Summer 2021

Characterizing Anxiety and Physiological Correlates in Preschool Children with Neurodevelopmental Disabilities

Jordan Ezell

Characterizing Anxiety and Physiological Correlates in Preschool Children with Neurodevelopmental Disabilities

by

Jordan Ezell

Bachelor of Arts
Samford University, 2013

Master of Arts
University of South Carolina, 2018

Submitted in Partial Fulfillment of the Requirements

For the Degree of Doctor of Philosophy in

School Psychology

College of Arts and Sciences

University of South Carolina

2021

Accepted by:

Jane Roberts, Major Professor

Kimberly Hills, Committee Member

Jessica Bradshaw, Committee Member

Connor Kerns, Committee Member

Tracey L. Weldon, Interim Vice Provost and Dean of the Graduate School

ABSTRACT

This dissertation is comprised of two original research manuscripts broadly examining anxiety and potential correlates in young children with neurodevelopmental disabilities. The first manuscript assessed cardiac regulation during an auditory startle paradigm in young children (3-6 years old) with autism spectrum disorder (ASD), fragile x syndrome (FXS), and neurotypical peers. The second manuscript utilized a new measure developed to capture anxiety in individuals with ASD in order to assess the rate of typical and atypical anxiety and potential risk factors (ASD severity, sex, cognitive ability) for anxiety in preschool children with ASD contrasted to neurotypical preschool children. Collectively, these two manuscripts address the limited understanding and many challenges of measuring anxiety disorders in young children with developmental disabilities and what individual characteristics may serve as risk or protective factors for these children. Additionally, the comparison of children with ASD to children with FXS in the first study can inform both shared risk and divergent features of each disorder. Overall, these studies provide insight into the complex presentation of anxiety in young children with comorbid developmental disorders. Further, this work can inform the early identification of risk factors to develop more targeted interventions in early life to improve long-term outcomes in children with neurodevelopmental disabilities.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ASD ..... Autism Spectrum Disorder

FXS ..... Fragile X Syndrome

ID ..... Intellectual Disability

IBI ..... Inter-Beat-Interval

RSA ..... Respiratory Sinus Arrythmia

CHAPTER 1

CARDIAC STARTLE RESPONSE AND CLINICAL OUTCOMES IN PRESCHOOL CHILDREN WITH FRAGILE X SYNDROME AND AUTISM SPECTRUM DISORDER

Physiological regulation during threat is a critical adaptive response formed early in development. When a threat is experienced, the autonomic nervous system (ANS) activates the sympathetic branch in response, while the parasympathetic branch supports the body in recovering to a baseline state after the threat has passed. Well-integrated physiological regulation is related to a range of positive outcomes including better language skills, increased social responsiveness, peer engagement, emotion recognition, healthy social attachment, and social approach (see Beauchaine, 2001; see Klusek, Roberts, & Losh, 2015). In contrast, physiological dysregulation is linked to a litany of maladaptive outcomes including emotion dysregulation, social deficits, delayed adaptive skills, and a range of psychological disorders (Patriquin, Scarpa, Friedman, & Porges, 2013; Schmitz, Krämer, Tuschen-Caffier, Heinrichs, & Blechert, 2011). Specifically, heightened physiological response and reduced modulation in response to threat, like an auditory startle, can be indicative of anxiety (Bakker, Tijssen-De Koning, Van Der Meer, Koelman, & Boer, 2009; Levine, Fleming, Piedmont, Cain, & Chen, 2016). Individuals with neurodevelopmental disabilities are at an elevated risk for physiological dysregulation and emotional difficulties, despite differing etiologies. Because there is a clear relationship between physiological regulation and developmental outcomes

(Porges, 1992; Thayer, Friedman, & Borkovec, 1996), studying this phenomenon in individuals One way to capture physiological regulation is to measure heart activity in response to threat. Two cardiac indices of physiological regulation are inter-beat interval (IBI), defined as the time between heartbeats and an indicator of heart rate, and respiratory sinus arrhythmia (RSA), the temporal variation in IBI synced with respiration. When faced with an unpredictable or sudden threat, a specific pattern of physiological reactivity, known as the startle response, is often observed. This startle response is considered a primitive, elicited response to intense or sudden stimuli that prepares the body for "fight or flight" (Davis, 2006). During a startle response, the sympathetic nervous system is activated, allowing blood flow to move more rapidly to the extremities and breathing to increase, which allows the body to mobilize a response. During this phase, both reduced IBI (i.e., less time between heartbeats), and reduced RSA (i.e., less variability in time between heartbeats) are typically observed. After the threat has passed, the parasympathetic nervous system becomes activated, which causes breathing to slow and blood flow to return to central organs (Porges, 1995). Additionally, IBI and RSA both increase as they return to baseline "resting state" levels.

Individuals with anxiety often show an atypical physiological startle response. For example, adults and children with anxiety exhibit lower resting RSA, which leads to the sympathetic system over-responding to threat, causing an exaggerated startle reflex of increased muscle tension, blink response, RSA withdrawal, and galvanic skin response. Individuals with lower resting RSA also show a slower return to baseline state after a threat (Bakker et al., 2009; Gorka et al., 2013; Levine et al., 2016). Additionally, children with anxiety often exhibit a shorter IBI at resting state and a slower IBI recovery than

non-anxious peers, suggesting that they have restricted autonomic flexibility in response to threatening stimuli (Paniccia, Paniccia, Thomas, Taha, & Reed, 2017; Schmitz et al., 2011; Thayer et al., 1996). Evidence also suggests that lower RSA during a resting state is predictive of a heightened startle response in typical adults (Gorka et al., 2013). Taken together, these studies provide compelling evidence that physiological dysregulation, particularly in response to threat, may underlie vulnerability to anxiety in neurotypical individuals. However, little work has examined IBI and RSA during a startle paradigm in children with neurodevelopmental disorders, such as autism spectrum disorder (ASD) or fragile X syndrome (FXS).

