The Role of Comorbid Anxiety Symptoms in Children and Adolescents with Autism Spectrum Disorders (ASD) on an Emotional Perception Task

Kayla Sargent
ABSTRACT

The focus of the present study is to investigate the relationship between anxiety symptoms and neural outcomes of emotion processing in children and adolescents with autism spectrum disorders (ASD) and neurotypical controls. Eighteen child and adolescent participants (9 ASD, 3 female) completed questionnaires, behavioral testing, and an fMRI scan. Participants with ASD demonstrated significantly higher scores on parent-reported anxiety measures than typically developing (TD) participants; however, observed amygdala activations were not related to parent-reported anxiety scores and OFC activation was not observed during emotional stimuli for either group. ASD and TD participants demonstrated significant bilateral responses in the superior temporal sulcus during emotional stimuli. The current study was unable to demonstrate
potential functional neuropathology in the amygdala as a mechanism for apparent differential
diagnostic patterns in ASDs.

INDEX WORDS: Autism spectrum disorders, fMRI, Amygdala, Anxiety, Children, Emotion processing
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by

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DEDICATION

This project is dedicated to my friends and family who have supported me endlessly in my education.
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My graduate advisor, Diana, and the team of colleagues, students, volunteers, families, and children who made this project possible are acknowledged. Thank-you to my supporting committee members, Tricia and Robin, for offering guidance and support throughout the writing process.
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1 INTRODUCTION

Autism Spectrum Disorders (ASD) are neurodevelopmental disorders characterized by restricted interests and stereotyped behavior as well as deficits in communication and social interaction. The Centers for Disease Control and Prevention report that the current prevalence of ASD is 1 in every 88 cases (CDC MMWR, 2012). Given lost productivity in the job force and the cost of adult care (Ganz, 2007), ASDs reportedly cost the United States $35 billion per year (Ganz, 2006). Currently, the approach to treatment with the most empirical support is behavioral intervention following an early diagnosis (Dawson, Rogers, Munson, Smith, Winter, & Greenson et al., 2010; Eaves & Ho, 2004; Harris and Handleman, 2000), but treatment methods and protocols are often variable and highly specialized due to the heterogeneous symptomology of individuals with ASD.

Despite decades of research, a limited number brain-behavior relationships have been established in conceptualizing this extremely complex spectrum of disorders. For example, we currently do not understand why some children with ASD develop language whereas others do not, some have intellectual disabilities and others do not, some demonstrate very early delays while others appear developmentally typical until a period of “regression,” and a high degree of variability is evidenced among repetitive, obsessive-compulsive, and social behaviors. At present, there are no established theories or identified neurological mechanisms which can account for the entire triad of ASD symptomology. However, a number of theories exist which attempt to explain components of ASDs. Some theories identify neuroanatomical regions of interest that relate to specific symptoms, such as the amygdala theory of autism (Baron-Cohen, Ring, Bullmore, Wheelright, Ashwin, & Williams, 2000; Schumann et al., 2004), which targets the amygdala as a site of disruption in the social brain network. Other theories focus on
behavioral markers to infer neurological deviance, like the executive functioning theory of autism (Bennetto, Pennington, & Rogers, 1996; Minshew, Webb, Williams, & Dawson, 2006) which conceptualizes known deficits in working memory, planning, inhibition, cognitive flexibility, and self-monitoring as the foundation that leads to social behavior and deficits in ASD.

Schultz (2005) proposes that the search for a neurological etiology of ASDs should begin with examining social deficits. Because ASDs are behaviorally defined and diagnosed, individual differences result in a spectrum of disorders that have heterogeneous presentations. Highlighting the importance of social deficits in ASDs is relevant given that researchers describe them as the most fundamental and pervasive symptoms in ASD; thus, they represent the uniting characteristics that are seen collectively in this heterogeneous group. Osterling and Dawson (1994) demonstrated that when coding videotapes from birthdays, children with ASDs were distinguishable from typically developing children based on four social behaviors: pointing, showing objects, looking at others, and orienting to their names. Subsequently, children with ASDs were distinguishable from 1-year-olds with later diagnoses of an intellectual disability by looking at others and orienting to their names significantly less frequently (Osterling, Dawson, & Munson, 2002). Numerous studies have documented the widespread difficulties children with ASD face within the social domain: social and affective relatedness, difficulty developing and maintaining peer relationships, use of social language, use of non-verbal social behaviors like gesture, and emotional awareness and expression (for reviews, see Loveland, 2005; Loveland & Tunali-Kotoski, 2005; Volkmar et al., 1997). These behavioral data corroborate what Schultz (2005) described: children with ASDs may have diverse impairments in more than one area, but social deficits appear to be the most fundamental, pervasive, and easily identifiable behavioral
manifestation of these disorders. Regarding the trajectory and development of ASD, the social deficits theory hypothesizes that language and communication deficits are natural consequences of a child with ASD’s inability to glean socially useful information through the known impairments reported above. This sequence of symptoms indicates deviant rather than delayed development, further corroborating the need for early detection and treatment. Not surprisingly, these are the areas targeted most by empirically supported behavioral interventions.

1.1 Brain-Behavior Relationships in Autism Spectrum Disorders

As the cognitive abilities of infants increase with age, increased sophistication of more complex social skills is expected, such as burgeoning expertise for perceiving, recognizing, and discriminating faces. Children with autism have difficulty recognizing faces, even when they perform similarly on other verbal and nonverbal tasks (Klin, 1999). One hypothesis for understanding the mechanisms underlying these deficits in face perception is an underlying tendency for individuals with ASDs to rely on a part-based vs. holistic perceptual approach (Joseph & Tanaka, 2002; Schultz et al., 2000). Typically developing children continually perform better at recognition tasks (Joseph & Tanaka) when “parts” of the face (i.e. mouth, nose, eyes) are within the context of the entire face and are particularly skilled at tasks that rely heavily on the eyes for recognition. However, Joseph and Tanaka demonstrated that deficits seen in children with ASD could not be fully explained by the part vs. whole hypothesis, given that children with ASD in their sample relied heavily on the mouth region of the face but remained markedly impaired at recognizing faces, which relied heavily on the eyes. Recognizing human emotion is also difficult for individuals with autism (Hobson, Ouston, & Lee, 1988). Compared to typically developing (TD) adults, men with autism have shown significant difficulties recognizing emotions despite being able to recognize gender (Hall, Szechman, & Nahmias, 2003). Children with ASDs have also demonstrated this deficit, performing significantly worse
than typically developing children and other clinical groups (conduct disorder and dysthymia) on emotion recognition and theory of mind tasks (Buitelaar, Van der Wees, Swaab-Barneveld, & Van der Gaag, 1999).

