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Emergence Of Stranger Fear In Preschoolers With Fragile X Syndrome And Idiopathic Autism Spectrum Disorder

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EMERGENCE OF STRANGER FEAR IN PRESCHOOLERS WITH FRAGILE X SYNDROME AND IDIOPATHIC AUTISM SPECTRUM DISORDER

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ABSTRACT

This study investigated multiple behavioral indicators of social fear and their relationship to symptoms of autism in preschool boys with atypical and normative development using a stranger approach design. Participants consisted of 101 male preschoolers that were categorized into four groups: boys with fragile X syndrome (FXS; N= 29), FXS with elevated autism symptoms (fxASD; N = 25), idiopathic autism spectrum disorder (iASD; N = 11), and typically developing boys (TD; N = 36). Results indicated specific behavioral responses to a stranger differentiated preschoolers with more severe symptoms of autism (e.g. fxASD and iASD groups) from those with low autism symptomology (e.g. FXS and TD groups). Cross-group comparisons demonstrated that preschoolers with FXS displayed more avoidant gaze from the stranger and their parent during the stranger approach with boys with fxASD exhibiting the greatest proportion of avoidant gaze patterns. The fxASD group also referenced their parent less during the stranger approach in comparison to the FXS and TD groups. The iASD group displayed elevated facial fear in response to the stranger that differentiated them from the FXS, fxASD, and TD groups. No group differences were observed in escape behaviors. Overall, results from this study indicated specific behavioral patterns of social fear in response to a stranger using a normative and cross-syndrome approach. Given the high prevalence of anxiety in FXS and iASD, it is critical to identify how anxiety emerges in atypical and normative development to determine shared and

distinguishable traits of anxiety that can be used to inform targeted assessment, prevention and treatment efforts.

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CHAPTER 1

INTRODUCTION

Fragile X syndrome (FXS) is a single-gene disorder and the most common genetic cause of autism spectrum disorder (ASD; Hagerman, 2008; Crawford et al., 2002). Accordingly, 60% of individuals with FXS will also meet clinical criteria for an ASD (Harris et al., 2008). FXS and ASD are highly comorbid with anxiety, which often compounds the behavioral and emotional impairments exhibited in each individual disorder (Cordeiro et al., 2011; Muris et al., 2008). Cross-syndrome comparisons of anxiety in children with FXS and ASD to typically developing children are critical in order to distinguish how anxiety is expressed similarly and differently across both atypical and normative development. In order to examine these relationships, this paper will first discuss methodological considerations for examining anxiety in young children and various ways to measure anxiety behaviorally that go beyond traditional parent and self-report methods. Then we will describe anxiety in typical development, followed by the expression of anxiety in ASD and FXS. Finally, stranger approach paradigms will be discussed in relation to behavioral profiles of social fear in FXS and ASD.

1.1 MEASUREMENT OF ANXIETY: CHALLENGES OF EARLY CLINICAL POPULATIONS

Evidence-based assessment of social anxiety in children contains methods of collecting data that include diagnostic interviews, rating scales, observations, and self-monitoring forms (Silverman & Ollendick, 2005). However, these methods to study

social anxiety in young children are challenging due to a number of developmental factors. For example, anxiety and fear represent both normative and atypical responses in development based on age-related experiences and the nature of the setting. A clinical disorder is implied when anxiety or fear leads to excessive somatic arousal or worry beyond normative expectations associated with impairment (Silverman & Ollendick, 2005). The measurement of anxiety in young children is complex and self-report measures in children may be less reliable given that young children often lack or display limited insight to understand or communicate their symptoms of anxiety. Finally, methods used to study anxiety in children often have been extended from previous measures intended and used with adults or adolescents without consideration if underlying constructs are developmentally similar across age groups (Silverman & Ollendick, 2005). Therefore, there is a need to study both existing features and prodromal traits that may predispose young children to anxiety to more accurately identify and assess traits that are normative in comparison to traits that are maladaptive or prodromal features of anxiety.

One framework for identifying characteristics associated with social anxiety is a temperamental approach through behavioral observation (Rapee, Kennedy, Ingram, Edwards, & Sweeney, 2005; Turner, Beidel, & Wolff, 1996). Behavioral observation is considered part of the gold standard when assessing clinical levels of social anxiety given the limitations with parental and self-report for young children (Silverman & Ollendick, 2005). Embracing temperament is one viable model to understand how anxiety disorders develop and explain how individual features may represent either risk or resilient factors for the development of later psychopathology (Durbin, Klein, Hayden, Buckley, &

Moerk, 2005; Lonigan & Phillips, 2001; Rapee & Coplan, 2010). Temperament has been defined as "individual differences in reactivity and self-regulation" (p. 123) in response to varying environmental contexts (Rothbart, Ahadi, & Evans, 2000) and is biologically based with traits emerging early in infancy and remaining relatively stable across time (Clark & Watson, 1999; Durbin et al., 2005; Rothbart, Derryberry, &Hershey, 2000). Features of temperament may be influenced both by genetic predispositions, as well as environmental triggers, and have been studied both physiologically (Brooker et al., 2013; Tagle, Donzella, Gunnar, 2008) and behaviorally (De Rosnay, Cooper, Tsigaras, & Murray, 2006; Durbin et al., 2005, Sroufe, 1977). According to Rothbart and colleagues (2000), temperament is composed of three main factors measuring an individual's negative affectivity, extraversion/surgency and effortful control. There are also 16 sub-domains within the three main components of temperament that measure individual differences with sub-domains of fear and shyness being two of the most robust indicators for the development of anxiety in children (Degnan et al., 2010; Goldsmith & Lemery, 2000; Kagan & Snidman, 1999).

Previous studies have tied in temperamental aspects to elicit behavioral responses during observation periods in order to identify certain characteristics that may predispose young children to experience anxiety (Brooker et al., 2013; De Rosnay et al., 2006; Greenberg & Marvin, 1982; Tagle et al., 2008). One strong predictor of the later development of social anxiety in young children is fearfulness to novel situations or people (Buss & Goldsmith, 2000; Colennesi et al., 2014). One way to study temperamental traits of fearfulness in children is to examine the behavioral responses to the approach of an unfamiliar person or a stranger (Goldsmith & Rothbart, 1996). For

example, Pisula (2004) studied preschoolers with ASD and their fear responses to a novel person (e.g. a stranger), as well as responses to their mother during a social approach task. Specific behavioral responses that have been studied using a temperamental framework during an approach from a stranger include measures of fearfulness and inhibitory responses (Brooker et al., 2013; Greenberg & Marvin, 1982; Tonnsen, Shinkareva, Deal, Hatton, & Roberts, 2013). In a longitudinal study, infants with high temperament traits of reactivity or fear to novel stimuli exhibited more symptoms of anxiety later in childhood (Kagan, Snidman, Zentner, & Peterson, 1999). In addition to reactivity or fear responses, early measures of temperamental shyness have also been studied in relation to negative emotional responses, such as anxiety (Chariva, Stein, & Malcarne, 2002; Volbrecht & Goldsmith, 2010). High levels of shyness, such as increased behaviors of avoidance and gaze aversion, in children have been found to be related with elevated symptoms of anxiety (Buss & Goldsmith, 2000; Colonnesi, Naploeone, & Bogels, 2014; Henderson & Zimbardo, 1998).

One method that has been utilized to study fearfulness in children in response to the approach of an unfamiliar person is the Stranger Approach from the Laboratory Temperament Assessment Battery (Goldsmith & Rothbart, 1996). The Stranger Approach is part of a standardized assessment battery that allows for the comparison of behavioral measures of fear during an observation where a stranger approaches a child and a parent (Goldsmith & Rothbart, 1996). In order to assess social withdrawal and temperamental traits of fearfulness within the child, multiple behaviors are examined directly and indirectly. Direct measures of fear include assessing the intensity of a behavior, such as escape behavior or facial fear. Indirect measures of social fear, such as

a child's gaze patterns, during the stranger approach also provide useful information in regards to attentional patterns. Gaze patterns have frequently been studied in relation to social anxiety in response to novel situations and people (e.g. strangers) through methods examining social aversion or avoidance (e.g. looking away), social referencing (e.g. looking to a parent), and selective attention (e.g. looking at the fearful stimulus) (De Rosnay et al., 2006; Vasey, El-Hag, & Daleiden, 1996). Although it is adaptive to direct attention and process information in situations that are both novel and threatening; maladaptive patterns emerge when selective attention creates biases or distortions in the way individuals process an environmental cue (Vasey et al., 1996).

According to attentional bias theory, individuals pay greater attention to stimuli that are more threatening and, consequently, cause them more anxiety (Cisler & Koster, 2010; MacLeod, Mathews, & Tata, 1986). Therefore, children that spend longer durations of time looking towards a stranger, a potentially fear-provoking stimulus, may be displaying increased behaviors of anxiety. Alternatively, attention orienting theory, which involves the detection and processing of a novel stimulus (Posner, 1980) suggests that attention directed towards a stimulus represents interest and cognitive processing. Attention and emotion are often interrelated and linked through motivational systems (Bradley, 2009). For example in response to unfamiliar situations, we often direct our attention to process the novelty of the stimulus and to determine if that stimulus is threatening. After information has been processed, motivational systems react either with a defensive or appetitive response which is crucial to survival (Bradley, 2009). According to these competing models of attention, a child's visual attention to the stranger may represent fear (attention bias) or interest (attention orienting) or a

combination of both. Thus, a comprehensive experiment of social fear needs to include multiple behavioral representations of social fear along with complementary and potentially validating measures against which the behavioral indicators can be compared.

Specific behavioral variables that have been studied using the stranger paradigm include examining directed attention during the observation (i.e. at a parent, the stranger, or away from the stranger and parent), facial fear, vocalizations, escape behaviors, and attachment behaviors towards a caregiver (De Rosnay et al., 2006; Greenberg & Marvin, 1982; Tonnsen et al., 2013). Various populations have been studied using the stranger approach design including children with ASD (Pisula, 2004; Sigman & Mundy, 1988), FXS (Tonnsen et al., 2013), Down syndrome (Pisula, 2004), and typically developing (TD) children (Brooker et al., 2013). Specifically, Brooker et al., (2013) examined the trajectories of stranger fear and behavioral inhibition in 6-to-36-month-old TD children and observed increased levels of fearfulness to a stranger led to more inhibition over time. Additionally these relationships extended into early childhood; such that infants who displayed greater stranger fear responses over time were at a greater risk for anxiety into toddlerhood (Brooker et al., 2013).

1.2 ANXIETY IN TYPICALLY DEVELOPING CHILDREN

Anxiety disorders are common childhood mental disorders with symptoms often emerging early in life with impact across the lifespan (Egger & Angold, 2006; Merikangas et al., 2010; Rapee, 2002; Rapee, Schniering, & Hudson, 2009). Estimates indicate that 9-20% of preschoolers will meet diagnostic criteria for an anxiety disorder with far more preschoolers exhibiting features of anxiety (Chavira, Stein, Bailey, & Stein, 2004; Egger & Angold, 2006). Symptoms of anxiety disorders can be detected in

neurotypical children as young as the first year of life through parent report (Carter, Briggs-Gowan, & Davis, 2004; Mian, Godoy, Birggs-Gowan, & Carter, 2012) with a cluster of behavioral responses including social withdrawal, shyness, fearfulness, and avoidance of novel or unfamiliar situations as primary indicators (Degnan, Almas, & Fox, 2010; Hudson & Dodd, 2012; Rapee, 2014). Anxiety disorders in childhood are highly comorbid with the development of depression, externalizing disorder, as well as other anxiety disorders in later periods of adolescence and adulthood (Aina & Susman, 2006; Bittner et al., 2007; Cassana, Rossi, & Pini, 2003; Gorrman, 1996). Anxiety has negative influences on development and is related to difficulties in school (Mazzone et al., 2007), peer relationships (La Greaca & Lopez, 1998), and cognitive performance (Eysenck, Derakshan, Santos, & Calvo, 2007; Vytal, Cornwell, Arkin, & Grillon, 2012). Given the pervasiveness and chronicity of symptoms and outcomes related to anxiety disorders, focus has been directed on the early emergence of anxiety and its developmental trajectory over time with the aim of directing early intervention and treatment efforts.

As previously highlighted, measurement is fraught with challenges necessitating consideration of both symptom and diagnostic aspects of impairment. Therefore, consideration of multiple indicators and inclusion of early emerging potential prodromal features is beneficial to efforts aimed at early identification of risk factors for anxiety disorders in young children. Despite support for early temperamental indicators leading to the development of anxiety disorders, the continuity or stability of temperament is limited (Goldsmith & Lemery, 2000). While elevated fear or shyness may result in a later diagnosis of an anxiety disorder for many children, these temperamental responses

also reflect normal and adaptive responses that help young children learn about their environment and how to regulate their responses (Muris, 2010). Typically, around 6 months of age, infants are able to discriminate strangers from familiar adults and begin to regulate fear responses that will continue to develop during the first few years of life (Brooker et al., 2013; Waters, Matas, & Sroufe, 1975). Inhibitory and fearful behaviors may indicate increased risk, not deterministic pathways, for anxiety disorders and highlight the importance to identify early characteristics that are associated with the emergence of anxiety in order to intervene and provide better outcomes (Kagan & Snidman, 1999). Buss (2011) highlights the heterogeneity of fearful behavior within temperament and cautions for the consideration of multiple aspects (e.g. facial expression, flight or freezing, social withdrawal, etc.) using multiple behavioral indicators. Studying different behavioral indicators of fearfulness or inhibition in young children allows us to gain a better understanding in how underlying symptoms of anxiety develop and change over time both in normative and at-risk populations.

Given the high prevalence and substantial impact of anxiety on individuals across the lifespan, recent interest has focused on the identification of potential subgroups that may confer increased risk for psychiatric disorders given their cognitive and genetic predispositions (Frank et al., 2006; Strang et al., 2012). Individuals that have intellectual disabilities or cognitive impairment are an identified high risk group given their limited emotional insight and difficulty regulating their emotions (Einfeld & Tonge, 1996; Gray et al., 2011). However, children with intellectual impairments are often not identified or treated for anxiety due to a failure to recognize impairments in this group and challenges associated with the diagnostic process (Wallander, Dekker, & Koot, 2003). Genetic

factors should also be considered as a factor associated with anxiety and may impact a child's predisposition to develop emotional and behavioral difficulties (Frank et al., 2006; Trzaskowski, Zavos, Haworth, Plomin, & Eley, 2011). For example, parents that have higher anxiety tend to have children that display more behavioral inhibition and develop anxiety disorders themselves later in childhood (Hudson, Dodd, Lyneham, & Bovopoulous, 2011; Muris, van Brakel, Arntz, & Schouten, 2011) Genetic factors may also negatively influence a child's physiological response to stress and increase their vulnerability to develop anxiety (Merikangas, Avenevolie, Dierker, & Grillon, 1999). For example, children who have parents with anxiety disorders have been shown to display increased sensitivity to stress through heightened startle reflexes and autonomic responses (Merikangas et al., 1999). Therefore, children with genetic predispositions for anxiety may have a unique biological profile that plays a bidirectional role in the development and maintenance of anxiety disorders due to dysregulation of physiological mechanisms and responses to environmental cues. Consistent with the recognition of biological predispositions that place subgroups at accelerated risk for anxiety disorders, autism spectrum disorder (ASD) and fragile X syndrome are two disorders that represent known or suspected genetic syndromes at elevated risk for anxiety.

