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Biobehavioral Measurement of Heterogeneity In Autism Spectrum Disorder

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BIOBEHAVIORAL MEASUREMENT OF HETEROGENEITY IN AUTISM SPECTRUM DISORDER

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ABSTRACT

Autism spectrum disorder (ASD) is a highly prevalent neurodevelopmental disorder characterized by the presence of social-communication deficits and restricted and repetitive interests and behaviors (American Psychiatric Association, 2013b; Baio et al., 2018). It is characterized by high genotypic and phenotypic heterogeneity, which complicates both diagnosis and treatment efforts (Jeste & Geschwind, 2014; Kim, Macari, Koller, & Chawarska, 2016). Well-studied sources of heterogeneity in ASD include age, sex, intellectual ability, temperament, physiological arousal, social attention, and the presence of genetic syndromes that are highly penetrant with ASD (Campbell, Shic, Macari, & Chawarska, 2014; Harris et al., 2008; Klusek, Roberts, & Losh, 2015; Macari, Koller, Campbell, & Chawarska, 2017).

Symptoms of ASD can resemble those of other diagnoses, especially social anxiety and intellectual disability (ID; Kerns & Kendall, 2012; Roberts et al., 2018; Thurm, Farmer, Salzman, Lord, & Bishop, 2019). These phenotypic overlaps greatly complicate the diagnostic process in children with neurodevelopmental disorders. As a result, researchers have begun to use biobehavioral measurement markers to support the differentiation of social anxiety and ASD in children with neurodevelopmental disorders. A biobehavioral framework integrates behavioral observations and biological data as indicators of complex characteristics or processes. By so doing, it has the potential to supplement current diagnostic procedures by providing quantifiable information about a child's overall functioning that takes into account a variety of developmental systems.

Towards this end, this dissertation employed biobehavioral measurement procedures to inform our understanding of the ASD phenotype across preschoolers with varied genetic risk for ASD and social anxiety.

Although ASD in itself is highly heterogeneous, it can also occur concomitantly with additional disorders and psychological problems. One of the most common genetic disorders associated with ASD is fragile X syndrome (FXS). Fragile X syndrome is a rare monogenic disorder caused by expansions of a CGG repeat on the *FMR1* gene (R. Hagerman, Turk, Schneider, & Hagerman, 2014). This mutation leads to an under-expression of fragile X mental retardation protein (FMRP) and results in several associated physical features and psychological characteristics, including ASD symptoms, social anxiety, and intellectual disability (Bailey, Raspa, Olmsted, & Holiday, 2008).

Given the complicated nature of heterogeneity in ASD, robust phenotypic characterization of ASD is critical to adequately assessing the various profiles of this disorder for both diagnostic and treatment purposes. In particular, much work needs to be done to understand which features are potentially indicative of comorbid disorders and which are part of the ASD phenotype. The proposed dissertation furthers our understanding of heterogeneity in ASD through a series of two studies aimed at cataloging phenotypic profiles among children with etiologically distinct (e.g., syndromic versus non-syndromic) risk factors for ASD.

The first study seeks to understand how two biological mechanisms, temperamental negative affect and baseline physiological arousal inform our understanding of ASD and other comorbid clinical symptoms. It addresses the following research questions: how do physiological regulation and temperamental negative affect

differ in preschoolers with non-syndromic ASD (nsASD), FXS, and typical development (Research Question 1); and do physiological regulation and temperamental negative affect predict ASD symptomology and social anxiety symptoms within all groups (Research Question 2)? The second study aimed to clarify how social attention impairments manifest in nsASD, FXS, and TD and how social attention, a neurally-based biobehavioral process, relates to clinical symptoms. This study asks: how do preschoolers with nsASD, FXS, and typical development differ in their allocation of attention to faces in response to social and non-social scenes? (Research Question 1); and how does attention to faces during social scenes relate to developmental ability, ASD symptom severity, and social anxiety within groups? (Research Question 2)?

Taken together, these studies leverage biobehavioral methods to understand the influence of genetics, biology, and behavior on the presentation of ASD and related comorbidities. Results will address critical gaps in our understanding of the entire spectrum of ASD and have the potential to inform more targeted intervention techniques.

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CHAPTER 1

THE CONTRIBUTIONS OF NEGATIVE AFFECT AND PHYSIOLOGICAL AROUSAL TO SOCIAL ANXIETY AND AUTISM SPECTRUM DISORDER[1]

[1]Wall, C. A. & Roberts, J. E. To be submitted

Abstract

Autism spectrum disorder (ASD) is a highly heterogeneous disorder that presents with diagnostic challenges, pointing to a need for robust, objective markers of symptomology. These challenges are exacerbated by the phenotypic overlap between ASD and other diagnoses, including social anxiety and genetic disorders like fragile X syndrome (FXS). Biobehavioral measurement approaches integrate behavioral and biological data, and by so doing have the potential to address diagnostic challenges and shed light on the mechanisms underlying social impairments. The present study utilized a biobehavioral approach to evaluate 1) how physiological regulation, indexed by baseline respiratory sinus arrhythmia (RSA) and temperamental negative affect differ in a sample of 120 preschoolers with non-syndromic ASD (nsASD), FXS, and typical development, and 2) whether physiological regulation and temperamental negative affect predict ASD symptomology and social anxiety symptoms. Results indicated that children with nsASD, but not those with FXS, differ from typically developing children in their negative affect. Additionally, findings corroborated prior work indicating that negative affect predicts social anxiety but not ASD severity in children with FXS and established the same relation in children with nsASD. Finally, this study found that baseline RSA was a unique predictor of ASD severity for nsASD, but not those with FXS or typical development. Taken together, the present study provides evidence that biobehavioral markers can differentiate the presence of social anxiety and ASD in different genetic risk groups. In addition to providing diagnostic clarity using objective measurement, these findings can inform our understanding of the mechanisms behind these disorders.

Introduction

Autism Spectrum Disorder (ASD) is a highly prevalent and impairing neurodevelopmental condition characterized by impairments in social communication and the presence of restricted and repetitive interests and behaviors (American Psychiatric Association, 2013b; Baio et al., 2018). Social anxiety is a specific category of anxiety that, much like ASD, is associated with social symptoms including avoidance, anxious anticipation, or distress in social situations (American Psychiatric Association, 2013b). Because both ASD and social anxiety have significant overlap in behavioral symptoms, differentiating when these disorders occur independently or jointly poses challenges (Kerns & Kendall, 2012). These challenges are magnified when trying to differentially diagnosis ASD and social anxiety in young children who have limited language and poor insight which makes self-report measures inappropriate. However, differential diagnoses of ASD and social anxiety are critical to initiate targeted treatments known to reduce severity (Dawson & Bernier, 2013; Rapee, 2013). To address these challenges, a biobehavioral measurement approach that utilizes multiple sources of functioning and recognizes that social behavior (such as ASD and anxiety symptoms) is driven by biological underpinnings can improve diagnostic clarity. We apply a biobehavioral framework to the present study by examining physiological and temperament as biological factors that provide insight to ASD and social anxiety diagnoses in two distinct groups of preschoolers at high genetic risk for ASD. We accomplish this in two ways. First, we evaluate differences in variables known to be biologically based (i.e., physiology and temperament) across the two groups at high risk for ASD and social anxiety. Second, we determine the association of the biologically

based variables to ASD and social anxiety behavioral symptoms and potential group distinction across these high-risk groups.

Integrating a biobehavioral framework with a genetic model of ASD has the potential to accelerate discoveries regarding the underpinnings of ASD and the association of ASD with social anxiety. Both ASD and social anxiety have clear genetic underpinnings (Jeste & Geschwind, 2014; Scaini, Belotti, & Ogliari, 2014); however, they are complex genetic disorders. The high comorbidity of ASD with social anxiety suggests shared genetic influences, and studies that examine the co-occurrence of multiple disorders within an identified genetic syndrome can advance the field (Willcutt, 2019). One of the most promising genetic models of ASD is fragile X syndrome (FXS; Abrahams & Geschwind, 2010). Fragile X syndrome is a rare monogenic disorder caused by mutations on the *FMR1* gene and the ensuing under-expression of its associated fragile X mental retardation protein (FMRP; Verkerk et al., 1991). Fragile X syndrome is associated with a range of social impairments that are highly associated with both ASD and social anxiety, including social avoidance and reduced eye contact (Roberts et al., 2019; Roberts, Weisenfeld, Hatton, Heath, & Kaufmann, 2007). Furthermore, FXS has the highest penetrance for any single gene disorder associated with ASD; 50-75% of males and 25% of females with FXS meet diagnostic criteria for ASD (Cordeiro, Ballinger, Hagerman, & Hessl, 2011; Klusek, Martin, & Losh, 2014). Individuals with FXS also have a high risk for anxiety disorders with current estimates that 86% of males and 77% of females with FXS have an anxiety disorder (Cordeiro et al., 2011; Ezell et al., 2019; Kaufmann et al., 2004). The high co-occurrence of ASD and anxiety make FXS an ideal candidate for the study of the diagnosis and measurement of both social anxiety and

ASD and their independence in a discreet single gene disorder. Contrasting temperament and physiological indices and their relationship to ASD and social anxiety features in children with FXS to children with ASD not associated with a known syndrome (nsASD) will help disentangle the role of genetics to understand comorbidity in both groups.

Temperament and Physiological Indices in nsASD and FXS

Temperamental negative affect refers to a biological tendency towards negative emotions such as fear, anger, or sadness (Rothbart & Bates, 2007). It has been linked to neural functioning, especially amygdala activation, as well as autonomic and behavioral responses; it is also present in infancy and persists through toddlerhood (Rothbart & Bates, 2007). Negative affect is reliably measured using parent-report, making it a useful and feasibly deployed biobehavioral marker in young children with and without neurodevelopmental disorders (Rothbart, Ahadi, Hershey, & Fisher, 2001; Wall et al., 2019). However, most research has focused on negative affect in neurotypical children with increased application to clinical groups observed more recently.

Research has begun to identify the ways in which children with FXS, those with nsASD, and typically developing (TD) children differ in their expression of negative affect. Macari, Koller, Campbell, & Chawarska (2017) found that 26-month-old toddlers with nsASD have higher negative affect than their TD peers; by contrast, a matched group of developmentally delayed toddlers without ASD did not present with elevated negative affect. Studies of negative affect in children with FXS and TD have taken a developmental approach to studying how the trajectory of negative affect compares in these two groups, with children with FXS demonstrating an accelerated increase in negative affect over time (Tonnsen, Malone, Hatton, & Roberts, 2013; Wall et al., 2019),

despite cross-sectional studies showing children with FXS having lower negative affect at certain ages (Shanahan, Roberts, Hatton, Reznick, & Goldsmith, 2008). No study to date has directly compared children with nsASD and with FXS in terms of their negative affect, leaving an important gap in our understanding of the role negative affect plays in the characterization of these populations.

Physiological regulation is another useful biobehavioral marker for ASD and social anxiety as there is clear evidence that physiological regulation contributes to both cognitive and social competency (Patriquin, Scarpa, Friedman, & Porges, 2013; Porges, 2009). Physiological regulation can be reflected through autonomic nervous system (ANS) activity, including both the sympathetic and parasympathetic nervous systems. Both can be easily measured non-invasively through cardiac indices, including respiratory sinus arrhythmia (RSA). Respiratory sinus arrhythmia indexes parasympathetic nervous system function by measuring the beat-to-beat variability in heart rate associated with respiration and plays an important role in the physiological regulation of stress and social engagement (Porges, 2001; Symons & Roberts, 2013). Parasympathetic nervous system function works to slow heart rate and lower blood pressure in order to allow resources necessary for social engagement and promote behavioral regulation (Porges, 2007). For example, adaptive RSA suppression has been associated with fewer childhood internalizing and externalizing problems and greater emotion regulation (Hastings et al., 2008). Because RSA indexes systems of social engagement and the ability to regulate in response to stressors, it can a useful objective measure of social anxiety responses (Porges, 2007). Atypical infant RSA has been shown to predict social fear in neurotypical children (Brooker et al., 2013).

