI Analysis

Psychophysiological interaction analyses (PPI) were conducted using 6mm seeds in left and right voice-selective cortex to examine differences in task-based functional connectivity between the CIN and C+N conditions. For the left pSTS seed, TD youth showed reduced connectivity with frontal and parietal attention areas (bilateral frontal pole, superior frontal gyrus, middle frontal gyrus, supramarginal gyrus), as well as left auditory and visual cortex during the CIN condition relative to C+N (i.e., CIN<(C+N); Figure 5, top row; Supplementary Table 4). ASD

youth demonstrated reduced connectivity between the left pSTS and the right auditory cortex, precentral gyrus, and central opercular cortex during CIN compared to C+N. The direct between group comparison showed that, compared to ASD youth, TD youth showed greater reductions in left pSTS connectivity with the precuneus, middle frontal gyrus, middle temporal gyrus, inferior temporal gyrus, and visual cortex during the CIN condition. For the left pSTS seed, neither TD or ASD participants showed increased connectivity during the CIN condition relative to the alone conditions (i.e., CIN>(C+N)). Functional connectivity of the right pSTS was not modulated by task condition in TD youth, whereas the ASD youth showed reduced connectivity between the right pSTS and left auditory cortex during CIN relative to C+N (i.e., CIN<(C+N)), as well as increased connectivity between the right pSTS and the right postcentral gyrus moving into the superior parietal lobule during CIN compared to C+N (i.e., CIN>(C+N); Figure 5, bottom row; Supplementary Table 4). The direct between group comparison showed that, relative to TD youth, ASD youth showed significantly greater connectivity between the right pSTS and right postcentral gyrus/superior parietal lobule during the CIN condition.

DISCUSSION

The goal of the current study was to investigate neural mechanisms underlying selective attention to speech in the context of background noise in youth with and without ASD. To do so, we employed a novel conversation-in-noise paradigm in which participants heard two-person conversation in the context of common environmental noises. In addition, we related behavioral measures of auditory sensory over-responsivity and attention (i.e., post-scan recall task) to brain activity in TD and ASD youth. Finally, we assessed how task-based functional connectivity of voice-selective cortex might vary when conversations were presented together with environmental

noises. Our behavioral results are consistent with previous studies investigating sensory-over responsivity in youth with and without ASD, [24] as we found that ASD youth had significantly elevated auditory sensory over-responsivity compared to TD youth. Also, in agreement with previous studies investigating the ability to recall verbal information presented in conjunction with distracting background auditory stimuli, [21,22] we found that both TD and ASD youth had better recall of topics heard when listening to conversation in the absence of background noise than when they heard conversation masked in noise. With regard to brain responses, we found significant differences in brain activity between ASD and TD youth when comparing conversation-in-noise to either alone, and that brain response was modulated sensory over-responsivity and attention. In addition, we found that connectivity of the left and right voice-selective cortex was modulated by task condition in both ASD and TD youth. Below we discuss each of these findings in further detail.

While the groups showed overall similar activity when comparing across conditions, during exposure to simultaneous conversation and noise (above and beyond either one alone) ASD youth showed greater activity in right hemisphere auditory cortex compared to TD youth. A large body of previous research has shown that increased activation in right hemisphere language homologues is associated with complex speech processing in individuals with ASD, for example when interpreting ironic remarks,[48–50] and when recognizing prosody.[51] Furthermore, the right temporal parietal junction has been associated with selective listening in neurotypical adults,[47] suggesting that increased activity in this region, as was observed here in ASD youth, may be a compensatory mechanism allowing for improved selective listening to conversation in the presence of distracting noise. In addition to increased right auditory cortex activity, we also observed increased activity in the right insula in ASD youth. The insula is a key node of the

salience network, a group of brain regions involved in identifying the most relevant information in one's internal and external environment,[52–55] making it a key brain region underlying allocation of auditory attention.[56] Our data are in agreement with other research suggesting that insula activity is involved in both speech perception and production,[57] and reports that increased activity in this region is associated with neural processing of difficult language stimuli such as distorted speech.[58] Notably, the observed diagnostic group differences in right auditory areas and insula were driven by greater neural activity observed in ASD youth with high auditory SOR compared to TD youth when listening to conversation-in-noise; we did not observe differences in these regions when comparing ASD youth with low SOR to TD youth. Furthermore, in only ASD youth with high SOR, increased activity in these regions was related to better recall for topics of conversation heard in the simultaneous condition. All together, these findings in ASD youth with high SOR indicate that increased right hemisphere auditory and insula activity in response to exposure to conversation-in-noise may serve as a compensatory attentional mechanism, possibly reflective of more effortful neural processing, ultimately with positive effects on the ability to hone-in on social stimuli in the presence of non-social distractors.

Notably, we also saw increased activity in the bilateral auditory thalamus specifically in youth with ASD and high auditory SOR when listening to conversation-in-noise relative to conversation and noise alone. Previous work investigating the neural basis of SOR in youth with ASD has documented heightened thalamic activity in response to aversive visual, tactile, and auditory sensory stimuli in youth with ASD,[30,34] and children with ASD display hyperconenctivity between thalamus and temporal cortex in resting-state.[32] Our results extend these findings as they suggest that the thalamus is also over-active in situations in which both social and non-social sensory stimuli are presented simultaneously. It has recently been suggested that increased

connectivity between the thalamus and auditory cortex may serve as a compensatory mechanism in ASD, relating to less severe social and repetitive behavior symptomatology.[59] While we did not observe a relationship between thalamic response and ASD symptomatology in this study, we did find that ASD youth with the highest thalamic activity (i.e., the ASD group with high SOR) also had the highest right auditory cortex activity when comparing conversation-in-noise to conversation and noise alone, which was, in turn, related to better recollection of the topics of conversation heard in the presence of noise. Thus, the hypothesis that greater thalamic-auditory communication may serve a compensatory purpose, perhaps in a subset of individuals with ASD, remains an intriguing possibility.