1.3 AUTISM SPECTRUM DISORDER

Autism spectrum disorder (ASD) is a chronic neurodevelopmental disorder characterized by impairments listed in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) that include deficits in social-emotional reciprocity, nonverbal communicative behaviors, and difficulty developing, maintaining, and understanding social relationships (American Psychiatric Association [APA], 2013). In

addition to social difficulties, an individual with ASD may also display stereotyped motor movements, restricted interests, ritualized patterns of behavior, or hyper or hypo-reactivity to sensory input (APA, 2013). ASD impacts 1 in every 68 children (Center for Disease Control and Prevention, 2014) with boys being at a five times greater risk at developing the disorder than girls (Baio, 2014). Alarmingly, the prevalence rates for ASD have been steadily increasing with a reported 78% increase since the year 2002 (Baio, 2014). Although the precise causal mechanisms for how ASD develops are unknown, there is clear evidence that genetic influences and environmental mechanisms put an individual at risk (Geschwind 2008; Geschwind & Levitt, 2007; Geschwind, 2011; Gurrieri & Neri, 2009). This strong genetic link has been documented in infant sibling studies of individuals with ASD in that 19% of infants that have an older sibling with ASD are later reported to develop the disorder themselves (Ozonoff et al., 2011). Therefore, ASD is likely the result of numerous genetic factors and epigenetic interactions that influence the expression of the disorder (Schaefer & Mendelsohn, 2013). This etiological variability of ASD makes it difficult to understand and treat the underlying mechanisms that cause behavioral and biological symptoms, because these factors are complex and likely not impacted by a single factor.

ASD is characterized by a unique phenotype with cognitive, behavioral, and emotional traits associated with this complex disorder (Charman, Jones, Pickles, Simonoff, Baird, & Happe, 2011; Gray et al., 2012; Horiuchi et al., 2014). Children with ASD are reported to have poor inhibition skills (Adams & Jarrold, 2012 and increased emotional reactions (Georgiades et al., 2011), which consequently cause impairments in social, academic, and behavioral functioning. Children with ASD are at risk for

behavioral and emotional problems because symptoms are highly prevalent and may exaggerate or overlap with existing symptoms of ASD (e.g. social impairment, communication difficulties, etc.; Lainhart, 1999; Lecavalier et al., 2014; Leyfer et al., 2006). Tseng et al., (2011) investigated the prevalence of emotional and behavioral problems in children with autism and found that 73% of their sample had at least one syndrome scale of the Child Behavior Checklist (CBCL; Achenbach, 1991) in the clinically significant range, which highlights the high prevalence of behavioral problems in this clinical population.

It is not surprising given the heterogeneous presentation of ASD and high prevalence of clinical behavioral symptoms that there is a high degree of comorbidity with other disorders including attention deficit/hyperactivity disorder (ADHD), oppositional defiant disorder, aggression, and internalizing disorders (Gadow, De Vincent, Pomeroy, & Azizian, 2004; Mayes, Calhoun, Mayes, & Molitoris, 2012; Simonoff et al., 2008). Anxiety disorders are one of the most prevalent comorbid disorders within ASD making differential diagnosis challenging (Kreiser & White, 2014; Van Steensel, Bogels, & Perrin, 2011;Vasa et al., 2013; White et al., 2014; White, Oswald, Ollendick, & Scahill, 2009). Symptoms of anxiety and ASD often overlap or look similar in presentation to anxiety disorders making it difficult to measure anxiety in ASD (Lecavalier et al., 2014). Past research has focused on distinguishing early identifiers of ASD, but the importance of examining early comorbid factors in ASD is gaining attention in order to improve future outcomes (Matson & Goldin, 2013). It is critical to identify how anxiety emerges in this complex population in order to provide individualized and targeted treatments.

One of the challenges associated with identifying comorbid conditions, such as anxiety disorders, and their emergence in ASD is the vast heterogeneity within this complex disorder. Individuals with ASD present with variable cognitive functioning, language abilities, and adaptive functioning (Kanne et al., 2011; Wing, 1981) causing professionals in the field to describe ASD not as a singular disorder, but as a collection of various etiologies more appropriately termed "the autisms" (Geschwind & Levitt, 2007). Perhaps most predictive of comorbid mental health disorders in ASD is intellectual functioning (Hill & Furniss, 2006; Matson & Shoemaker, 2009; McCarthy, 2007). Individuals with ASD that also have lower intellectual functioning display high rates of co-occurring psychopathology with anxiety disorders being one of the most common comorbid conditions (Bradley, Summers, Wood, & Bryson; 2004; Hill & Furniss, 2006). Reduced intellectual ability in ASD is associated with greater vulnerabilities to develop comorbid conditions, particularly anxiety disorders; therefore, consideration of how these relationships emerge in younger children should be explored.

One of the most common comorbid conditions that is particularly debilitating in ASD are anxiety disorders (e.g., social anxiety, specific phobia, separation anxiety, etc.; Kreiser & White, 2014; van Steensel, Bogels, & Perrin, 2011;Vasa et al., 2013; White et al., 2014; White, Oswald, Ollendick, & Scahill, 2009). Individuals with ASD are reported to exhibit increased symptoms of anxiety (Eussen et al., 2013; Kim, Szatmari, Bryson, Streiner, & Wilson, 2000; Vasa et al., 2014) and as many as 84% of males with ASD between 2 and 18 years meet diagnostic criteria for at least one anxiety disorder (Muris, Steerneman, Merckelbach, Holdrinet, & Meesters, 1998). Age and cognitive ability may influence the presentation and prevalence of anxiety within ASD (Hill &

Furniss, 2006; Kim, Szatmari, Bryson, Streiner, & Wilson, 2000; White et al., 2009). There is mixed evidence as to how intellectual ability is related to anxiety in ASD with some studies reporting that higher cognitive abilities are associated with greater symptoms of anxiety in ASD (Mazurek & Kanne, 2010; Sukhodolsky et al., 2008) and others reporting that increased rates of anxiety disorders, in general, are associated with lower cognition (Van Steensel et al., 2011). For example, in their sample of adolescents with ASD and severe intellectual disability (e.g. IQ < 40), Bradley et al. (2004) found a specific vulnerability to anxiety in participants with autism compared to a control group with similar intellectual impairments. Similarly, in a sample of 69 children and adults with ASD and intellectual impairments, 77% percent displayed elevated symptoms of anxiety compared to 39% in the comparison group with just intellectual impairment (Hill & Furniss, 2006). MacNeil et al. (2009) argue that the disparities in prevalence rates between high and lower intellectual functioning individuals with ASD might be due to challenges in both recognizing and having specific ways to measure symptoms of anxiety in individuals with lower cognitive functioning. Younger children and individuals with lower intellectual abilities typically display more behavioral symptoms (e.g. social avoidance, irritability, etc.) associated with anxiety, whereas older adolescents and individuals with higher intellectual abilities may present with more cognitive symptoms associated with anxiety (e.g. maladaptive thought patterns, etc). Nonetheless, these results highlight a need for more research to examine the emergence of anxiety in populations that are young and at risk for cognitive difficulties in manners that go beyond self-report measures.

Features associated with anxiety are often similar and are associated with core symptoms of ASD (White et al., 2009). For example, Lecavalier et al. (2014) highlights the challenges that are encountered when measuring anxiety in ASD that include: 1) a high overlap in symptoms between the disorders 2) lower cognitive abilities in children with ASD 3) the manifestations of anxiety may be unique and 4) and more than one anxiety disorder may be present which makes differential diagnosis difficult. However, recent research has started to examine how anxiety can be distinguished from ASD by studying various measurement tools and the latent structures behind underlying mechanisms of symptom presentation (Helverschou & Martinsen, 2011; Lecavalier et al., 2014; White et al., 2014). This work has shown that individuals with ASD may have trouble recognizing the physiological or somatic symptoms associated with anxiety, which may lead clinicians to overlook symptoms of anxiety in favor of behaviors associated with ASD (Helverschou & Martinsen, 2011). Treatment most likely will vary depending on the unique characteristics and etiology of symptom presentation that are specific to each individual disorder.

Social anxiety is characterized by symptoms of anxiety surrounding contexts that involve interpersonal encounters or social situations (e.g. speaking in public, expressing thoughts, etc.; APA, 2013; Bellini, 2006; Kuusikko et al., 2008; Stein & Stein, 2008). Symptoms of social anxiety are high in ASD with 49% of adolescents' self-reporting elevated symptoms of social anxiety compared to same aged peers on a self-report measure (Bellini, 2004). A positive relationship between symptoms of ASD and social anxiety has been documented with higher symptoms of anxiety indicating more symptoms of ASD and overall greater social deficits (Cath et al., 2008). Early symptoms

of social anxiety often lead to clinical levels of impairment and it is estimated that 17% of children under the age of 18 with ASD meet diagnostic criteria for a comorbid social anxiety disorder (SAD; van Steensel et al., 2011). Social impairments in individuals with ASD are often better categorized under the diagnostic criteria of ASD than that of SAD, which may not adequately describe all the symptoms present in that individual especially if social impairments are due to underlying symptoms of anxiety (White et al., 2009). The presentation of SAD and ASD together is often more impairing than either disorder separately and are difficult to differentiate. Taken together, there is a need to identify how symptoms of social anxiety emerge in children with ASD to accurately identify targeted intervention efforts and improve later outcomes.

The overlap of symptoms of SAD and ASD contributes to the complexity of distinguishing comorbid conditions in ASD. For example, individuals with ASD may avoid social situations because of a lack of social reciprocity or desire to engage in social interactions with others, which is a core diagnostic symptom associated with ASD (APA, 2013; Kreiser & White, 2014). However, in cases where an individual with ASD has comorbid SAD they may avoid social contexts because of symptoms of anxiety directly related to the interpersonal situations or the fear of being rejected or humiliated in some way. Although the behavior is similar (e.g. social avoidance) the mechanism may be different for how and why social impairments are manifested. The developmental interplay of how social anxiety emerges in individuals with ASD is dependent on various temperamental and biological factors, as well as socially-mediated environmental factors (Bellini, 2006). Additionally, the relationship between symptoms of social anxiety and ASD is bidirectional in that symptoms of ASD may cause a lack of insight or awareness

in social contexts that cause social impairments, while social anxiety may further contribute to an individual avoiding social interaction thus enhancing the effects of each individual disorder. The interplay of social anxiety and the social impairments inherent in ASD warrants a developmental approach to studying how co-occurring conditions emerge in childhood in order to distinguish how these disorders impact functioning to target treatments to treat each disparate disorder.

There is evidence that autism symptom severity within ASD may be associated with specific anxiety disorders (Van Steensel, Bogels, & Perrin, 2011) leading to a debate as to whether SAD in ASD is indeed a distinct disorder or should be conceptualized as part of the heterogeneous presentation of ASD (Kreiser & White, 2014;Wood & Gadow, 2010). Research has begun to examine the relationships of how anxiety disorders develop in neurotypical children compared to children with ASD. Evidence suggests that the latent constructs of anxiety disorders in children with ASD are different than neurotypical children and these unique features should be considered more in depth (Ollendik & White, 2013; White et al., 2014). For example, when latent factors associated with symptoms of anxiety (e.g. separation anxiety, panic, physical anxiety, social anxiety and harm avoidance) were compared in a sample of children with comorbid anxiety and ASD against typically developing children with anxiety disorders, clusters of interrelated items were similar within each group (White et al., 2014). However, when these relationships were examined between groups, latent factors did not match across groups which suggests that there may be underlying differences in how anxiety is manifested and presented in children with ASD compared to TD children (White et al., 2014). These findings suggest that anxiety in ASD may be "atypical" in

comparison to "traditional" representation of anxiety in neurotypical populations (Kerns et al., 2014). A recent study found that children with ASD express symptoms of anxiety both similarly and differently than established DSM diagnostic criteria for anxiety (Kerns et al., 2014). These disparities suggest that some symptoms of anxiety within ASD may emerge and develop differently over time and may be influenced by the co-occurrence of ASD. There is a need to study how specific behavioral symptoms associated with ASD and anxiety are both distinct and shared in order to begin to conceptualize the underlying factors that maintain each disparate disorder.

Much of the literature studying social anxiety in individuals with ASD has examined relationships with high functioning adolescent and adult populations (Bellini, 2004; Kuusikko et al., 2008; Mattila et al., 2010; White, 2009). Older samples with ASD have been studied because the measures used to assess social anxiety often rely on the participant's ability to self-report symptoms through interview questions or responses on rating scales. This requires the participant to be able to identify and express symptoms they are feeling and may not be appropriate for children with ASD because of their difficulty with introspection, which is necessary for accurately self-reporting symptoms of anxiety (Baren-Cohen, 1985).

Few studies have examined the relationship of social anxiety and ASD in younger children (Cervantes et al., 2013; Davis et al., 2011; Gadow et al., 2004; Vasa et al., 2013), potentially because SAD is typically diagnosed in late childhood and adolescence in community samples, with an average age of onset at 8 years of age (Beesdo et al., 2007; Simonoff et al., 2008; Vasa et al., 2013). Despite later diagnosis of SAD, symptoms of anxiety often emerge in early childhood in typical samples and in children

with ASD as well. For example, Davis et al. (2011) found that anxiety tends to rise from toddlerhood to childhood, decrease from childhood to young adulthood, and then rise again from young adulthood to older adulthood in individuals with ASD. These developmental trends suggest that there are specific factors that put an individual with ASD at risk for experiencing more anxiety during toddler years, and the emergence of these factors may be expressed differently depending on the developmental and cognitive level of the child. For example, preschool age children may have limited abilities to communicate their emotions and feelings and may display more behavioral symptoms associated with anxiety. Cervantes et al. (2013) demonstrated that toddlers with ASD who displayed more severe anxiety behaviors had overall higher rates of challenging behaviors including increased aggression/destruction, stereotypies, and self-injurious behaviors than children that had little to no anxiety. Despite these differences in behavioral symptoms associated with developmental level and anxiety, it is critical to distinguish comorbid disorders early in a child's development in order to address the most impairing symptoms and provide treatments that are appropriately targeted to prevent symptoms from progressing or worsening over time.

1.4 FRAGILE X SYNDROME

Fragile X syndrome (FXS) is a neurodevelopmental disorder and the most common genetic cause of ASD (Hagerman, 2008; Crawford et al., 2002). FXS is a single gene disorder caused by expanded CGG trinucleotide repeats (>200 CGG repeats) on the X chromosome (Hagerman & Hagerman, 2002). This expansion impacts the fragile X mental retardation 1 (FMR1) gene, which leads to reduced FMR1 protein (FMRP) production (Verkerk et al., 1991). FMRP is important for brain development and growth

and negatively influences development in individuals with FXS in multiple domains including their cognitive and emotional functioning (Loesch, Huggins, & Hagerman, 2004; McLennan, Polussa, Tassone, & Hagerman, 2011; Schneider, Hagerman, & Hessl, 2009). FXS has an estimated prevalence rate of approximately 1 in 2,500 males (Cordeiro, Ballinger, Hagerman, & Hessl, 2011; Hagerman, 2008). Females with FXS are often less affected than males (1 in 6,000 females) because they have two X chromosomes and may not display significant intellectual impairments (Bennetto et al., 2001). However, males with FXS are typically mild to severely affected intellectually and have distinct cognitive profiles (Baker et al., 2011; Kogan et al., 2004; Munir, Cornish, & Wilding, 2000). Recent attention has been given to how various biological and behavioral factors influence cognitive and emotional development in this unique population (Cornish et al., 2009; Roberts, Tonnsen, Robinson, & Shrinkareva, 2012).