Although behavioral research has historically dominated the field, neuroimaging methodologies and other sophisticated neuropsychological techniques have emerged in the past decade, offering more depth and breadth to our understanding of ASDs. Understanding social behavior as it arises in TD individuals is helpful in conceptualizing social deficits in ASD and hypothesizing about potential disruption of typical mechanisms that may result in ASD-like symptoms. Schultz (2005) proposed that typically developing infants are born with a perceptual bias for faces at birth which leads to learning and an enhanced salience for faces throughout infancy. This developing salience for faces carefully sculpts perceptual skill and ultimately provides scaffolding for language and complex social skill development. These skills appear to follow an interactive specialization model for brain-behavior development (Johnson & de Haan, 2011), given that complex behaviors like social skills and language are first represented in the brain through wide interconnected networks of cortico-limbic regions but become increasingly focal as the skills are honed and automatized. Schultz hypothesized that a specific congenital anomaly in ASDs disrupts or impairs the innate bias for faces leading to a sequence of neurologic and behavioral consequences that ultimately impair social development, then language and sensory development.

For TD individuals, a set of interconnected “core” and “extended” brain regions regulate face perception (Haxby, Hoffman, & Gobbini, 2002). The “core” system can be conceptualized as being responsible for analyzing visual stimuli, utilizing inferior occipital gyri for early visual perception which projects to the superior temporal sulcus (STS) for perception of eye gaze and expression and the lateral fusiform gyrus (FG), also known as the fusiform face area (FFA;
Kanwisher, 2000) for perception of faces as unique and invariant. From there, the “extended system” assumes responsibility for more complex social processing. While multiple “extended” brain regions are implicated for specific aspects of face perception, the amygdala is highlighted as being directly and reciprocally connected to the FFA (Freese & Amaral, 2005; 2006), focusing on emotion processing and emotional response. Consequently, the STS is implicated in increasingly complex, integrative social perception (Winston, Henson, Fine-Goulden, & Dolan, 2004) than the FFA which is recruited for more basic facial perception. Thus, the core system, including the FFA, is responsible for integrating visual information while the extended system (amygdala), in concert with the core system, determines the visual information’s social relevance (Ishai, 2008; Figure 1). Within this framework, social perception becomes possible.

![Figure 1 FFA-Amygdala Model of Face Processing](image)

Anatomical studies in primate research have clearly demonstrated strong reciprocal connections between the amygdala and corresponding FFA regions (Freese & Amaral, 2005; 2006). Recent human anatomical research has provided evidence that this fusiform-amygdala connection may be disrupted (Dziobek, Bahnemann, Convit, & Heekeren, 2010) for individuals with ASDs. Specifically, Dziobek and her colleagues found increased cortical thickness of the FFA for ASD patients vs. TDs; however, volumetric covariance between the amygdala and FFA was demonstrated to be decreased for ASD participants in addition to being associated with impairments in facial recognition. This suggests that participants with ASD have disrupted...
reciprocal connections between these social brain regions. By age two, children with ASD are already showing significantly increased amygdalar volume associated with lack of joint attention when compared to NTs (Mosconi et al., 2009), suggesting that these neural circuits are disrupted early on in the trajectory of these disorders.

Integrating the findings of these anatomical data with Schultz’s (2005) theory of innate social impairments, it is hypothesized that a congenital anomaly in the amygdala might interrupt the biological “face bias” from birth, leading to a lack of salience or apathy for faces. This facial “apathy” would then disrupt facial expertise and FFA activations, resulting in hypoactivation of the amygdala and FFA and reduced white matter connections between the two regions.

A large body of literature also documents abnormalities in functional activations of individuals with ASD compared to NT individuals, not just anatomical discrepancies; among this research is an established but growing demonstration of amygdala and FFA disruption with impairment on a number of experimental social tasks, such as face processing (Adolphs, Sears, and Piven, 2001; Ashwin et al., 2006; Critchley et al., 2000; Monk et al., 2010; Schultz et al., 2000), face recognition (Klin, Sparrow, and de Bildt et al., 1999), emotion recognition (Bal et al., 2010; Hall, Szechtman, and Nahmias, 2003), and social attribution (Schultz et al., 2003). Amygdala dysfunction may also be specifically related to observed difficulties in perception of negatively valenced emotions (Ashwin et al., 2006) relative to positively valenced emotions.

Deficits in face perception are of particular importance given that faces represent an integral primary source of social information; successfully and fluidly decoding faces is the foundation for more complex social inference and behavior. While the FFA is clearly linked to face perception, a growing body of literature has proposed that FFA activations are actually mediated by expertise and eye gaze (e.g. Hadjikhani, Joseph, Snyder, & Tager-Flusberg, 2007). For example, individuals with ASD can demonstrate FFA activations comparable to NT
participants when prompted to look directly at stimuli, thus controlling for gaze. Participants with ASD have also demonstrated difficulty maintaining eye gaze fixations (Dalton et al., 2005), which were associated with atypical amygdala activations. However, the amygdala abnormalities presented in these projects are equivocal; individuals with ASD demonstrate increased (Dalton et al., 2005; Kleinhans et al., 2009; 2010; Monk et al., 2010; Tottenham et al., 2013), decreased (Baron-Cohen et al., 1999; Critchley et al., 2000; Pierce, Muller, Ambrose, Allen, & Courchesne, 2001; Schultz, 2005), and comparable (Wang, Dapretto, Hariri, Sigman, & Bookheimer, 2004) amygdala activations compared to NT participants, suggesting that an additional variable must be accounted for to precisely understand the role of the amygdala in the social deficits of autism.

Whereas lack of emotional salience and “apathy” for faces (Schultz, 2005) is a logical and empirically supported explanation for ASD patients who demonstrate amygdala hypoactivation, it appears that this theory falls short in generalizing to the majority of this heterogeneous clinical group. It is plausible that amygdala hyperactivations found in other studies (Dalton et al., 2005; Kleinhans et al., 2010) are caused by a hyperarousal and aversion to social stimuli. Essentially, for some, faces may lack emotional salience (amygdala hypoactivation, decreased eye gaze), resulting in apathy toward social interaction. This sequence is illustrated by the top portion of Figure 2. For others, faces may be overstimulating (amygdala hyperactivation, decreased eye gaze), causing overarousal, aversion, and anxiety during social interactions, illustrated by the lower portion of Figure 2. Therefore, it is possible that both hypoactivation and hyperactivation of the amygdala could lead to impaired performance on social tasks, resulting in widespread equivocal findings throughout the current body of literature on emotion perception in ASDs.
Examining heterogeneity in amygdalar-fusiform responses to social perceptions addresses relevant issues regarding the widespread variability seen in the behaviors and symptoms of children with ASDs. Bachevalier and Loveland (2006) highlight an important distinction that is not commonly addressed in the literature regarding ASD, which is that understanding mechanisms behind social action are equally important as understanding the mechanisms behind social perception. In other words, the breakdown of social skills in children with ASD is a two-fold process including a failure to perceive or infer accurately what others know, feel, or intend and also a failure to modify one’s own behavior appropriately in light of accurate information. Therefore, in evaluating neuropsychological models of ASD, it is important to capture structures and systems that underlie social awareness and emotion perception but also those that underlie the regulation of behavior as a result of social perception. In primates, the orbitofrontal cortex (OFC) has been implicated in these components of social behavior in such as monitoring of emotions, social cognition, and behavioral self-regulation (Barbas, 1995; Brothers, 1995). Bachevalier and Loveland (2006) proposed that a breakdown of the OFC network connections with the amygdala may be responsible for the impairments in social action demonstrated in ASDs. The OFC, particularly the lateral network, is interconnected with and relies heavily on input received from the amygdala and thalamus (Barbas, 1995). Integrating these findings with the data suggesting that amygdala hyperactivation and
hypoactivation will lead to social aversion and social apathy, respectively, pose an interesting challenge for those seeking to understand the neurological etiology of these symptoms. These data imply that dysfunction of the orbitofrontal cortex and its ability to accurately regulate social cognitions and behaviors is a natural consequence of amygdala dysfunction, whether it be hyperactive or hypoactive in response to social situations.