Brain activity during exposure to conversation-in-noise predicted post-scan accuracy in recollecting the topics of conversations heard in both ASD and TD youth. In ASD youth, better ability to recognize topics of conversation heard during simultaneous presentation of conversation and noise was related to increased activity in right hemisphere auditory and language regions implicated in discourse processing,[60,61] as well as interpretation of the general intent or purpose of a conversation, such as affect, tone, and patterns of stress in speech (i.e., prosody).[62] In ASD youth, we further observed that post-scan accuracy was related to visual cortex activity when listening to conversation-in-noise relative to conversation and noise alone. As previously suggested,[63] co-activity of visual and auditory cortex during perceptual processing may be reflective of atypical cross-talk between sensory systems in youth with ASD. In contrast to our findings in ASD youth, in TD controls increased accuracy in the post-scan test was related to higher activity in canonical language regions during simultaneous exposure to conversation and noise relative to conversation and noise alone. Thus, to hone attention to auditory social information in the context of non-social background noise, neurotypical youth engaged frontal and temporal regions (i.e., inferior frontal

gyrus, frontal orbital cortex, superior temporal gyrus) associated with different aspects of language processing.[64,65] Activity in left hemisphere language regions, and the inferior frontal gyrus in particular, has been shown in neurotypical adults to correlate with the ability to distinguish speech-in-noise[66] and the degree to which ASD youth show reduced activity in the inferior frontal gyrus when listening to speech-in-noise has been demonstrated to correlate with degree of ASD symptomatology.[67] Our results in ASD and TD youth are in agreement with a recent fMRI study examining interactions between sensory and social cognition in which youth with and without ASD were presented with sarcastic language in the presence of aversive sensory stimuli (had their arm rubbed with a scratchy fabric); here too the authors found that TD youth showed increased activity in left frontal cortex and left-hemisphere language regions when attending to sarcastic speech – a pattern that was not observed in youth with ASD.[23] Together, these data suggest that the ability to "hone-in" on speech in the presence of background noise depends on the sustained activity of left hemisphere canonical language areas in TD youth, whereas ASD youth rely on compensatory activity in right hemisphere auditory regions.

Lastly, we mapped task-based functional connectivity of the voice-selective cortex in ASD and TD youth, focusing on changes in functional connectivity between the conversation-in-noise and noise or conversation alone conditions. In conditions in which conversation and noise were presented simultaneously, the TD brain showed reduced connectivity between left voice-selective cortex (pSTS) and a number of attention-related areas in frontal and parietal cortex. Our results suggest that, in TD youth, connectivity between specifically left-hemisphere voice-selective cortex (i.e., in the hemisphere dominant for language) and attentional brain networks is disrupted during presentation of conversation-in-noise. Interestingly, relative to ASD youth, TD youth showed significant decreases in connectivity between voice-selective cortex and front-parietal attention

regions during conversation-in-noise processing. One other study has reported changes in connectivity between the temporoparietal junction (TPJ; a brain region adjacent to the voice-selective-cortex) during speech-in-noise listening in neurotypical adults, finding positive connectivity between the TPJ and nodes of the attention network during selective listening to speech compared to baseline.[47] These findings would seem to contradict those observed here in neurotypical youth; however, our analyses focused not on differences in connectivity during conversation-in-noise compared to baseline (as in Puschmann et al. (2017)), but rather differences in connectivity related specifically to *simultaneous* exposure to conversation and noise versus conversation and noise *alone*. Taken together with previous findings in the literature, it appears that the superior temporal cortex does indeed show positive functional connectivity with fronto-parietal attention systems during simultaneous exposure to conversation and noise in neurotypical individuals, although the strength of this connectivity during conversation-in-noise listening is reduced as compared to when listening to conversation and noise alone. Conversely, in ASD youth, functional connectivity was reduced between the voice-selective cortex and contralateral auditory, pre-motor, and sensory brain regions during conversation-in-noise listening compared to conversation and noise alone. These results in ASD youth suggest that there may be altered cross-hemisphere connectivity between left and right auditory cortex in ASD possibly reflective of broader disruptions of sensory-system connectivity in this population.[33,63,68]

In sum, we find that youth with and without ASD engage different neural systems to "hone-in" on language when listening to conversation-in-noise. In TD youth, the ability to sustain activity in canonical language-related brain areas during exposure to conversation-in-noise predicted better memory for what was heard. By contrast, in ASD youth better recall for conversation-in-noise was related to greater activity in auditory cortex and right hemisphere language homologues. We

speculate that increased activity in the thalamus, right language and auditory cortex in youth with ASD may serve as a compensatory mechanism, allowing for increased neural processing of difficult language stimuli. Importantly, however, this may be the case for only a subset of individuals with ASD who have heightened auditory sensory over-responsivity; ASD participants in our study were high-functioning, with verbal IQ in the normal range. It is possible that in this subgroup of individuals with ASD, heightened auditory sensitivity enhances attention to all auditory stimuli. More broadly, these findings suggest that the neural mechanisms leading to increased selective attention to speech in neurotypical youth involve increased activity in brain regions subserving language processing. Conversely, in youth with ASD speech stimuli may not be deemed as salient, making the task of selectively attending to speech more difficult and requiring the recruitment of additional neural resources outside of the canonical language network. In future studies it will be crucial to extend these findings to individuals with more severe ASD phenotypes and lower IQ, as well as to younger children on the autism spectrum. To this end, prospective studies of infants at high risk for developing ASD will be essential to track the longitudinal co-development of sensory responsivity, language acquisition, and ASD symptomatology.

	ASD	TD	P-Value
Gender (% male)	69%	64%	
Handedness (% right handed)	96%	93%	
Age	13.8 (3.0)	13.8 (2.7)	0.97
Full IQ	102.4 (14.9)	113.1 (13.0)	<0.01*
Nonverbal IQ	108.0 (17.6)	112.6 (12.7)	0.27
Verbal IQ	97.4 (14.3)	110.6(13.4)	<0.01*
Mean Absolute Motion	0.4 (0.3)	0.4 (0.3)	0.72
Max Absolute Motion	1.8 (1.6)	1.4 (1.2)	0.36
Mean Relative Motion	0.1 (0.1)	0.1 (0.1)	0.98
Max Relative Motion	1.2 (1.1)	0.9 (0.8)	0.27
SSP Auditory Filtering	18.1 (5.6)	9.0 (0.4)	<0.01*
SensOR Auditory Count	5.0 (4.4)	0.7 (2.1)	<0.01*
Auditory SOR Composite	0.6 (0.8)	-0.6 (0.5)	<0.01*

Table 1. Descriptive Statistics. Data shown are Mean (Standard Deviation). ASD = Autism Spectrum Disorder; TD = Typically Developing; IQ = Intelligence Quotient; SSP = Short Sensory Profile; SensOR = Sensory Over-Responsivity Scales; SOR = Sensory Over-Responsivity Composite.