Children with neurodevelopmental disorders appear to be at elevated risk for both physiological dysregulation and anxiety. Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social communication skills and the presence of restricted, repetitive behaviors (American Psychiatric Association, 2013). Current estimates suggest that 1 in 54 children have an ASD, and approximately 40-50% will develop a co-occurring anxiety disorder (Maenner et al., 2020; F. J. A. van Steensel, Bögels, & Perrin, 2011; White, Oswald, Ollendick, & Scahill, 2009). Fragile X Syndrome (FXS) is a monogenic disorder characterized by atypical social and communication skills, repetitive behaviors, and intellectual disability. Approximately 1 in 4000 males and 1 in 8000 females have the full mutation of FXS, which results from a mutation on the *FMR1* gene of >200 CGG repeats (Cohen, 1995; Oostra & Willemsen, 2003). Similar to children with ASD, children with FXS are also at a heightened risk for developing comorbid anxiety disorders (50-86%; Cordeiro, Ballinger, Hagerman, & Hessl, 2011; Ezell et al., 2018). Children with FXS also exhibit a behavioral phenotype

that is strikingly similar to ASD, with approximately 60% of children with FXS also meeting diagnostic criteria for ASD (Abbeduto et al., 2019; Roberts et al., 2020b). For instance, repetitive speech and behaviors, social avoidance, aberrant eye contact, and physiological dysregulation are features common to both ASD and FXS. Given the similar behavioral difficulties but divergent etiology between non-syndromic ASD (nsASD) and FXS, cross-population studies can provide insight into genetic contributions to physiological and emotional dysregulation (see Klusek et al., 2015).

Studies of children with ASD suggest that autonomic dysregulation is common in this population with findings showing a persistent state of hyperarousal, though the pattern of findings are not consistent. For example, at baseline, most studies have found that individuals with ASD are hyper-aroused showing lower RSA and higher heart rate than peers, yet some studies found no differences (see Klusek et al., 2015; Patriquin, Hartwig, Friedman, Porges, & Scarpa, 2019). Physiological responses to threat also appear to be atypical but varied in ASD, as evidenced by hyperarousal during cognitive tasks (elevated heart rate, lower RSA), no differences in heart rate during social interactions, and a blunted heart rate during social performance tasks (lower during stress) (Dijkhuis, Ziermans, van Rijn, Staal, & Swaab, 2019; Hollocks, Howlin, Papadopoulos, Khondoker, & Simonoff, 2014; Corbett, Muscatello, & Baldinger, 2019).

Some evidence suggests that features comorbid with ASD can also influence physiological response to threat, including anxiety and intellectual disability (ID). The role of anxiety in physiological responses to stress within ASD is limited and not well understood. In studies of children and adolescents with ASD and comorbid anxiety, a blunted physiological response to threat (i.e., lower heart rate, less electrodermal activity)

has been found compared to individuals with ASD only and typically developing peers (Hollocks et al., 2014; Panju, Brian, Dupuis, Anagnostou, & Kushki, 2015). In addition to anxiety, differences are also evident in individuals with ASD and ID in that some studies indicate that these individuals are hyper-aroused and show little variation in HR in response to stimuli (Patriquin et al., 2019). Combined, these findings suggest that the coordination of the sympathetic and parasympathetic nervous system's threat response is disrupted in some way, particularly in those children who also have anxiety or ID alongside ASD. Understanding the characteristics and outcomes of children with poor physiological regulation is important because reduced resting RSA has been associated with poorer language skills and social responsiveness in children with ASD. Overall, evidence suggests that physiological regulation has critical implications for downstream social-communicative functioning (Edmiston, Jones, & Corbett, 2016; Patriquin et al., 2013; Patriquin et al., 2019).

Evidence for physiological dysregulation is generally more consistent in children with FXS than in children with ASD. Overall, individuals with FXS show a developmental affect that becomes more pronounced with age, from hypo-arousal in the first two years of life towards hyperarousal thereafter, exhibiting reduced IBI and RSA during resting state (see Klusek et al., 2015; Roberts, Tonnsen, Robinson, & Shinkareva, 2012). In response to threat, one study found that infants with FXS showed reduced RSA during a stranger approach paradigm, a task designed to elicit social fear where an examiner dresses in a disguise and approaches the child (Tonnsen, Malone, Hatton, & Roberts, 2013). Another study found no differences in change in IBI in response to an auditory startle between boys with FXS and neurotypical boys, but did find that older

boys with FXS showed a stronger startle response than younger boys with FXS (Roberts et al., 2013). Similarly, in another study examining heart activity during rest and stress, adolescents with FXS remained in an aroused state throughout rest and stress periods (i.e., reduced RSA, shorter IBI) compared to typically developing peers (Boccia & Roberts, 2000). Thus, it appears that children with FXS exhibit chronic hyperarousal through baseline arousal as well as response to cognitive or social threats, which becomes more pronounced over age. However, the link between the physiological startle response and anxiety has not been investigated in FXS. Further, information regarding the potentially compounding impacts of ASD on physiological dysregulation in FXS is limited and a direct comparison of startle response between children with FXS with and without ASD has not been conducted, although there is evidence for decreased cognitive and adaptive functioning in children with FXS and ASD (Abbeduto et al., 2019; Hernandez et al., 2009).