Baseline RSA, or RSA measured during a neutral, unaroused state, is thought to be a measure of individual differences in arousal (Hastings et al., 2008), It has been used to understand underlying differences in physiological regulation in individuals with nsASD, FXS, and TD (see Klusek, Roberts, & Losh, 2015 for a comprehensive review). Studies have shown conflicting evidence for atypical RSA in nsASD, with some studies finding no evidence for differences between with nsASD and those without (e.g., Daluwatte et al., 2013), and some studies finding reduced RSA in nsASD (e.g., Guy, Souders, Bradstreet, Delussey, & Herringto, 2014). Longitudinal work has shown that infants who go on to develop nsASD show a smaller increase in RSA over time relative to their TD peers, suggesting that there may be developmental processes at play (Sheinkopf et al., 2019). Taken together, this evidence points to the need for additional research. A paucity of work has compared RSA in nsASD and neurodevelopmental disabilities including FXS, and it has only been conducted in older children with a wide age range, but those studies suggest that these groups do not differ (Daluwatte et al., 2013; Klusek, Martin, & Losh, 2013). A large body of work has documented maladaptive (i.e., lower) baseline RSA values in FXS compared to TD from infancy (Roberts, Tonnsen, Robinson, & Shinkareva, 2012) through adolescence (Hall, Lightbody, Huffman, Lazzeroni, & Reiss, 2009). Notably, no studies have included preschool females with FXS, leaving critical gaps in our understanding of physiological regulation in this population. The present study addresses multiple gaps in the current literature by directly comparing nsASD, FXS, and TD in a population of preschool-aged males and females.

Temperament and Physiological Indices as Predictors of ASD and Social Anxiety

In addition to providing descriptive information about underlying differences in genetic risk groups, biobehavioral measures have predictive utility in determining the presence and severity of disorders like ASD and social anxiety. Many studies have investigated the role of negative affect as a useful predictor of later ASD diagnosis in children with nsASD (Clifford, Hudry, Elsabbagh, Charman, & Johnson, 2013; Garon et al., 2009; Zwaigenbaum et al., 2005). In longitudinal prospective studies of infant siblings of children with nsASD, higher parent-reported negative affect in infancy has been associated with ASD outcomes in toddlerhood in multiple independent studies (Clifford et al., 2013; Garon et al., 2009; Zwaigenbaum et al., 2005). Importantly, the association of higher negative affect to increased ASD diagnoses in children with nsASD applies to both males and females (Garon et al., 2009). Recent work has not demonstrated a correlation between ASD severity and negative affect in toddlers with nsASD, and this relation has not been examined in TD or non-ASD DD toddlers, leaving an important avenue for future research (Macari et al., 2017). Some studies have investigated RSA as a predictor of ASD severity, but this work is also limited (see Klusek et al., 2015 for a review). In school-age children and older, there is mixed evidence for the relation between baseline RSA and ASD severity in children with a diagnosis; however, no work has examined these relations in pre-school children.

Negative affect has been shown to predict the onset of childhood anxiety throughout early development (Brooker et al., 2013; Gartstein et al., 2010; Grant, Bagnell, Chambers, & Stewart, 2009; Van Hulle, Moore, Lemery-Chalfant, Goldsmith, & Brooker, 2017). Early social fear, a component of negative affect, has also been found to

predict social anxiety symptoms later in childhood (Brooker, Kiel, & Buss, 2016; Buss et al., 2013). Notably, although negative affect predicts anxiety in neurotypical populations, no studies to our knowledge have examined the prediction of anxiety from negative affect in a sample of children diagnosed with nsASD. Because between 42-79% of individuals with ASD have comorbid anxiety, this component of the ASD phenotype is an important avenue for future research (van Steensel, Bögels, & Perrin, 2011). In the one study that examined the relation between RSA and anxiety in nsASD, FXS, and TD, Klusek and colleagues (2013) did not find a significant association. Other work has shown a relation between internalizing symptoms and RSA in nsASD (Neuhaus, Bernier, & Beauchaine, 2014). However, this work was done with school-aged children and adolescents, and little is known about these relations in young children.

There is an accumulating body of work utilizing biobehavioral measurement to predict ASD and social anxiety within FXS as well. Interestingly, despite the connection between early negative affect and ASD symptoms in children with nsASD no such relation seems to exist in children with FXS (Tonnsen et al., 2013; Wall et al., 2019). However, within FXS, there is evidence to suggest that RSA becomes increasingly atypical across early development, a trajectory that mirrors the onset of ASD symptoms (Roberts et al., 2012). In older boys with FXS, RSA dysfunction is associated with communicative deficits common in ASD, but not ASD severity (Klusek et al., 2013).

Negative affect has consistently predicted anxiety symptoms in children with FXS despite the lack of relation to ASD symptoms, pointing to negative affect's utility as a distinguishing diagnostic marker in this population. Infants and toddlers with FXS exhibit atypical longitudinal patterns of facial and behavioral social fear and these patterns are

associated with withdrawal symptoms associated with social anxiety (Tonnsen, Scherr, Reisinger, & Roberts, 2017). Further, prospective longitudinal studies have shown that early negative affect predicts anxiety symptoms in males (Tonnsen, Malone, et al., 2013) and females (Wall et al., 2019) with FXS. In older children, RSA dysfunction is associated with pragmatic language deficits common in ASD in older boys with nsASD, but not ASD severity (Klusek et al., 2013). Infants with FXS demonstrate a blunted RSA response to a social stressor in comparison to TD infants, suggesting that RSA may be a marker of social anxiety in FXS (Black, Hogan, Smith, & Roberts, under review).

The Present Study

The challenge of quantifying social behavior in very young children, along with significant comorbidities in ASD, social anxiety, and FXS, complicate differential diagnoses in neurodevelopmental disorders. Furthermore, ASD and social anxiety are primarily diagnosed and characterized by the identification of behavioral atypicalties, despite evidence showing that they have clear and observable biological underpinnings. Therefore, there is great utility in utilizing measures that disentangle specific phenotypic profiles in children with neurodevelopmental disorders. Towards this end, the present study aims to address the following research questions: 1) how do baseline RSA and temperamental negative affect differ in preschoolers with nsASD, FXS, and typical development and 2) do baseline RSA and temperamental negative affect predict ASD symptomology and social anxiety symptoms within all groups?

Methods

Participants

We used observational data from male and female preschoolers with non-syndromic ASD (n = 47, n_{males} = 40), FXS (n = 41, n_{males} = 29), and typical development (n = 32, n_{males} = 25). Participants were drawn from existing longitudinal studies of early development at the University of South Carolina. As a part of this larger study, children were assessed on several developmental outcomes, including physiology, temperament, and ASD and anxiety symptomatology. A subset of the current FXS (n = 29) and TD sample (n = 24) overlap with previous work examining negative affect in relation to anxiety and ASD (Wall et al., 2019). In addition, 61.7% of this sample overlapped with the participants in Chapter 2.

Participants were included in the present study if they had gestational age >37 weeks, English as the primary language in the home, and no other known medical conditions. Participants were recruited primarily from research and medical sites as well as social media sites specializing in nsASD or FXS. Assignment to risk group was made at intake using existing genetic or psychological reports. Participants enrolled in the TD group had no family history of ASD, and they were confirmed not to have ASD or developmental delay (i.e.., Mullen Early Learning Composite scores greater than 70) through study participation. Those in the FXS group were confirmed through a review of a genetic report of greater than 200 repeats of the CGG sequence on the FMR1 gene, and those in the non-syndromic ASD group had an existing community diagnosis of ASD. ASD diagnoses for the FXS and ASD groups were confirmed through study participation. Groups were matched by age such that each group comparison was not significantly

different at α =.5 (Kover & Atwood, 2013). Participant characteristics can be found in Table 1.1.

Table 1.1. Participant demographic information

	nsASD	FXS	TD
n (% Male)	47 (85.1)	41 (70.7)	32 (78.1)
Age [months]	42.9 (5.5)	45.4 (5.7)	45.6 (5.7)
Ethnicity			
%Not Hispanic/Latino	95.7	95.1	96.9
%Hispanic/Latino	2.2	4.9	3.1
%Unknown	2.1	0	0
Race			
%White	78.7	65.9	87.5
%Black	10.6	4.9	6.3
%Other	2.1	4.9	0
%More than One Race	6.4	24.4	6.2

Procedures

Data were drawn from two existing studies of early developmental markers in neurodevelopmental disorders, including nsASD and FXS. The existing studies are approved by the Institutional Review Board at the University of South Carolina. Parents provided written informed consent before enrollment. All procedures were approved by the Institutional Review Board at the University of South Carolina. Assessments included a battery of behavioral measurements, including measures of physiological arousal during a baseline activity and ASD diagnostic procedures. Parent-report measures were completed using paper and pencil format and were mailed to parents before each assessment. Behavioral measures were conducted by trained lab staff. For the current study, data were used from participants' 48-month assessment unless those data were unavailable; in these cases, their 36-month assessment was used. For the nsASD group,

17 participants' data were from the 36-month visit and 30 from the 48-month visit; for FXS, 18 participants' data were from the 36-month visit and 23 from the 48-month visit; and for the TD group, 18 participants' data were from the 36-month visit and 14 from the 48-month visit. There was no difference between groups in the assessment used (H = .295; p = .863).

Autism Spectrum Disorder diagnoses were assigned or ruled out through the larger longitudinal study using Clinical Best Estimate (CBE) procedures (Hogan et al., 2017). Cases were reviewed by a team including a licensed psychologist who is also an independent trainer for the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2) and at least two other team members who were both research reliable on the ADOS-2 one of whom was the primary evaluator of the child. Data reviewed during the CBE process included ADOS-2 scores and videos, clinical interviews, and behavioral ratings across the assessment, in addition to performance on cognitive and adaptive behavior assessments.

Measures

Baseline RSA. Baseline physiological regulation was indexed by mean RSA during a baseline video condition. During this time, the child was seated and watched an engaging, five-minute video without words. Heart activity was collected via electrocardiogram (ECG) while the child was sitting still. The ECG signal was measured by an Actiwave Cardio Monitor (CamNtech Ltd., Cambridge, UK) at 1024 Hz, and heart rate was extracted using a threshold detection method in QRSTool software (Allen, Chambers, & Towers, 2007). A trained research assistant edited ECG data to correct false heart periods and artifacts using CardioEdit software, and data were only included if they

met a threshold of 10% or fewer beats edited (Brain-Body Center, 2007b). CardioBatch was then used to sample sequential heart periods in 250ms epochs and de-trend the data with a 21-point moving polynomial algorithm (Brain-Body Center, 2007a). Next, the data were filtered to remove variance associated with respiration rate (.3-1.3 Hz), and RSA was estimated by transforming the variance to its natural logarithm. The mean RSA averaged across 30 second epochs during the baseline period was then taken as the independent variable of interest. Data were missing from 15% of participants due to the inability to tolerate the heart rate monitor, greater than the allowed threshold for editing, or other technical malfunctions. There were no identified patterns of missingness suggesting that groups differed on this metric, but it is worth noting that clinical groups were more represented. Data were missing from 19.1% of the nsASD group, 10.5% of the FXS group, 3.1% of the TD group.

Negative Affect. Temperamental negative affect was assessed using the negative affect composite score from the Childhood Behavior Questionnaire (CBQ), a standardized measure of temperament from children aged three to seven years (Rothbart et al., 2001). The negative affect composite includes items related to anger, frustration, fear, sadness, soothability, and the ability to recover from distress. For the scales comprising the Negative Affect Composite in 4- to 5-year-old children, internal consistency estimates (coefficient α) ranged from .66 to .80 (Rothbart et al., 2001). The factor structure of this temperament framework has been evaluated in populations with FXS, and it was largely retained for the negative affect composite (Roberts, Tonnsen, Robinson, McQuillin, & Hatton, 2014). Negative affect is relatively stable in children

with FXS across this time frame, changing by less than half a point (Wall et al., 2019). Data were missing from 5% of participants due to incomplete return of parent forms.

Social Anxiety Symptoms. Social anxiety was measured using the Spence Preschool Anxiety Scale (PAS; Spence, Barrett, & Turner, 2003). The PAS is a parent-report screening and diagnostic questionnaire for anxiety disorders in young children. Raw scores for the social anxiety scale were used in the present study because T-scores had a restricted range in the sample. Although the PAS was not designed for use in populations with neurodevelopmental disabilities, it has been used in studies of children with ASD, intellectual disabilities, FXS, and other genetic syndromes (Crawford, Waite, & Oliver, 2017; Rzepecka, McKenzie, McClure, & Murphy, 2011). Social anxiety items have strong factor loadings on their hypothesized scale, and an adaptive version of the PAS shows strong internal consistency for this scale as well ($.74 < \alpha < .77$; Nauta et al., 2004; Spence et al., 2001). Data were missing from 7.5% of participants due to incomplete return of parent forms.