		Typically Developing				Autism Spectrum Disorder			
		(N) Condition MNI Peak			Max	MNI Peak (mm)			Max
	R/L	x	y	z	*Z*	x	y	z	*Z*
Noise (N) Condition									
Angular Gyrus	L	-62	-56	12	2.77				
Frontal Pole	R	54	44	0	2.68				
Heschl's Gyrus	L	-44	-20	4	6.22				
Inferior Frontal Gyrus, pars triangularis	R	40	28	4	4.43				
Insular Cortex	R	40	-4	-12	3.36				
Insular Cortex	L	-38	2	-18	2.74				
Planum Temporale	R	58	-26	12	6.94	58	-26	12	9.06
Planum Temporale	L	-48	-38	16	3.24	-44	-30	12	6.57
Superior Temporal Gyrus, anterior division	L	-64	-6	-2	5.11				
Superior Temporal Gyrus, posterior division	R	68	-10	0	5.49				
Superior Temporal Gyrus, posterior division	L	-68	-24	10	5.36	-66	-30	16	4.02
Supramarginal Gyrus, posterior division	L	-64	-44	28	3.28				
Temporal Pole	R	58	14	-8	4.82				
						42	6	-16	3.84
Conversation (C) Condition									
Angular Gyrus	L					-54	-50	16	4.09
Frontal Medial Cortex	R					6	42	-22	4.75
Frontal Orbital Cortex	L					-46	26	-12	3.28
Frontal Pole	L	-8	52	34	4.91				
Frontal Pole	R	12	44	48	3.17				
Heschl's Gyrus	L	-40	-24	10	7.15				
Heschl's Gyrus	R					48	-14	4	7.23
Inferior Temporal Gyrus, posterior division	L					-42	-12	-32	3.32
Middle Temporal Gyrus, anterior division	R					66	0	-14	4.71
Middle Temporal Gyrus, posterior division	R	50	-10	-18	5.24	54	-32	-6	4.05
Middle Temporal Gyrus, posterior division	L					-68	-22	-20	3.48
Middle Temporal Gyrus, temporooccipital part	L	-52	-58	12	3.88				
Parahippocampal Gyrus, anterior division	L					-16	-18	-24	4.32
Planum Temporale	L					-56	-28	8	7.23
Superior Frontal Gyrus	L	-12	36	46	3.02	-2	52	30	3.79
Superior Temporal Gyrus, anterior division	L	-58	-12	-2	7.37	-56	0	-14	6.36
Superior Temporal Gyrus, posterior division	R	66	-20	2	6.96	68	-18	4	6.71
Superior Temporal Gyrus, posterior division	L	-60	-32	4	5.67				
Temporal Pole	L	-44	6	-18	6.71				
Temporal Pole	R	52	14	-18	6.63	52	12	-26	5.38
Temporal Pole	L	-48	22	-30	5.18				
Thalamus	L					-6	-8	0	4.19

Supplementary Table 1. Peak coordinates of brain activity for Conversation-In-Noise, Conversation, and Noise ($z>2.3$, $p<0.05$, cluster corrected for multiple comparisons at $p<0.05$).

		Typically Developing				Autism Spectrum Disorder			
		(N) Condition MNI Peak			Max	MNI Peak (mm)			Max
	R/L	x	y	z	*Z*	x	y	z	*Z*
Conversation-In-Noise (CIN) Condition									
Amygdala	L					-18	-8	-14	4.17
Angular Gyrus	L	-42	-54	20	5.30				
Angular Gyrus	R					44	-48	18	4.09
Frontal Medial Cortex	R	6	46	-20	5.40				
Frontal Orbital Cortex	L	-46	32	-12	3.72				
Frontal Orbital Cortex	R	44	26	-14	4.59				
Frontal Pole	L	-10	48	34	4.90				
Frontal Pole	R	12	52	34	3.90	6	48	50	3.21
Heschl's Gyrus	L	-40	-24	10	6.79	-48	-20	6	6.22
Heschl's Gyrus	R	46	-22	8	5.86	50	-22	6	7.33
Hippocampus	R					20	-12	-18	3.97
Lateral Occipital Cortex, superior division	L					-58	-62	26	3.18
Middle Temporal Gyrus, anterior division	R					58	0	-26	3.42
Middle Temporal Gyrus, posterior division	L	-48	-36	-4	5.15				
Middle Temporal Gyrus, temporooccipital part	R	62	-48	4	3.20				
Subcallosal Cortex	R	4	20	-26	5.54				
Superior Frontal Gyrus	L	-14	32	54	4.51				
Superior Frontal Gyrus	R					4	54	30	5.25
Superior Temporal Gyrus, anterior division	L					-50	-4	-18	6.29
Superior Temporal Gyrus, anterior division	R	60	4	-14	6.35	62	-6	-6	5.94
Superior Temporal Gyrus, posterior division	L	-56	-10	-8	6.89	-66	-28	10	6.65
Superior Temporal Gyrus, posterior division	R	66	-20	2	6.72	68	-32	12	4.59
Supramarginal Gyrus, posterior division	L	-62	-46	12	5.39	-54	-44	14	5.15
Temporal Fusiform Cortex, posterior division	L	-40	-16	-26	3.94	-40	-24	-20	2.54
Temporal Pole	L	-46	8	-20	6.49	-44	16	-20	5.58
Temporal Pole	R	50	16	-38	3.74	42	14	-24	5.92

Supplementary Table 1 Continued. Peak coordinates of brain activity for Conversation-In-Noise, Conversation, and Noise ($z>2.3$, $p<0.05$, cluster corrected for multiple comparisons at $p<0.05$).