1.1 PRESENT STUDY

Although a link between physiological response to threat and anxiety has been established in neurotypical populations, it remains understudied in clinical groups at elevated risk for anxiety, such as ASD and FXS. A clearer understanding of the cardiac startle response in individuals with neurodevelopmental disabilities and how it relates to clinical features can provide insight into the mechanistic underpinnings of negative behavioral outcomes and guide the development of targeted prevention and intervention programs. The present study is the first to assess the cardiac startle response in preschool-aged children with nsASD, FXS with comorbid ASD (FXS+ASD), and FXS only (FXS-

Only) compared to neurotypical peers (NT). Our specific research questions are as follows:

1a.) Are there differences in cardiac response (IBI) to threat during an auditory startle paradigm between preschool children with nsASD, FXS+ASD, and FXS-Only compared to NT controls? 1b.) Does pre-startle RSA predict startle IBI differentially across groups?

2. Does cardiac response to startle (IBI) relate to nonverbal mental age, ASD severity, or parent-reported anxiety, and do these relationships differ by group?

Given the current evidence for cardiac dysregulation in both FXS and ASD, it is predicted that the clinical groups will demonstrate an exaggerated physiological response to threat (shorter IBI) after an unexpected auditory stimulus compared to the neurotypical (NT) controls. Additionally, it is expected that higher baseline RSA will be associated with longer IBI during the auditory stimulus, regardless of group. Exaggerated startle is predicted to be related to clinical outcomes like low nonverbal mental age, high ASD severity, and high anxiety symptoms, given the connection between poor regulation and negative outcomes.

CHAPTER 2

METHODS

2.1 PARTICIPANTS

Participants for this study were drawn from an ongoing NIMH study (1R01MH107573-01A1; PI: Roberts) that is focused on the emergence of anxiety symptoms in young children with neurodevelopmental disabilities. The present sample consisted of 107 children between 36-72 months of age divided into four diagnostic groups: non-syndromic ASD (nsASD; n = 42, 36 males), FXS with comorbid ASD ruled out (FXS-Only; n = 21, 11 males), FXS with comorbid ASD (FXS+ASD; n = 17, 13 males), and neurotypical controls (NT; n = 27, 20 males). Participants were excluded if they were born premature (gestational age < 37 weeks) or had a history of seizures. Participants with FXS were confirmed to have the full mutation (>200 CGG repeats) of the *FMR1* gene through genetic records. The nsASD group had no known genetic disorders, but genetic testing was not confirmed in the present study. Autism diagnoses for the nsASD and FXS+ASD groups were confirmed through a Clinical Best Estimate (CBE) review process (Roberts et al., 2020a). The NT sample had no known diagnoses nor family history of ASD. The FXS-Only and NT samples were confirmed to not have ASD through the CBE process. Both males and females were included in the study in order to reflect the heterogeneity of the populations.

2.2 MEASURES

Auditory Startle Paradigm. The Anxiety Dimensional Observation Schedule (Anx-DOS; Mian, Carter, Pine, Wakschlag, & Briggs-Gowan, 2015) is an observational measure that consists of a variety of tasks designed to elicit anxious and fearful behaviors in preschool-aged children. During the auditory startle task, each participant watched a two-and-a-half minute silent children's movie while wearing a heart rate monitor. Approximately halfway through the movie, a 200ms white noise burst occurs at approximately 98 decibels.

Physiological Regulation. Heart activity data was recorded continuously through two electrodes placed onto the child's chest using the Actiwave Cardio Monitor (CamNtech Ltd., Cambridge, UK) at 1024 Hz. To ensure uniformity among participants, a trained research assistant identified the heart activity data period of interest as 30sec prior to the auditory stimulus through 90sec after. The cropped heart activity data was visually inspected and edited off-line for artifacts, arrhythmias, and false heart periods by trained research assistants using CardioEdit software (Brain-Body Center, University of Illinois at Chicago). Mean values for RSA and IBI were extracted using CardioBatch software (Brain-Body Center, University of Illinois at Chicago). To calculate RSA values, CardioBatch samples sequential heart periods at 250ms epochs and then de-trends the data with a 21-point moving polynomial algorithm (Porges & Bohrer, 1990). The data was then bandpassed filtered to extract variance associated with spontaneous breathing parameters (0.24-1.04 Hz). The variance was then changed to its natural logarithm to provide an estimate of RSA.

Pre-Stimulus RSA was defined as the mean RSA for the 30 seconds prior to the auditory stimulus as a measure of baseline RSA. Stimulus IBI was the mean IBI extracted from the 1-second interval that began at the onset of the auditory stimulus. Post-Stimulus IBI was the mean IBI extracted from the 1-second interval at 10 seconds post-stimulus (Figure 1). Pre-stimulus RSA assessed the capacity for regulation during the startle, while stimulus and post-stimulus IBI captured the acute cardiac startle response.