Individuals with FXS have a unique behavioral phenotype that puts them at elevated risk for emotional problems and developing comorbid conditions. Behavioral features associated with FXS include increased symptoms of inattention, aggression, and hyperactivity (Cornish, Cole, Longhi, Karmiloff-Smith, & Scerif, 2013; Hessl et al., 2008; Wheeler et al., 2014). Boys with FXS display higher rates of maladaptive behavior such as compulsive behavior and self-injurious behavior (Bailey et al., 2008; Hall, Lightbody, & Reiss, 2008). These problem behaviors often are disruptive and impact an individual's functioning in a variety of domains including academics, social relationships, and adaptive functioning (Hatton et al., 2002; Hatton, Bailey, Hargett-Beck, Skinner, & Clark, 1999). The behavioral phenotype associated with FXS also overlaps with other clinical disorders such as anxiety, ADHD, intellectual disability, and ASD, which makes

differential diagnosis and treatment challenging (Bailey, Hatton, Mesibov, Ament, & Skinner, 2000; Bailey, Raspa, Olmsted, & Holiday, 2008; Cornish et al., 2013; Lesniak-Karpiak, Mazzocco, & Ross, 2003; Rogers, Wehner, & Hagerman, 2001; Sullivan et al., 2006).

Perhaps the most impairing of the comorbid behavioral conditions within FXS is that of ASD (Kaufmann et al., 2004; Lewis et al., 2006). The genetic etiology of FXS allows for a more controlled way to examine biological mechanisms behind the development of ASD. Therefore, ASD symptoms have been studied in individuals with FXS in hopes to isolate biological mechanisms that may help explain causal factors of ASD. ASD and FXS share similar behavioral features, despite having distinct etiological mechanisms, with 90% of individuals with FXS displaying at least one symptom associated with ASD (Hagerman, 2002). Increased symptoms of ASD put individuals with FXS at risk for meeting diagnostic criteria of a comorbid ASD. Discussion to whether ASD in FXS is similar to idiopathic autism spectrum disorders (iASD; ASD that is not associated with FXS in this study) has led to the examination of how these groups are both similar and different in symptom presentation. It has been proposed that that "the clinical heterogeneity of ASDs might be reduced when subgroups based on a specific genotype are extracted from the overall genetically heterogeneous ASD population" (Bruining et al., 2010, p. 3), which suggests that comorbid ASD in FXS (fxASD may be the result of a distinct etiological genetic mechanism. Moreover, fxASD may have a specific behavioral phenotype associated with it than what would be expected in iASD.

There have been mixed findings to whether individuals with fxASD display more impairments than individuals that have FXS without ASD (McDuffiet et al., 2010; Wolf et al., 2012). Social behavior has been frequently studied as a differential factor between FXS and fxASD and a positive relationship between symptoms of autism and social impairments has been observed (Bailey et al., 2001; Kaufmann et al, 2004; Roberts et al., 2007). However, some studies have not found differences in social behavior between FXS and fxASD. For example, McDuffie et al. (2010) used the ADI-R to study differences in autism symptoms in children and adolescents with FXS, with and without autism, and did not find any group differences in social reciprocity after controlling for cognitive impairment. Individuals with fxASD often display greater cognitive impairments and are at an increased risk to exhibit more psychopathology than individuals with FXS (Cordeiro et al., 2010; Lewis et al., 2006). Cordeiro and colleagues (2010) found that their sample of older children and adolescents with fxASD displayed more diagnostic symptoms of social anxiety and specific phobia than children and adolescents with FXS. Given their cognitive and behavioral vulnerabilities, individuals with fxASD may be at a greater risk for developing anxiety compared to individuals with FXS, despite their similar genetic etiological backgrounds.

Few studies have examined the different behavioral profiles between fxASD and those with iASD. Of the studies that have been conducted, most compare ASD diagnostic criteria of children with FXS to those with iASD from the two domains including social and communicative functioning and repetitive behaviors and interests (Hall, Lightbody, Hirt, Rezvani, & Reiss, 2010; Rogers et al., 2001; Wolf et al., 2012). For the first ASD diagnostic domain of social impairment, there does appear to be

differences observed between individuals with fxASD and iASD (Hall et al., 2010; Wolf et al., 2012). For example, Hall and colleagues (2010) studied the behavioral profiles of individuals with FXS using two common diagnostic measures, the Social Communication Questionnaire (SCQ; Rutter, Bailey, & Lord, 2003) and the ADOS (Lord, 2002) to determine if autism symptoms were similar in FXS and iASD. Results indicated that individuals with FXS had lower rates of impairment in social (e.g. social smiling, range of social expressions, quality of social overtures, and joint attention) than individuals diagnosed with iASD (Hall et al., 2010). Additionally, Wolf et al. (2012) investigated symptoms of autism using the ADOS in 3-to-5-year-old boys with fxASD compared to boys with iASD and found that the FXS group had less severe symptoms of ASD related to gaze integration, social overtures, social smiling, and facial expressions. However, differences are less definite between fxASD and iASD for the second diagnostic domain concerning repetitive behaviors and interests (Wolf et al., 2012). Wolf et al. (2012) highlighted in their sample that an area of overlap between fxASD and iASD were related to their restricted, repetitive behaviors rather than specific social impairments. In summary, although individuals with fxASD display behaviors consistent with diagnostic criteria for ASD, in comparison to individuals with iASD their behaviors may be less severe in certain diagnostic domains that involve social behavior. Some argue that ASD in FXS is driven more by impairments from anxiety than true symptoms of ASD (Cordeiro et al., 2011; Talisa, Boyle, Crafa & Kaufmann, 2014). Therefore, careful consideration of factors that are both similar and disparate to FXS, fxASD, and iASD in regards to measures anxiety and autism should be studied to understand how these groups overlap and can be differentiated.

Given that FXS has a well-defined, genetic, etiological basis, some studies have examined neurobiological and physiological factors associated with FXS and iASD (Belmonte & Bourgeron, 2006; Garcia-Nonell et al., 2008; Hoeft et al., 2011). Potential biological factors may explain differences in FXS and account for variability in the presentation with the co-occurrence of other disorders, such as ASD and anxiety disorders. Patterns of brain growth and development have been studied in preschoolers with FXS and iASD and neurological differences that distinguish these groups have been identified (Hazlett et al., 2012; Hoeft et al., 2011). For example, abnormal frontal and temporal regions—those involved in social cognition—have been observed in toddlers with fxASD compared to TD controls (Hoeft et al., 2011). Biological and physiological factors may play a role in how anxiety develops and presents in these disparate disorders. The fragile X mental retardation protein (FMRP) has been studied in relation to autistic behavior in FXS (Bailey, Hatton, Skinner, & Mesibov, 2001; Harris et al, 2008; Hatton et al., 2006). There have been mixed results regarding the role that FMRP plays in ASD in FXS with one study reporting a negative relationship between symptoms of ASD and FMRP (Hatton et al., 2006), while other studies have reported no relationship (Bailey et al., 2001; Harris et al., 2008). Even though FXS and iASD are both highly comorbid with anxiety disorders, differences in brain structure and physiological mechanisms suggest that there are behavioral idiosyncrasies between the groups that are functionally disparate. These biological differences may influence the development and presentation of anxiety in FXS, fxASD, and iASD.

One physiological measure that has been studied in FXS and iASD as a biomarker for anxiety is cortisol, a hormone associated with psychological stress and arousal (Hessl

et al., 2006; Hollocks, Papadopoulos, Howlin, & Simonoff; Spratt et al., 2012). Roberts et al. (2009) found differences in cortisol in boys with fxASD and those with FXS that was negatively related to social interactions. Boys with fxASD that exhibited more severe symptoms of autism displayed less reactivity, as measured by cortisol (Roberts et al., 2009). Collectively, these results suggest that neurobiological and physiological differences might are apparent in iASD and FXS, which may influence the expression of anxiety.

Anxiety disorders are common within FXS with 86% of males meeting DMS-IV diagnostic criteria for one or more anxiety disorders (Cordeiro et al., 2011) and 70% receiving treatment for anxiety symptoms (Bailey et al., 2008). Despite these elevated prevalence rates in FXS for comorbid anxiety disorders, research examining anxiety in FXS is limited (Bailey et al., 2008; Cordeiro et al., 2011; Hall et al., 2012; Lesniak-Karpiak et al., 2003). To date, only one study has used diagnostic methods to clinically assess the prevalence of anxiety disorders in FXS using DSM-IV criteria (Cordeiro et al., 2011). Cordeiro et al. (2011) sampled 97 males and females with FXS and found the most prevalent anxiety disorders included specific phobia and social phobia. Also, the co-occurrence of ASD in FXS increased these relationships signifying that individuals with fxASD are more at risk for anxiety. Parent report also indicates that children with FXS are at risk for social withdrawal and anxiety that collectively may negatively influence their ability to learn (Bailey et al., 2008).

Associations between physiological arousal and anxiety, in relation to social stimuli, have also been investigated in FXS (Boccia & Roberts, 2000; Hall et al., 2012). Hall et al (2012) examined the effects of oxytocin on alleviating symptoms of anxiety

during a social task through measuring cortisol and heart activity. Oxytocin was related to improved eye contact and a decrease in cortisol suggesting that oxytocin may be beneficial in reducing some of the symptoms associated with anxiety. Anxiety has been recognized as a considerable feature within the behavioral phenotype of FXS; however, the mechanisms to how anxiety emerges in this high-risk population have not been studied in depth.

Among the anxiety disorders comorbid with FXS, social anxiety is particularly impairing. Individuals with FXS often display high amounts of social anxiety with behavioral symptoms including shyness, avoidance of social situations, difficulty understanding social cues, fearfulness, poor socioemotional processing, and poor social skills during interpersonal interactions (Cordeiro et al., 2011; Lesniak-Karpiak et al., 2003; Tonnsen, Malone, Hatton, & Roberts, 2013; Williams, Porter, & Langdon, 2014). Cordeiro et al., 2011 found that 58% of their sample of male and females with FXS displayed clinical symptoms of a social phobia. Distinct factors that are associated with the unique genetic and behavioral profiles of FXS have been studied to explain the highly prevalent symptoms of anxiety that often accompany this population.

Challenges arise when trying to identify social anxiety in FXS. Individuals with FXS often have cognitive impairments that reduce their ability to self-report or have insight into the symptoms they are experiencing. Parents may also have a limited awareness of the symptoms of anxiety that their children with FXS are experiencing. For example, Lesniak-Karpiak et al. (2003) found that parents did not indicate elevated levels of social anxiety despite reporting higher levels of social difficulty in females with FXS.

These results highlight that although parents may be aware of social impairments that their children with FXS are displaying, they are not attributing the difficulties to anxiety. Physiological vulnerabilities within FXS may provide a way to distinguish biological mechanisms that play a role in the presentation of social anxiety (Hall, DeBernardis, & Reiss, 2006; Hall, Lighbody, McCarthy, Parker, & Reiss, 2012; Hessl et al., 2002; Hessl et al., 2006; Wisbeck et al., 2000). The physiological systems of individuals with FXS are dysregulated with abnormal functioning of the sympathetic nervous system causing them to display more behaviors relating to hyperarousal (Boccia & Roberts, 2000; Hall et al., 2009; Hessl et al., 2006; Porges, 1996). Hyperarousal in FXS is strongly associated with anxiety and social avoidance (Boccia & Roberts, 2000; Hall et al., 2009). Therefore, children with FXS may be at risk for developing symptoms of anxiety given their unique biological etiology, which is different than what would be expected in neurotypical children or in other populations, such as children with ASD.

Few research studies have examined how comorbid symptoms of anxiety emerge in FXS, compared to iASD or neurotypically developing youth. The work that has been done uses a temperamental framework. Relationships between negative affect and the development of anxiety over time suggest that children with FXS that display more severe behavioral indicators of fear and sadness are at greater risk for having increased anxiety (Tonnsen et al., 2013). However, it is unclear if children with FXS display comorbid anxiety in similar ways to neurotypical children or children with iASD given that the etiological pathways to developing comorbid conditions are different. In order to better understand how comorbid conditions emerge in individuals with FXS attention has to be given to how these factors are both similar and dissimilar among groups that are

at risk for developing comorbid conditions, such as ASD. Despite these findings that individuals with FXS are displaying clinical levels of symptomology associated with social anxiety, few studies have examined how social anxiety emerges in groups that are particularly at risk (Hall, Lightbody, Huffman, Lazzeroni, & Reiss, 2009; Hessl et al., 2006, Tonnsen et al., 2013). Early behavioral indicators of anxiety should be studied more in depth in order to target treatment and alter the development or progression of later anxiety in FXS.

1.5 SOCIAL APPROACH IN FXS AND ASD

As previously discussed, anxiety is highly prevalent in FXS (Bailey et al., 2008)and is associated with increased impairment in social situations making it difficult to distinguish symptoms of ASD from symptoms of anxiety (Roberts et al., 2007; Wolf et al., 2012). Although individuals with FXS have been documented to have poor peer relationships, communication, and social skills, these impairments may be due to more underlying symptoms of anxiety rather than symptoms of ASD (Budimirovic & Kaufmann, 2011). For example, an individual with FXS may have poor eye contact due to social anxiety rather than lack of nonverbal communication skills or social reciprocity. Individuals with FXS may also be "slow to warm up" and demonstrate increased social anxiety initially in response to novel conditions, but improve over time. Roberts et al. (2007) showed that the eye contact of boys with FXS improved over the course of the assessment, despite displaying similar initial levels of avoidant eye contact to boys with fxASD. However, boys with fxASD sustained the avoidance of their eye contact during the entire course of the observation. Similarly, Cohen et al. (1991) demonstrated differences in gaze patterns in children with FXS compared to children with iASD and

reported that children with FXS had less gaze avoidance when interacting with their mothers versus a stranger. Children with iASD, on the other hand, avoided gaze regardless of whether the person was familiar or not (Cohen et al., 1991). Collectively these results suggest that the gaze avoidance seen in FXS may be due to anxiety from a novel situation (e.g. the stranger) rather than global impairments in social reciprocity as seen in ASD across all conditions (e.g. mother and stranger). Therefore, it is important to distinguish behavioral features in FXS and iASD that may be disparate due to underlying genetic, or biological mechanisms, or underlying features of another comorbid disorder, such as anxiety, because diagnosis and intervention would likely look different in these populations depending on the mechanism for why the behaviors are occurring.