1.2 Comorbid Anxiety Symptoms in Autism Spectrum Disorders

Anxiety symptoms are frequently comorbid with ASDs (White, Oswald, Ollendick, & Scanhill, 2009), and Simonoff et al. (2008) reported that at least 29% of individuals with ASD have a comorbid Social Anxiety Disorder. More recently, Gobrail and Raghavan (2012) reported prevalence of 32.6% in children and adolescents with ASD using the Glasgow Anxiety Scale. Pediatric studies demonstrate that children with clinical anxiety and children with ASD report similar levels of anxiety (Kuusikko et al., 2008); however, Russell and Sofronoff (2004) demonstrated that individuals with ASD reportedly experience more obsessive-compulsive symptoms and fears about physical injury than individuals with clinical anxiety. Children clinically described as having ADHD or ASD were reported by their biological mothers and teachers to experience clinically significant levels of generalized anxiety (Guttman-Steinmetz, Gadow, DeVincent, & Crowell, 2010) when compared to typically developing children. Additionally, there is evidence of some overlap in the clinical presentations of children with ASD and children with social anxiety; for example, socially anxious individuals may evidence difficulties with social awkwardness, non-verbal communication, and emotional expression (Tantam, 2000), and it is common for these individuals to also create stereotyped routines and rituals to ease anxiety symptoms. More recently, Towbin et al. (2005) reported that 47% of children with preexisting diagnoses of depression or anxiety scored in the ASD-likely range on measures like the Social Communication Questionnaire (SCQ; Berument, Rutter, Lord, Picles, &
Bailey, 1999), the Children’s Communication Checklist, (CCC-2; Bishop, 1998), and the Social Reciprocity Scale (SRS; Constantino, Przybeck, Friesen, & Todd, 2000).

Poor social skills and empathic reasoning are related to elevated anxiety symptoms in ASD (Bellini, 2004). Children with ASD who demonstrate higher-than-average levels of physiological arousal paired with poor social skills demonstrate the highest levels of social anxiety (Bellini, 2006), suggesting that measuring anxiety in this population offers a way to measure neurobiological differences using simpler behavioral techniques. Kuusikko et al. (2008) found that not only did children with ASD score higher on measures of anxiety when compared to TD control participants, they also evidenced increasing levels of behavioral avoidance with age, whereas the typical trajectory for avoidance decreases with age. These findings corroborate the hypothesis that an additional pathway in the development of social impairments in ASD may occur via hyperarousal for social stimuli and social aversion, as measured by anxiety (Figure 2).

The presence and quality of restricted interests present in individuals with ASD may also overlap with symptoms of anxiety. Symptomatic expressions of these behaviors have demonstrated significant overlaps with ASD seen in conditions like Obsessive Compulsive Disorder (OCD; Spiker, Lin, van Dyke, & Wood, 2012). It has been suggested that restricted interests may represent a coping response to negative emotional experiences raised by comorbid anxiety symptoms.

Kleinhans et al. (2010) were the first to corroborate the relationship between social aversion and the amygdala using fMRI in ASDs. Using only angry and fearful faces, they employed a task requiring participants to match faces or shapes. Results from their analyses indicate that as anxiety scores increase, amygdala hyperactivation occurs for their ASD group. Even in non-ASD populations, individuals with anxiety disorders alone demonstrate amygdala
hyperactivation compared to NTs (Shin & Liberzon, 2009). Understanding how anxiety symptoms play a role in ASDs will help to distinguish between these two subgroups.

1.3 The Current Study

The purpose of the proposed study is to examine the neural mechanisms underlying social deficits in ASDs. Given that there is evidence for two potential behavioral mechanisms underlying social perception, social apathy and social aversion, the current study aims to corroborate the findings of Kleinhans et al. (2010), which is well grounded in the literature and documents the need to understand how social perception in ASDs are impacted by anxiety. Specifically, measurement and surveillance of anxiety symptoms in a sample of children and adolescents with ASD may serve as a behavioral marker for understanding two separate neural mechanisms of social deficits, as children with co-morbid anxiety will likely demonstrate amygdala hyperactivation and social aversion, whereas children without anxiety will likely demonstrate relative amygdala hypoactivation and social apathy. In addition to teasing apart the mechanisms which contribute to inaccurate or disabled social perception, it is also important to understand the subsequent neurological mechanisms which contribute to poor self-monitoring and social behavior (Bachevalier & Loveland, 2006), particularly given the well-researched role of the orbitofrontal cortex within this context. Expanding on Kleinhans et al.’s approach, the proposed study will utilize an fMRI task encompassing a wider range of emotions; specifically, positively (i.e. happy) and negatively (i.e. angry) valenced emotional stimuli will be used to understand the impact of anxiety on amygdala activations for a wider range of emotions that are more likely to be encountered in actual social situations.
1.3.1 **Hypothesis 1**

Participants with ASD will demonstrate higher parent-reported anxiety scores, on average, relative to TD participants. An independent samples *t* test will be used to confirm this relationship using SPSS (IBM, 2010).

1.3.2 **Hypothesis 2**

Across both groups, amygdala activations during an emotion perception task will predict increased parent report anxiety scores. A multiple linear regression model will be used to evaluate the relationship between observed amygdala activations and anxiety scores using SPSS (IBM, 2010).

(A). As amygdala activation increases during the emotional stimuli vs. rest contrast, parent-reported anxiety scores will increase.

(B). Because anxiety symptoms are hypothesized to impact ASD participants more, diagnostic group will be included in the regression model to evaluate whether amygdala hyperactivation for the emotional stimuli vs. rest contrast influences the relationship between diagnosis and parent-reported anxiety scores.

(C). Because using rest conditions as a functional baseline can be problematic in fMRI and the amygdala has demonstrated sensitivity to negatively valenced emotions in the literature, additional contrasts will be explored to investigate amygdala activations. First, negatively valenced emotional stimuli (angry and fearful) will be contrasted vs. rest. Second, negative (angry and fearful) vs. positive (happy) emotional stimuli will be explored.
1.3.3  *Hypothesis 3*

Participants with ASD demonstrate neurological correlates of poor self-monitoring skills and self-regulation of social and emotional behaviors. A whole-brain, random effects general linear model will be conducted in Brain Voyager QX (Goebel, 2003) to evaluate whether differences in functionally defined orbitofrontal cortex are observed.