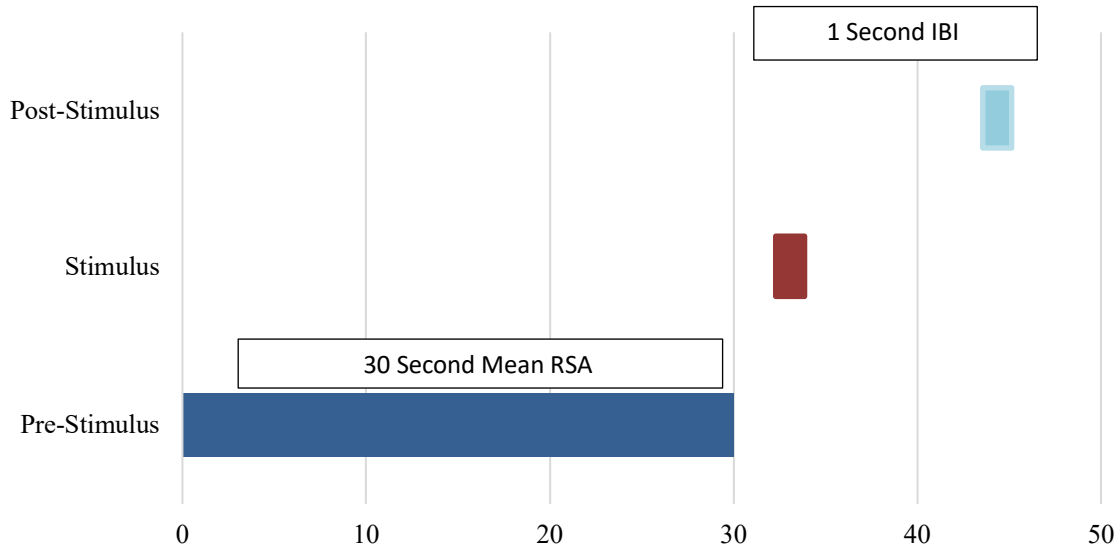

Figure 2.1 Three Phases of the Auditory Stimulus Paradigm

Autism Diagnosis and Severity. The *Autism Diagnostic Observation Schedule- 2nd Edition (ADOS-2)* is a semi-structured, play-based observational measure assessing the presence of autism symptomology (Lord et al., 2012). The tasks in the ADOS-2 are designed to elicit social-communication skills, and the presence of restricted, repetitive behaviors. The ADOS-2 has four modules (1-4), ranging from nonverbal to fluent verbal abilities. The ADOS-2 was administered and scored by research reliable, graduate-level professionals and reviewed by a licensed psychologist through the CBE process to confirm an ASD diagnosis in the ASD group. Site reliability was conducted on 20% of the ADOS-2 administrations (item-level inter-rater agreement=83.3%). The Calibrated Comparison Score (CSS) is an overall severity composite relative to children of similar language abilities, ranging from 1-10. The CSS was used as a continuous variable of ASD severity across groups for analysis in the present study.

Anxiety Symptoms. The *Spence Preschool Anxiety Scale (PAS)* (Spence, Rapee, McDonald, & Ingram, 2001) is a 34-item caregiver report of anxiety symptoms in children aged 2.5-6.5 years. Item scores range from 0-4 and summary scores are computed for Generalized Anxiety, Social Anxiety, Obsessive-Compulsive disorder, Physical Injury Fears, Separation Anxiety, and Total Anxiety. For this study, the Total Anxiety raw score was used as a measure of overall anxiety symptoms. While the PAS was developed for typically developing children, studies suggest that it is an appropriate tool for parent-reported anxiety for children with ASD because many questions target observable behaviors (Carruthers, Kent, Hollocks, & Simonoff, 2020; Wigham, Rodgers, South, McConachie, & Freeston, 2015; Zainal et al., 2014).

Developmental Level. The *Mullen Scales of Early Learning (MSEL, Mullen, 1995)* is a standardized measure designed to assess development from birth to 68 months across gross and fine motor skills, receptive and expressive language, and visual reception. Children with ASD and FXS often have language delays that can overshadow cognitive skills, particularly before age 5 (Lord, Risi, & Pickles, 2004). Evidence suggests that nonverbal IQ is a more stable and accurate representation of cognitive ability in young children with ASD (Akshoomoff, 2006). Thus, nonverbal mental age (NVMA) was used as an index of nonverbal cognitive ability for the present study. NVMA was computed by averaging the visual reception age equivalent and the fine motor age equivalent ($\frac{\text{VR age} + \text{FM age}}{2}$) (Stephens et al., 2018). The MSEL shows good internal consistency for each subscale (0.75 to .08), and test-retest reliability (.70 to .80).

CHAPTER 3

RESULTS

3.1 ANALYTIC PLAN

Statistical analysis was conducted through three phases: preliminary analysis, stimulus analysis (RQ1), and correlates of startle response (RQ2). First, in the preliminary analysis, groups were compared through one-way ANOVAs to assess for group differences in chronological age, NVMA, ADOS-2 CSS, and PAS Total Anxiety scores. Pre-stimulus IBI was also compared through a one-way ANOVA to assess for group differences in IBI prior to the auditory stimulus (i.e., baseline IBI). Second, to evaluate the cardiac response to the auditory stimulus (RQ1a), multilevel regression models were run to assess group differences in IBI during the 1-second period that began at the onset of the auditory stimulus and the 1-second period occurring 10 seconds post-stimulus to test time by group interactions. The interaction was probed by centering time at the stimulus and at 10 seconds post-stimulus to determine any points of significant divergence in IBI trajectories (Cohen et al, 2014). Because IBI increases during the preschool developmental period, groups were matched on chronological age. Then, a multiple regression model was run to assess if pre-startle RSA predicted startle IBI with group as a covariate (RQ1b). Finally, a within group correlations were used to determine whether PAS Total Anxiety scores, ADOS-2 CSS, or NVMA predicted IBI at the stimulus and post-stimulus for each group (RQ2).