Attention has been given to identify the early social impairments that emerge in fxASD (Ozonoff et al., 2011; Tonnsen et al., 2013; Watson et al., 2013; Zwaigenbaum, Bryson, & Garon, 2013). However, only a few studies have examined or compared behaviors associated with social anxiety in a stranger approach paradigm with children that have iASD, FXS, and/or fxASD and no study has conducted cross-syndrome comparisons in children of each respective group (Hobson & Lee, 1998; Pisula, 2004; Sigman & Mundy, 1988; Tonnsen et al., 2013; Williams et al., 2014). Williams et al. (2014) demonstrated that individuals with FXS performed worse on a social approach task in regards to emotion recognition compared to a group of chronologically and mental age matched controls, which are consistent with patterns of behaviors congruent with social anxiety. Physiological mechanisms related to social anxiety may also explain relationships of greater fear responses towards a stranger in FXS given the unique biological and genetic etiology associated with the disorder. For example, Tonnsen et al.

(2013) found that young children with FXS display more facial fear and greater distress vocalizations in the presence of a stranger compared to TD controls. Also, this relationship was associated with increased measures of cardiovascular activity related to hyperarousal, rather than symptoms of ASD in children with FXS (Tonnsen et al., 2013). Hobson and Lee (1998) performed a study that examined social engagement between individuals with and without iASD and found that those with iASD are less likely to maintain eye contact with a stranger during an interpersonal exchange. Some studies have also looked at adaptive responses to stranger approach and looked at parent-child interactions through gaze patterns and social referencing (Cohen et al., 1991; Pisula, 2004; Sigman & Mundy, 1988). An early study examined social comprehension but studying affective responsiveness and awareness to both familiar (e.g. a parent) and unfamiliar stimuli (e.g. a stranger) and found that children with iASD were more likely to direct social behavior to their mothers compared to a stranger and these behaviors increased after a separation period from their mothers (Sigman & Mundy, 1988). Collectively these results demonstrate variable responses to a stranger that are dependent on ASD symptomology and levels of attention or arousal.

1.6 PRESENT STUDY

ASD and FXS are highly comorbid, and overlap in symptom presentation (Hagerman, 2002; Lewis et al., 2006). Additionally, ASD and FXS are both comorbid with social anxiety (Bellini, 2004; Cordeiro et al., 2011; White et al., 2009). Early research has focused on identifying early indicators of iASD and FXS (Bailey, Raspa, Olmsted, & Holiday, 2008; Cornish et al., 2013; Gray et al., 2012), but recent research has recognized the importance of identifying how social anxiety develops in young children with FXS

and iASD (Bailey et al., 2008; Cordeiro et al., 2011; Hall, Lightbody, & Reiss, 2008; Matson & Goldin, 2013). Given the etiological differences between FXS and iASD, it is important to identify how anxiety emerges in ways that are both similar and disparate in order to provide targeted assessment and interventions practices that will improve future outcomes. Few studies have compared behavioral responses of social fear or anxiety in both typically developing populations, as well as atypical populations, such as FXS and iASD (Hobson & Lee, 1998; Tonnsen et al., 2013; Williams et al., 2014). To our current knowledge, no study has examined the early emergence of behavioral indicators of social fear using cross-syndrome comparisons between FXS, fxASD, and iASD groups.

1.7 PURPOSE OF STUDY

The over-arching purpose of this study is to contrast behavioral indicators (e.g. facial fear, escape behaviors, and gaze patternos) of social fear in four groups of preschool boys; (1) FXS with low autism symptoms (FXS) (2) FXS with high autism symptoms (fxASD) (3) idiopathic autism spectrum disorder (iASD), (4)and typically developing boys (TD). Secondarily, this study will investigate the relationship of autism symptomology across a continuum to social fear for the iASD and fxASD groups.

1.8 RESEARCH QUESTIONS

Do behavioral profiles of social fear differ among iASD, FXS, and fxASD and TD groups? We hypothesize that preschoolers with higher autism symptoms will display more behaviors of social fear to a stranger with the fxASD group showing the most behavioral responses of fear, followed by the iASD group, then the FXS group, and the typically developing control group displaying the least amount of fear compared to the other atypical groups. Preschoolers that spend a greater percentage looking at the

stranger, the potential fear- provoking stimulus, will display the most anxiety, followed by gaze patterns of looking at a parent. Finally, preschoolers that spend the greatest amount of time looking away (e.g. not at the stranger or parent) are displaying the least amount of anxiety to the stranger. Groups that display more escape behaviors, as well as facial fear, exhibit more social fear in response to the stranger. We predict that the fxASD, followed by the iASD, then the FXS groups will display the most escape behaviors and facial fear in response to a stranger in comparison to the typically developing control group.

What is the relationship between behavioral indicators of social fear and autism symptoms in preschool boys with iASD compared to those with fxASD? We first hypothesize that autism symptoms are positively associated with all behavioral measures of social fear (e.g. gaze, escape behaviors, and facial fear). Additionally, we predict that there will be an interaction between our iASD and fxASD groups and symptoms of autism on social fear. We predict that the fxASD group will display a stronger relationship of symptoms of ASD associated with more behaviors of social fear to the stranger in comparison to the iASD group.

CHAPTER 2

METHOD

2.1 PARTICIPANTS

Participants included a total of 101 male preschoolers between the ages of 2 and 5 years of age categorized into the following four groups: FXS with low ASD symptoms (FXS; N = 29), FXS with high ASD symptoms (fxASD; N = 25), idiopathic ASD (iASD; N = 11), and a typically developing control group (TD; N= 36). Data were collected from participants across two associated studies examining temperament and early development in FXS. The first study is a completed project from the University of North Carolina (UNC; PI: Bailey) that focused on preschool aged males with FXS. The second study is from an ongoing study at the University of South Carolina (USC; PI: Roberts) focused on infant and preschool development in children with FXS. Data obtained from the completed study at UNC represented a total of 75 participants that included 28 boys with FXS, 17 with fxASD, and 30 TD boys. Data collected at USC are part of an ongoing study led by the final author (JER), who also was an investigator at UNC. The USC study added a total of 26 participants that included 1 boy with FXS, 8 boys with fxASD-, 6 TD boys, and 11 boys with iASD. Table 2.1 lists the distribution of participants across each respective study.

Data collected at USC were obtained using the same standard assessment protocols as the UNC study. Site differences in the participants' chronological age, cognitive ability, and total level of autism symptoms were examined by completing a

series of independent samples t-tests for the groups with fxASD and TD but not for the iASD and FXS given that only 1 participant with FXS was from the USC site and no participants with iASD were from the UNC site. Results indicate that the participants from the UNC study were older across the groups (FX+ASD, and TD) with average ages of 4.9, and 3.7 years respectively contrasted to the USC participants with average ages of 2.1, and 2.0 years. Cognitive ability was higher in the USC site (51.25 vs. 48.95) for the fxASD group as was autism symptomology (16.42 vs. 15.22) for the TD group. While these site differences are statistically different, they do not represent large differences based on the mean values and are of minimal clinical significance (e.g., the cognitive abilitystandard scores are within the same range for both sites and the CARS scores for the TD are far below clinical cutoffs for both sites). Also, these site differences are expected given the younger developmental focus at the USC site and that younger age is associated with elevated cognitive ability in FXS (Roberts et al., 2009) and more variability in typical development. So site differences are not controlled for in the analyses as they are redundant with age and cognitive ability. Site descriptive statistics and results from the t-tests are provided in Table 2.1.

When multiple assessments were available, the youngest age point for each participant was used in the current study (i.e., between 2-5 years) due to our focus on very early behavioral development. Participants were included in our study if they were born full-term, currently lived with their biological mother, and if English was the primary language spoken in their home. Participants from all groups were excluded from the study if they had been previously diagnosed with another known medical or genetic condition (e.g., seizure disorder) that may interfere with the results of this study.

Participants were recruited through a national registry for research, support groups, or advertising through community centers near the universities.

Participants in the FXS and fxASD groups together comprised 54 boys who had previous genetic testing to confirm the presence of the disorder. Participants were recruited nationally through support groups and current national research databases. Within the collective FXS groups, 8 boys (15%) had an ADOS-2 completed with 100% having a CARS score. Thus, the CARS scores were used to determine groups based on high and low ASD features with the ADOS-2 scores used to validate the CARS scores(r = .86). Based on the CARS, 25 boys (46% of the sample) had a total score of a 30 or greater indicating increased symptoms of autism reaching the established clinical cutoff. This subgroup of boys with FXS and elevated symptoms of autism were categorized as the fxASD group. The FXS group included 29 boys (54% of the sample) and was determined by CARS total scores below 30 indicative of low autism symptomology. Of the fxASD group, 7 (28%) boys and 1(17%) boy within the FXS group had an ADOS-2 completed. Additionally, of the fxASD and FXS groups that had ADOS-2 data, 7 (100%) of the boys with fxASD displayed elevated autism symptomology on the CARS (e.g. a total score above 30), compared to none of the boys with FXS. Both groups were matched on cognitive ability with the fxASD displaying an average ELC standard score on the Mullen Scales of Early Learning of 49.68, which was similar to the FXS average ELC standard score of a 51.25. Further descriptive and demographic information about the FXS groups are provided in Table 2.2.

The iASD group consisted of 11 preschool aged boys with an average chronological age of 4.4 years. Inclusion into the iASD group for this study was based

on having met DSM-criteria for ASD documented through a diagnostic evaluation by a qualified community professional (e.g., psychologist; N=10) or by meeting threshold on the ADOS-2 (N=1) as well as receiving a total score higher than 30 on the CARS through participation in the USC study (N=11). Also, one participant with both CAR-2 and ADOS-2 data met diagnostic criteria on both measures. Community diagnoses were confirmed by CARS scores calculated as part of study participation. Participants with iASD were excluded from participating if there were known genetic (e.g. FXS) or medical conditions reported in the family history report or during the initial screening. The iASD group was matched on cognitive ability to the FXS and fxASD groups and had an average Early Learning Scale standard score of a 52.91 on the Mullen Scales of Early Learning. Demographic and descriptive information concerning the iASD group is provided in Table 2.2.

Boys in the typically developing (TD) group included 36 participants with an average age of 3.33 years and an average CARS score of 15.42. Participants were included in the TD group if they were reported to have no developmental concern by parental report. Additionally, participants in the TD group were required to perform within the average range on developmental measures of functioning as measured by the Mullen Scales of Early Learning. Descriptive and demographic data for the TD group are provided in Table 2.2.

2.2 MEASURES

The Stranger Approach observation from the *Laboratory Temperament Assessment Battery* (Lab-TAB; Goldsmith & Rothbart, 1996) was used to elicit behavioral indicators of stranger fear from the participants. The Stranger Approach from

the Lab-TAB paradigm is designed to allow for cross-lab comparisons of temperament in children using standardized experiments. Other studies have looked at the Lab-TAB Stranger Approach in relation to temperamental measures of social anxiety and found associations with increased behavioral inhibition (Brooker, Buss, Lemery-Chalfant, Aksan, Davidson, & Goldsmith, 2013) and increased physiological stress reactivity (Talge, Donzella, & Gunnar, 2008) in children, as well as a positive association of mood disorders in mothers (Durbin, Klein, Hayden, Buckley, & Moerk, 2005). The Stranger Approach was embedded in a larger series of standardized epochs from the Lab-TAB and followed a non-demanding, engaging task that measured the participants' attention. Per standardized procedures, the "stranger" was a female examiner that wore black sunglasses, a baseball hat, an oversized gray sweatshirt, and a long black skirt. The Stranger Approach has three distinct phases: approach, kneel, and recovery. Prior to the approach of the stranger, the preschoolers were in an affectively neutral state and positioned so that they were seated on a caregiver's lap or seated beside their caregiver on a chair in an empty room. During the approach phase, the stranger appeared in the room then slowly walked toward the child for 10 seconds. After approaching the child, the stranger kneeled for approximately 2 minutes in front of the child with a neutral affect then exited the room for a recovery period of 10 seconds. The total duration of the Stranger Approach episode lasted approximately 2.5 minutes from the approach to the withdraw of the stranger.

Following the LabTAB manual, the following behavioral variables were coded: child's gaze, intensity of escape, and intensity of facial fear during the Stranger Approach phase. The child's gaze pattern included looking at the stranger, at the parent, or

nonsocial gaze (e.g. not looking at a social stimulus, such as the stranger, parent, or examiner). Escape behaviors reflected head turns and whole body movements (e.g., twisting away). Per LabTAB procedures (Brooker et al, 2013), facial fear was coded using Carol Izard's (1979) *Maximally Discriminative Facial Movement Coding System* which defined three specific facial regions (eyebrows/forehead, eyes, and mouth) and the intensity of movement associated with fear to the stranger. All observations of stranger fear were coded only if the behavior was readily visible (e.g., facial fear was coded as obscured if more than 50% of the facial region was not able to be seen clearly). Given the natural variability in the availability of behavior that can occur due to either obscured behavior (e.g., child placing hands over eyes that obscures gaze determination) or poor video angles or quality, we calculated the mean duration across each behavior and excluded any sessions that were outside two standard deviations of the mean duration to control for potential confounding effects related to duration. Detailed descriptions of how the behavioral variables of facial fear, escape, and gaze were coded are provided in Table 2.3 and Table 2.4.

Data were coded by trained research assistants that established reliability standards with a master coder across all behavioral variables (e.g. facial fear, escape, and gaze). In order to achieve reliability on each of the behavioral variables, training involved reviewing the coding schemes with the master coder and consensus coding 3 videos together while verbally discussing the behavioral codes. After coding together, the master coder and the research assistant established reliability standards by coding 3 consecutive videos separately and receiving 80% agreement between the codes on each of the videos. Reliability was determined by using Cohen's kappa coefficient of 0.80

and was conducted on 20% of all of the data coded. Cumulative kappa coefficients were recorded for gaze (.83), escape behaviors (.83) and facial fear (.89). Figure 3.1 and Figure 3.2 display mean group differences across each of the behavioral variables.

To obtain the intensity score of a behavioral variable, calculations were conducted using procedures described by Gagne and colleagues (2011) that include: weighting each level of intensity of a behavioral variable (e.g., no behavior= 0, highest level of behavior=3), multiplying the weight of each level of intensity against proportion of time spent at each level, and dividing that number across the total observation time to produce an average intensity score for each of the behavioral variables that were coded. The participants' gaze was not analyzed on an intensity scale but as a percentage of time during the observation of where they were looking (e.g. at stranger, parent, or nonsocial gaze) based on the total duration of a level of a behavior (e.g. looking at the stranger) divided by the total observation time (e.g. the entire Stranger Approach observation duration).

In our sample, a subset of our participants (N = 93) also had data from the Child Behavior Checklist (CBCL/1.5-5; Achenbach & Rescorla, 2001), a measure often used to assess internalizing symptoms, such as anxiety, in preschool aged children. Raw scores from the DSM-Anxiety subscale on the CBCL demonstrated a modest positive relationship (r =15) with gaze towards parents in our study that is similar to reports of the correspondence between observed stranger fear and parental ratings in young TD children (r = .22; Brooker et al., 2013). A total of 99 participants (TD N = 24; FXS N= 29; FxASD N = 25; iASD N = 11) had CBCL data. CBCL anxiety t-score indicate that one participant with FXS fell within the subclinical range (e.g. t-score of 65-69) of

anxiety, while 2 participants with FXS and one participant with fxASD displayed clinical levels (e.g. t-scores greater than 69) of anxiety. Correlations between the CBCL and observations of stranger fear for the combined sample and each of the individual groups are provided in Table 2.5. Figures 3.3-3.7 also demonstrate scatterplots of these relationships.