ASD Symptoms. ASD symptom severity was measured using the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2; Lord et al., 2012). The ADOS-2 is a semi-structured play-based, semi-structured measure to elicit social interaction. Module 1, Module 2, or Module 3 of the ADOS-2 was administered by lab-reliable or research-reliable examiners, and module selection was determined by the child's age and expressive language abilities. Calibrated severity scores (CSS) were utilized in the present study to account for symptom severity across modules and to provide a validated, continuous measure of ASD symptomatology (Gotham, Pickles, & Lord, 2009).. The Overall Total Scores were used to derive CSS scores, and those have

been shown to have high test-retest (ICC > .94) and interrater reliability (ICC > .83). Scores range from 1 to 10, with higher numbers reflecting more severe ASD symptoms.

Developmental Ability. Developmental ability was measured with the Mullen Scales of Early Learning (MSEL), standardized assessments of early development for children birth through 68 months (Mullen, 1995). It evaluates development in the following five categories: Gross Motor, Visual Reception, Fine Motor, Receptive Language, and Expressive Language. Early Learning Composite (ELC) scores are derived from Visual Reception, Fine Motor, Receptive Language, and Expressive Language scores and utilized as a standardize metric of developmental ability.

Analytic Plan

Preliminary analyses. All proposed analyses were conducted in SPSS 26 with the Missing Values package. The data were analyzed for the percent of missingness using the Missing Values add-on package for SPSS 26, and multiple imputation was used to account for missing data. Based on current recommendations, twenty imputed datasets were used, and results were based on the average pooled estimates (Graham, Olchowski, & Gilreath, 2007). Data were screened for outliers and violations of assumptions. Descriptive analyses, including means and standard deviations, were calculated for all variables of interest. Group differences in covariates, including developmental ability and biological sex, were evaluated, and correlations between all study variables were conducted for all groups individually.

Research question 1. Our first research question asked whether 1) baseline RSA and 2) temperamental negative affect differed in preschoolers with nsASD, FXS, and typical development. First, a one-way analysis of covariance (ANCOVA) was used to test

for group differences in RSA, with group as the factor and RSA as the dependent variable. In line with prior work, developmental ability was included in the model as a covariate in RSA due to its potential effect on the dependent variable (Tonnsen, Shinkareva, Deal, Hatton, & Roberts, 2013). Second, a one-way analysis of variance (ANOVA) was used to test for group differences in negative affect, with group as the factor and CBQ negative affect composite score as the dependent variable. Developmental ability was not included as a covariate for this analysis, because we have shown that is it a construct independent of temperament (Wall et al., 2019).

Research question 2. The second research question asked how baseline RSA and temperamental negative affect and predict ASD symptomology and social anxiety symptoms within all groups? To assess this, six separate multiple regressions were run to evaluate whether negative affect and RSA together predict a) social anxiety symptoms and b) ASD severity in preschoolers with 1) nsASD, 2) FXS, and 3) typical development. For each group, clinical symptoms (i.e., social anxiety and ASD severity) were regressed on RSA and negative affect scores, with developmental ability and gender included in all models as covariates.

Results

Preliminary Analyses

Data were screened for violations of assumptions. Kolmogorov-Smirnov test were significant for both the PAS social anxiety score ($p < .0001$) and ADOS CSS ($p = .044$), suggesting that they were were non-normally distributed; however, regression is robust to violations of this assumption at the given sample size. Study variables were then analyzed for patterns of missingness. Overall, data were missing for RSA, negative affect, social

anxiety, and developmental ability measures, In addition, 21.7% of cases, and 5.67% of values had incomplete data. However, the data were analyzed for missing value patterns, and it was determined that the data were missing completely at random (MCAR; Heitjan & Basu, 1996). An automatic data scan was conducted to confirm this, and a fully conditional specification method of multiple imputation was conducted. conducted on the pooled data.

Descriptive statistics are presented in Table 2. A univariate ANOVA indicated that the groups did differ in developmental ability ($F(2, 117) = 99.2$, $p < .001$). Post hoc analyses demonstrated that the TD group was different from both clinical groups ($ps < .001$), but the nsASD and the FXS groups did not differ ($p = .884$). A Kruskal-Wallace test indicated that groups did not significantly differ in the distribution of sex *(H(*2) = 2.64, $p = .266$). Means and standard deviations for all independent and dependent variables are shown in Table 1.2.

Table 1.2. Descriptive means (SD)

	nsASD	FXS	TD
MSEL ELC	58.36 (13.5)	59.15 (15.6)	101.3 (16.2)
Baseline IBI	565.6 (65.2)	581.2 (74.4)	575.4 (58.2)
CBQ Negative Affect	3.96 (.754)	3.77 (.619)	3.50 (.691)
PAS SA Score	2.71 (2.85)	3.08 (3.21)	1.91 (2.09)
ADOS-2 CSS	7.4 (1.65)	5.46 (2.69)	1.78 (1.24)

Note. Results based on average pooled estimates. MSEL = Mullen Scales of Early Learning. ELC = Early Learning Composite. NVDQ = Nonverbal Developmental Quotient. CBQ = Childhood Behavior Questionnaire. PAS = Preschool Anxiety Scale. SA = Social Anxiety. ADOS-2 = Autism Diagnostic Observation Schedule, Second Edition. CSS = Calibrated Severity Score.

Exploratory Pearson correlations amongst all key study variables for each group were conducted to understand the relation between RSA, negative affect, social anxiety, and ASD severity prior to conducting the main study analyses. Because of the

exploratory nature, they were not corrected. Baseline RSA and ASD severity were significantly negatively correlated in both the nsASD group (r = -.340, p = .033) and the FXS group (r = -.326, p = .048). For the TD group, no correlations were significant.

Research Question 1

The first research question aimed to address whether 1) baseline RSA and 2) temperamental negative affect differed in preschoolers with nsASD, FXS, and typical development. To address this question, two separate models were run. First, a univariate ANCOVA with group as the factor, controlling for developmental ability, and RSA as the dependent variable indicated that groups were not significantly different in their RSA (F(3, 116) = .253, p = .859; partial η^2 = .004). Next, a univariate ANOVA with group as the factor and negative affect as the dependent variable indicated that there was a significant difference in negative affect among the groups (F(2, 117) = 4.33, p = .015; partial η^2 = .063). Post hoc analyses suggested that the TD group had significantly lower negative affect than the nsASD group (p = .007), but not the FXS group (p = .120); the nsASD and FXS groups did not differ (p = .261).

Research Question 2

The second research question asked whether baseline RSA and temperamental negative affect predicted social anxiety and ASD severity within all groups. Altogether, six models were run (two outcomes X three groups), and developmental ability and gender were included in all models as covariates. Results from these multiple linear regression models are shown in Table 1.3.

Table 1.3. Summary of linear regressions predicting social anxiety and ASD

	PAS SA			ADOS-2 CSS		
	B (SE)	*t*	R^2	B (SE)	*t*	R^2
ASD			.233*			.263*
Intercept	-6.46 (3.34)	-1.94		13.36 (1.83)	7.28**	
RSA	-.025 (.347)	-.071		-.461 (.194)	-2.37*	
CBQ NA	1.39 (.585)	2.37*		-.218 (.319)	-.686	
ELC	.066 (.035)	1.90		-.043 (.017)	-2.59*	
Male	-.409 (1.23)	-.333		.190 (.641)	.296	
FXS			.374**			.389**
Intercept	-12.2 (4.19)	2.90**		11.6 (3.56)	3.56**	
RSA	.135 (.381)	.356		-.373 (.289)	-1.29	
CBQ NA	3.06 (.874)	3.50**		.447 (.670)	.667	
ELC	.061 (.038)	1.61		-.096 (.037)	-2.61*	
Male	-2.07 (1.19)	-1.75		.057 (1.02)	.056	
TD			.124			.095
Intercept	.251 (3.40)	.074		-1.25 (2.05)	-.612	
RSA	.272 (.432)	.630		.072 (.261)	.275	
CBQ NA	.808 (.582)	1.39		.232 (.351)	.660	
ELC	-.028 (.023)	-1.15		.018 (.015)	1.20	
Male	.018 (.924)	.020*		.071 (.557)	.127	

Note. Results based on average pooled estimates. IBI = Inter-beat-interval. CBQ = Childhood Behavior Questionnaire. NA = Negative Affect. PAS = Preschool Anxiety Scale. SA = Social Anxiety. ADOS-2 = Autism Diagnostic Observation Schedule, Second Edition. CSS = Calibrated Severity Score. ELC = Mullen Early Learning Composite. *$p < .05$. ** $p < .01$.

Analyses predicting social anxiety from RSA and CBQ negative affect scores were significant for the nsASD ($F(4, 42) = 2.84$, $p = .011$; $R^2 = .233$) and FXS groups ($F(4, 35) = 4.60$, $p = .004$; $R^2 = .374$), but not the TD group ($F(4, 27) = .095$, $p = .450$; $R^2 = .124$). However, these findings seem to largely be driven by the effect of negative affect, as RSA was not a significant individual predictor. In non-syndromic ASD, a unit

increase in CBQ score resulted in a 1.39-point increase in social anxiety score (p = .018). In FXS, negative affect was also a significant individual predictor, where a unit increase in CBQ score resulted in a 3.06-point increase in social anxiety score (p < .001). Figure 1.1a illustrates the modeled relations between negative affect and social anxiety for all three groups, and Figure 1.1b shows a scatterplot of this relation using the original, unimputed dataset.

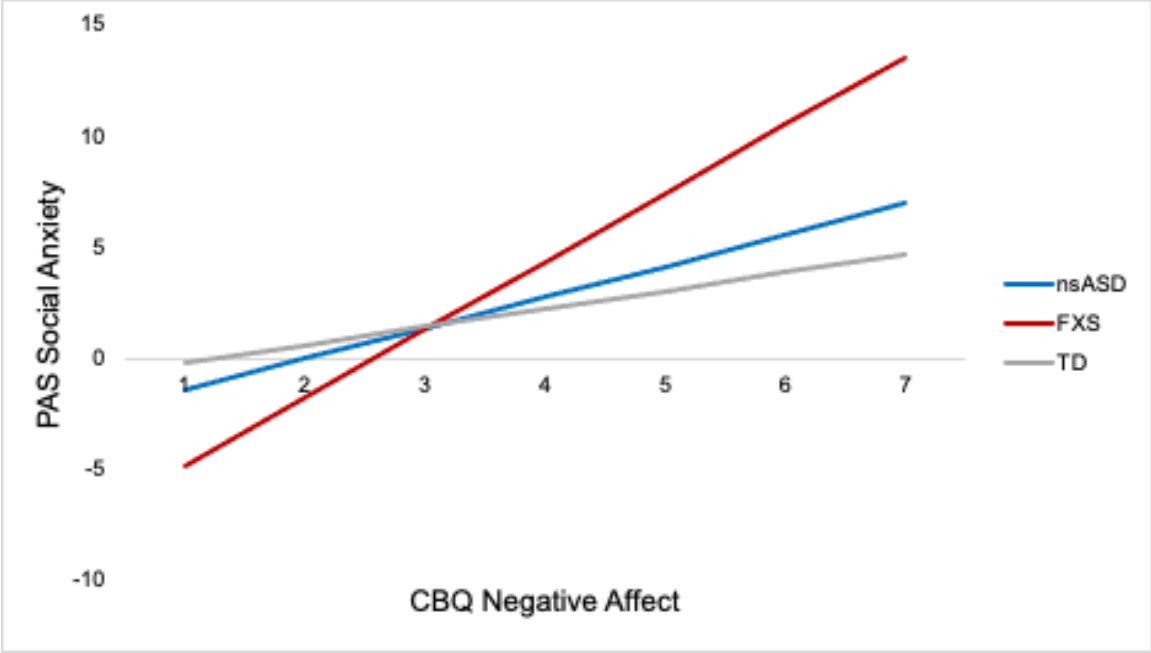

Figure 1.1a. Modeled relation between negative affect and social anxiety for all groups.