3.2 PRELIMINARY ANALYSIS

Results of the first one-way ANOVA showed no significant group differences for chronological age ($F(3, 106) = 0.14$; $p = .94$). As anticipated, significant differences were found for ADOS-2 CSS between groups ($F(3, 92) = 78.8$; $p < .001$), in that the nsASD and FXS+ASD groups showed significantly higher severity scores than the FXS-Only and NT groups, and the FXS-Only group showed higher severity scores than the NT group. Results of the one-way ANOVA for NVMA also showed significant differences between groups ($F(3, 102) = 24.3$; $p < .001$). As expected, the NT group demonstrated significantly higher NVMA than the FXS+ASD, ASD, and FXS-Only groups, and the FXS+ASD group showed significantly lower NVMA than all groups. The nsASD and FXS-Only groups were not significantly different on NVMA. For the PAS Total Anxiety Raw score, no significant group differences were observed ($F(3,92) = 1.48$, $p = .23$). Lastly, results of the one-way ANOVA indicated that groups did not differ on pre-stimulus IBI ($F(3,102) = 1.25$, $p = .29$). Table 3.1 depicts means and standard deviations for each group.

Table 3.1 Descriptive Data by Group

	Gender M:F	**Age in Months** Mean (*SD*)	**ADOS-2 CSS** Mean (*SD*)	**NVMA** Mean (*SD*)	**Spence Raw Total** Mean (*SD*)	**Pre-Stimulus IBI** Mean **(***SD***)**	**Pre-Stimulus RSA** Mean **(***SD***)**
nsASD (*n* = 42)	36:6	46.08 (8.0)	7.09 (1.4)$_a$ (n = 35)	30.24 (11.2)$_b$ (n = 40)	12.17 (10.0) (n = 35)	574.06 (73.48)	5.90 (1.44)
FXS-Only (*n* = 21)	11:10	47.46 (7.7)	3.56 (1.8)$_b$ (n = 18)	35.08 (10.3)$_b$ (n = 20)	14.44 (11.1) (n = 18)	577.18 (57.56)	6.08 (1.25)
FXS+ASD (*n* = 17)	13:4	46.10 (9.3)	7.33 (1.5)$_a$ (n = 15)	22.50 (5.9)$_c$ (n = 16)	15.47 (9.3) (n = 15)	583.71 (105.29)	5.20 (1.99)
NT **(***n* = 27)	20:7	46.55 (9.2)	2.0 (1.2)$_c$ (n = 25)	47.59 (10.7)$_a$ (n = 27)	9.8 (6.2) (n = 25)	584.89 (76.40)	6.30 (1.39)

Note. Group differences ($p < .05$) are indicated by different subscripts.

3.3 STARTLE ANALYSIS

Multilevel regression results with time centered at the stimulus (30 seconds) indicated that, the nsASD group exhibited a significantly shorter IBI than the NT group (b = -33.856, p = 0.027). Neither the FXS-Only group nor the FXS+ASD groups showed significant trajectories from the NT group with time centered at the stimulus (see table 3.2). Results from the model probing at 10 seconds post-stimulus (40 seconds) indicated that the nsASD group continued to display a shorter IBI than the NT group (b = -35.120, p = 0.026). Further, the FXS-Only group and the FXS+ASD groups continued to show similar IBIs to the NT group with time centered at 10 seconds post-stimulus. The interaction of IBI by time was not significant indicating that this group difference is present regardless of time (see table 3.3). Post-hoc multilevel regressions were then used to assess for group differences between the nsASD group and the FXS+ASD and the FXS-Only group. Results indicated no significant differences between the three clinical groups for IBI at the stimulus or post-stimulus (ps > 0.594).

Table 3.2 Regression Model Centered at Stimulus

	b	SE(*b*)	*p*
Intercept	609.445	13.190	< .001
Epoch 30s	0.014	0.014	0.90
FXS+ASD	-25.850	21.22	0.23
FXS-Only	-27.508	19.94	0.17
nsASD	-35.501	16.91	0.038
FXS+ASD x Epoch 30s	-0.003	0.17	0.99
FXS-Only x Epoch 30s	0.060	0.16	0.71
nsASD x Epoch 30s	-0.126	0.14	0.36

Table 3.3 Regression Model Centered at Post-Stimulus Regression

	b	SE(*b*)	*p*
Intercept	609.59	13.25	< .001
Epoch 40s	0.014	0.11	0.90
FXS+ASD	-25.88	21.31	0.23
FXS-Only	-26.91	20.03	0.18
nsASD	-36.76	16.98	0.032
FXS+ASD x Epoch 40s	-0.003	0.17	0.99
FXS-Only x Epoch 40s	0.060	0.16	0.71
nsASD x Epoch 40s	-0.126	0.14	0.36