		Typically Developing				Autism Spectrum Disorder				ASD > TD			
		(N) Condition MNI Peak			Max	MNI Peak (mm)			Max	MNI Peak (mm)			Max
	R/L	x	y	z	*Z*	x	y	z	*Z*	x	y	z	*Z*
CIN > (C+N)													
Angular Gyrus	L	-42	-52	20	4.44								
Angular Gyrus	R	48	-58	28	3.20	54	-58	20	4.60				
Frontal Medial Cortex	R	4	42	-22	5.31								
Frontal Orbital Cortex	R					52	28	-12	3.05				
Frontal Pole	R					2	58	22	3.67				
Insular Coretx	R									42	8	-8	2.47
Lateral Occipital Cortex, superior division	L	-40	-70	36	2.91	-56	-62	26	3.92				
Left Amygdala	L	-28	-6	-22	4.70								
Middle Temporal Gyrus, anterior division	L	-64	-8	-14	7.82								
Middle Temporal Gyrus, posterior division	L	-66	-28	-18	2.97								
Middle Temporal Gyrus, posterior division	R					52	-12	-16	5.46				
Middle Temporal Gyrus, temporooccipital part	R	66	-46	6	4.05								
Parahippocampal Gyrus, posterior division	L	-18	-26	-16	3.09								
Planum Polare	R	48	2	-16	6.10					40	-20	-2	4.00
Planum Temporale	L	-62	-18	6	6.33	-34	-32	14	3.35				
Planum Temporale	R					42	-36	18	3.00	42	-30	10	2.65
Precuneous Cortex	R					2	-54	40	3.15				
Subcallosal Cortex	L	-2	16	-24	5.30								
Superior Frontal Gyrus	L	-6	46	34	5.09	6	46	40	4.41				
Superior Frontal Gyrus	R					10	22	58	3.42				
Superior Temporal Gyrus, posterior division	L					-64	-26	2	5.47				
Superior Temporal Gyrus, posterior division	R	58	-20	-2	6.06	68	-36	12	5.43				
Supramarginal Gyrus, posterior division	L	-60	-44	14	8.26	-54	-44	14	5.20				
Temporal Pole	L	-42	16	-30	5.72	-44	16	-20	5.58				
Temporal Pole	R	50	22	-26	3.70	46	16	-36	5.09	38	14	-20	2.98
CIN < (C+N)													
Central Opercular Cortex	R	54	4	4	5.57								
Cingulate Gyrus, anterior division	R	2	28	28	5.80								
Cingulate Gyrus, posterior division	L	-6	-40	26	4.71								
Cingulate Gyrus, posterior division	R	8	-34	48	3.08								
Cuneal Cortex	R	14	-72	22	2.92								
Frontal Operculum Cortex	L	-34	26	4	3.30								
Frontal Pole	R	24	58	18	4.67								
Frontal Pole	L	-30	52	28	4.41								
Insular Cortex	L	-36	12	-12	3.42								
Insular Cortex	R	36	12	-16	3.27								
Paracingulate Gyrus	L	-8	16	48	3.01								
Planum Polare	R	40	-20	-2	3.95								
Precentral Gyrus	L	-52	6	4	5.04								
Precuneous Cortex	R	12	-62	44	4.42								
Precuneous Cortex	L	-10	-66	28	4.00								
Supramarginal Gyrus, anterior division	L	-62	-32	50	2.59								

Supplementary Table 2. Peak coordinates of brain activity for Conversation-In-Noise vs. Conversation and Noise ($z>2.3$, $p<0.05$, cluster corrected for multiple comparisons at $p<0.05$.

		Typically Developing				Autism Spectrum Disorder				ASD > TD			
		MNI Peak (mm)			Max	MNI Peak (mm)			Max	MNI Peak (mm)			Max
	R/L	x	y	z	*Z*	x	y	z	*Z*	x	y	z	*Z*
CIN > (C+N)													
Angular Gyrus	R					40	-52	30	3.04				
Frontal Orbital Cortex	L	-46	22	-14	3.73								
Heschl's Gyrus	R									48	-24	6	3.39
Inferior Frontal Gyrus, pars opercularis	L	-54	18	14	4.03								
Inferior Temporal Gyrus, temporooccipital part	L									-42	-50	-8	3.35
Lateral Occipital Cortex, inferior division	L					-48	-74	-6	4.13				
Lateral Occipital Cortex, inferior division	R					50	-64	-12	2.89	40	-90	2	4.26
Lateral Occipital Cortex, superior division	L					-40	-84	16	2.91	-50	-64	26	3.53
Lateral Occipital Cortex, superior division	R					30	-74	20	2.97	28	-74	20	3.54
Middle Temporal Gyrus, posterior division	L	-62	-30	-4	2.84								
Middle Temporal Gyrus, posterior division	R					50	-26	-6	3.79				
Middle Temporal Gyrus, temporooccipital part	L	-64	-52	8	3.75	-54	-52	8	4.94				
Middle Temporal Gyrus, temporooccipital part	R									40	-56	10	3.36
Occipital Fusiform Gyrus	L					-18	-88	-16	3.14				
Occipital Fusiform Gyrus	R									30	-64	-8	2.81
Occipital Pole	L					-28	-92	2	4.50	-32	-94	-12	3.92
Occipital Pole	R					28	-90	2	5.13	10	-98	12	3.87
Parietal Operculum Cortex	R									36	-28	22	2.87
Planum Polare	L	-48	0	-16	3.30	-50	4	-6	3.36				
Planum Temporale	L					-36	-30	12	3.02				
Planum Temporale	R					54	-36	22	3.95				
Supramarginal Gyrus, posterior division	R									54	-38	20	3.76
Temporal Pole	L	-46	10	-34	4.71	-52	6	-28	5.01				
Temporal Pole	R	42	10	-36	3.96								

Supplementary Table 3. Peak coordinates of brain activity related to post-scan test accuracy ($z>2.3$, $p<0.05$, cluster corrected for multiple comparisons at $p<0.05$).

		Typically Developing				Autism Spectrum Disorder				TD > ASD				ASD > TD			
		MNI Peak (mm)			Max	MNI Peak (mm)			Max	MNI Peak (mm)			Max	MNI Peak (mm)			Max
	R/L	x	y	z	Z	x	y	z	Z	x	y	z	Z	x	y	z	Z
Left pSTS Seed - CIN < (C+N)																	
Cuneal Cortex	L	-20	-72	24	3.41												
Frontal Pole	L									-26	56	14	2.90				
Frontal Pole	R	40	56	4	4.77												
Heschl's Gyrus	R					52	-14	2	2.75								
Inferior Frontal Gyrus, pars triangularis	R	42	34	14	3.50												
Lateral Occipital Cortex, inferior division	L	-46	-74	2	3.56												
Lateral Occipital Cortex, superior division	L	-16	-64	48	3.98					-52	-74	8	4.36				
Lingual Gyrus	L	-28	-46	-6	3.17					-14	-64	52	3.75				
Middle Frontal Gyrus	L	-42	34	20	4.62												
Middle Temporal Gyrus, posterior division	L																
Middle Temporal Gyrus, temporooccipital part	L	-54	-44	2	3.42					-40	36	20	3.63				
Occipital Pole	L	-16	-92	30	2.91					-62	-18	-20	4.42				
Planum Polare	L	-56	-8	4	4.12					-62	-56	-8	3.26				
Planum Polare	R					60	-2	2	2.96								
Planum Temporale	L	-60	-28	14	3.14												
Postcentral Gyrus	L	-52	-32	60	3.71												
Precentral Gyrus	R					54	4	14	3.65								
Superior Parietal Lobule	L	-42	-54	60	3.23												
Supramarginal Gyrus, anterior division	L	-50	-38	40	3.45												
Temporal Occipital Fusiform Cortex	L	-40	-56	-20	2.69												
Right pSTS Seed - CIN < (C+N)																	
Heschl's Gyrus	L					-54	-16	8	3.12								
Planum Temporale	L					-40	-14	-8	3.51								
Right pSTS Seed - CIN > (C+N)																	
Superior Parietal Lobule	R					22	-42	66	3.60					30	-34	54	4.75