(A). Participants with ASD will demonstrate weaker activations in the orbitofrontal cortex for emotional stimuli vs. rest.

(B). If additional significant whole-brain group differences in response to emotional stimuli vs. rest are observed, they will be considered for exploratory analysis.
2 \hspace{1cm} \textbf{METHOD}

2.1 Participants

Archival data were used for the present study. Participants were recruited from the metropolitan Atlanta area as part of a larger research project investigating neural correlates of adaptive functioning and emotion perception in fMRI. As part of this project, 108 total participants were enrolled. Of those, 18 initially enrolled but subsequently failed to continue participation with the basic behavioral components of the study. Of the remaining participants, 44 met criteria for an ASD, and 45 met criteria as typically developing. Only 18 typically developing and 9 ASD were able to successfully complete the neuroimaging portion of the study. Difficulty laying still and fear of the fMRI scanner itself were the primary reasons for the significant amount of attrition in participation; however, three participants who successfully completed the study were excluded due to their age (above 21). Subsequently, nine age-matched participants were selected from the typically developing sample ($M_{\text{age}} = 14.64$, $SD = 4.50$; 2 females) to match the nine participants in the ASD sample ($M_{\text{age}} = 13.22$, $SD = 3.33$; 1 female).

Researchers often recruit adolescent and adult participants with ASD (Shriberg et al., 2001). Given that adolescents with autism often evidence adaptive behaviors at a developmental level below their chronological age (Bellini, 2004; Charman et al., 1997; Gilotty, Kenworthy, Sirian, Black & Wagner, 2002), individuals up to age 21 were included for analysis. Age criteria for inclusion required that participants be between the ages of 7 and 21, with parent consent and assent for those with legal guardians, and informed consent for legal adult participants.

The Autism Diagnostic Interview – Revised (ADI-R; Lord, Rutter, & Couteur, 1994) and the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 1989) and the Social Communication Questionnaire (SCQ; Berument, Rutter, Lord, Picles, & Bailey. 1999) were used to determine whether ASD and NT participants met inclusion criteria. Specifically, participants
who met criteria for ASD on the ADI-R and ADOS were included in the ASD group (see Materials section for more detail). NT participants scored within normal limits on the SCQ. Participants in this reduced sample were matched with respect to age. According to Dennis et al. (2009), matching IQ across patient and control groups in neurodevelopmental research can be problematic: it is possible that it can result in overcorrected or unusual findings. At the time of data collection, participants were matched on IQ to ensure that the functional and anatomical MRI changes observed between groups were due to fundamental characteristics of ASD rather than underlying cognitive deficits.

2.2 Materials

2.2.1 Dynamic Audiovisual Emotion (DAVE) Movies

DAVE stimuli (see http://www2.gsu.edu/~wwwpsy/robins.html) were developed at the Yale Child Study Center (Robins, Hunyadi, & Schultz, 2004). Each stimulus consists of an actor presenting one of 10 sentences in one of four emotions (angry, fearful, happy, and neutral). Paid actors delivered emotionally-ambiguous sentences using these emotions; for example, “the door is open” can be easily interpreted as angry, fearful, happy, or neutral depending on context which is not provided. Audio and video tracks were spliced while maintaining lip synchrony, creating 320 stimuli, 80 of which are congruent (face and voice portray the same emotion) and 240 of which are incongruent (face and voice portray different emotions). For the current study, 144 stimuli were congruent (angry, fearful, or happy), 144 stimuli were combinations of two emotions, and 72 stimuli consisted of a neutral face paired with an emotional voice. Incongruent neutral stimuli and neutral voice stimuli were not used in the study give that behavioral and functional responses to neutral and incongruent stimuli are not well understood; furthermore, incongruent stimuli in particular lack external validity in terms of real world emotional
experiences. Stimulus duration was 1.5-2.5 s. All unimodal stimuli were detected correctly by 85% or more of a pilot sample. The ability of the pilot sample to detect incongruence was variable across stimuli.

2.2.2 Autism Diagnostic Interview-Revised (ADI-R)

The ADI-R (Lord, Rutter, & Couteur, 1994) is a 2-hour, structured parent interview which provides cut-off scores for the classification of Autistic Disorder. Algorithm cut-offs were established after developing ROC curves to indicate the optimal intersection of sensitivity and specificity with both exceeding .90. Three major domains are assessed: social, communication, and restricted, repetitive, and stereotyped behavior with respect to the age at which symptoms emerged. Using these symptoms, the ADI-R classifies individuals as having Autistic Disorder using a cut-off of 10, 8, and 2 on the social, communication, and repetitive behavior domains, respectively. In order to also include higher functioning participants with ASD, individuals were classified as having ASD through one of the following: a.) minimum score of 10 on Social only or b.) minimum score of 8 on Social AND minimum score of 6 on Communication OR 1 on Restrictive/Repetitive Behaviors. The ADI-R was developed based on the parent interviews of 10 children with autism and 10 children with other mental handicaps and then tested for validity against 25 additional children with autism and mental handicaps, demonstrating good reliability and validity. Inter-rater reliabilities for each domain were high: for participants aged 5-29, correlations were well above .70, except for a few individual items (items 51, 64, 34, 56, 61, and 68), which ranged from .31 to .69. Test-retest correlations were above .93 for all domains. The ADI-R was able to discriminate between children with language and intellectual disabilities and children with ASD on the social \( F(1,48) = 243.38, p < .001 \), communication [nonverbal: \( F(1,25) = 28.91, p < .001 \); verbal: \( F(1,21) = 69.60, p < .001 \)], and restricted behavior domains
[\( F(1,48) = 37.56, p < .001 \)]. All but one of the 25 clinically diagnosed children with autism met the ADI-R criteria.

### 2.2.3 Autism Diagnostic Observation Schedule (ADOS)

The ADOS (Lord, Rutter, DiLavore, & Risi, 2002) is a standardized play and interview session lasting approximately one hour. The ADOS relies on interactions that are social and/or toy-based and are developmentally appropriate given the participant’s current communicative and cognitive level. Four modules are available to ensure each administration is developmentally appropriate: Module 1: nonverbal children; Module 2: children with phrase speech; Module 3: children exhibiting conversational and abstract language; Module 4: adolescents and adults exhibiting conversational and abstract language. Using social and communication algorithms, the ADOS classifies participants as having autism, autism spectrum disorders, or no ASD diagnosis. Interclass correlations for interrater and test-retest reliability were between .59 and .93 for each domain. In a validation sample, ADOS diagnoses consistently agreed with independent clinical diagnoses 90% of the time for Autistic Disorder and 80% of the time for Pervasive Development Disorder – Not Otherwise Specified (PDD-NOS). Participants scoring the ASD range (social-communication score > 6) were included for participation. Like the ADI-R, the ADOS is a widely used, state-of-the-art, and stringent diagnostic tool for research.