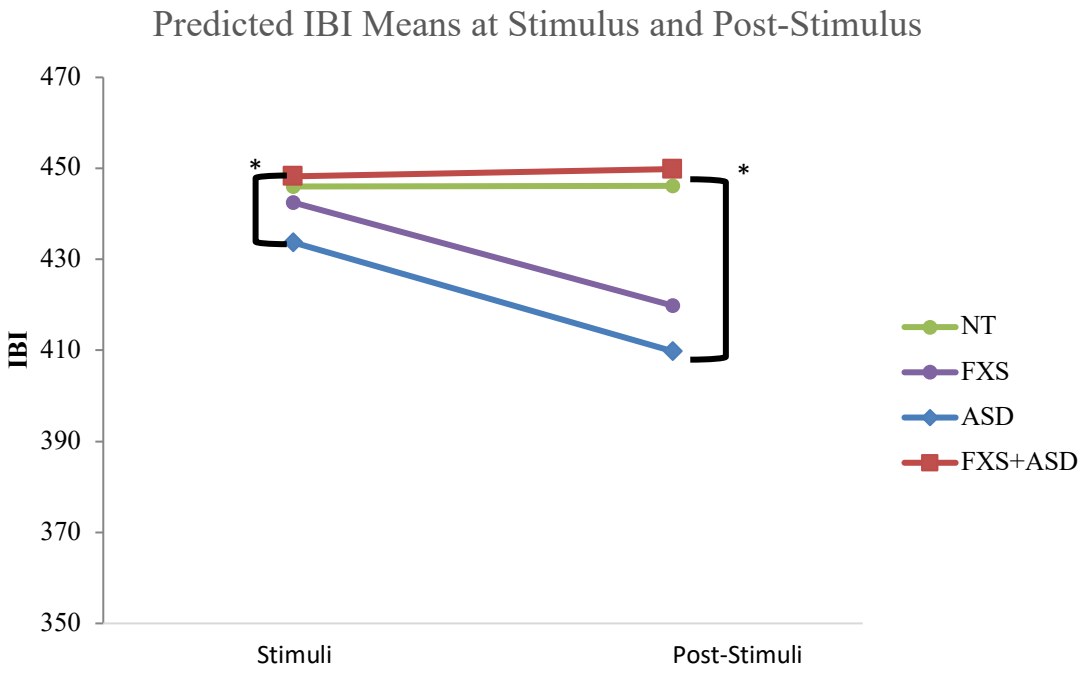

Figure 3.1 Epoch by Group Comparison of Inter-Beat Intervals during Startle Paradigm

Note. Significant differences were seen between the ASD and the NT groups for stimulus (p = .03) and post-stimulus IBI (p = .02).

3.4 RELATIONSHIP BETWEEN PRE-STIMULUS RSA AND STIMULUS IBI

Results from the multiple regression model assessing differential associations of pre-stimulus RSA to stimulus IBI as a function of group accounted for approximately 49% of the variance. Additionally, parameter estimates indicated that accounting for group, pre-stimulus RSA was a predictor for stimulus IBI ($b = 41.68$; $p < 0.001$) such that for each unit increase in pre-stimulus RSA, there was an associated increase of 41.7ms in IBI. Further the effect of pre-stimulus RSA on stimulus IBI did not differ as a function of group (see table 3.4).

Table 3.4 Regression Model of Pre-Stimulus RSA to Stimulus IBI

	b	SE(*b*)	*p*
Intercept	352.40	56.52	< .001
Pre-Stimulus RSA	41.68	8.80	< .001
FXS+ASD	-69.71	70.88	0.33
FXS-Only	42.34	88.65	0.63
nsASD	28.69	69.54	0.68
FXS+ASD x Pre-Stimulus RSA	18.74	11.70	0.11
FXS-Only x Pre-Stimulus RSA	-11.03	14.10	0.44
nsASD x Pre-Stimulus RSA	-8.13	11.05	0.46

3.5 RELATIONSHIP BETWEEN STARTLE RESPONSE AND CLINICAL OUTCOMES

Lastly, correlation analysis was used to assess if nonverbal mental age, ASD severity, or parent-reported anxiety predicted stimulus IBI or post-stimulus IBI within each group. NVMA was significantly correlated with stimulus IBI for the NT group (r = .63, p < .001) and FXS-Only group (r = .50, p = 0.022) and for post-stimulus IBI for the NT group (r = .53, p =.004) and for the ASD group (r = .36, p = 0.022). The FXS+ASD group did not show an association between NVMA and cardiac response to stimulus (p > 0.273). ADOS-2 CSS was moderately correlated with stimulus IBI (r = .44, p = 0.07) and significantly correlated with post-stimulus IBI (r = .53, p = 0.02) for the FXS-Only group, but no relationship was found for any other groups. Lastly, PAS Total Anxiety Raw Score was not significantly correlated with stimulus IBI or post-stimulus IBI for any groups (Table 3.5).

Table 3.5 Post-Hoc Correlations

		ADOS-2 CSS	NVMA	Spence PAS
nsASD	Startle IBI	r = -0.13	r = 0.26	r = 0.22
	Post-startle IBI	r = -0.10	r = 0.36*	r = 0.23
FXS-Only	Startle IBI	r = 0.44	r = 0.50*	r = -0.17
	Post-startle IBI	r = 0.53*	r = 0.42	r = -0.08
FXS+ASD	Startle IBI	r = -0.19	r = 0.12	r = -0.07
	Post-startle IBI	r = -0.28	r = 0.29	r = -0.02
NT	Startle IBI	r = 0.27	r = 0.63**	r = -0.13
	Post-startle IBI	r = 0.22	r = 0.53**	r = -0.23

CHAPTER 4

DISCUSSION

Poor physiological regulation in response to threat is linked to a range of negative developmental outcomes including anxiety, behavioral difficulties, and low adaptive skills, which are highly prevalent and impairing in young children with neurodevelopmental disabilities, like FXS and ASD (see Klusek et al., 2015; Patriquin et al., 2013; S. Porges, 2013; Schmitz et al., 2011). In this study, we examined the cardiac response to an auditory startle in an age-matched sample of preschool children with FXS, ASD, and typical development. We also investigate clinical features that are thought to be associated with poor physiological regulation. Our results indicate that the children with ASD exhibit an exaggerated cardiac response to a sudden auditory stimulus that differentiated them from NT children, but no other significant group differences were observed. Elevated pre-startle RSA was associated with longer IBI at the stimulus across all the groups. In contrast, more severe ASD symptoms were associated with reduced cardiac startle only in the group with FXS that did not have ASD. Also, elevated non-verbal ability was related to reduced cardiac startle in the NT, nsASD and FXS group without ASD but not in the FXS group with ASD. Finally, parent-reported anxiety symptoms were not associated with cardiac startle in any group.