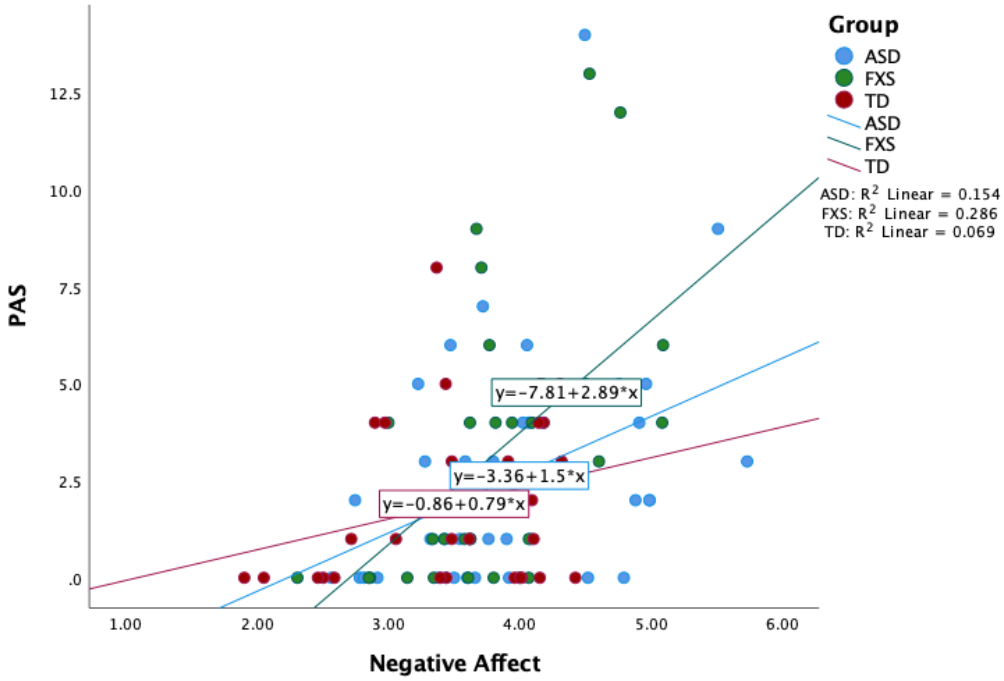

Figure 1.1b. Scatterplot of relation between negative affect and social anxiety with the original, unimputed dataset.

Analyses predicting ADOS CSS from RSA and negative affect were also significant for the nsASD ($F(4, 46) = 3.75$, $p = .010$; $R^2 = .263$) and FXS groups ($F(4, 40) = 5.96$, $p = .0007$; $R^2 = .389$), but again not for the TD group ($F(4, 31) = .707$, $p = .593$; $R^2 = .095$). In examining the individual predictors, RSA was significant for the nsASD group only, whereby a unit increase in RSA (i.e., more typical regulation) was associated with a .461-point decrease in CSS score ($p = .018$). Mullen ELC scores were also significantly related to ASD severity in both the FXS and nsASD groups ($ps < .05$). Figure 1.2a illustrates the modeled relations between RSA and ASD severity for all three groups, and Figure 1.2b shows a scatterplot of this relation using the original, unimputed dataset.

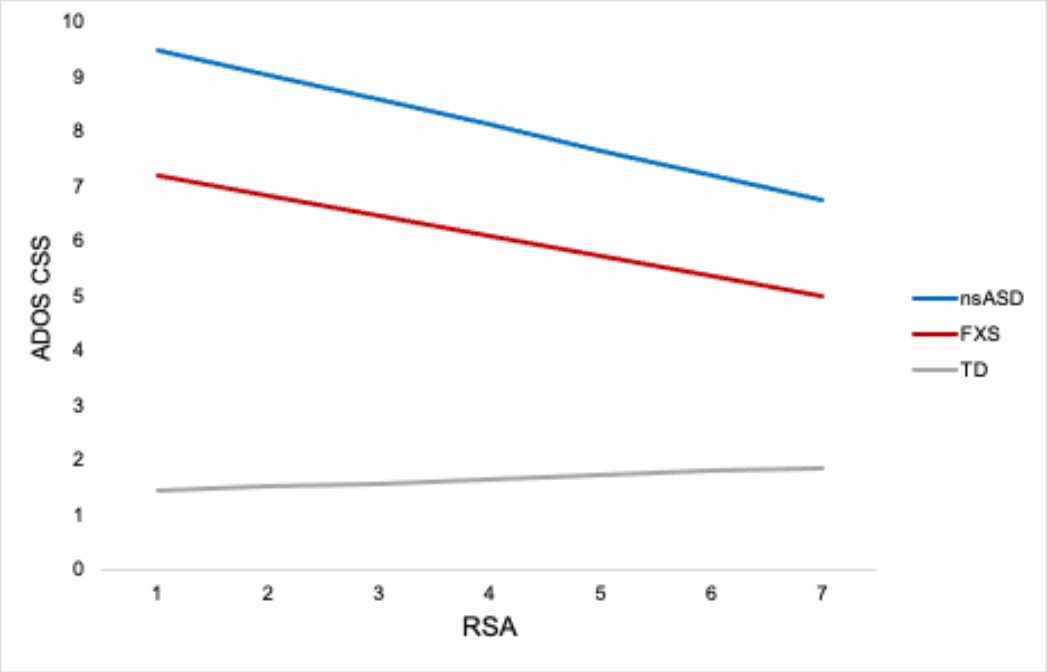

Figure 1.2a. Modeled relation between RSA and ASD severity for all groups.

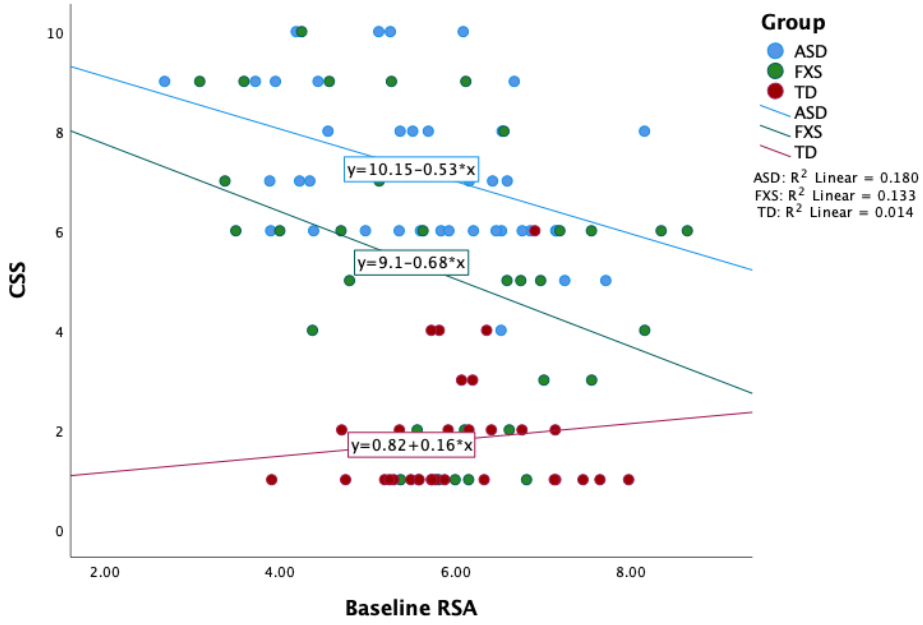

Figure 1.2b. Scatterplot of relation between RSA and ASD severity with the original, unimputed dataset.

Discussion

The present study aimed to advance our understanding of certain biobehavioral underpinnings of social anxiety and ASD symptoms in young children with nsASD and FXS. Given the high prevalence and significant impairment of social anxiety that overlaps with ASD features in both nsASD and FXS, this work is critical to identify discrete targets and timing for intervention. However, this work is challenging given difficulties measuring symptoms in young children when features of social anxiety may be less clear. These challenges are magnified when studying young children with nsASD and FXS who present with intellectual and communication impairments that preclude the use of self-report measures and for whom the presentation of social anxiety may differ from neurotypical controls. As such, implementing a biobehavioral approach including objective markers that are detectable early in development and identify the potential cause, rather than focus on symptom presentation, are critical. In this study, we employ temperament measures of negative affect that reflect behavioral indices of reactivity and regulation to environmental and social challenges complemented with physiological responses of RSA that capture biological competence to manage challenges (Porges, 2007; Rothbart & Bates, 2007). Both negative affect and RSA have been linked to ASD symptoms and social anxiety in both clinical and neurotypical populations (Brooker et al., 2013; Klusek et al., 2015; Macari et al., 2017; Wall et al., 2019); however, the integration of these relationships have not been examined in young children with nsASD or contrasted to FXS. The present study addresses this gap in the field, and our findings indicate patterns of atypicality that are unique across clinical groups.

Research Question 1

Our initial research question aimed to evaluate differences in biobehavioral markers (i.e., RSA and negative affect) among children with and without neurodevelopmental disorders. This study is one of only a few to evaluate group differences in RSA between FXS and nsASD; it the first to do so in preschoolers (Klusek et al., 2013). Consistent with this other work, our findings indicated that there were no group differences in baseline RSA. Other studies that compare baseline RSA in nsASD and other ASD-associated neurogenetic conditions have found a similar lack of group differences (Daluwatte et al., 2013)**.** Of note, our results did not replicate previous findings of decreased baseline RSA in males with FXS; however, the present study is the first to our knowledge that included both males and females with FXS (Klusek et al., 2015). Given the inclusion of both males and females within our sample, our current findings may suggest that atypical baseline RSA may be specific to the genetic profile or elevated level of impairment associated with males with the disorder, rather than a characteristic of the broader FXS phenotype. More work including females is needed to understand how sex may play a role in the relation between physiological regulation and broader symptomatology.

Our findings did reveal differences in negative affect, whereby children with nsASD had elevated negative affect as compared to their TD peers; however, no differences were observed between FXS and TD children or between nsASD and FXS. Taken together, these findings comport with and expand upon the existing literature on temperamental negative affect in neurodevelopmental disabilities. Prior work has demonstrated that 26-month-old toddlers with nsASD have higher negative affect than

their TD peers, a trait that is not shared with non-ASD developmental delayed toddlers (Macari et al., 2017). Our study found a similar pattern of differences in 45-month-old children with nsASD. Our finding that FXS and TD children are similar in their negative affect is in line with other work demonstrating that around 36 months of age and older, children with FXS do not differ from their TD peers (Wall et al., 2019). In effect, this body of work suggests that elevated negative affect may be representative of a nsASD-specific temperamental vulnerability that persists throughout development. Our findings extend this literature to suggest that elevated negative affect also distinguishes preschool children with nsASD from those with FXS.

Research Question 2

Predicting Social Anxiety Symptoms. Our second research question evaluated how baseline RSA and temperamental negative affect predict social anxiety symptoms and ASD severity within all groups. Our first set of analyses focused on the prediction of social anxiety symptoms from RSA and negative affect. Building off of an existing body of work in FXS, the present study found that negative affect significantly predicted anxiety symptoms in children with nsASD (Tonnsen, Malone, et al., 2013; Wall et al., 2019). Although we did not have the power to test for interactions between groups, we found a similar relation between negative affect and social anxiety across nsASD and FXS; furthermore, these patterns are different from those observed in our TD controls. Much work has documented the similarities and differences among those with FXS and nsASD, and both diagnoses are associated with heightened risk for comorbid anxiety (Boyle & Kaufmann, 2010; R. J. Hagerman & Harris, 2008; Roberts, Tonnsen, McCary, Caravella, & Shinkareva, 2016). Negative affect is a key theoretical mechanism of the

development of anxiety in neurotypical pediatric populations; our findings provide evidence that this mechanism is preserved in children with neurodevelopmental disorders (American Psychiatric Association, 2013b; Joiner, Catanzaro, & Laurent, 1996). The absence of this relation in our typical group may be due to very low levels of social anxiety in our sample.

However, the present study did not ascertain a unique relation between baseline RSA and social anxiety in any group. Nevertheless, this finding comports with prior literature that did not find associations between baseline RSA and parent-reported anxiety symptoms in older children with nsASD and FXS (Klusek et al., 2013). Furthermore, our lack of evidence for a relation between RSA and social anxiety is congruent with other work in males with FXS that suggests that physiological indicators in adolescent males may not be predictive of social anxiety (Roberts et al., 2018b). Although there is work to suggest that RSA may be an important infant marker of social fear, our findings suggest this may not be the case later in development among children with neurodevelopmental disorders (Brooker et al., 2013).