### 2.2.4 Behavior Assessment System for Children, 2nd Edition (BASC-2)

The BASC-2 (Reynolds & Kamphaus, 2006) is a parent rating scale created to assess the behavior of children as young as 2 to adults up to age 25. Depending on the reading level of the parent, it takes roughly 10-20 min to complete the entire questionnaire. The BASC-2 subscales are informed by DSM-IV and DSM-IV-TR criteria and are well correlated with other instruments, and it was normed using both general population and clinical samples of American
children and adolescents (ages 4-18). It measures diverse aspects of behavior, personality, and emotion using multiple subdomains: Anxiety, Depression, Withdrawal, Attention problems, Hyperactivity, Adaptability, Somatization, Atypicality, Conduct Disorder, Activities of Daily Living, Leadership, Functional Communication. Test-retest reliability was strong, with correlations at .80 or above for composite scores and between .70 and .80 for subdomain scales. Interrater reliabilities for composite and individual scales were at .70 or above. The BASC-2 is highly correlated with other similar behavioral scales like the Connors’ Teacher Rating Scale – Revised (CTRS-R; Conners, 1998), the Child Behavior Checklist (CBCL; Achenback, 1992), and the Behavior Rating Inventory of Executive Functioning (BRIEF; Gioia, Isquith, Guy, & Kenworthy, 2000). Individual anxiety scores load heavily on the BASC-2 internalizing problems composite; individually, it demonstrates strong inter-rater reliability and moderate correlations with similar measures. The proposed study used standard $T$ scores from the Anxiety subscale as an indirect, parent-report measure of anxiety symptomology in this sample. Following the manual, $T$ scores between 50 and 59 will be considered “at risk” for becoming clinically anxious, and $T$ scores of 60 or higher will be considered as clinically significant levels of anxiety.

### 2.2.5 Neuroimaging

Scanning was performed on a 3T Siemens Trio research-dedicated scanner at Emory University Hospital. A standard birdcage head coil was used. Anatomical scans included 2D (T1 flash, axial oblique plane through the AC-PC, 34 slices, 4mm$^3$ isotropic voxels, no gap, TR = 300, TE = 2.47, flip angle = 60 deg) and 3D (MPRAGE, 176 slices, 1 mm$^3$ isotropic voxels, TR = 2530, TE = 3.66, flip angle = 7 deg), both full cortex coverage. Eight functional runs were acquired in the axial AC/PC plane, using a gradient echo, single-shot echoplanar sequence (TR = 1950, TE = 25, flip angle = 60 deg, 34 slices, 4mm$^3$ isotropic voxels, no gap), lasting roughly six minutes each.
2.3 Procedure

During eight functional runs, participants responded to stimuli using a standard four button controller, similar to a videogame controller. Each run consisted of nine blocks that began with a 4 s emotion cue (i.e. Angry?; Happy?; Fearful?, in addition to a “yes/no?” prompt), followed by five DAVE stimuli. Each block included one congruent stimulus that matched the cue, one congruent stimulus that did not match the cue, one incongruent stimulus whose face matched the cue, one incongruent stimulus that did not match the cue in either face or voice, and one stimulus consisting of a neutral face with a voice that matched the cue. After each stimulus, participants pressed a button to indicate whether the DAVE movie matched the cue or not. Participants were not aware that some stimuli were incongruent, and they were not cued to attend to either face or voice specifically, but just to note whether the actor portrayed the cued emotion or not. Only congruent emotional movies were used in the current study; across an entire 8-run scan with 9 blocks per run, participants responded to a total of 48 congruent angry, fearful, and happy stimuli each, resulting in 144 active fMRI data points per participant. Incongruent movies were excluded from analyses given that the current study is attempting to elicit functional responses that are likely to be experienced in live social situations. Each of the nine blocks within a run was followed by a 10 s resting period. The interstimulus interval between stimuli was jittered between 2500 ms and 4100 ms to maintain the mixed event-related and block design. Following the functional runs, high resolution 3D anatomical and diffusion tensor imaging scans were collected.

All participants underwent a number of individual sessions in the following order: (a) behavioral and/or diagnostic testing lasting approximately two hours, (b) parent-rated questionnaires (filled out before or during behavioral testing session), (c) psychophysiological session measuring facial electromyography and short behavioral post-task lasting approximately
90 minutes (d) practice MRI in a “mock” scanning environment lasting approximately 30 minutes, and (e) functional magnetic resonance imaging (fMRI) scanning session including anatomical, functional, and diffusion tensor images lasting 90 minutes. Parts A-D took place on campus at Georgia State University; part E took place at Emory University Hospital. In concert with the larger research project, a number of additional measures were collected that are not being used in the current study in addition to part D above. Collected within part A, the following measures were not used within the current study: Vineland Adaptive Behavior Scale – 2nd Edition, Seashore Rhythm test, Benton Facial Recognition Task, Edinburgh Handedness Questionnaire, and the Diagnostic Analysis of Nonverbal Accuracy. Two fMRI button boxes were used with respect to handedness as responses were recorded using the participant’s dominant hand. Testing was generally completed within 2 to 3 sessions on separate days, depending on scheduling conflicts and whether or not individual participants required breaks. Portion C of the study occasionally occurred after portions D and E due to scheduling conflicts.

2.4 Data Analysis

Brain Voyager QX (Goebel, 2003) was used to process and analyze the fMRI data. Data were pre-processed using scripts that first rename raw DICOM files compatible with Brain Voyager, subsequently creating compiled 3D anatomical and functional files. Preprocessing included spatial smoothing, 3-dimensional (3D) motion correction, and temporal filtering of the functional scans. Given that small structures in the limbic system are easily over-smoothed, spatial smoothing was conducted at 4mm in order to maintain the integrity of the amygdala as much as possible, and voxel-wise analyses accounted for minor artifacts and changes in head movement.

Time course data were integrated using protocol files created in Microsoft Excel, creating four conditions (Angry, Happy, Fearful, Neutral Face, and Incongruent) each containing time
course data for their respective DAVE stimuli from each block within the run. Protocol files were organized as an event-related design based on stimulus type only, irrespective of block cue. For example, all congruent angry stimuli were compiled across all blocks into one condition, “Angry”, regardless of whether the stimulus was presented during a block with an angry cue.

Aligning the functional brain onto the 3D brain is a two-step process, whereby Brain Voyager initially automatically aligns the two images before an additional manually chosen fine-alignment adjustment is needed. Before creating a general linear model, each brain was manually constructed into Talairach space using Anterior Commissure and Posterior Commissure points. For group analyses, brains were averaged together to form a group brain using a random effects general linear model; however, each participant’s data will also be processed independently to better understand how individual variability may contribute to observed group differences.

In addressing Hypothesis 1, an independent samples t test will be conducted to evaluate diagnostic group mean differences in parent-reported anxiety scores.