The Childhood Autism Rating Scale (CARS; Schopler, Reichler, & Renner, 1986) is a well-established measure used as part of the autism diagnostic process (Rellini, Tortolani, Trillo, Carbone, & Montecchi, 2004). The CARS measures the severity of symptoms associated with autism spectrum disorders across 15 behaviors. Scores for each item are rated on 4-point scale ranging from within normal limits to severely abnormal for the age. Each item on the CARS is summed together to obtain a total score, which is used to classify the child as having minimal to no symptoms of an autism spectrum disorder (e.g., total score below 30), mild to moderate symptoms of an autism spectrum disorder (e.g., total score between 30-36.5), and severe symptoms of an autism spectrum disorder (e.g., total score higher than a 36.5). Ratings and scores are given to a child based on behavioral observations by a clinician. The CARS is considered a reliable and valid measure of autism symptom severity for children as young as 2-years of age. The CARS has an internal consistency of a .94 and a test-retest stability of a .88 (Schopler, Reichler, & Renner, 1988).

For this study, the CARS total score was used as a measure autism symptom severity in each of the participants and scored by consensus by the investigators. The CARS has high agreement with the Autism Diagnostic Observation Schedule (ADOS; Lord, Rutter, DiLavore, & Risi, 2000), another diagnostic measure used in assessing

symptoms associated with autism spectrum disorders (Ventola et al., 2006). For a subset of participants in this study (N=20; iASD = 1, fxASD = 7; FXS = 5; TD = 7), the ADOS was administered as a portion within the assessment battery. We observed a high correlation (r = .90) between the CARS total score and the ADOS-2 total raw score in our sample supporting a strong agreement between these two measures. Also, results from an independent t test suggest no significant group differences on CARS scores between the fxASD and the iASD groups; t (33) = 4.52, p = .96.

The Mullen Scales of Early Learning (MSEL; Mullen, 1995) is a developmental measure used to assess cognitive abilities in young children. Five domains measure Gross Motor, Visual Reception, Fine Motor, Expressive Language, and Receptive Language. The MSEL has established high test-retest reliability (.70- .80), median split-half internal consistency ranges for each of the scales (0.75- .83), and interrater reliabilities (.91-.96). The Early Learning Composite (ELC; M = 100, SD = 15) was used as a measure of cognitive ability in this study. The atypical groups (e.g. FXS, fxASD, iASD) were matched on their ELC scores prior to data analysis to control for cognitive ability. Table 2.2 lists descriptive data for each of the groups regarding cognitive performance on the MSEL.

2. 3 PROCEDURES

The Stranger Approach episode was completed within a larger battery of behavioral and developmental assessments. Written consent was obtained from the parents of all the participants and background information was provided about the study. Individual assessments were conducted either in the participants' homes or at the university's research laboratory based on the age, preference, and location of the families.

Since the larger battery of behavioral assessments tested other aspects of temperament, behavioral tasks during each assessment were administered using a standard order at similar times of day to control for reactivity and carry over effects. The assessments were conducted by examiners who were trained research assistants or Ph.D.-level investigators. All data were collected and coded offline from videotaped recordings of the behavioral assessments using Noldus The Observer XT 10.0 (Noldus International Technology,Wageningen, the Netherlands). Following the behavioral assessments, the examiners completed the CARS based on observations of the participant during the assessment period.

Table 2.1 Group Means of Variables Across Sites

	Total		UNC		USC		Sig.
	N	M (SD)	N	M (SD)	N	M (SD)	p=
TD							
Age	36	3.42 (0.83)	30	3.69 (0.58)	6	2.02 (0.05)	.000
Cog. Ability	36	108.22 (15.11)	30	109.37 (16.04)	6	102.50 (7.77)	.317
CARS	36	15.42 (0.88)	30	15.22 (0.67)	6	16.42 (1.20)	.001
FXS Total							
Age	54	4.63 (1.31)	45	5.13 (0.70)	9	2.11 (0.11)	.00
Cog. Ability	53	50.51 (2.88)	44	50.27 (2.53)	9	51.67 (4.21)	.188
CARS	54	29.50 (6.18)	45	28.38 (5.79)	9	35.11 (5.13)	.002
fxASD							
Age	25	4.00 (1.44)	17	4.89 (0.72)	8	2.12 (0.11)	.000
Cog. Ability	25	49.68 (2.61)	17	48.94 (0.56)	8	51.25 (4.30)	.036
CARS	25	35.14 (3.35)	17	34.71 (2.66)	8	36.06 (4.56)	.355
FXS							
Age	29	5.17 (0.89)	28	5.28 (0.66)	1	1.99 (--)	--
Cog. Ability	28	51.25 (2.95)	27	51.11 (2.91)	1	55.00 (--)	--
CARS	29	24.64 (3.13)	28	24.54 (3.14)	1	27.50 (--)	--
iASD							
Age	11	4.42 (1.22)	--	--	11	4.42 (1.22)	--
Cog. Ability	11	52.91 (5.49)	--	--	11	52.91 (5.49)	--
CARS	10	35.05 (6.93)	--	--	10	35.05 (6.93)	--

Table 2.2 Descriptive and Demographic Data of Participants

	TD	FXS	fxASD	iASD
	N (%)			
Race				
Caucasian	36 (100%)	25 (86%)	20 (80%)	7 (64%)
African American	0 (0%)	3 (10%)	0 (0%)	0 (0%)
Other	0 (0%)	1 (3%)	5 (20%)	4 (36%)
	M (SD)			
Chronological Age	3.42 (0.83)	5.17 (0.89)	4.00 (1.44)	4.42 (1.22)
CARS	15.42 (0.88)	24.64 (3.13)	35.14 (3.35)	35.05 (6.93)
Mullen ELC	108.22 (15.11)	51.25 (2.95)	49.68 (2.61)	52.91 (5.49))
Mullen Nonverbal Composite	51.21 (9.20)	20.93 (2.39)	20.26 (1.30)	27.77 (13.64)
CBCL Anxiety Raw Score	2.26 (1.91)	3.96 (2.90)	3.90 (2.51)	3.56 (2.70)
CBCL Anxiety T-score	52.00 (3.06)	55.41 (7.30)	55.29 (5.95)	54.89 (5.64)

Table 2.3 Facial Expression Coding Definitions

Facial Region:	Movement:
Forehead/Brow	Entire Brow should be raised and drawn together; Brows may also look straighter across than usual; faint horizontal furrows may be present in forehead
Eyes/Nose/Cheek	Upper eyelids raise making the eyes appear wider; eye have tense appearance
Mouth/ Lips/ Chin	Lip corners are drawn straight back; mouth is usually less than wide open

Table 2.4 Behavioral Variables Coded for Facial Fear, Escape Behaviors, and Gaze

Behavior	Level	Behavioral Description
Facial Fear	0	No facial region shows codable fear
	1	One facial region shows codable fear/low intensity fear
	2	Two facial regions show codable fear or one region show very clear distinct facial fear
	3	Appearance change occurs in all three facial regions/impression of strong facial fear
Escape Behaviors	0	No escape behavior or social referencing
	1	Mild or fleeting escape behavior (e.g. turning away, sinking into chair)
	2	Moderate escape behavior resulting in significant, but not extreme attempts to get away or resist. Full body movements such as arching back, twisting away, and leaning away are included, as well as hitting, pushing and/or slapping.
	3	Vigorous escape behavior, usually involving linked, intense full-body movements like those found in "2". These usually last the entire epoch.
Gaze		Looking at parent; must be looking at the parent from above the shoulders
		Looking away; or nonsocial gaze; gaze behaviors not directed at the parent, stranger, or examiner.
		Looking at stranger; must be looking at the stranger from above the shoulders

Table 2.5 Correlations Among Behavioral Variables and CBCL Raw Scores

Group	Gaze-Stranger	Gaze-Away	Gaze-Parent	Escape Behaviors	Facial Fear
Total Participants	-.13	.12	.15	.02	-.03
TD	.00	.09	.02	.38*	-.08
FXS	-.05	-.02	.24	.00	-.20
fxASD	.04	-.10	.60**	.05	.11
iASD	-.10	.02	-.10	-.20	.45

CHAPTER 3

RESULTS

3.1 PRELIMINARY ANALYSES

Preliminary analyses were conducted to test the assumptions of normality, absence of outliers, homogeneity of variance, and linearity of the data. Two of the dependent variables, percentage of time gazing at parent and the facial fear composite score, were transformed due to a non-normal distribution across groups. A log and square root transformation were performed respectively to satisfy the assumption of normality. Additionally, because the dependent variables used in the analyses were based on either a proportion of time (e.g. percent of time gazing during stranger approach) or a composite score derived from durations of time, cases that had a total duration during the Stranger Approach for any behavior coded greater or less than 2 standard deviations from the mean were removed from analyses. A total of 6 cases (fxASD N= 1, FXS N= 2, iASD N= 1, and TD N= 2) were removed from analyses due to extreme total durations of time during the stranger approach. To test the assumption of homogeneity of variance, the Levene's test was performed to examine whether the variances of the groups differed across each dependent variable. Results from the Levene's test indicated that assumption of homogeneity of variance was satisfied for all variables under consideration (p's >.05). Furthermore, inspection of bivariate scatterplots between variables allow for the assumption of linear relationships across groups. To control for the cognitive ability of the atypical group, the fxASD, FXS, iASD groups were matched on their Mullen ELC

scores prior to data analyses. Descriptive data concerning each group's cognitive ability are presented in Table 2.2. All statistical analyses were performed using SPSS (Version 22; SPSS Inc., 2013-2014).

3.2 RESEARCH QUESTION 1: GROUP DIFFERENCES IN SOCIAL ANXIETY

A one-way analysis of variance (ANOVA) was conducted to evaluate the relationship between group and each measure of observed stranger fear. The independent variable, group, included four levels: the fxASD, FXS, iIASD, and TD groups. The dependent variables included behavioral measures of stranger fear including: gaze behaviors (e.g. percentage of time looking at the stranger, looking away, and looking at parent), escape behavior, and facial fear. Follow-up tests were conducted to evaluate pairwise differences among group means using Scheffe post-hoc comparisons.

Three separate one-way ANOVAs were performed to examine the relationship between group and percentage of time each participant spent gazing at the stranger, away, and at their parent during the Stranger Approach. The one-way ANOVA between group and the percentage of time spent looking at the stranger was significant F (3, 95) = 5.32, p = .002, η^2 = .15. The TD group (M = 45.41, SD = 19.74) spent a significantly higher percentage of time gazing at the stranger in comparison to the fxASD (M= 27.22, SD = 18.29) and iASD (M = 24.96, SD = 13.11) groups. The FXS, fxASD and iASD groups were not different in the proportion of time gazing at the stranger. The one-way ANOVA examining group differences for the percentage of time looking away was also significant F(3, 95) = 8.94, p = .000, η^2 = .23. The TD group (M= 41.98, SD = 17.95) spent a lower percentage of time looking away from the stranger than the fxASD (M= 66.99, SD = 19.27), iASD (M = 65.10, SD = 21.89), and the FXS (M = 56. 83, SD = 23.41) groups.

The FXS, fxASD and iASD groups did not differ in the proportion of time gazing away. The one-way ANOVA examining the relationship between group and gazing at parent was significant $F(3, 95) = 4.37$, $p = .006$, $\eta^2 = .13$. The fxASD group (M = .43, SD = .45) spent significantly less time looking at their parent than the FXS (M = .87, SD = .46) and the TD (M= .82, SD = .54) groups. The fxASD and iASD did not differ in the proportion of time gazing at the parent. These pairwise differences and significance levels for their corresponding gaze behaviors are listed in Table 3.1.

A one-way ANOVA was performed to examine the relationship between group and escape behavior during the Stranger Approach. No significant group differences were observed for escape behaviors $F(3, 100) = 1.56$, p = n.s. The one-way ANOVA between group and the behavioral composite of facial fear revealed significant findings $F(3, 94) = 6.60$, $p = .000$, $\eta^2 = .18$. The iASD group (M = .68, SD = .32) displayed more facial fear than both the TD (M = .32, SD = .30) and the FXS (M = .16, SD = .24) groups. The FXS and fxASD did not differ in the degree of facial fear displayed. Table 3.1 lists these pairwise differences and corresponding significance levels for the behavioral composite of facial fear.

3.3 RESEARCH QUESTION 2: AUTISM SYMPTOMS AND STRANGER FEAR

Multiple regression models were tested to examine the relationship between the two groups with high autism symptoms (e.g. the fxASD and the iASD groups) and symptoms of autism (e.g. CARS score) on each measure of stranger fear (e.g. gaze behavior, escape behavior, and facial fear). After centering the CARS scores and computing the group-by-CARS interaction term, the two predictors and the interaction were entered into a sequential regression model. The two main effects of group and

CARS scores were entered into the first model, followed by the interaction term in the second model (Keith, 2014).

Results indicated no significant group differences between the fxASD and the iASD groups on gaze behaviors of looking at the stranger, looking away, and looking at the parent. There was not a significant main effect of the combined groups CARS scores and gaze behaviors of looking at the stranger, away, and at the parent. The interactions between group and autism symptoms for all gaze behaviors were not statistically significant. Table 3.2 lists results of regression models for gaze behaviors.

Results indicated that there were no main effects of group or CARS scores on behavioral measures of escape behaviors during the Stranger Approach. Additionally, the interaction between group and CARS scores for escape behaviors was not statistically significant. Table 3.2 lists these results. Results indicated that there were statistically significant differences between the fxASD and the iASD group on measures of facial fear (b = -.320, SEb = .145, β = -.351, p =.036). The iASD group (M = .678, SD = .318) displayed significantly higher amounts of facial fear than the fxASD group (M = .328, SD = .430). Although CARS scores did not statistically significantly explain facial fear as a main effect, there was a positive trend observed (b = .017 SEb = .019, β = .182, p =.066) indicating that higher CARS are associated with greater amounts of facial fear during the Stranger Approach across both groups. The interaction between group and autism symptoms for facial fear was not statistically significant. Table 3.3 further describes the results of the regression model for facial fear.