Predicting ASD Symptom Severity. The second aim of this research question was to predict ASD severity from baseline RSA and negative affect. Our results indicated that negative affect was not associated with concurrent ASD symptoms in any group. This finding concurs with other studies indicating that negative affect does not predict ASD symptoms in FXS populations (Tonnsen, Malone, et al., 2013; Wall et al., 2019). Furthermore, work has shown that negative affect and ASD severity are uncorrelated in toddlers with nsASD (Macari et al., 2017). Taken together, these findings provide support for the notion that although nsASD is associated with elevated negative affect,

temperamental traits measure early personality characteristics that are distinct from ASD symptoms. Interestingly, research on temperament in nsASD has shown that temperamental regulation (i.e., effortful control) is associated with ASD severity, pointing to another potential category of regulatory dysfunction that is associated with ASD (Macari et al., 2017). This literature suggests that for those with nsASD, it may not be temperamental negativity, but rather the underlying regulation of emotions that is most indicative of symptom severity.

Interestingly, we found that RSA predicted ASD symptom severity in the nsASD group only such that lower baseline RSA was associated with higher ADOS scores. The present study is the first of our knowledge to ascertain a unique relation between baseline RSA and ASD symptom severity in nsASD, although many studies have demonstrated a relation between RSA and ASD-associated impairments (Klusek et al., 2015). Of note, this is the first study to measure ASD symptoms using the ADOS-2, a highly validated tool for measuring ASD severity, in preschoolers with nsASD. Although the analyses in present study did not directly test whether this relation was unique to nsASD in comparison to the other groups, this finding have important implications for understanding the heterogeneity with nsASD itself. Given the theorized relation between RSA and social engagement, this result suggests a mechanism through which underlying levels of arousal relate to greater social impairments in observed contexts (Porges, 2007). Our findings underscore the importance of taking both individual differences, as well as developmental stage, into account when considering candidates for biobehavioral markers.

Limitations and Future Directions

The present study benefited from a large, well-matched, and controlled sample of children across all groups of interest that allowed us to test for unique biobehavioral mechanisms at a specific time. However, one limitation is that the cross-sectional nature of this design also precludes any examination of the progression of symptoms in neurodevelopmental disorders. Future work examining biological or physiological underpinnings of the relation between negative affect and anxiety would do well to consider how developmental trajectories impact the onset of symptoms in young individuals with neurodevelopmental disorders. In addition, due to power limitations, our FXS sample comprised children with and without concomitant ASD diagnoses. It is yet unknown whether a relation between negative affect and later ASD symptoms also exists in children with FXS who have a stable ASD diagnosis, and this question offers an important avenue for future research. Finally, we did not control for medications, which could have an impact on our RSA values. Nevertheless, exploratory analyses indicated similar patterns of results across our FXS group regardless of ASD status.

In sum, this work confirms and extends the existing literature in several important ways. First, it suggests that between 3-4 years of age, children with ASD differ from their typically developing peers in their negative affect, but children with FXS do not. Second, it verifies prior work in FXS indicating that concurrent negative affect predicts social anxiety symptoms but not ASD severity; it also extends this finding to a cohort of children with nsASD. Finally, our findings indicate that baseline RSA predicted ASD severity for nsASD only, which is useful given the potential consequences of early regulatory atypicality on later symptoms in nsASD and FXS (Macari et al., 2017; Roberts

et al., 2012; Wall et al., 2019). The present study provides evidence for between group as well as within-group heterogeneity in neurodevelopmental disorders, and future work should also consider how biobehavioral markers can help refine our understanding of individual disorders. Our findings can be used to further disentangle that ways that biological markers index the relation between the presence of underlying diagnoses with neural underpinnings and observed symptom severity.

CHAPTER 2

SOCIAL ATTENTION AS AN INDEX OF PHENOTYPIC HETEROGENEITY IN AUTISM SPECTRUM DISORDER[2]

[2]Wall, C. A, Shic, F., & Roberts, J. E. To be submitted

Abstract

Social attention, or attention to the gaze, facial expressions, and social cues of others. is one of the earliest-emerging and most critical skills for learning and development. Social attention deficits are highly impairing and present in developmental disorders, especially autism spectrum disorder (ASD). Our understanding of the nature and impact of social attention impairments in ASD is complicated by the high heterogeneity of the disorder, including the presence of comorbid clinical symptoms. The present study aimed to clarify 1) how social attention impairments manifest in a sample of 76 preschoolers with non-syndromic ASD (nsASD), FXS, and TD and 2) how social attention relates to comorbid clinical symptoms. Findings revealed that young children with nsASD and FXS did not differ in their attention to an overall social scene, but children with FXS looked significantly more at an actress's face than those with nsASD. Correlational analyses suggested similar relations among clinical symptoms in nsASD and FXS. In both groups, increased attention to a social scene was correlated with higher verbal and nonverbal developmental ability but not ASD or social anxiety symptoms. These findings suggest that children with FXS allocate more attention to faces than those with nsASD, despite attending to social scenes for a similar amount of time. Further, the relation between social attention and clinical symptoms is similar among children with nsASD and FXS. These results further our understanding of the mechanisms behind social deficits in children with a variety of neurodevelopmental disorders and underscore their nuanced role in early development.

Introduction

Social attention, or attention to the gaze, facial expressions, and social cues of others, is one of the earliest-emerging skills in infancy, and it is critical to learning and development across multiple domains over time. Many studies have documented how social attention, in the form of social orienting or joint attention, is present very early in infancy and facilitates the development of higher-order social skills such as social cognition, language, and theory of mind (Charman et al., 2000; Mundy & Newell, 2007). Social attention has been linked to molecular and neural reward circuitry in the brain, making it a deeply-rooted biological process that can be measured through both behavioral and neurophysiological means (Dawson, Bernier, & Ring, 2012). Evidence from neural imaging has highlighted the developmental, interactive specialization of discrete neural regions in response to dynamic social stimuli that takes place from childhood through adulthood highlighting the developmental importance of this skill (Johnson, Grossmann, & Kadosh, 2009). Because of the early and far-reaching importance of social attention, early disruptions of social attention can have profound consequences for concurrent and later developmental outcomes (Barrett, Dadds, & Rapee, 1996; Schreibman et al., 2015).

Social Attention in ASD

Disrupted social attention is well-documented in specific clinical groups including neurodevelopmental disorders such as autism spectrum disorder (ASD) (Chita-Tegmark, 2016; Dawson et al., 2012; Frazier et al., 2017). Autism spectrum disorder is a pervasive developmental disorder resulting in impairments in social communication and restricted and repetitive interests and behaviors (American Psychiatric Association, 2013a). A large

meta-analysis has demonstrated that children with ASD exhibit poor social attention as they show a deficit in their ability to select and attend to socially relevant stimuli, particularly faces (Frazier et al., 2017). Impaired social attention is one of the earliest and most robust markers of social-communicative deficits in ASD, often preceding formal diagnoses and persisting throughout early development (Chawarska, Macari, & Shic, 2012; Jones & Klin, 2013; Pierce et al., 2016). Given the profound importance of social attention and its relevance to ASD, scholars have attempted to quantify it using a variety of neuroscience techniques including behavioral coding, eye-tracking, and neuroimaging (Dawson et al., 2012; Falck-Ytter, Bölte, & Gredebäck, 2013). This paper will focus on eye-tracking studies of social attention, given the robustness of research in the area and its utility in capturing social attention impairments in our population of interest.

Although impaired social attention is recognized as a primary deficit in ASD, there is tremendous heterogeneity in other features of ASD that creates important sources of variation reflected in research on social attention. Age is one important contributor to the manifestation of social attention deficits, and research has highlighted the importance of social attention as it relates to the developmental progression and clinical presentation of ASD across various ages and stages of early development. For example, when presented with dynamic social scenes depicting an actress engaging in child-directed speech, infants who are later diagnosed with ASD (Shic, Macari, & Chawarska, 2014) and toddlers with confirmed ASD diagnoses (Chawarska et al., 2012) looked less at socially relevant stimuli than their typically developing peers.

In addition to age-based variability that affects social attention in ASD, there are a number of sex effects that also impact social attention. The overall prevalence of males to

females with ASD is 4:1; however, this ratio varies based on cognitive ability, and in low-functioning individuals is more equally distributed (Halladay et al., 2015; Maenner et al., 2020). The profile of males with ASD often differs from that in females, who appear to have greater variability in some domains. In the social domain, differences in looking patterns are reported in females with ASD or who are at risk for ASD. For example, females with ASD seem to display more gender-typical looking behavior than their male peers with ASD (Harrop et al., 2018). In addition, female infant siblings of a child with ASD display increased social looking compared to both males and females with no ASD risk (Chawarska, Macari, Powell, DiNicola, & Shic, 2016). However, more work is needed to understand whether and how sex differences affect social attention in preschool-aged children with ASD as there is little work in this area despite its importance as the age at which most children are reliably diagnosed with the disorder.

Social Attention and Comorbidities in ASD

Individuals with ASD often present with multiple psychiatric and genetic comorbidities, making it challenging to identify patterns of impairment that are applicable across the spectrum (Abrahams & Geschwind, 2010; Simonoff et al., 2008). Thirty-one percent of individuals with ASD have comorbid intellectual disability (ID), and yet, despite the growing body of research utilizing eye-tracking methods to understand social attention in ASD, very little of this work has included children with comorbid ID (Maenner et al., 2020). Indeed, children with ASD and ID are considered "the neglected end of the spectrum" because they are perceived as more difficult to assess in research protocols, despite recommendations designed to increase compliance (Tager-Flusberg & Kasari, 2013; Tager-Flusberg et al., 2016).

Given the well-documented heterogeneity in ASD (Kim et al., 2016) and the difficulties in distinguishing this disorder from broader intellectual and developmental delay (Thurm et al., 2019), investigations of social attention can play a critical role in furthering our understanding of early neurodevelopmental disorders, and the emerging work examining the relation between social attention and intellectual disability in ASD has tremendous promise. Among toddlers with ASD, decreased social attention was associated with both lower IQ and greater ASD severity (Campbell et al., 2014; Chawarska et al., 2016). Interestingly, this association was not observed in non-ASD developmentally delayed toddlers. Further, recent research suggests that interventions shaping social attention in young children with ASD may be most effective for children with lower cognitive abilities (Wang et al., 2019). Thus, there is a great need to increase our understanding of social attention in a sample of children with nsASD and comorbid ID.

Co-morbid anxiety disorders are another very common occurrence in ASD with 17% of individuals with ASD meeting diagnostic criteria for social anxiety (van Steensel et al., 2011). Social attention plays an important role in understanding social anxiety in both clinical (e.g., ASD) and non-clinical populations. Indeed, avoidant eye contact is a hallmark symptom of social anxiety in neurotypical populations (Schneier, Rodebaugh, Blanco, Lewin, & Liebowitz, 2011), and orienting to emotional faces has been linked to clinical anxiety symptoms in typically developing individuals (e.g., Mogg, Garner, & Bradley, 2007). Despite the connection between social attention and anxiety in neurotypical individuals, work investigating this connection within those with ASD is mixed; furthermore, this research has primarily been conducted in adults. For example,

one study found no relation between social attention and anxiety in individuals with ASD (Hong et al., 2019) while another reported that reduced attention to the eyes is associated with greater social anxiety symptoms (Corden, Chilvers, & Skuse, 2008). No study to our knowledge has examined the relation between social anxiety and looking at social information in young children with ASD. This is important given that the presence of both ASD and anxiety clearly reduces quality of life and early treatment has been shown to reduce these negative consequences (Barrett et al., 1996).

Social Attention in Fragile X Syndrome

Although studies suggest there is a clear genetic component to ASD, evidence establishes that multiple genes are likely involved highlighting the complicated biological basis of the heterogeneity in presentation and etiology of ASD (Jeste & Geschwind, 2014). Single gene models, however, have emerged as promising genetic models with which to study variability within ASD, especially within the context of social attention (Abrahams & Geschwind, 2010; Tick, Bolton, Happé, Rutter, & Rijsdijk, 2016). Fragile X syndrome (FXS) is the leading known genetic cause of ASD, with recent prevalence rates indicating 73% of preschool males and 29% of preschool females meet diagnostic criteria (Roberts et al., 2020), and approximately 1-2% of all ASD diagnoses accounted for by FXS (Abrahams & Geschwind, 2008). In addition to the high prevalence of ASD diagnoses in FXS, ASD features are present in nearly all individuals with FXS independent of an ASD diagnosis. Avoidant eye contact is one of the most highly prevalent and well-documented social attention deficits in FXS that also represents a core symptom of ASD (Farzin, Rivera, & Hessl, 2009; Harris et al., 2008; Roberts et al., 2019). Significant comorbidities are also present in FXS; up to 96% of males and 64% of

females have ID (Bailey et al., 2008), and 86% of males and 77% of females with FXS have an anxiety disorder (Cordeiro et al., 2011; Ezell et al., 2019; Kaufmann et al., 2004).