Supplementary Table 4. Peak coordinates for PPI analyses of the voice-selective cortex ($z>2.3$, $p<0.05$, cluster corrected for multiple comparisons at $p<0.05$).

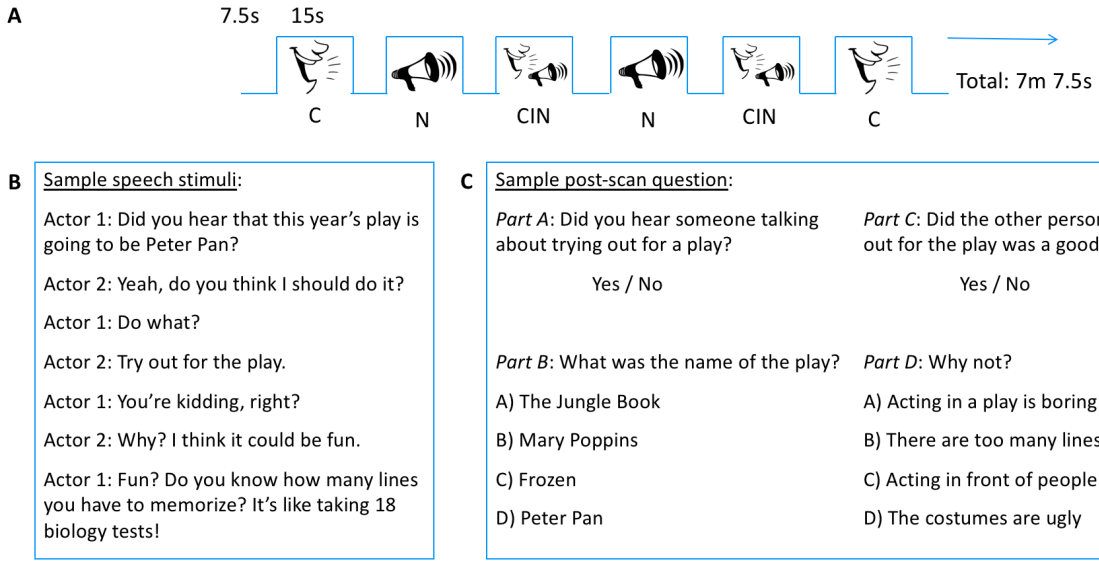


Figure 1. Experimental design. A) Block design fMRI task. B) Example of a conversation heard during fMRI data acquisition. C) Sample of post-scan questions. CIN = Conversation-In-Noise; C = Conversation; N = Noise.