Regarding Hypothesis 2, a fixed-effects, z transformed t-test individually contrasted emotional stimuli (Angry, Fearful, and Happy) vs. rest for each participant. The right and left amygdala were anatomically defined using spheres of 7mm radius in Talairach space (Talairach & Tournoux, 1988; RA: 21, -5, -16; LA: -21, -5, -16); however, the left amygdala was excluded from inclusion after none of the participants demonstrated significant BOLD activations for explored contrasts. Right amygdala contrasted t-values for the emotion vs. rest comparison for each participant, as well as their respective BASC-2 anxiety t-scores were exported into SPSS. T-values can be extracted from Brain Voyager using various methods. If significant group differences were to occur in the amygdala between ASD and TD participants, a volume-of-interest (VOI) map would have been created based on the functional cluster observed, assuming it fell within 7mm of the specified Talairach coordinates. This map would have been used collect
average ts in the same functional cluster and anatomical area for each participant, individually. Given an absence of amygdala activations, both individually or by group, the average t at the specified amygdala Talairach coordinate was exported. Emotionally congruent stimuli alone were included in each contrast. All contrasts were numerically balanced within Brain Voyager’s general linear model to account for uneven condition distributions. A multiple linear regression model was created in SPSS to determine whether increases in amygdala activations for emotional stimuli lead to increases in parent-reported anxiety symptoms. Diagnostic group was included in the model to evaluate possible amygdala activations as a potential mediator of the relationship between diagnosis and anxiety. This approach was repeated for two additional exploratory contrasts: negatively valenced emotional stimuli (angry and fearful) vs. positively valenced emotional stimuli (happy) and negatively valenced emotional stimuli vs. rest.

For Hypothesis 3, a random-effects, z transformed t test was used in a whole brain, between groups analysis to evaluate whether diagnostic group differences in orbitofrontal cortex were observed for emotional stimuli vs. rest. Given group differences, exploratory analyses were continued by using two individual diagnostic group brain analyses (1 combined ASD and 1 combined TD), which also produced random-effects, z transformed t values. Considered exploratory contrasts were as follows: negative emotional stimuli (angry and fearful) vs. rest as well as negatively valenced emotional stimuli (angry and fearful) vs. positively valenced emotional stimuli (happy). Negatively valenced emotions were isolated for these exploratory analyses because they have been particularly salient in behavioral studies (Ashwin, Chapman, Colle, Baron-Cohen, 2006; Dalton et al., 2005) and may be relatively more effective at eliciting amygdala BOLD activations.
3 RESULTS

3.1 Demographics

ASD ($n = 9$, 1 female) participants did not differ significantly from TD ($n = 9$, 2 females) participants with respect to age or IQ (see Table 1). Both groups included primarily Caucasian participants (see Table 2). Diagnostic scores are also reported with TD participants demonstrating typical-range SCQ scores and ASD participants demonstrating ASD-range ADOS and ADI scores.

Table 1 Demographic Scores

<table>
<thead>
<tr>
<th></th>
<th>ASD M (sd)</th>
<th>TD M (sd)</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>13.22 (3.33)</td>
<td>14.64 (4.50)</td>
<td>t(16) = .76, $p = .46$</td>
</tr>
<tr>
<td>range</td>
<td>10 - 21 years</td>
<td>9 - 21 years</td>
<td></td>
</tr>
<tr>
<td>Verbal IQ</td>
<td>96.89 (21.23)</td>
<td>114.67 (19.05)</td>
<td>t(16) = 1.87, $p = .80$</td>
</tr>
<tr>
<td>range</td>
<td>71 - 127</td>
<td>75 - 148</td>
<td></td>
</tr>
<tr>
<td>Perf. IQ</td>
<td>105.11 (23.73)</td>
<td>105.56 (12.3)</td>
<td>t(16) = .05, $p = .96$</td>
</tr>
<tr>
<td>range</td>
<td>61 - 133</td>
<td>87 - 124</td>
<td></td>
</tr>
<tr>
<td>FSIQ</td>
<td>101.00 (23.16)</td>
<td>111.22 (14.53)</td>
<td>t(13.45) = 1.12, $p = .28$</td>
</tr>
<tr>
<td>range</td>
<td>66 - 131</td>
<td>78 - 128</td>
<td></td>
</tr>
<tr>
<td>Anxiety</td>
<td>64.67 (16.52)</td>
<td>49.33 (8.80)</td>
<td>t(16) = -2.46, $p = .03$</td>
</tr>
<tr>
<td>range</td>
<td>36 - 89</td>
<td>37 - 62</td>
<td></td>
</tr>
</tbody>
</table>
Table 2 Participant Characteristics

<table>
<thead>
<tr>
<th></th>
<th>ASD</th>
<th>TD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td>1 female</td>
<td>2 females</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td>1 African American</td>
<td>1 African American</td>
</tr>
<tr>
<td></td>
<td>1 Hispanic American</td>
<td>1 Hispanic American</td>
</tr>
<tr>
<td><strong>M(sd) SCQ</strong></td>
<td>N/A</td>
<td>1.00 (1.53)</td>
</tr>
<tr>
<td><strong>M(sd) ADOS (soc+comm)</strong></td>
<td>12.89 (5.06)</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>M(sd) ADI-R (soc)</strong></td>
<td>21.33 (5.12)</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>M(sd) ADI-R (comm)</strong></td>
<td>16.44 (4.93)</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>M(sd) ADI-R (rest. behaviors)</strong></td>
<td>5.00 (2.24)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

### 3.2 Hypothesis 1

Individuals with ASD demonstrated significantly higher parent-reported anxiety scores, $t(16) = -2.46, p = .03$. On average, ASD participants’ scores were within the at-risk range, whereas TD participants’ scores were within normal limits (see Table 1). Three participants with ASD demonstrated clinically significant anxiety scores ($t > 69$), and three demonstrated at-risk scores ($t = 60-69$). Therefore, two thirds of ASD participants were experiencing symptoms of anxiety. Alternatively, only one TD participant evidenced symptoms of anxiety that were in the at-risk range.

### 3.3 Hypothesis 2

$T$-scores representing contrasted (emotional vs. rest) amygdala activations were regressed onto anxiety scores in a linear regression model using SPSS. Descriptive data on each contrast are presented in Table 3. No significant left amygdala activations were observed in individual
contrasts of participants in either group; therefore, only right amygdala contrasts were included in the following analyses. Group differences were not observed for emotional stimuli vs. rest (see Figure 3).