Although there is a growing interest in examining social attention in FXS, findings are somewhat complicated and limited. Indeed, only two studies in FXS have employed dynamic, naturalistic social scenes as stimuli (Crawford et al., 2016; Hong et al., 2019). These studies found that, relative to their typical peers, individuals with FXS do not show diminished attention to social information. However, social attention may be associated with elevated autism and anxiety symptoms (Crawford, Moss, Oliver, & Riby, 2017; Hong et al., 2019). For example, evidence suggests that adolescents with FXS may display an attentional bias to threat that is related to anxiety, but these results are complicated by age (i.e., when in development anxiety disorders emerge) and the intensity of the stimuli utilized (Kelleher et al., 2020). However, other work has found the opposite: that longer looking to the eyes is associated with less social avoidance behaviors (Klusek, Moser, Schmidt, Abbeduto, & Roberts, 2020). Despite the increasing focus on social attention in FXS, very little work has been conducted with young children or has employed naturalistic, social scenes rather than static images as indices of social attention (e.g., Dalton, Holsen, Abbeduto, & Davidson, 2008; Farzin et al., 2009).

Despite the existence of social attention impairments in children with non-syndromic ASD (henceforth referred to as nsASD) and FXS, relatively few eye-tracking studies have compared these two groups directly (Dalton et al., 2008; Hong et al., 2019). These initial studies found no differences in attention to faces between individuals with nsASD and those with FXS, and the relation between social attention and anxiety observed in FXS was not observed in individuals with nsASD (Hong et al., 2019).

Importantly, these studies were limited in that they included a broad age range of individuals that tended to be adolescents or young adults, used predominantly male samples, and did not explicitly test for a relation between social attention and intellectual ability. Further, in examining differences in gaze to faces among clinical groups, these studies utilized static, emotional face stimuli. Given the importance of high social content in understanding the level of impairments in nsASD, more stimulating methodology should be utilized in studies that include individuals with FXS (Chita-Tegmark, 2016). Taken together, the current body of work suggests that the behavioral social impairments that are prevalent in FXS may be functionally and mechanistically different than those observed in nsASD. However, further research is still essential to understand how these mechanisms function in the course of early development.

In sum, social attention is a vital prerequisite for learning and development that is linked to molecular and neural reward circuitry (Dawson et al., 2012; Johnson et al., 2009). Although much work has been done independently examining developmental patterns of social attention in nsASD, FXS, and typical development, cross-syndrome comparisons that combine all three groups early in life are needed. Further, understanding how social attention may relate similarly and differentially to clinical features (e.g., ASD and social anxiety symptoms and developmental ability) across groups provides insight into the mechanisms underlying social impairments. These efforts are important to direct the timing and targets of intervention. Towards this end, the present study addresses the following research questions:

1) How do preschoolers with nsASD, FXS, and typical development differ in their allocation of attention to faces in response to social and non-social scenes?

2) How does attention to faces during social scenes relate to developmental ability, ASD symptom severity, and social anxiety within groups?

Methods

Participants

Data were collected from preschoolers with nsASD (n = 30), FXS (n = 24), and typical development (n = 22). Participants were drawn from longitudinal studies of early development at the University of South Carolina. Individuals were included in this study if they had complete data on all measures. Inclusion criteria included gestational age >=37 weeks, English as the primary language in the home, and no known medical conditions (e.g., seizures, vision or hearing impairments) other than a diagnosis of FXS for that group. Participants were recruited primarily from research and medical sites as well as social media networks specializing in ASD or FXS. Assignment to group was made at intake using existing genetic or psychological reports. Participants enrolled in the TD group had no family history of ASD, and they were confirmed not to have ASD or developmental delay (i.e., Mullen Early Learning Composite scores greater than 70) through study participation. Those in the FXS confirmed were confirmed through a review of a genetic report of greater than 200 repeats of the CGG sequence on the FMR1 gene, and those in the non-syndromic ASD group had an existing community diagnosis of ASD. Although no formal genetic testing was done to rule out comorbid syndromes in the ASD group, parents did not report any genetic abnormalities. ASD diagnoses for the FXS and ASD groups were confirmed through study participation. Participant characteristics can be found in Table 2.1. A large portion (81.5%) of this sample overlapped with the participants in Chapter 1.

Table 2.1. Participant demographic information

	nsASD	FXS	TD
n (% Male)	30 (83.3)	24 (54.1)	22 (86.3)
Age [months]	55.1 (11.0)	59.7 (12.5)	66.2 (14.1)
Ethnicity			
%Not Hispanic/Latino	90.0	100	95.5
%Hispanic/Latino	0	0	4.5
%Unknown	10.0	0	0
Race			
%White	83.3	75.0	90.9
%Black	6.7	0	4.5
%Other	3.3	4.2	0
%More than One Race	3.3	16.7	4.5

Procedures

As a part of their participation, children were assessed on several developmental outcomes including developmental ability, anxiety and ASD symptomatology. In addition, they completed an eye-tracking experiment focused on social attention at a single time point. Behavioral measures were conducted by trained lab staff and paper and pencil measures were mailed to parents and completed before assessment visits. All procedures were approved by the Institutional Review Board at the University of South Carolina, and parents provided written informed consent before enrollment in the study.

Autism Spectrum Disorder diagnoses were assigned or ruled out using Clinical Best Estimate (CBE) procedures (Hogan et al., 2017). Cases were reviewed by a team including a licensed clinical psychologist and independent trainer for the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2), and at least two other team members who were both research reliable on the ADOS-2, one of whom was the primary evaluator of the child. Data reviewed included ADOS-2 scores and videos, clinical

interviews, cognitive and adaptive behavior assessments, and behavioral ratings across the assessment.

Measures

Social Attention. Attention to faces is the primary social attention variable in this study. These data were collected on an SR Eyelink 1000 Plus eye tracker that deployed a 3-minute dynamic video scene depicting an adult female actress seated at a table surrounded by four mechanical toys (Chawarska et al., 2012). Four conditions were depicted: *Dyadic Bid*, the actress engages the viewer in child-directed speech; *Sandwich,* the actress makes a sandwich without direct gaze; *Joint Attention*, the actress looks at the viewer and then towards one of the toys; and *Moving Toys,* the actress looks towards the viewer while the mechanical toys activate. The stimulus was built and presented with SR Experiment Builder using monocular, remote eye tracking on a 20-inch widescreen LCD monitor with a 60-Hz refresh rate. Children were shown a cartoon to adjust to the experimental setting. Five-point calibration preceded the experiment for all participants, and five-point validation was completed if children remained attentive and compliant throughout calibration.

In order to probe for difference in social attention, two conditions were examined as exemplars of social and non-social events during the video scene based previous literature: *Dyadic Bid* was selected as a social condition because it is a robust marker of differences in social attention in nsASD, and *Moving Toys* was selected as a non-social comparison event because it directs attention away from the face using highly salient visual and auditory cues (Chawarska et al., 2012; Wang, Campbell, Macari, Chawarska, & Shic, 2018). The primary dependent variables of interest for measuring social attention

were and the proportion of the looking time (i.e., the summation of all fixation durations) at any point on the screen over the total duration of the conditions of interest (%Scene) and the proportion of looking time to the actress' face (%Face) as a proportion of the summation of fixations durations to the total scene during the conditions of interest. That is, %Face was taken as a subset of %Scene. Participants were excluded from analyses at the condition level if %Scene was less than 20 for a particular condition.

ASD Symptoms. Severity of ASD symptomatology was measured using the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2; Lord et al., 2000). The ADOS-2 is a diagnostic instrument for ASD, a play-based, semi-structured measure to elicit social interaction. Module 1, Module 2, or Module 3 of the ADOS-2 was administered by lab-reliable or research-reliable examiners, and module selection was determined by the child's age and expressive language abilities. Calibrated severity scores (CSS) were utilized in the present study, which range from 1 to 10. Greater CSS indicates more severe ASD symptoms.

Social Anxiety Symptoms. The Spence Preschool Anxiety Scale (PAS) was used as an index of social anxiety symptoms (Spence et al., 2001). The PAS is a 38-item, parent-report questionnaire designed as a screening and diagnostic instrument for anxiety disorders in young children. Raw scores for the social anxiety scale were utilized in the present study, with higher scores indicating more anxiety symptoms. Although this measure was not designed for use in populations with neurodevelopmental disabilities, it has been used in studies of children with ASD, intellectual disabilities, FXS, and other genetic syndromes (Crawford, Waite, et al., 2017; Rzepecka et al., 2011).

Intellectual/Developmental Ability. Intellectual/developmental ability was measured using one of two standardized assessments of early development, the Mullen Scales of Early Learning (MSEL) or the Differential Ability Scales, Second Edition (DAS-II; Elliott, Murray, & Pearson, 1990; Mullen, 1995). The MSEL was used for participants up to 68 months of age. It captures development in the following five domains: Gross Motor, Visual Reception, Fine Motor, Receptive Language, and Expressive Language. Nonverbal developmental quotient scores (NVDQ) were calculated by averaging age equivalents for the Visual Reception and Fine Motor domains and dividing that value by chronological age. The verbal developmental quotient scores (VDQ) were calculated by averaging age equivalents for the Receptive and Expressive Language domains and dividing that value by chronological age. The DAS-II was used for participants greater than 68 months, and it captures cognitive development in verbal and nonverbal reasoning, as well as spatial abilities. The Verbal Reasoning and Nonverbal Reasoning composite scores were used, and they have a mean of 100 and a standard deviation of 15. To account for the different measures utilized in the present study, Z-scores were calculated for each domain and used in the present analyses.

Analytic Plan

Data Processing. Eye-tracking data were processed with custom Matlab software that accommodated standard techniques for processing eye-tracking data including blink detection, data calibration, and Region of Interest (ROI) analysis (Shic, 2009). Data reduction and analysis were carried out through programs written in R (v3.5.1; R Core Team, 2016).

Statistical Analyses. Descriptive analyses, including means and standard deviations, were derived for all variables of interest. Distributions of variables were also examined. Preliminary univariate ANOVAs and a Kruskal-Wallis test were run to evaluate group differences in primary study variables. To determine whether groups differ in the allocation of attention during social versus nonsocial conditions (Research Question 1), three analyses of covariance (ANCOVAs) were run in SPSS 25. The first evaluated overall attention to a social scene (%Scene during *Dyadic Bid*); the second evaluated attention to a face during a social scene (%Face during *Dyadic Bid*); the third evaluated attention to a face during a nonsocial comparison scene (%Face during *Moving Toys*). To determine how attention to faces during a social scene relates to ASD severity and social anxiety, separate Pearson correlations were run between %Face and developmental ability, ADOS CSS, and PAS Social Anxiety raw scores during the *Dyadic Bid* condition only (Research Question 2).

Results

Preliminary Analyses

All analyses were conducted using SPSS 25. Data were screened for violations of assumptions. The Social Anxiety raw score was nonnormally distributed, but ANCOVA is robust to violations of this assumption at this sample size, so analyses proceeded as planned. Descriptive statistics, including means and standard deviations for clinical variables, are presented in Table 2.2.

Table 2.2. Descriptive means (SD)

	nsASD	FXS	TD
Combined NVDQ	69.5 (18.1)	68.5 (21.2)	101.0 (8.52)
Combined VDQ	58.7 (21.5)	61.1 (29.0)	101.5 (13.9)
ADOS-2 CSS	6.63 (1.71)	4.67 (2.50)	1.95 (1.32)
PAS SA Score	2.54 (3.08)	3.25 (4.8)	3.05 (2.67)
Dyadic Bid %Scene	.39 (.22)	.44 (.24)	.60 (.22)
Dyadic Bid %Face	.38 (.24)	.53 (.25)	.57 (.22)
Moving Toys %Face	.14 (.13)	.19 (.15)	.21 (.13)

Note. MSEL = Mullen Scales of Early Learning. NVDQ = Nonverbal Developmental Quotient. VDQ = Verbal Developmental Quotient. DAS = Differential Ability Scales. NVSS= Nonverbal Standard Score. VSS = Verbal Standard Score. ADOS = Autism Diagnostic Observation Schedule. CSS = Calibrated Severity Score. PAS = Preschool Anxiety Scale. SA = Social Anxiety.