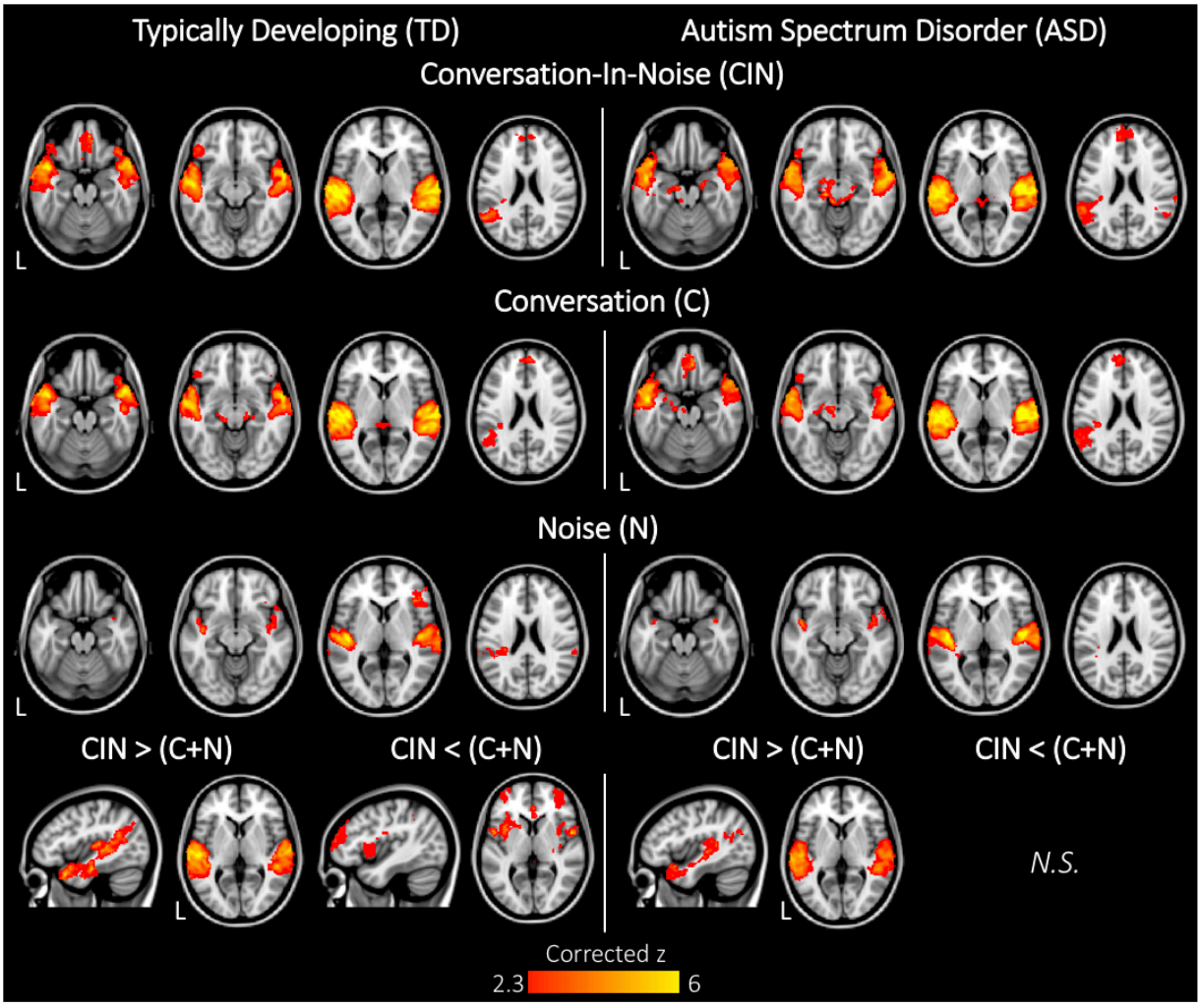


Figure 2. Within-Group Results. Maps are presented at z>2.3 (p<0.01), corrected for multiple comparisons at the cluster level (p<0.05). CIN = Conversation-In-Noise; C = Conversation; N = Noise; L = Left.

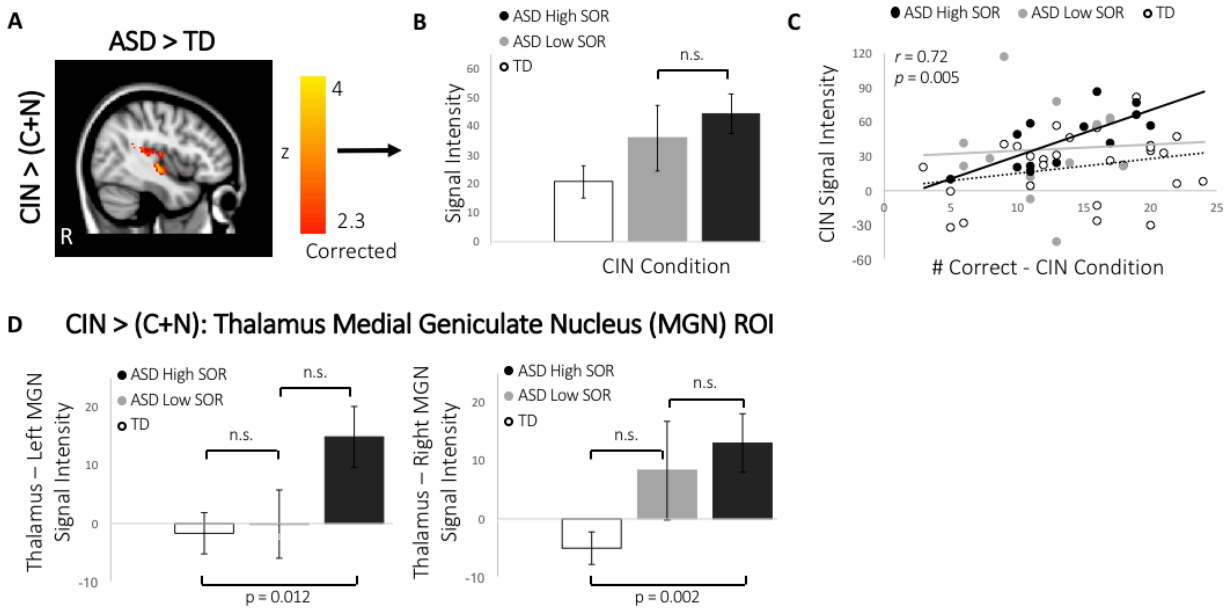


Figure 3. Between-group results for the contrast of CIN>(C+N). A) Whole-brain results showing right hemisphere brain regions where ASD youth had greater activity relative to TD youth. B) Activity from brain regions shown in (A) extracted from the CIN condition. B) Parameter estimates were extracted from regions shown in (A) and plotted against post-scan test accuracy for topics of conversation heard in the CIN condition for each of the 3 SOR groups. C) BOLD signal intensity extracted from the medical geniculate nucleus of the thalamus. ASD = Autism Spectrum Disorder; TD = Typically Developing; SOR = Sensory Over-Responsivity; MGN = Medial Geniculate Nucleus; CIN = Conversation-In-Noise; C = Conversation; N = Noise.

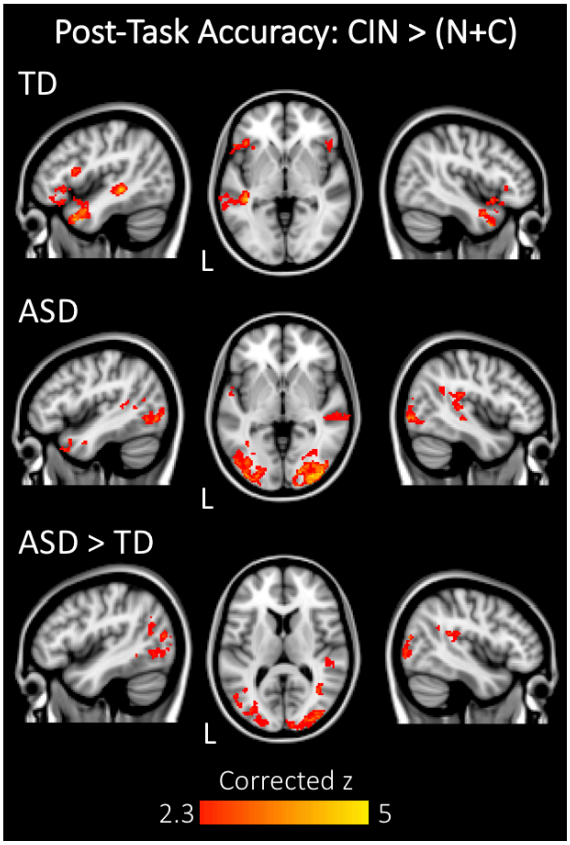


Figure 4. Relationship between accuracy on post-scan questions about conversations heard in the CIN condition and differential brain response to CIN and C+N. ASD = Autism Spectrum Disorder; TD = Typically Developing; CIN = Conversation-In-Noise; C = Conversation; N = Noise.