Table 3 Ms, SDs, and Range of Right Amygdala t-values by Contrast and Diagnosis

<table>
<thead>
<tr>
<th>Contrast</th>
<th>M(sd)</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASD Emot. vs. Rest</td>
<td>0.98 (1.84)</td>
<td>-1.32</td>
<td>4</td>
</tr>
<tr>
<td>SD vs. Rest</td>
<td>2.08 (2.45)</td>
<td>-1.43</td>
<td>5.87</td>
</tr>
<tr>
<td>SD vs. Pos.</td>
<td>-0.36 (0.99)</td>
<td>-2.3</td>
<td>0.68</td>
</tr>
<tr>
<td>TD Emot. vs. Rest</td>
<td>-0.90 (2.33)</td>
<td>-4.81</td>
<td>3</td>
</tr>
<tr>
<td>SD vs. Rest</td>
<td>0.59 (1.89)</td>
<td>-2.96</td>
<td>3.67</td>
</tr>
<tr>
<td>SD vs. Pos.</td>
<td>-0.46 (1.49)</td>
<td>-3</td>
<td>1.94</td>
</tr>
</tbody>
</table>

Figure 3 Group Differences (ASD vs. TD) in the Amygdala for Emotion vs. Rest
Contrasted right amygdala t-values were unrelated to parent-reported anxiety scores, regardless of diagnostic group (see Table 4). Only two out of nine participants with ASD demonstrated significant right amygdalar responses to emotional vs. rest contrasts; of those two participants, one participant also demonstrated clinically significant parent-reported anxiety. No TD participants demonstrated significant amygdalar activations in response to emotional stimuli.

### Table 4 Model Summary for Regression Model of Amygdala Activations and Diagnosis on Parent-Reported Anxiety Scores (Emotional vs. Rest)

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Std. Error</th>
<th>Beta</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>49.81</td>
<td>4.77</td>
<td>N/A</td>
<td>10.44</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>14.34</td>
<td>7.11</td>
<td>0.49</td>
<td>2.02</td>
<td>0.06</td>
</tr>
<tr>
<td>Amygdala</td>
<td>0.53</td>
<td>1.62</td>
<td>0.08</td>
<td>0.33</td>
<td>0.75</td>
</tr>
</tbody>
</table>

While parent-reported anxiety scores differed significantly between diagnostic groups as reported in Hypothesis 1, amygdala activations for emotional stimuli vs. rest did not, $t(16) = -1.90, p = .076$ ($M_{ASD} = .98, SD = 1.84; M_{TD} = -.90, SD = 2.33$).

Because this contrast failed to elicit expected amygdala activations, two additional contrasts were explored in the right amygdala both individually by participant and in a group contrast. First, negative emotionally valenced stimuli (angry and fearful) vs. rest were explored (Figure 4); group differences were not observed, and amygdala activations were unrelated to parent-reported anxiety scores (Table 5). Individually, one TD and two ASD participants demonstrated significant observed amygdala activations for negatively valenced stimuli vs. rest. Second, negatively valenced stimuli (angry and fearful) were contrasted against positively valenced stimuli (happy) to evaluate whether amygdala activations could be more effectively elicited by negative emotions. Group differences were not observed (Figure 5), and amygdala activations were unrelated to parent-reported anxiety scores (Table 6).
Table 5 Model Summary for Regression Model of Amygdala Activations and Diagnosis on Parent-Reported Anxiety Scores (Neg vs. Rest)

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Std. Error</th>
<th>Beta</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>48.7</td>
<td>4.57</td>
<td>N/A</td>
<td>10.65</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>13.71</td>
<td>6.75</td>
<td>0.47</td>
<td>2.03</td>
<td>0.06</td>
</tr>
<tr>
<td>Amygdala</td>
<td>1.08</td>
<td>1.54</td>
<td>0.16</td>
<td>0.71</td>
<td>0.49</td>
</tr>
</tbody>
</table>
The amygdala can be difficult to image in long fMRI tasks and is prone to habituation over time (Wright et al., 2001). A method commonly employed in studies attempting to isolate the amygdala involves using the first functional run only rather than the entire eight-run task in an attempt to elicit amygdala activations pre-habituation. Despite having relatively few participants to employ this method with adequate power, each of the aforementioned contrasts were explored between diagnostic groups. Significant between group amygdala activations were not present in any of the three contrasts.
3.4 Hypothesis 3

Across group, in a whole brain analysis of the emotional vs. rest contrast, both ASD and TD participants demonstrated significant activity in the bilateral superior temporal sulcus (STS) during processing of emotional stimuli, right STS: $t(17) = 8.028, p < 0.001$, left STS: $t(17) = 7.860, p < 0.001$ (Figure 6). Comparable activations were observed in the additional negative vs. rest and negative vs. positive contrasts.

![Figure 6 Accros Group Activity in STS (Emotion vs. Rest)](image)

Between-group, z transformed, whole brain random-effects analyses were used in a two-sample $t$-test contrasting emotional stimuli (angry, fearful, happy) vs. rest to evaluate potential OFC differences and exploratory regions between ASD and TD groups. It was expected that OFC activations would be smaller during emotional vs. rest contrasts for ASD than for NT participants. Significant OFC activations were not observed when contrast ing emotional vs. neutral stimuli between diagnostic groups. TD participants demonstrated significantly stronger activations in parahippocampal, inferior parietal, superior parietal, and fusiform regions relative to ASD participants during rest conditions. These activations suggest that TD participants were
recruiting significantly more cognitive resources between tasks and indicate that using rest as a baseline condition may be inappropriate for comparison to experimental tasks. The exploratory contrasts evaluated in Hypothesis 2 were also evaluated, which corroborated previous results indicating no OFC involvement in emotional decision making for these stimuli. See Figures 7-9 for screenshots of OFC for each respective contrast.

Figure 7 Group Differences in OFC (Emotion vs. Rest)
Figure 8 Group Differences in OFC (Negative vs. Rest)

Figure 9 Group Differences in OFC (Negative vs. Positive)
4 DISCUSSION

Social deficits have been identified as a starting point for inferring fundamental neurological deviations from neurotypical (NT) development in ASDs. Specifically, marked social impairment may lead to impairment in communication and occurrence of repetitive and stereotyped behaviors commonly identified in ASD. Previous literature has highlighted the amygdala as playing an important role in early social development; the amygdala has clearly been implicated in ASD (Kleinhans, 2010) with regard to processing of emotions.

Individuals with ASD have demonstrated an increased prevalence of symptoms of anxiety relative to typically developing peers (Simonoff et al., 2008; White, Oswald, Ollendick, & Scahill, 2009); given the amygdala’s involvement in modulation of fear responses and implications in general social behavior, understanding possible linkages between anxiety and social behavior in children with ASD is an intuitive next step to understanding this diverse and behaviorally defined condition.

Children in the current sample demonstrated similar patterns of anxiety symptomatology to those evidenced in the literature (Gobrail & Raghavan, 2012), with 33% (3 out of 9) of participants with ASD experiencing clinical levels of anxiety and an additional 33% (3 out of 9) of participants with ASD experiencing at-risk level symptoms of anxiety. In the TD group, only 11% (1 participant) demonstrated at-risk level anxiety symptoms, and none were in the clinically significant range. Mechanisms for understanding why anxiety symptoms are more prevalent in ASD vs. TD populations may have to do with the nature of how ASDs are diagnosed. Behaviorally speaking, a number of similarities have been observed demonstrating symptomatic overlap between ASDs and other conditions. For example, children with comorbid ASD and anxiety tend to have observed symptoms that overlap with conditions like Obsessive Compulsive
Disorder, mimicking compulsions through stereotyped behaviors, routines, and need for consistent structure. (Russel & Sofronoff, 2004). Typically developing individuals with social anxiety alone have demonstrated difficulties with social awkwardness, non-verbal communication, emotional expression, and stereotyped routines and rituals (Tantam, 2000).