Table 3.1 Post Hoc Results for Gaze Behaviors and Facial Fear

Group	Stranger Gaze Mean (SD)	Stranger Gaze Mean Differences Between Groups 1	2	3	4
1. fxASD	27.22 (18.29)	--			
2. FXS	31.94 (23.58)	4.71	--		
3. iASD	24.96 (13.11)	-2.27	-6.98	--	
4. TD	45. 40 (19.74)	18.18*	13.47	20.45*	--

Group	Away Gaze Mean (SD)	Away Gaze Mean Differences Between Groups 1	2	3	4
1. fxASD	66.99 (19.27)	--			
2. FXS	56.83 (23.41)	-10.16	--		
3. iASD	65.10 (12.98)	-1.89	8.27	--	
4. TD	41.98 (17.95)	-25.00***	-14.85*	-23.12*	--

Group	Parent Gaze Mean (SD)	Parent Gaze Mean Differences Between Groups 1	2	3	4
1. fxASD	0.43 (0.45)	--			
2. FXS	0.87 (0.46)	0.44*	--		
3. iASD	0.61 (0.42)	0.17	-0.27	--	
4. TD	0.82 (0.54)	0.38*	-0.06	0.21	--

Group	Facial Fear Mean (SD)	Facial Fear Mean Differences Between Groups 1	2	3	4
1. fxASD	0.33 (0.43)	--			
2. FXS	0.16 (0.24)	-0.17	--		
3. iASD	0.68 (0.32)	0.35*	0.52***	--	
4. TD	0.32 (0.30)	-.01	0.16	-0.36*	--

Table 3.2 Predictors of Gaze Behaviors

	Stranger		Away		Parent	
Variable	Model 1 *B*	Model 2 *B*	Model 1 *B*	Model 2 *B*	Model 1 *B*	Model 2 *B*
Group	0.08	0.08	0.06	0.06	-0.22	-0.22
CARS	-0.20	-0.18	0.15	0.08	-0.03	0.17
GroupXCARS		-0.02		0.12		-0.33
R^2	.04	.04	.03	.04	.05	.12
F	0.71	0.46	0.43	0.37	0.77	1.29
ΔR^2		.00		.01		.07
Δ F		0.01		0.26		2.28

Table 3.3 Predictors of Escape and Facial Fear Behaviors

	Escape		Facial Fear	
Variable	Model 1 *B*	Model 2 *B*	Model 1 *B*	Model 2 *B*
Group	-0.05	-0.05	-0.35*	-0.35*
CARS	0.11	0.11	0.30	0.18
GroupXCARS		0.01		0.20
R^2	.02	.02	.21	.24
F	0.25	0.16	4.22	3.11
ΔR^2		.00		.02
Δ F		0.00		0.92

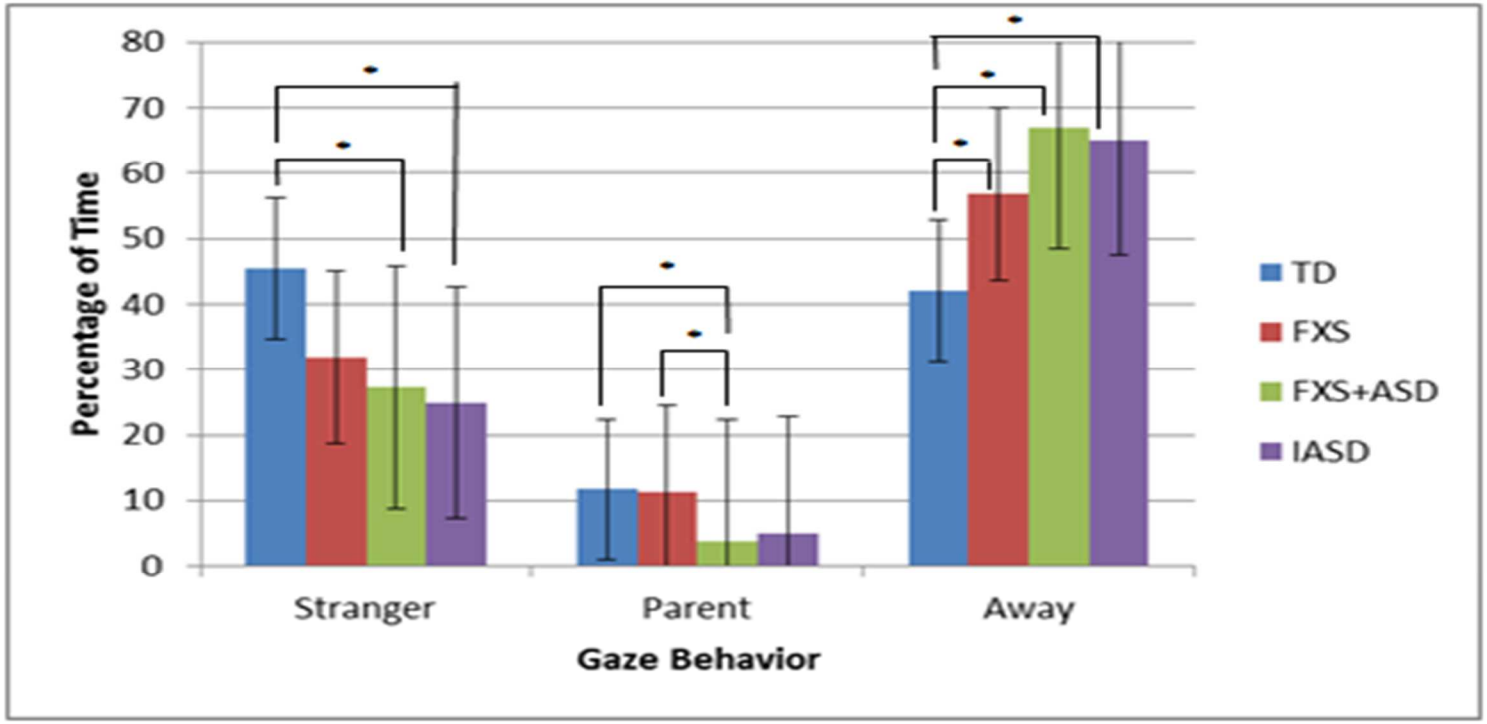

Figure 3.1 Group Differences in Average Levels of Gaze Behaviors.

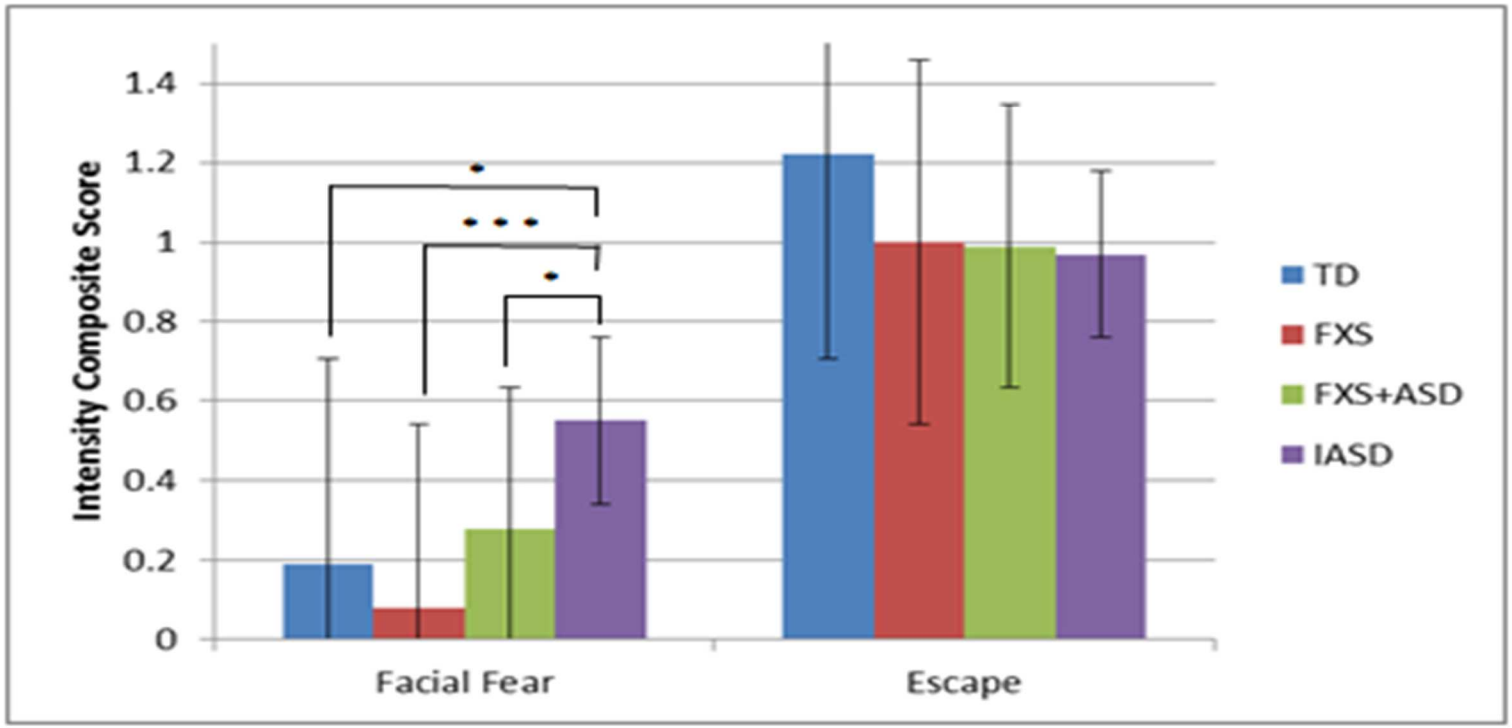

Figure 3.2 Group Differences in Average Levels of Facial Fear and Escape Behaviors.

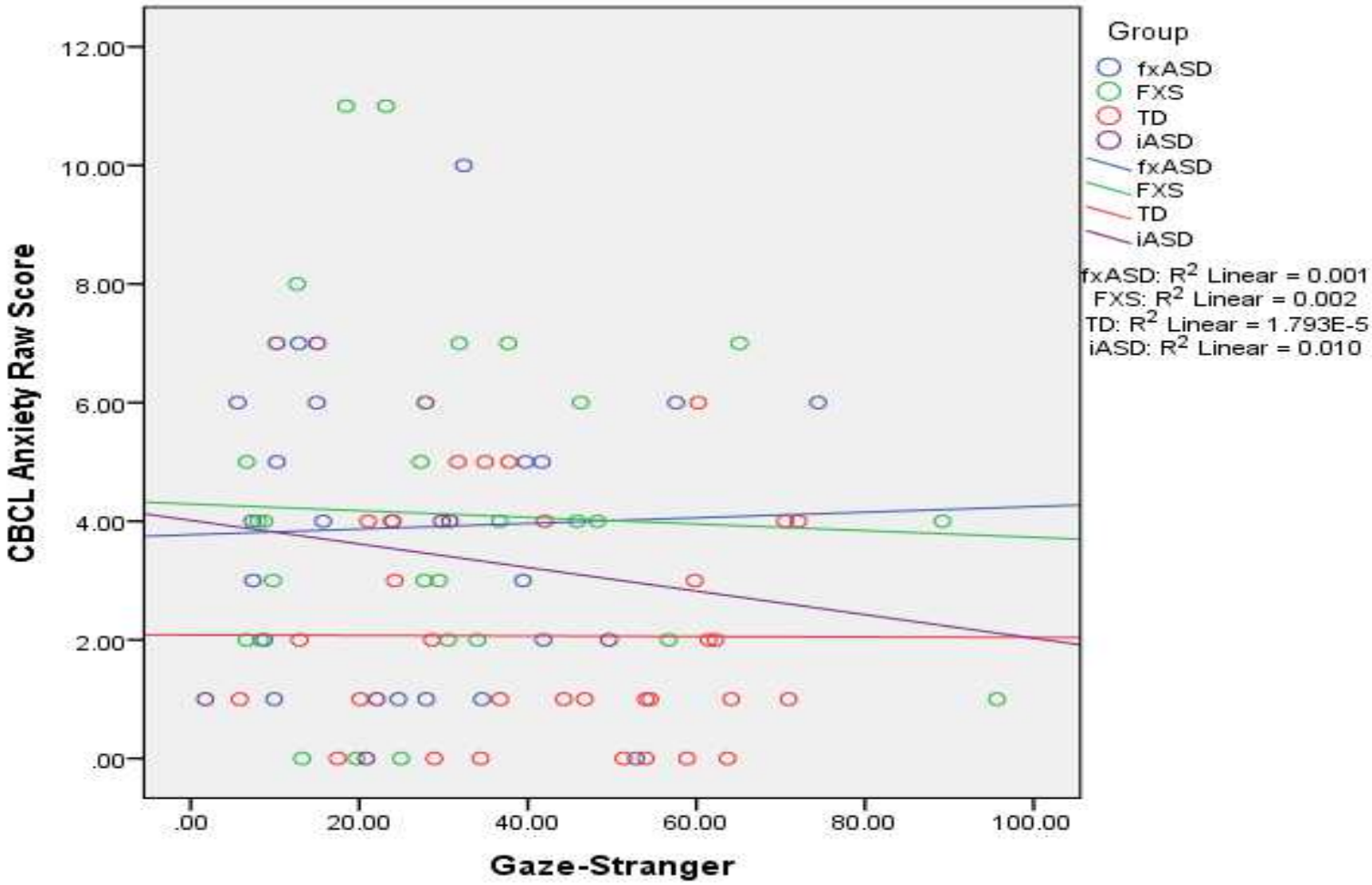

Figure 3.3 Scatterplot of CBCL Anxiety Subscale and Stranger Gaze Behavior.

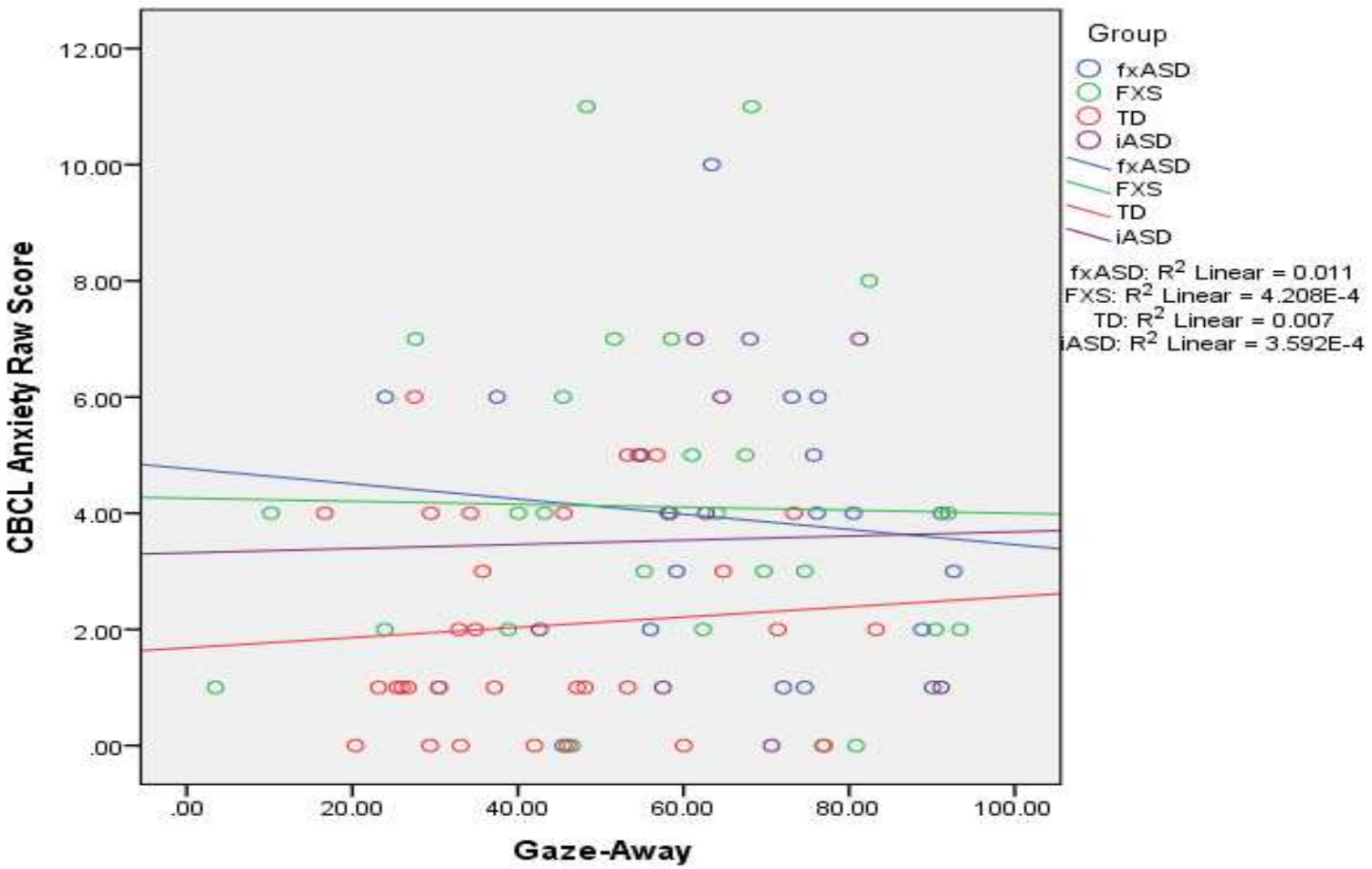

Figure 3.4 Scatterplot of CBCL Anxiety Subscale and Gazing Away Behavior.