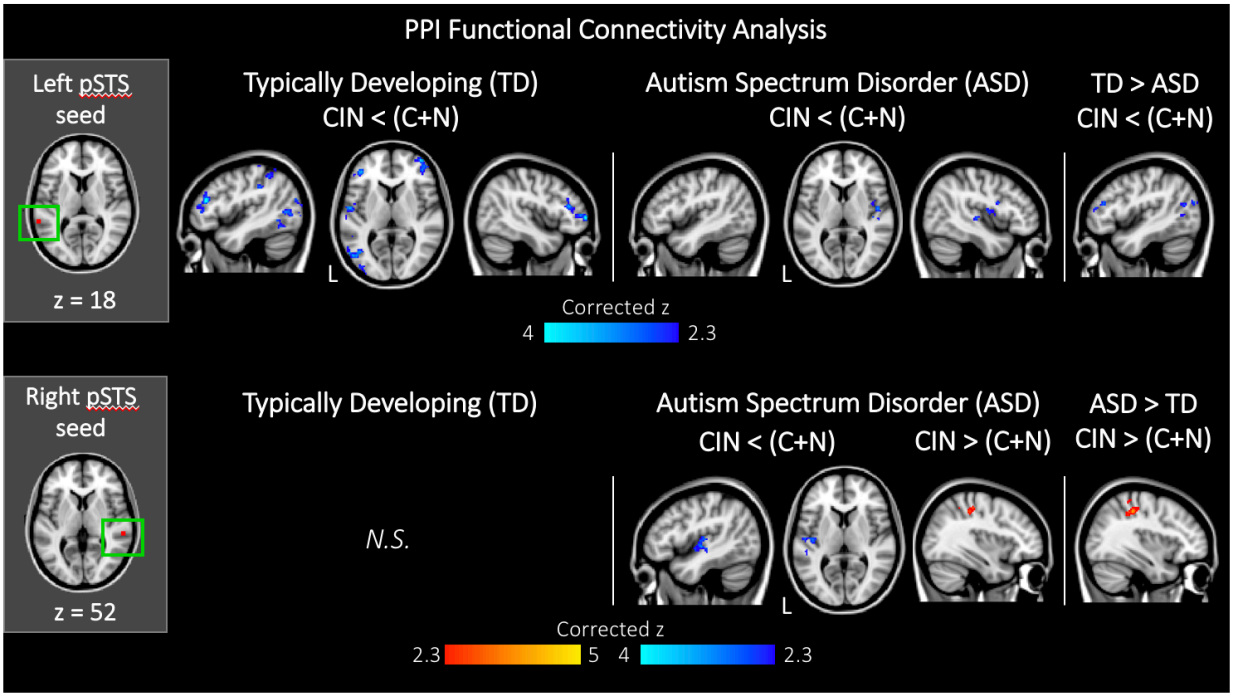
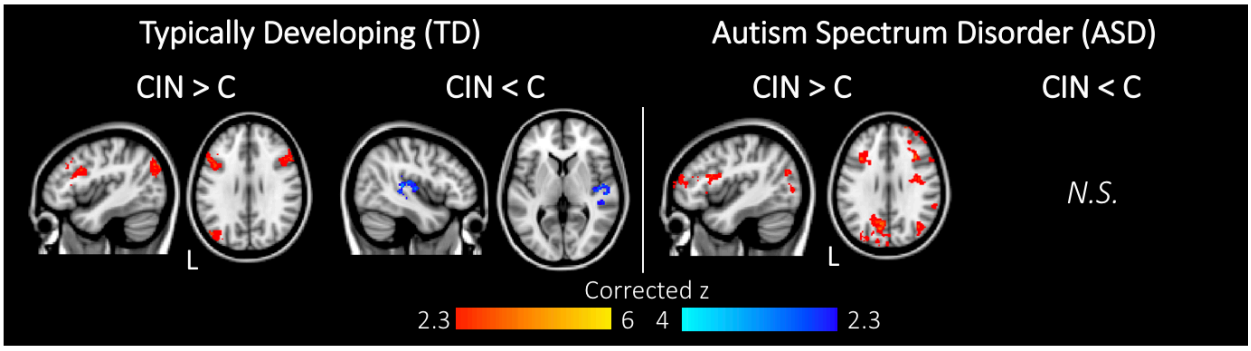


Figure 5. PPI Analyses. Within and between group results for PPI analyses. Top row: results for the left pSTS seed. Bottom row: results for the right pSTS seed. N.S. = not significant; CIN = Conversation-In-Noise; C = Conversation; N = Noise.



Supplementary Figure 1. Within-Group Results. Maps are presented at z>2.3 (p<0.01), corrected for multiple comparisons at the cluster level (p<0.05). C, conversation; CIN = Conversation-In-Noise; C = Conversation; L = Left.

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CONCLUSION

Autism spectrum disorder (ASD) is a genetically and behaviorally heterogeneous neurodevelopmental disorder, making identification of individuals at risk for developing the disorder and designing effective treatments a significant challenge. While there is still much to be learned about the genetic and neurobiological factors that underlie the etiology of ASD, it is clear that brain function and connectivity are altered. This dissertation advances our understanding of how heterogeneity in ASD neuroendophenotypes is related to variability in genetic risk for ASD, gender, and common behavioral symptoms of ASD such as atypicalities in sensory processing. Importantly, the identification of intermediate biomarkers at the level of the brain can help to identify subgroups of individuals likely to express specific ASD-associated phenotypes, as well as to isolate brain networks to target for individualized pharmacological and/or behavioral interventions.

In Chapter 1, resting-state functional connectivity of the brain's reward network was examined in a predominantly male sample of youth with and without ASD. While overall ASD and neurotypical youth showed a similar pattern of positive and negative reward network connectivity, genetic risk in the oxytocin receptor gene (*OXTR)* stratified neuroendophenotypes, and differentially so in each diagnostic group. In ASD youth, inheriting higher numbers of ASD-associated risk variants in the *OXTR* was associated with reduced connectivity within the reward network itself. Conversely, in neurotypical youth, having higher numbers of *OXTR* risk alleles was related to increased connectivity between the reward network and frontal brain regions important for mentalizing. Increased reward-frontal connectivity in neurotypical youth was associated with better behavioral measures of social functioning, suggesting that increased connectivity serves as a compensatory mechanism in neurotypical individuals at higher genetic risk for ASD. This study

was the first to examine how aggregate genetic risk for ASD, across several loci, are related to neuroendophenotypes and behavioral symptomatology, showing for the first time that neural heterogeneity in ASD can be explained by individual differences in genetic dosage. Ultimately, considering interactions between individual genetic factors and neuroendophenotypes may help to explain some of the neural heterogeneity so often observed across neuroimaging studies of ASD.