A series of univariate ANOVAs indicated that the groups did differ in their chronological age ($F(2, 76) = 5.05$, $p = .009$), verbal ($F(2, 73) = 25.9$, $p < .001$) and nonverbal developmental ability ($F(2, 73) = 26.1$, $p < .001$), and ASD severity ($F(2, 76) = 38.2$, $p < .001$), but not Social Anxiety scores ($F(2, 67) = 0.247$, $p = .782$). Post hoc analyses demonstrated that the ASD group was significantly younger than either the FXS or TD group (ps < .05), but the FXS and TD groups did not differ. For both verbal and nonverbal ability, the TD group was significantly higher than either clinical group (ps < .001), but FXS and ASD did not differ. For ASD severity, all groups were significantly different from each other (ps < .001), with ASD having the highest scores, followed by FXS, and then TD.

A Kruskal-Wallis test also indicated that groups also significantly differed in the distribution of males to females ($H(2) = 8.074$, $p = .018$). Post hoc analyses illustrated that the distribution was different between FXS and both other groups (ps < .05), but

ASD and TD did not differ. Chronological age and sex were included in subsequent models to account for group differences.

Research Question 1

The first research question evaluated whether groups differed on their allocation of attention to the actress's face during social (*Dyadic Bid)* versus nonsocial *(Moving Toys)* conditions. To assess this, three univariate analyses of covariance (ANCOVAs) were conducted, and model results are presented in Table 2.3.

Table 2.3. Results from ANCOVAs examining group differences in social and nonsocial attention

	SS	df	*F*	*p*
Dyadic Bid: %Scene				
Intercept	.428	1	14.2	<.001
Group	.343	2	5.71	.005
Sex	.004	1	.119	.732
Age	.087	1	2.89	.094
Dyadic Bid: %Face				
Intercept	.707	1	13.6	<.001
Group	.323	2	3.12	.050
Sex	.069	1	1.34	.251
Age	.0001	1	.003	.956
Moving Toys: %Face				
Intercept	.054	1	3.33	.072
Group	.032	2	.998	.374
Sex	.033	1	2.06	.156
Age	.003	1	.197	.658

In the first analysis, we evaluated overall attention to a social scene. The dependent variable was %Scene during the social *Dyadic Bid* condition, diagnosis was the between-groups factor, and chronological age and sex were included as covariates. Results indicated a significant effect of diagnosis ($F(2,76) = 5.71$, $p = .005$; partial $\eta^2 =$

.139). Post hoc analyses revealed that the nsASD (p = .005) and FXS (p = .003) group both looked significantly less at the scene than the TD group, but they did not differ from each other. A graph of the estimated marginal means is presented in Figure 2.1.

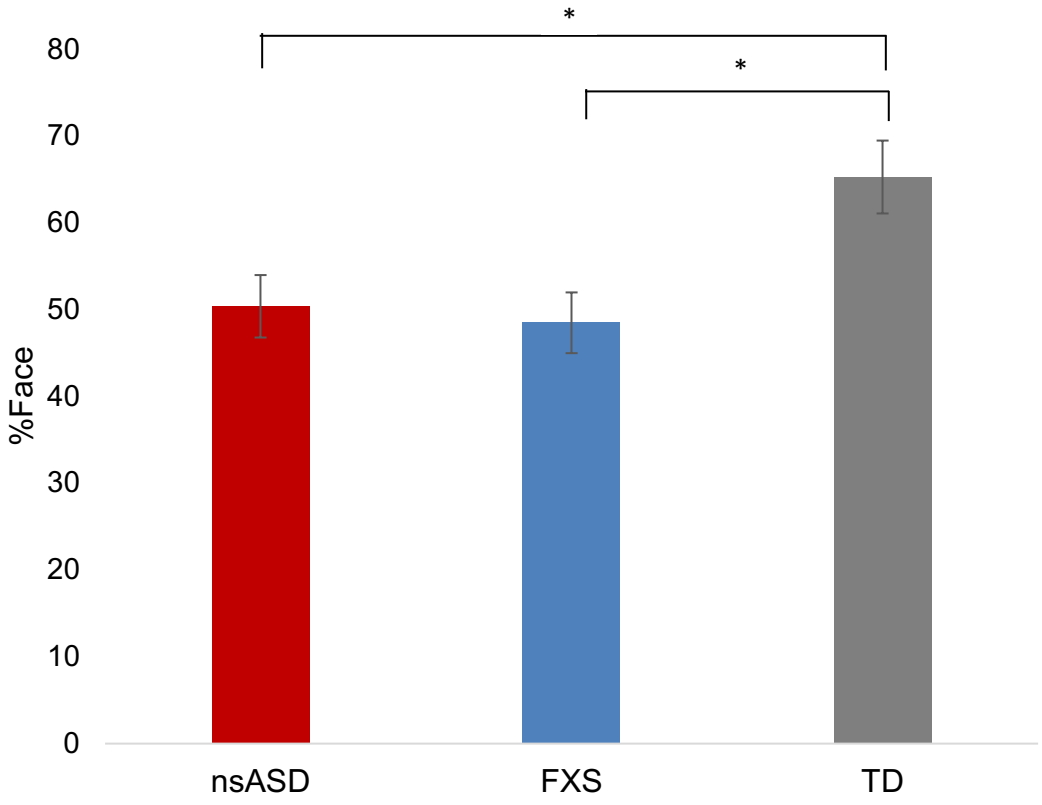

Figure 2.1. Estimated marginal means for attention to the scene during the social Dyadic Bid condition.

Secondly, we evaluated attention to a specific social partner (i.e., the face of an actress engaging in child directed speech). In this analysis, the dependent variable was %Face during the social *Dyadic Bid* condition, diagnosis was the between-groups factor, and chronological age and sex were included as covariates. A significant effect of diagnosis was found ($F(2,76) = 3.12$, p = .050; partial η^2 = .081). Post hoc analyses revealed that this difference was concentrated with the nsASD group, who looked at the

face during the social condition significantly less than both the FXS (p = .036) and TD (p = .042) groups. The FXS and TD groups did not differ (p = .994). A graph of the estimated marginal means is presented in Figure 2.2.

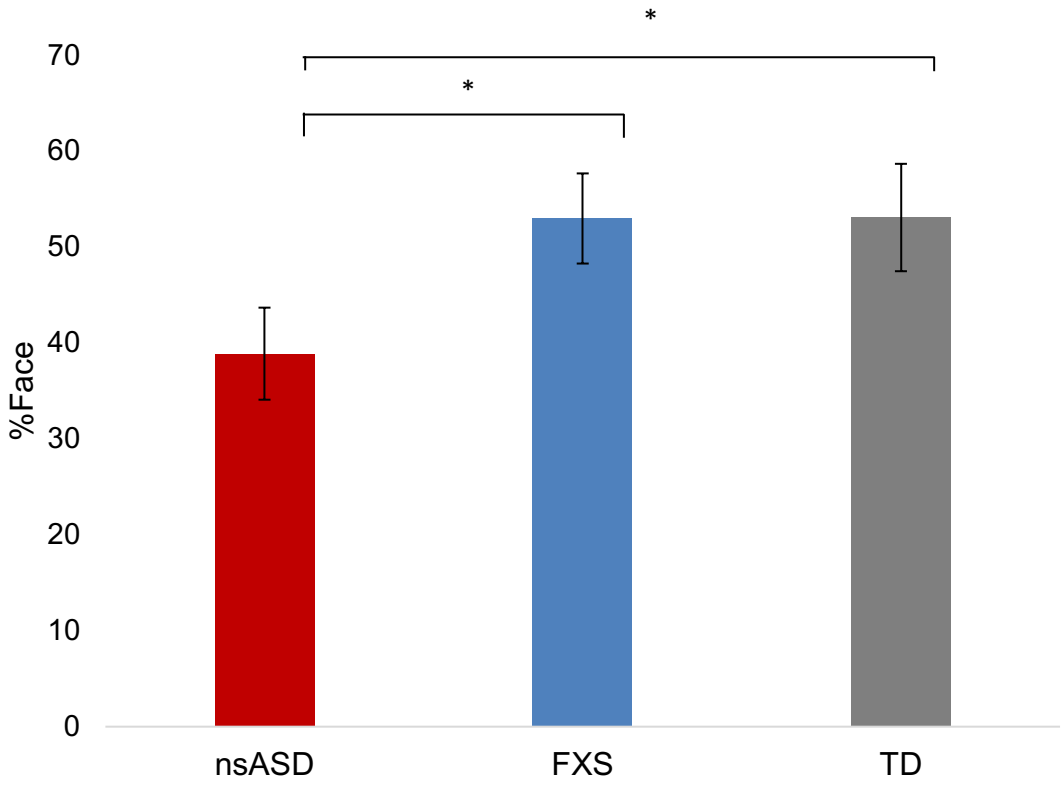

Figure 2.2. Estimated marginal means for attention to the face during the social Dyadic Bid condition.

The final ANCOVA evaluated attention to the actress's face during a nonsocial comparison scene. In this analysis, the dependent variable was %Face during the *Moving Toys* condition, diagnosis was the between-groups factor, and chronological age and sex were again included in the model. This analysis found no difference in %Face between groups during the non-social condition (F(2,71) = .998, p = .374; ; partial η^2 = .029). A graph of the estimated marginal means is presented in Figure 2.3.

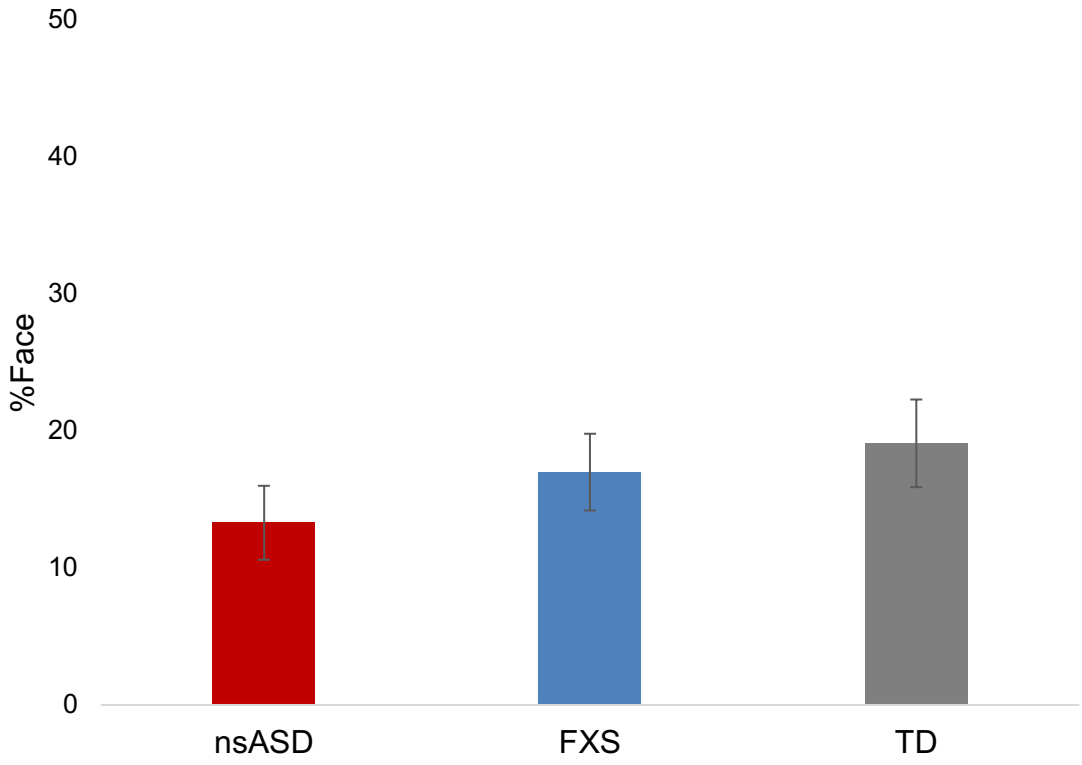

Figure 2.3. Estimated marginal means for attention to the face during the nonsocial Moving Toys condition.