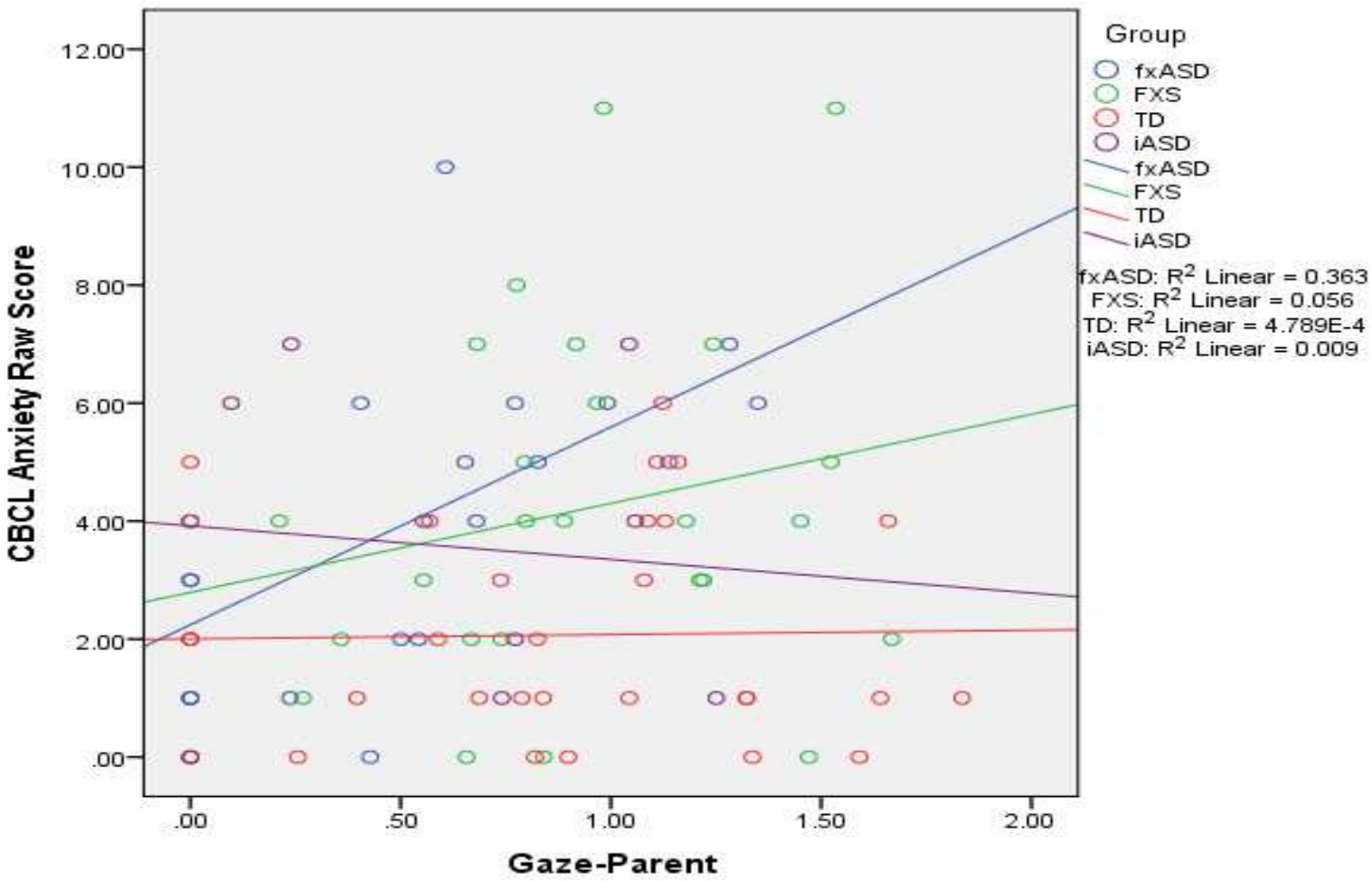

Figure 3.5 Scatterplot of CBCL Anxiety Subscale and Parent Gaze Behavior.

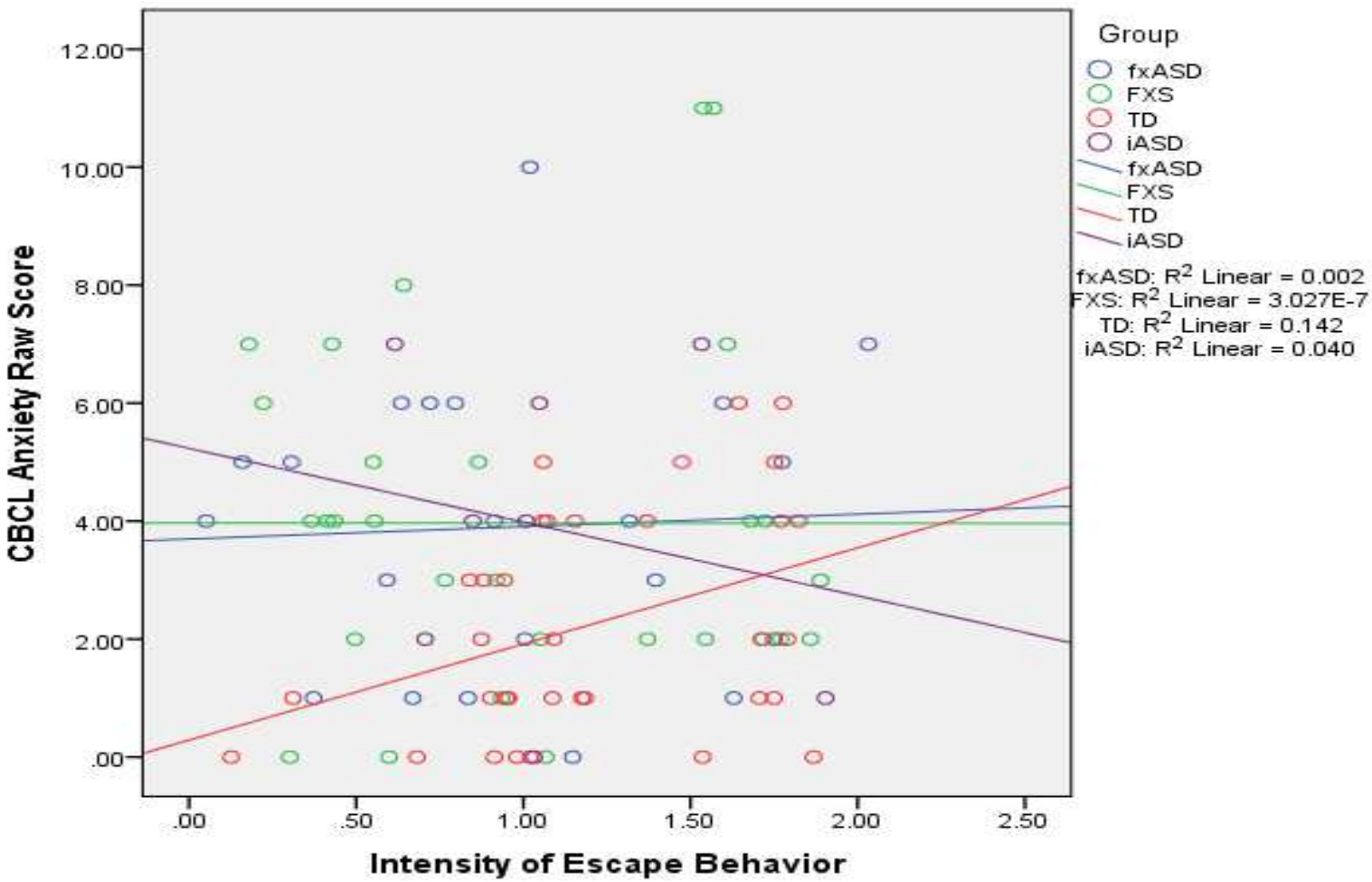

Figure 3.6 Scatterplot of CBCL Anxiety Subscale and Escape Behavior.

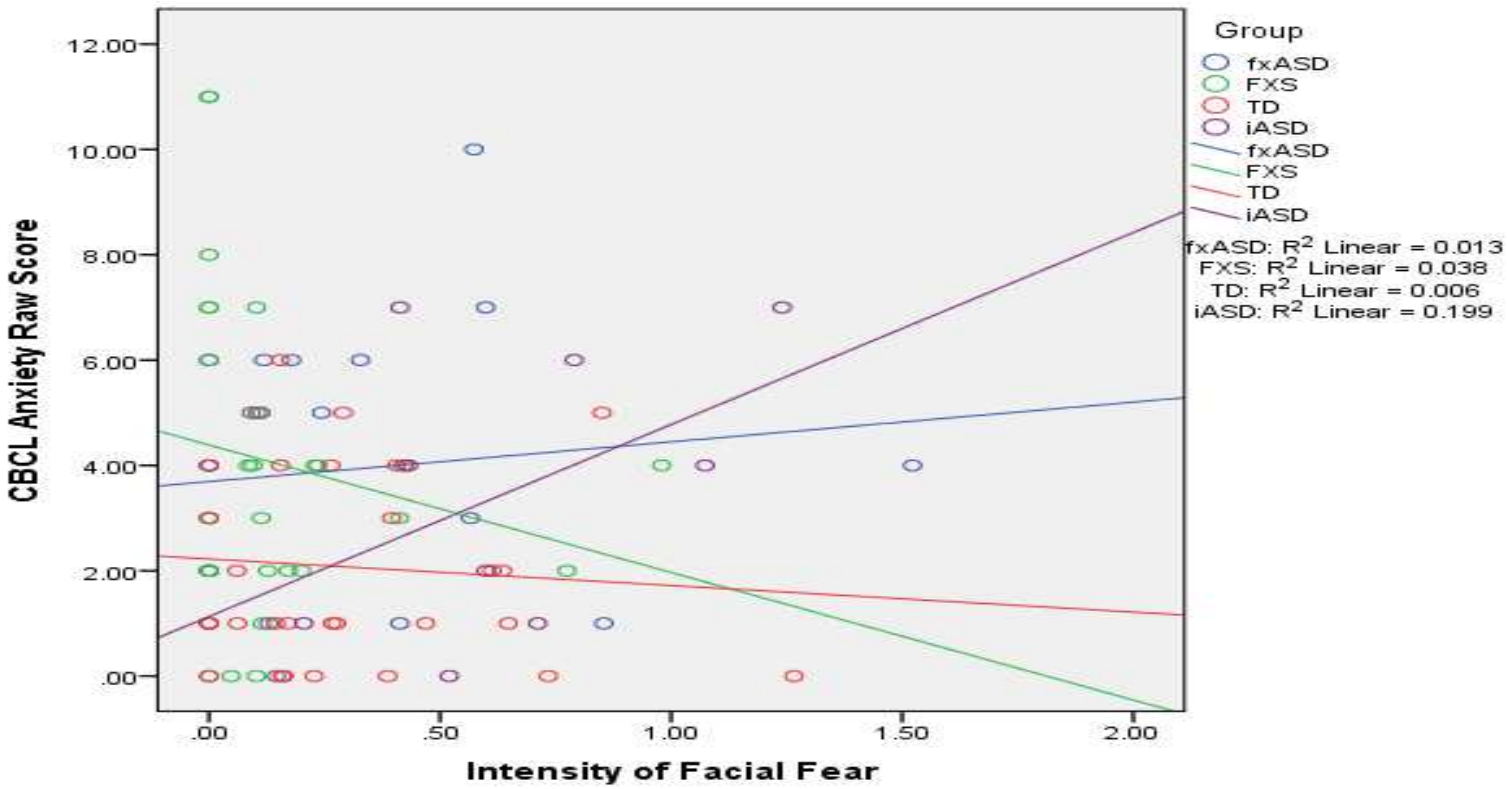

Figure 3.7 Scatterplot of CBCL Anxiety Subscale and Facial Fear.

CHAPTER 4

DISCUSSION

Fragile X syndrome is a single gene disorder associated with an increasingly well-defined phenotype including intellectual impairment and anxiety (Cordeiro et al., 2011; Muris et al., 1998). In addition, autism traits are very common in FXS with increased impairment associated with these co-occurring and overlapping disorders making differential diagnosis challenging but important (Hagerman, 2002; Harris et al; 2008). Despite the prevalence and impact of anxiety in FXS, little work has examined how anxiety features emerge in FXS or other clinical disorders. Moreover, no study has examined behavioral observations of fear and its association with autism traits in young children with FXS or disassociated patterns in FXS from children with idiopathic autism spectrum disorder. This work is important not only to contribute to our understanding of individual differences that predict anxiety in young children but cross-syndrome studies such as this one provide important information to the FXS and ASD fields where attention to latent heterogeneity is often lacking. The early detection of specific behavioral anxiety factors that may convey elevated risk to groups at-risk for anxiety is important given the impact that differential diagnosis and targeted treatments can have on improving the performance and changing the trajectory of the later development of anxiety disorders (Rapee et al., 2010). Fear of strangers has been shown to be a particularly robust predictor of the emergence and severity of anxiety in young children (Brooker et al., 2013; Kagan, 2000).

In this study, we examined multiple behavioral indicators of stranger fear and their relationship to autism symptom severity in FXS using a normative and cross-syndrome approach. Well-controlled standardized observations of stranger fear were conducted with preschool boys with FXS contrasted to TD boys to generate information regarding deviations from normative standards. In addition, we contrasted observations of stranger fear within the group with FXS by contrasting those with elevated symptoms of ASD to those with a low degree of ASD symptomology as well as conducting comparisons to boys with idiopathic (non-FXS) ASD to detect features that may convey shared or unique patterns across these groups. In order to explain how individual factors are influenced by novel environmental contexts, multiple behaviors of social fear were studied within a temperamental framework by looking at gaze patterns, escape behaviors and facial fear. Our results indicate important group distinctions with normative differences being more pronounced in the fxASD group than FXS group and specific fear responses differentiating those with elevated ASD symptoms (iASD and fxASD) from those with fewer ASD symptoms (FXS and TD).