In Chapter 2, this work was extended by exploring genetic and neural heterogeneity in females with and without ASD, using the same seed-based functional connectivity methods to examine reward network connectivity and associations with *OXTR* genetic variants. Similar to the findings reported in Chapter 1 (which focused on a predominantly male sample), females with ASD and neurotypical females showed overall similar patterns of whole-brain reward network connectivity; aggregate *OXTR* risk-allele dosage also modulated functional connectivity in both groups, albeit differentially so. In ASD females, inheriting higher numbers of ASD-associated *OXTR* variants was associated with greater connectivity between the reward network and subcortical brain regions; in turn, this was related to higher restricted interest and repetitive behavior scores. Conversely, neurotypical females showed increased functional connectivity between the reward network and frontal brain regions involved in the process of mentalizing (i.e., thinking about the mental states of oneself and others). Importantly, when examining the effect of sex and *OXTR* risk on reward network connectivity in individuals with ASD, we found that, relative to males with ASD, females with ASD showed significantly more reward network connectivity with subcortical brain areas, as well as the frontal pole. Furthermore, this sex-specific increased connectivity with the frontal pole observed in females with ASD and higher *OXTR* genetic risk was associated with better behavioral measures of social functioning, mirroring the brain-behavior relationship we observed previously in neurotypical males. Taken together, these

imaging-genetics studies in males and females with ASD indicate that not only do common genetic variants stratify individual neuroendophenotypes, but they do so in a sex-specific manner. More generally, these data also point to the critical need to include females in studies of ASD, as they are likely to display distinct neurogenetic profiles compared to their male counterparts, and the specific brain networks to be targeted by pharmacological and behavioral interventions may be sex-specific.

The study presented in Chapter 3 investigated how behavioral heterogeneity in auditory sensitivity and attention to speech stimuli modulate brain function in order to better understand how atypical sensory processing may affect the neural processing of socially relevant information. Using a novel fMRI paradigm, youth with and without ASD were exposed to a series of auditory stimuli including two-speaker conversation, common environmental noises, and conversation-in-noise. Relative to neurotypical youth, ASD youth showed higher activity in right language homologues when exposed to conversation-in-noise. Importantly this increased activity was associated with better recall for the content of the conversations (those presented together with distracting environmental noises) in children with ASD who had higher levels of sensory over-responsivity; interestingly, this group also showed increased activity in the auditory thalamus while listening to conversation-in-noise. Conversely, in neurotypical youth, better recall of these conversations was linked to increased activity in canonical left hemisphere language regions. These data suggest that ASD individuals with sensory processing atypicalities show distinct neuroendophenotypes relative to ASD youth without sensory over-responsivity, and importantly, that the neural network underlying optimal attention to socially relevant stimuli are different in these two ASD subgroups. More generally, these data speak to the importance of understanding

the neurobiological basis of ASD-associated symptoms to more fully understand how neural heterogeneity relate to behavioral outcomes.

Limitations and Future Directions

All of the studies discussed herein investigated neural heterogeneity in individuals with ASD at a single timepoint. Yet, importantly, ASD is a neurodevelopmental disorder whereby symptoms emerge over time, across the course of development. Thus, it will be essential to study the longitudinal developmental trajectories of brain function and connectivity in individuals with ASD, as well as how these trajectories may vary across individuals as a function of genetics, sex, emerging behavioral phenotypes, and ultimately clinical outcomes. Longitudinal studies of brain development can also elucidate causal associations between neural development and behavioral outcomes, which may help to identify critical time periods during which focused intervention efforts can lead to maximal neuroplasticity thereby lessening ASD symptomatology. Indeed, emerging research has shown that the brain of individuals who later go on to receive an ASD diagnosis are altered even in early infancy. Thus, longitudinal investigation of infants at high-risk for developing ASD can elucidate the neural mechanisms by which ASD emerges very early in life, even before clinically observable symptoms occur, potentially leading to earlier identification and intervention.

A second limitation is that the projects discussed herein focused on high-functioning individuals with ASD with full-scale IQs above 70. Thus, these data may not be readily generalizable to more severely affected individuals with ASD, whose IQ fall below the cut off required for participation in the neuroimaging studies. The choice to use a cutoff of 70 for IQ was made in order to increase the likelihood that participants would be able to successfully complete

the MRI scanning portion of the studies, which requires youth to stay still for a prolonged period of time in the MRI scanner, and to understand and cooperate with specific instructions for MRI and post-scanning task completion. It will be important for future studies to examine the effects of genetic and phenotypic variability on neuroendophenotypes in individuals with ASD and lower IQs, possibly using methods more suitable to this population such as electroencephalography (EEG).

The imaging-genetics studies in my dissertation were focused on how variability in single nucleotide polymorphisms in a single gene was related to brain connectivity. Importantly, however, there are hundreds of genes that contribute to the risk of developing ASD; thus, it will be critical for future research to investigate how polygenic risk for ASD affects brain function and connectivity. The choice to focus on the oxytocin receptor gene was driven by its association with social behavior in animal studies, as well as genetics studies suggesting a link with ASD. However, recent genome-wide association studies (GWAS) have not identified the oxytocin receptor as a locus related to increased rates of ASD. As the sample size of case and control data increases for GWAS studies investigating ASD, a growing number of genome-wide significant loci will likely be identified. Future imaging-genetics studies should focus on how these statistically significant genome-wide genetic variants affect neurodevelopment throughout the lifespan. By doing so, we may be able to stratify ASD sub-groups based on the genetic heterogeneity that contributes to the ASD phenotype and to build targeted treatments for individuals who have specific combinations of genetic risk factors.

Concluding Remarks

Autism Spectrum Disorder is heterogeneous, both in terms of genetic vulnerability, neuroendophenotypes, and behavioral phenotypes. A nuanced understanding of the factors that give rise to variability at the brain level is required to inform targeted treatment for individuals falling throughout the autism spectrum. This thesis used functional magnetic resonance imaging to investigate how variability in genetics, gender, and behavior affect brain connectivity and function. We found brain networks supporting social reward processing and social attention were altered in youth with ASD compared to neurotypical youth, and that that there is great variability even across individuals on the spectrum. This dissertation lays the groundwork for future investigations into the multiple neurobiological bases of ASD, and for the development of targeted individualized treatments in order to improve outcome and the quality of life for individuals on the autism spectrum and their families.