4.1 CARDIAC AUDITORY STARTLE RESPONSES ACROSS GROUPS

The present study found that preschool children with nsASD showed greater cardiac startle to an auditory startle relative to NT peers. Our findings are consistent with previous studies of individuals with ASD that indicate physiological dysregulation within this population (Garber, Visootsak, & Warren, 2008; Klusek et al., 2015; Patriquin et al., 2019; White et al., 2014b). While abnormalities in arousal are consistent in ASD, specific patterns of physiological response to stress are varied, possibly reflecting the heterogeneity of the ASD population. The present study is the first to assess cardiac startle response in young children with ASD and ID, as the majority of previous studies were conducted with individuals with higher cognitive and language abilities or older individuals with ASD. Given the heterogeneity of ASD populations, demonstrating hyperarousal in a relatively homogeneous sample of preschool children with ASD and ID compared to an age-matched sample shows that this trend towards hyperarousal begins early in life.

Understanding patterns of physiological activity in children with ASD is important, as hyperarousal has been theorized to underlie behavioral and learning difficulties often present in ASD. A review by White et al, 2015, posits that physiological hyperarousal in ASD is linked to emotion dysregulation, which can lead to social and psychological difficulties like anxiety (White et al., 2014). Emotional and behavioral problems can impact early learning and compound existing developmental delays and deficits. For instance, children with FXS and ASD often have more delays early in infancy and behavioral difficulties that cause long-term impairment, including the ability to become independent (Bailey, Hatton, Skinner, & Mesibov, 2001; Caravella & Roberts,

2017; Hahn, Brady, Warren, & Fleming, 2015; Hogan et al., 2017; Kaufmann et al., 2004; Klusek, Martin, & Losh, 2014). It is essential to understand the presentation of physiological abnormalities in ASD early in life in order to improve developmental outcomes, including social and emotional health (Lydon et al., 2016; Patriquin et al., 2019).

Our findings show interesting parallels with two studies assessing startle responses in individuals with FXS. First, a study by Cohen et al., 2015, compared physiological reactivity (electrodermal, heart activity, eye blink) in males aged 10-17 years old with ASD, FXS+ASD, FXS-Only, and NT controls as they viewed emotional stimuli (Cohen, Masyn, Mastergeorge, & Hessl, 2015). Similar to the present study, Cohen et al. found that the two FXS groups showed similar patterns of cardiac reactivity, despite the presence of ASD. In contrast, in the Cohen et al study, the FXS groups showed higher cardiac activity than the ASD group, whereas in the present study the nsASD group showed elevated cardiac activity compared to the FXS groups. Since the present study consisted of young children, one possible explanation for differences is increased arousal over age in individuals with FXS (see Klusek et al., 2015; Roberts et al., 2012).Further, the present study included females and children with intellectual disabilities, which warrants continued investigation into these factors and the influence they have on cardiac reactivity in individuals with ASD and FXS.

The second study assessed the cardiac response to an auditory startle in boys with FXS aged 1-10.5 years old compared to a NT age-matched group (Roberts et al., 2013). Similar to the present study, the Roberts et al. results did not find significant differences in cardiac response to startle between the boys with FXS and the NT boys. The study did

find that as children with FXS aged, their cardiac arousal to the startle increased, a shift not seen in the NT group. Although the developmental shift towards hyper-arousal with age for individuals with FXS was not seen in the present study, the present sample was limited to preschool aged children and was not a longitudinal study. Further, the present study examined the cardiac response in a sample of children with FXS divided into those with and without comorbid ASD, which may impact the trajectory of arousal in FXS. Future studies should examine the longitudinal patterns of cardiac response to threat in these two groups for nuances in trajectories as children age into adolescence and the potential impacts of comorbid ASD.

4.2 RELATIONSHIP BETWEEN CARDIAC AUDITORY STARTLE AND CLINICAL FEATURES

In the second aim, we examined whether clinical features of ASD and FXS are associated with cardiac startle responses within and across groups. Cognitive level is particularly important to consider, as autonomic regulation has been linked to aspects of development, like language ability and adaptive functioning, but has not been assessed directly with cardiac startle in young children with intellectual disabilities. Interestingly, NVMA was strongly correlated with post-startle IBI in that as nonverbal abilities increased, heart rate decreased. The NT group showed a moderate positive relationship between nonverbal ability and startle and post-startle IBI, suggesting that nonverbal abilities and the physiological regulation during an auditory startle are moderately linked in typical preschoolers. One hypothesis is that children with higher nonverbal cognitive abilities are able to regulate their cardiac response better than children with lower cognitive abilities because they can interpret the startle as non-threatening. Children with

lower cognitive abilities, however, showed difficulty modulating their cardiac responses to the startle, suggesting that cognitive delays negatively impact physiological regulation to threat.