Research Question 2

The second research question evaluated whether social attention related to developmental ability, ASD severity, and social anxiety, separate Pearson correlations were run between %Scene and %Face during the *Dyadic Bid* and ADOS-2 CSS and PAS Social Anxiety scores for each group individually. Results indicated a significant relation between nonverbal (r = .401; p = .031) and verbal ability (r = .372; p = .047) in nsASD. This finding was also observed in FXS, with significant correlations between %Scene and both nonverbal (r = .461; p = .027) and verbal ability (r = .453; p = .030). No

correlations were significant in the TD group. Complete results from this analysis are presented in Table 2.4, and scatterplots are presented in Figure 2.4.

Table 2.4. Pearson correlations between social attention during Dyadic Bid and clinical variables

	nsASD		FXS		TD	
	%Scene	%Face	%Scene	%Face	%Scene	%Face
Combined NVDQ	.401*	.246	.461*	.276	.246	-.217
Combined VDQ	.372*	.191	.453*	.293	.253	.106
ADOS-2 CSS	-.108	.006	-.330	.067	-.224	.-.200
PAS SA Score	-.139	-.201	-.025	.189	.263	.171

Note. *$p < .05$

Figure 2.4. Matrix scatterplot of clinical and eye-tracking variables

Discussion

Social attention is an early-emerging skill that has profound impacts on social and cognitive development (Charman et al., 2000; Mundy & Newell, 2007). Despite a broad literature of social attention deficits in nsASD, relatively little is known about how atypical social attention manifests within and across neurodevelopmental disorders and whether and how it is related to comorbid conditions. The present study aimed to increase the understanding of the nature of social attention impairments in nsASD compared to FXS and typical development and to understand the relation between social attention and clinical symptomology within these distinct diagnostic groups.

Research Question 1

Our first research question evaluated differences in looking at social and non-social information in children with nsASD, FXS, and TD. We found that children with nsASD looked significantly less at a social scene than their TD peers, and less to an actress' face during a dynamic attentional bid than either children with FXS or TD. These findings build upon a large literature documenting impairments in social attention in children with nsASD (Frazier et al., 2017). The present study also expands this body of work by replicating documented, context-dependent attentional impairments (i.e., present only in social conditions and not in nonsocial ones) in a group of children with nsASD and comorbid ID, as well as an older cohort of children than has previously been studied (Chawarska et al., 2012). No relation was found between sex and social attention in the present study, suggesting that, at this age and within these populations, male and females seem to allocate their attention similarly to social information.

Of particular interest are the conclusions drawn from the present study's FXS sample, which differs from nsASD in attention to faces, but not attention to overall social scenes. In terms of attention to faces, children with FXS do not differ from TD controls, but they do show diminished overall attention to social scenes. Our findings add to an emerging and nuanced body of work describing the nature of social attention impairments in FXS using eye-tracking methodologies. We found that young males and females with FXS did not differ in their attention to an overall social scene from their nsASD peers; however, both groups looked significantly less than the TD cohort. Although other studies found an increased preference for social information in older children with FXS compared to nsASD (Hong et al., 2019), our findings suggest that, when presented with a social scene and no other competing stimuli, children with nsASD and FXS perform similarly. The relative salience of social information may be increased in older children with FXS compared to those with nsASD; however, when viewing naturalistic and child-directed social scenes, young children with FXS allocate their attention in a way that mirrors the impairments found in their peers with nsASD.

Interestingly, we found that the above patterns changed when examining attention to faces. That is, children with FXS looked significantly more at the actress's face than their peers with nsASD, and they were similar in their attention to faces from the TD group. Essentially, although children with FXS spend a similar amount of time looking at a social scene compared to those with nsASD, they are allocating far more attention to faces. Much work in nsASD has documented impaired face processing in this group that distinguished this group from non-ASD developmentally delayed and TD children; our study provides further evidence for this effect in a specific phenotype of children with

delays (Adolphs, Sears, & Piven, 2001; Klin & Jones, 2008). Specifically, we found children with FXS do not differ from their TD peers in their attention to faces, and both groups looked at the face more than nsASD. Taken together with existing work in older individuals with FXS that also documents typical attention to faces, our results suggest that this specific social attention impairment may be unique to nsASD (Crawford et al., 2016). Our findings suggest that the mechanisms underlying similar behavioral phenotypes in nsASD and FXS may be quite distinct. This hypothesis is supported by research identifying activation of neural regions and structural differences in persons with FXS that are distinct from nsASD despite demonstration of similar behaviors (Dalton et al., 2008; Hazlett et al., 2009).

Research Question 2

Our second research question evaluated how social attention relates to clinical symptoms in nsASD, FXS, and typical development, including developmental ability, autism symptom severity and social anxiety. We found that attention to an overall social scene increased in association with higher developmental ability in both nsASD and FXS. Our findings are consistent with other research in nsASD that found a significant positive relation between verbal and nonverbal ability and total looking at a social scene, such that higher ability was associated with higher measured attention (Chawarska et al., 2012). Importantly, cluster analysis has identified a particular subgroup of children with nsASD who look significantly less at social scenes than their peers with nsASD and who have poorer developmental outcomes, especially nonverbal and verbal developmental ability; however, this study did not have a developmentally delayed control group, so it is unclear whether this finding is specific to the nsASD phenotype (Campbell et al., 2014).

Interestingly, the present study found a similar relation between attention to a social scene and verbal and nonverbal ability in FXS as compared to nsASD, and it is the first to our knowledge to demonstrate that social attention and developmental ability are correlated in preschoolers with FXS. One other study in older individuals with FXS found no correlation between IQ and social attention (Hong et al., 2019); however, given the importance of social attention to early language acquisition, it is important to understand that this relation may differ at various stages in development.

We did not find a relation between overall social attention and autism symptom severity in any group, and this is inconsistent with previous work that found a negative relation between total looking at a social scene during a dyadic bid and ASD severity in toddlers with nsASD (Chawarska et al., 2012). Given the relatively low developmental ability in the present study, our nsASD sample may be part of a homogenous subgroup, and therefore presents with less clinical variability related to social attention overall (Campbell et al., 2014). Our sample is also slightly older than these earlier studies. Similarly, our findings differ from those in much older individuals with FXS, where a relation has been found between ASD-related social communication deficits and attention to faces (Crawford, Moss, et al., 2017). It is worth noting that previous studies utilized total scores from the ADOS-G or parent-report measures of social communication rather than calibrated severity scores from the revised ADOS-2, so measurement differences may also play a factor in the present results. Nevertheless, taken in the context of the existing literature, the present study underscores the heterogeneity of social attention deficits, especially as they relate to developmental timelines.

Further, we did not find a relation between looking at social information anxiety symptoms in the nsASD and FXS groups. This finding is consistent with existing work that also found no relation between social attention and anxiety in older individuals with ASD, but further study is warranted as this is an area of emerging research (Hong et al., 2019). However, our results differ from previous studies that have shown either a positive (Crawford, Moss, et al., 2017; Hong et al., 2019) or negative relation between social attention and anxiety in FXS (Klusek et al., 2020). Notably, the stimulus used in the present study is non-threatening and child-directed by design, so anxious responses might be less likely to be evoked in this context. Furthermore, the present study includes a much younger and less variable age group of children with FXS, and the relation between social attention and clinical symptoms may not be present early in the development of these disorders. Indeed, these relations could be the result of learned experiences rather than innate differences present in children with FXS.

Limitations and Future Directions

Despite many novel findings and strengths of this study, some limitations warrant discussion. The present study did not isolate face looking to the eyes or mouth region and instead focused only on the face as a whole. Future work should examine whether differential attention to face regions is present in young children with FXS and whether it relates to clinical symptoms. In addition, we only examined two conditions among those presented in the video stimuli. Although the conditions of interest were selected using a principled approach, future work should examine how attention is differentiated between groups across the entire video, especially since significant differences were observed for total looking throughout the entire video, where individuals with nsASD looked less at

the total video than either FXS or TD, and FXS looked less than TD. Although this pattern is not unexpected based on the social nature of the stimuli, given the potential limitations of proportion metrics in this instance, future work should investigate how other metrics (e.g. cohesion models) can more robustly index group differences in looking pattens. (Wang et al., 2018; Yoder & Symons, 2010). Finally, the present study did not differentiate between children with FXS with and without comorbid ASD and instead took a dimensional approach to study ASD symptomology in this group. Further studies interested in the differences and similarities between nsASD, FXS, and FXS with ASD should examine these two groups dichotomously.

Conclusions

Although a growing body of literature has begun to unpack the nature of atypical social attention in nsASD and related conditions, a number of unexplored avenues of research remain. The present study aimed to clarify how social attention impairments manifest in nsASD, FXS, and TD and how social attention relates to clinical symptoms. The present findings revealed nuanced patterns of typical and atypical attention that have the potential to inform our understanding of the mechanisms behind social deficits in children with a variety of neurodevelopmental disorders.

CHAPTER 3

INTEGRATIVE DISCUSSION

The search for a biological marker of ASD and neurodevelopmental disabilities has been a major priority of the field (McPartland et al., 2020; Symons & Roberts, 2013; Zwaigenbaum & Penner, 2018). By pairing the measurement of biological mechanisms with behavioral phenotyping, this dissertation furthers our understanding of early phenotypic heterogeneity of ASD through biobehavioral techniques. These combined studies contribute to our understanding of how ASD and comorbid clinical symptoms (e.g., intellectual disability and social anxiety) manifest across different diagnostic and genetic risk groups using temperament, physiology, and social attention as predictors of interest. When reviewed in the context of the overarching aims of this dissertation, these two unique studies provide a nuanced understanding of the ways that heterogeneity functions among children with or at risk of ASD.

The first study aimed to understand 1) how physiological regulation and temperamental negative affect differ in preschoolers with non-syndromic ASD (nsASD), FXS, and typical development and 2) whether physiological regulation and temperamental negative affect predict ASD symptomology and social anxiety symptoms within all groups. Our findings are the first to indicate that children with nsASD differ from their typically developing peers in their negative affect, but children with FXS do not. Further, our results replicate prior findings that negative affect predicts social anxiety symptoms but not ASD severity in children with FXS and extends this finding to a cohort of children with nsASD. Finally, this study found that baseline RSA was a unique predictor of ASD severity for nsASD only. On the whole, this study provides evidence that biobehavioral markers have the potential to shed light on the nuanced presentation of social anxiety and ASD in different genetic risk groups.

The second study aimed to clarify 1) how social attention impairments manifest in nsASD, FXS, and TD and 2) how social attention relates to comorbid clinical symptoms. First, we found that young children with FXS did not differ in their attention to an overall social scene from their nsASD peers, but they looked significantly more at the actress's face than those with nsASD. Collectively, these findings suggest that children with FXS are allocating more attention to faces than those with nsASD, despite attending to social scenes for a similar amount of time. Our correlational analyses found similar relations among nsASD and FXS as well; for both groups, increased attention to a social scene was correlated with higher verbal and nonverbal developmental ability but not to ASD or social anxiety symptoms. This suggests that the relation between social attention and developmental ability, ASD symptoms, and anxiety is similar among young children with nsASD and FXS. These findings revealed nuanced patterns of typical and atypical attention that have the potential to inform our understanding of the mechanisms behind social deficits in children with neurodevelopmental disorders.

By taking a biobehavioral measurement approach, these studies leverage the importance of deep phenotyping towards improving our understanding of heterogeneity in ASD (McPartland et al., 2020). The biobehavioral measurement of phenotypic heterogeneity has the potential to make a tremendous impact on the field of ASD and other neurodevelopmental disorders. First, this process can inform differential diagnoses by illuminating the presence of underlying mechanistic differences between similar phenotypic presentations. It can also help identify specific therapeutic needs for children, thereby informing potential treatment targets that are individualized to each child's unique profile of strengths and weaknesses. Finally, biobehavioral measurement is

potentially more sensitive to change than traditional ASD measures, and accordingly has the potential to evaluate intervention success and advance clinical research. Early studies have already begun evaluating biobehavioral markers and demonstrated that they have the potential to measure subtle, biological changes as a result of treatments that are yet unobservable using behavioral indices alone (Hessl et al., 2019; McPartland et al., 2020; Murias et al., 2018).

This dissertation has illustrated, using a variety of indices, that there is much variability in temperament, physiology, and social attention both across and within diagnostic groups. Further, there are both similarities and differences in the way these biobehavioral indices predict clinical outcomes. Future work should build upon specific findings presented here to advance clinical research into the mechanisms underlying behavioral and clinical impairments across neurodevelopmental disorders.

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