Parent-reported anxiety scores, however, were not predicted by observed amygdala activations during social tasks in our study. A large body of literature suggest that poor social skills, poor empathic reasoning, social avoidance, and increased physiological arousal during social tasks have been related to anxiety symptoms in ASD (Bellini, 2004; 2006; Kuusikko et al., 2008), and Kleinhans et al. (2010) found that the amygdala is implicated in social aversions experienced by individuals with ASD. Given that the literature suggests that a relationship between amygdala responses and anxiety scores is to be expected, the lack of relationship observed in the current study may be attributable to apparent methodological limitations discussed below.

Orbitofrontal regions are important in social and emotional decision-making regardless of diagnostic group and have been demonstrated to rely heavily on input from the amygdala (Bachevalier & Loveland, 2006; Barbas, 1995; Brothers, 1995). Given the lack of amygdala activation in our sample for the current stimuli, it would not be expected that the orbitofrontal discrepancies proposed in hypothesis 3 would be observed. As such, group differences were not observed in orbitofrontal regions during emotional decision-making tasks. Lack of amygdala and orbitofrontal activations are possibly the result of limitations of using DAVE stimuli; specifically, lack of inclusion of an active comparison task (i.e., non-social/emotional stimuli as opposed to rest conditions) and ceiling effects in the behavioral task for the congruent stimuli may have contributed to weak BOLD signals in key emotion processing brain regions.
The superior temporal sulcus (STS) is commonly associated with social impairments in ASDs (Saitovitch et al., 2012). Both functional and anatomical STS deficits have been observed in ASD populations, implicated in social perception as well as atypical gaze fixations. In the current study, both TD and ASD participants demonstrated strong BOLD activity in the STS for emotional stimuli compared to rest, which is discrepant from what would be expected based on the literature. This shared variance in BOLD activity suggests that our small ASD sample may be not be representative of individuals with ASD at large. Confirmed ASD symptomatology via ADOS and ADI-R scores demonstrates that social deficits are present and pervasive in the ASD group. However, our group is relatively high-functioning with observed brief IQ scores in the average range. It is possible that ceiling effects mentioned above are present in congruent DAVE stimuli whereby the STS is able to be recruited for simple social emotional tasks but not for tasks with increasing cognitive demands or complexity.

Additionally, measurement of pediatric anxiety symptoms is complex. The BASC-2 is a broad screener for an assortment of potentially disruptive or problematic adaptive and social behaviors. The BASC-2 Anxiety scale is correlated with other anxiety scales on behavioral questionnaires (CBCL $r = .48$; Connors-R $r = .35$); however, using multiple reports of anxiety, such as self-, teacher-, and parent-report measures may offer a more comprehensive and thus accurate measure of pediatric anxiety. It is possible that the BASC-2 does not offer enough sensitivity to accurately measure anxiety in our sample given that very few items on the questionnaire address anxiety directly, and many share variance with items intended to measure pediatric depression.

Methodological issues may exist in the fMRI design. For example, problems have been identified with using baseline or rest conditions as comparison conditions in fMRI (Stark &
Squire, 2001). Using rest as a passive comparison in the current study was the optimal mechanism for investigating emotion processing of congruent DAVE stimuli given that congruent neutral stimuli were used in this study. Stark and Squire (2001) suggest that for memory tasks, elevated activity occurs during rest conditions and contributes to the reduction or elimination of activity during task conditions. This finding was consistent across both their longer block and shorter event-related designs. This appears to be a particularly relevant critique of the current study, given that TD participants demonstrated significant differences from ASD participants for the rest conditions. This finding calls into question whether “rest” in this design is representative of baseline BOLD signal. Additionally, significant habituation has been observed in the amygdala when repeated emotional stimuli are presented (Wright et al., 2001), which suggests that using the first run of a scan may most accurately elicit amygdala responses to emotional stimuli. Null results were observed when this approach was employed; however, given our limited sample size, additional participants are needed to effectively use that approach. Given methodological limitations, it is possible that with a more sensitive anxiety screener and a behavioral fMRI task that more accurately isolates amygdala activations (i.e. emotional/social stimuli vs. non-emotional/social stimuli) would more effectively allow for evaluation of a link between limbic dysfunction and behavioral anxiety symptoms.

Results from the current study corroborate the occurrence of increased anxiety symptoms in ASDs, which warrant further investigation in terms of how these symptoms impact assessment, diagnosis, and neuropathology in this diverse neurological condition. Larger samples of individuals with ASD are necessary to recruit enough ASD participants with clinical levels of comorbid anxiety. This approach, combined with more sophisticated measurement of anxiety and use of appropriate social vs. non social stimuli, is necessary to understand whether anxiety
symptoms can be used to understand complex social differences between individuals with ASD (i.e. presence of social aversion vs. social apathy).

For example, in a future study, ideally, four participant groups would be recruited, with sufficient sample sizes to maximize power: two groups of TD children and adolescents, half without the presence of significant anxiety symptoms and half with clinical levels of anxiety, and two groups of children and adolescents with ASD, half without the presence of significant anxiety symptoms and half with clinical levels of anxiety. Multi-rater measures are ideal for capturing conditions with high comorbidities like anxiety, including self-report measures like the Revised Children’s Manifest Anxiety Scale, Second Edition (RCMAS-2; Reynolds & Richmond, 2008). Delineating brain functions between clearly anxious and clearly non-anxious individuals will allow for more precise evaluation of impairments in social perception that may be influenced by amygdala activations and behaviorally screened for by anxiety scores (refer to Figure 2). Diagnostic group comparisons will also allow for exploration of neuropsychological and behavioral overlap among ASDs and anxiety disorders in typically developing children.

Effective fMRI stimuli are necessary for external validity and eliciting isolated ROIs; therefore, a simpler block design presenting clearly social and emotionally congruent stimuli in addition to clearly non-social stimuli would be ideal (i.e. emotional faces vs. musical instruments) to avoid use of questionable baseline rest conditions.

The current study aimed to understand potential functional neuropathology in the amygdala as a mechanism for apparent differential diagnostic patterns in ASDs. While the current sample mimicked literature demonstrating high comorbidity between ASD and symptoms of anxiety, amygdala responses to emotional stimuli were not related to parent-reported anxiety scores. Similar BOLD activations were observed in ASD and TD participants;
specifically, STS regions were recruited for emotion processing in both groups. OFC regions were not recruited for emotional decision making in either group. Given exhaustive literature on the amygdala’s involvement in emotional processes, it is likely that the emotional stimuli and fMRI design in this study were ineffective at eliciting the amygdala, thus interfering with our ability to accurately measure a relationship between BOLD activations and anxiety scores.
5 REFERENCES


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Differential prefrontal cortex and amygdala habituation to repeatedly presented emotional stimuli. *NeuroReport, 12*(2), 379-383.