4.1 GROUP DIFFERENCE IN STRANGER FEAR

Comparisons to the TD normative group indicated important distinctions across the two FX groups with increased deviation from the normative group observed in the fxASD group. Specifically, the group with FXS differed from the TD group only by demonstrating more time gazing away while the fxASD group differed in more time gazing away but also less time gazing at the stranger and at the parent. Our results support findings that indicate children with FXS have a specific profile of social behavior in response to situations and individuals that are novel (e.g. a stranger). Roberts and

colleagues (2009) demonstrated consistent patterns of avoidant gaze behavior in their social approach paradigm with individuals with both FXS and fxASD displaying similar levels of poor eye contact during the initial approach of a novel person in comparison to TD children. However, individuals with FXS improved their eye contact over the course of the assessment period, whereas boys with fxASD displayed avoidant eye contact across the entire assessment. We demonstrated consistent patterns between our preschoolers with FXS and fxASD and their avoidant gaze patterns to the initial approach of a stranger to previous work (McDuffie et al., 2010; Roberts et al., 2009; Roberts et al., 2007). In the current study, the total duration of the stranger approach consisted of two minutes, rather over the course of entire assessment period of a few hours; thus acting as a measure of "immediate" gaze behavior. However, in our brief observation we documented that preschoolers with fxASD displayed more avoidant gaze patterns of looking away from the stranger and parent in comparison to both the FXS and TD groups. Previous studies have found similar discrepancies in the modulation of eye contact between FXS and fxASD, which suggest that individuals with FXS may need time to "warm up" to social or novel situations (Roberts et al., 2009; Roberts et al., 2007).

The elevated avoidant gaze patterns in fxASD support previous work that suggest dysfunction in physiological mechanisms involved in regulating social fear in individuals with increased symptoms of autism in FXS (Hall et al., 2006; Hessl et al., 2002; Hessl, Glaser, Dyer-Friedman, & Reiss, 2006; Roberts et al., 2009). The hypothalamic-pituitary-adrenal (HPA) axis is involved as a putative mechanism for social withdrawal in FXS. The observed differences in gaze patterns between FXS and fxASD may be related

to the disruption of the hypothalamic-pituitary-adrenal (HPA) axis, with studies reporting unique relationships in abnormal social behavior and cortisol reactivity (Hessl et al., 2006; Roberts et al., 2009). For example, Roberts et al. (2009) found that boys with FXS and fxASD have discernable profiles with elevated cortisol and less cortisol reactivity being associated with more severe symptoms of autism. Therefore, increased cortisol as a result HPA dysfunction may be a biomarker for elevated symptoms of autism in FXS that contribute to avoidant behavior and social fear (Roberts et al., 2009). Collectively, our distinct profiles of social fear through gaze patterns support findings documented in the social behavior and physiological mechanisms associated with FXS and fxASD that differ from the normative TD group, as well as from each other.

Unlike gaze behavior, no group differences were detected between normative TD children and those with FXS and fxASD on behavioral measures of escape and facial fear. Our findings reflect that features of stranger fear are less sensitive to behavioral differences of escape behaviors and facial fear across our normative and atypical groups. Despite not finding any behavioral differences across groups for escape behaviors during the stranger approach, we observed a moderate positive relationship between the CBCL Anxiety subscale and escape behaviors ($r = .38$) in our TD group only. These results suggest that our TD preschoolers are displaying anxiety through increased behaviors of escape in comparison to our FXS and fxASD samples. This may be attributed to their immature developmental and cognitive level given the younger chronological age of the TD group ($M = 3$ years) in relation to the FXS ($M = 5$ years) and fxASD ($M = 4$ years) groups.

Although, Hall et al. (2006) observed that males with FXS are more prone to display problem behaviors in social situations that include escape behaviors of face-hiding, fidgeting, refusal, eye-rubbing, leaving the chair, and hand biting; the former study included older adolescents and used different methodology to elicit social anxiety (e.g. a face-to-face interview, a singing task, a silent reading task, and an oral reading task). In the current study, our sample was much younger (ages 2-5 years) reflecting that social fear exhibited through facial fear and escape behaviors may not be present yet or there may be more behavioral variability in these young groups as symptoms of anxiety or fear are beginning to emerge. Previous work has observed elevated profiles of fearful behavior in TD preschoolers in response to a stranger (Brooker et al., 2013). For example, Brooker et al. (2013) found that in their sample, infants that displayed more facial fear in response to a stranger exhibited more behavioral inhibition as preschoolers reflecting specific profiles of emerging anxiety over time. Although our findings are different from previous work that found infants with FXS displayed more facial fear over time in response to a stranger in comparison to TD infants (Tonnsen et al., 2013), disparities in results may be due to the age of the participants used in each respective study. For example, Tonnsen et al. (2013) sampled FXS participants that had an average age of 2 years, whereas the average age of the preschoolers with FXS in the current sample was 5 years of age. Stranger fear may be developmentally sensitive and reflect variable behavioral trajectories of escape behaviors and facial fear over time that is dependent on age.

The two groups with FXS displayed similar behavior in terms of their gaze at the stranger and away with no differences in facial fear and escape behavior during the

Stranger Approach. The only difference across the two groups with FXS was in the degree of parental referencing observed with boys with fxASD looking at their parent less than the FXS group. Of interest, the group with iASD also looked less at their parent than the FXS group and the two groups with elevated ASD features, iASD and fxASD, were not different from each other with both groups displaying reduced parental referencing. However the group with iASD displayed elevated facial fear that distinguished them from the FXS, fxASD and TD groups. Thus, we observed important group differences representing differential responses to a social stressor indicating both shared and unique profiles across our groups.

These distinctive patterns are consistent with relationships indicated through parent report of symptoms of anxiety on the CBCL and behavioral observations during the stranger approach. Correlational data suggest unique patterns in each of our groups in how anxiety is expressed towards a stranger. For example, our FXS groups displayed positive relationships of looking at their parent and parent-report of anxiety with our fxASD group displaying a stronger relationship ($r = .60$) than our FXS group ($r = .24$). However, our iASD group appeared to display anxiety through the expression of facial fear indicated by a moderately positive relationship ($r = .45$) between parent-report of anxiety and facial fear towards a stranger and their elevated levels of facial fear recorded in response to the stranger. Finally, as previously discussed, our TD group displayed a moderate positive relationship ($r = .38$) in escape behaviors to parent-report of symptoms of anxiety. These results suggest that behavioral profiles of anxiety towards a stranger may be expressed differently across both normative and atypical development.

Although individuals with FXS and fxASD both share similar genetic profiles with FMR1 gene dysfunction, social impairments associated with ASD may differentiate these groups and predispose those with higher autism symptomology to reference social stimuli less in novel or stressful situations. Previous work supports a positive relationship between autism symptom severity and social impairments (Bailey et al., 2001; Brock & Hatton, 2010; Roberts et al., 2007; Wolff et al., 2012). In our study, we observed that boys with fxASD spent less time looking at social stimuli (e.g. the stranger and their parent) and a greater proportion of time looking away than boys with FXS. These patterns of behavior in boys with greater symptoms of autism and FXS suggest a profile of aloofness or social withdrawal. Our patterns of similar social avoidant or withdrawn behavior towards a stranger in preschoolers with fxASD also correspond with previous studies that compared differences in social behavior in FXS and fxASD (Bailey et al., 2001; Brock & Hatton, 2010; Kaufmann et al., 2004; Roberts et al., 2009; Roberts et al., 2007). However, McDuffie et al. (2010) did not find any group differences in social impairments in their sample of individuals FXS and fxASD using the Autism Diagnostic Interview-Revised (ADI-R). Discrepancies in results may be due to methodology, as the ADI-R is a parent interview opposed to behavioral observations seen in the stranger approach design and ADOS.

Our results are also consistent with several studies that compared attachment behavior in relation to symptoms of autism using social stimuli with familiar (e.g. a parent) and /or novel (e.g. a stranger) condition in preschoolers with iASD compared to FXS, fxASD, and/or TD groups (Cohen et al., 1991; Pisula, 2004; Sigman & Mundy, 1988). Our work corresponds with findings that preschoolers with iASD looked less

frequently towards a stranger, reference their mother less often, and spent greater amounts of time avoiding social initiation by looking away (e.g. at a nearby door) than children that were typically developing during a social approach task (Pisula, 2004). Also, the patterns we observed in the iASD group of facial fear towards a stranger are consistent with literature that has documented individuals with iASD have trouble not only recognizing, but regulating their own facial expressions due to dysfunction of the amygdala (Baron-Cohen et al., 2000). Interestingly, in our sample, the TD group displayed the greatest proportion of time looking at the stranger, the potential fear-provoking stimulus, in comparison to the other groups. However, associations between parent-report of anxiety and behavioral responses of looking to the stranger suggest no relationship ($r = .00$) between the two measures of anxiety in the TD group. Instead, the TD group may be expressing more fear towards the stranger through escape behaviors rather than gaze patterns.

4.2 RELATIONSHIP OF AUTISM SYMPTOMS AND SOCIAL ANXIETY

Given the high overlap of ASD and anxiety in FXS (Cordeiro et al., 2011; Harris et al., 2008) a more comprehensive investigation of the relationship between continuous symptoms of autism and behavioral outcomes of stranger fear was studied in our iASD and fxASD groups using the Stranger Approach. Overall, we found that our iASD and fxASD groups displayed similar levels of stranger fear through gaze patterns and escape behaviors. Similar to our previous research question, we found that iASD displayed more facial fear than the fxASD group to the stranger but these relationships were not influenced by the severity of autism symptoms. Additionally, the severity of autism symptoms did not predict stranger fear in any of the behavioral variables, although a

positive trend was observed in facial fear. Our results correspond with findings from a study that investigated differences in social behaviors between iASD and fxASD in terms of profiles involving differences in facial expressions (Wolff et al., 2012). Conversely, the previous study also found differences in gaze patterns between the two groups with elevated symptoms of autism. Since anxiety is highly comorbid in individuals with FXS, some studies suggest differences in fxASD and iASD are the result of impairments from anxiety rather than autism (Cordeiro et al., 2011; Talisa, Boyle, Crafa, & Kaufmann, 2014; Tonnsen et al., 2013).

Contributing to the complexity, overlapping symptoms of anxiety in ASD makes it difficult to not only differentiate symptoms of anxiety from autism, but also to measure (Lecavalier et al., 2014). Although our results did not support that individuals with fxASD display more fear compared to iASD, one reason for this inconsistency may be due to the methodology employed. Previous studies used measures of autism (e.g. ADOS, ADI-R, etc) to compare differences between iASD and fxASD groups (Wolff et al., 2012), while in the present study we used behavioral responses of social fear to a stranger as outcome measures. Additionally, the methods in which we categorized our groups with high ASD symptoms used data from the CARS, while other studies have used the ADOS distinguish their groups with and without ASD (Wolf et al., 2012). Although there is high consistency between the CARS and the ADOS in our study ($r = .90$) and in others ($r = .43$; Reszka et al., 2013), there may be differences in the sample of participants that are categorized based on one measure over the other.

4.3 SUMMARY AND IMPLICATIONS

Overall, we found specific behaviors of social fear that were clear differentiating factors in both our cross-syndrome and normative groups. All of our atypical groups responded to the stranger by spending more time avoiding gaze with the stranger or parent in comparison to our TD group and those with more severe symptoms of autism displayed the greatest proportion of avoidant gaze patterns. These cross-syndrome differences indicate unique patterns of social fear that are influenced by autism symptomology. In the FXS groups, preschoolers with more severe symptoms of autism spent the least amount of time looking at their parent during the stranger approach. However, when comparing the groups with elevated autism symptomology, facial fear was the only behavior that differentiated fxASD from iASD with the iASD displaying more social fear through their facial expression. Collectively these results suggest that specific behavioral factors differentiate ASD in FXS, with parental social referencing when a novel person (e.g. a stranger) approaches as a particularly salient marker.

Similarly, fxASD and iASD can be distinguished through facial responses to a stranger. Both gaze patterns and facial fear have potential as distinguishing features in cross-syndrome comparisons. However, patterns of escape behavior provided little evidence in differentiating not only atypical groups from each other, but also atypical groups from normative development. No study has compared how social fear emerges in young children with FXS, fxASD, and iASD using behavioral methodology. Therefore, this study contributes to the literature by providing an increased understanding to how anxiety emerges in FXS in comparison to normative development and in iASD using behavioral indicators in response to a stranger approach. We also found a limited

association between the CBCL anxiety subscale and our stranger behavioral factors, which indicates that these two measures may be different in terms of what they are measuring or in their sensitivity to measure anxiety in this sample. Brooker et al. (2013) demonstrated similar relationships between parent-report measures of anxiety and behavioral responses to a stranger. Given this correspondence is similar across studies, it suggests that behavioral measures of social fear may be distinguishing factors of emerging anxiety that may not be easily captured through traditional parent-report measures.

4.4 LIMITATIONS AND FUTURE DIRECTIONS

Our study is not without limitations, irrespective of being one of the first studies to examine behavioral profiles of social fear in preschoolers with FXS contrasted to iASD. First, the current study was cross-sectional opposed to longitudinal in terms of data collection and analysis. Longitudinal studies would allow for trends to be seen over time and in relation to how social fear emerges and changes in development. Second, the participants included in our sample were only male, which may limit generalizations made from these results that include females. Finally, although there was high agreement between the CARS and ADOS-2, we were limited in using CARS data to categorize our groups. Clinical best estimates using the ADOS-2 is considered the gold-standard for diagnosing autism, and differs from the CARS in providing specific behavioral presses to measure autism symptomology rather than relying on naturalistic observation during a period of time (Lord et al., 2000).

Future studies should consider studying multiple observations or periods that measure social fear. Roberts et al. (2007) demonstrated a change over time in gaze

patterns the differentiated the FXS and fxASD groups. Therefore, breaking observation durations into initial and end states may reflect different patterns of behavior across groups with FXS and ASD. Also, because females with FXS are often less impacted than males with FXS, behavioral symptoms of social anxiety may be expressed differently due to gender differences associated with the unique genetic phenotype (Bennetto et al., 2001). The interplay of how gender influences the emergence of social fear in FXS over time should be investigated to better understand the development of this disorder. Additionally, given the genetic and physiological etiologies involved in the phenotypes of iASD and FXS, a biobehavioral model to study anxiety using biomarkers, such as cortisol or heart activity, and behavioral outcomes might be informative. The use of biomarkers provides information in children and individuals with lower cognitive ability, and thus impaired ability to self-report, by reflecting various states of arousal. Finally, although differential diagnosis is important, family systems impact child development as well (Kreppner & Lerner, 2013). Parental anxiety, as well as parent-child interactions during socially stressful situations should be studied in relation to comorbid conditions, since parents are often involved in treatment and a factor for positive developmental outcomes.

This study has specific contributions to behavioral research with children with developmental disabilities are that are unique in several regards. First, this is the first study to the knowledge of the authors, to examine the early emergence of behavioral indicators of anxiety at any age in boys with FXS and iASD. Additionally, our use of behavioral methodology goes beyond traditional parent and self-report methods that may differentiate individuals that are at risk for anxiety, such as those with ASD and FXS. Finally, few studies have examined the interplay of anxiety and autism using both

categorical and continuous methods in FXS and fxASD with young children and social approach paradigms. Collectively, we have found behavioral profiles of social fear that are both distinct yet similar in disorders that often overlap in symptom presentation, consequently make differential diagnosis and treatment difficult. Therefore, it is critical to identify underlying traits that distinguish symptoms of anxiety in ASD and FXS early in development to provide better future outcomes.

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