Within the clinical groups, the FXS-Only group also showed positive correlations between nonverbal ability and startle response like the NT group, while the nsASD group and the FXS+ASD group showed no relationship between NVMA and startle response. The shared behavioral diagnosis of ASD might indicate that features of ASD are confounding or influencing the relationship between cognitive ability and startle response. Some studies have found that cardiac flexibility is positively related to cognitive ability in ASD, whereas individuals with ASD and low cognitive abilities show higher physiological arousal and less flexibility in response to threat (see review Patriquin et al., 2019). Overall, the relationship between physiological response to startle and cognitive ability suggests that developmental delays are connected to cardiac startle in that higher nonverbal ability might support the ability to regulate physiologically because of the ability to cognitively cope in response to the startle.

Autism severity also significantly impacts development and the ability to regulate in response to stressors. The link between ASD severity and poor RSA is well established (Beauchaine, 2001; Geisler, Kubiak, Siewert, & Weber, 2013) and the clinical overlap of anxiety symptoms and features of ASD has been observed in neurotypical and ASD populations (Kerns et al., 2014; Kleberg et al., 2017; Skwerer, Joseph, Eggleston, Meyer, & Tager-flusberg, 2019). Even with these established connections, no studies have assessed the relationship between cardiac response to startle and ASD severity within ASD and FXS samples. In the present study, the FXS-Only group showed a significant

relationship between post-startle heartrate and ASD severity in that the higher the severity score, the less reactive the heartrate. This relationship was specific to the FXS-Only group, which uniquely represents a diagnostic group with ASD symptoms but not ASD. In the FXS-Only group, ASD severity might represent aspects of ASD behaviors like social avoidance or repetitive and restrictive behaviors, that suggest a link between specific ASD features and decreased cardiac startle. Interestingly, neither the ASD+FXS group nor the nsASD group showed a relationship between ASD severity and startle response, suggesting that having ASD might overpower any relationship between ASD severity and startle response.

Lastly, a relationship between parent reported anxiety and cardiac reactivity was not found for any group. The relationship between anxiety and cardiac activity is inconsistent across ASD and FXS, with some evidence suggesting that the interplay between anxiety, ASD severity, and cognitive level makes it difficult to isolate the impact of anxiety alone on autonomic functioning (see review Klusek et al., 2015; Kushki, Brian, Dupuis, & Anagnostou, 2014). Anxiety in young children, especially those with developmental delays, is very difficult to accurately distinguish from other behavioral difficulties when present (Freeman, Horner, & Reichle, 1999; Kerns et al., 2015; Kerns et al., 2014; Moskowitz et al., 2013). The reliance on parent report for interpreting pediatric anxiety is limiting, as many of these measures are designed for neurotypical children with classic presentations of anxiety. Previous research in anxiety in ASD has suggested that higher functioning individuals show higher levels of anxiety, but these studies often rely on classic presentations of anxiety. Individuals with ASD and ID have shown increased problem behavior, elevated heartrate, and decreased RSA in anxiety provoking situations,

which is difficult to characterize as anxiety without multiple sources of data or a functional behavior assessment (Moskowitz et al., 2013). Thus, to accurately understand and intervene in anxiety in individuals with ID, a multimethod approach that evaluates observed behaviors, physiological data, and clinical interviews is essential (Moskowitz et al., 2013, 2017).

Anxiety often becomes easier to identify and more distinct as a child ages and has the ability to use language to report internal feelings and experiences. Thus, anxiety in a preschool sample with intellectual disabilities is not only challenging to measure but may be subtle, idiosyncratic, or even absent at this stage of development. Additionally, physiological dysregulation has been posited to underlie emotion dysregulation, and thus, cardiac activity during the startle paradigm might better reflect general emotional dysregulation rather than anxiety specifically (White et al., 2014). Since, autonomic flexibility is associated with social functioning, language ability (Patriquin et al., 2013) and behavioral problems (Beauchaine, 2001), the startle paradigm might capture these aspects of development rather than anxiety. In the present study, ASD severity and nonverbal cognitive ability exhibited a correlation to cardiac startle response where parent-reported anxiety did not.

4.3 LIMITATIONS

Although this is the first study to directly compare heart activity in preschool children with ASD and FXS, some limitations should be considered. First, our groups varied in size and variability, which could mask patterns or effects in the smaller groups, particularly the FXS split sample. Additionally, while the Spence Anxiety scale has been

used in preschool children with ID, the measure was developed for typically developing children, and thus the nuances of atypical anxiety in children with ASD and FXS (Kerns et al., 2014) might be missed. Further, the Spence is a parent-reported measure, as self-report is very challenging in a young sample with ID. While parent-report is necessary during the assessment of children, it is limited to the parent's perceptions, observations, and conclusions about their child's behavior. The addition of behavioral observations and a clinician-led anxiety interview developed for children with ASD could clarify the relationship between physiological regulation and anxiety in preschool children with neurodevelopmental disabilities.

4.4 CONCLUSIONS AND FUTURE DIRECTIONS

Individuals with neurodevelopmental disabilities often show difficulties with autonomic flexibility in response to aversive stimuli and experience emotional and behavioral dysregulation alongside physiological dysregulation (see review Klusek et al., 2015; Kootz, Marinelli, & Cohen, 1982). The present study assessed cardiac startle response in children with varying risks for anxiety, ASD, and cognitive delays. The results demonstrate that physiological dysregulation begins early in childhood, during windows of time when children are particularly sensitive to intervention (Dawson & Burner, 2011; Rogers, 1996; Rogers & Vismara, 2008). Children with autonomic flexibility have better language, cognitive, and social skills (Patriquin et al., 2019, 2013), and thus, the relationship between behavioral and physiological functioning must be considered in early interventions. Evidence suggests that incorporating physiological components, like relaxation and neurofeedback, with learning social and cognitive skills can lead to more skill acquisition by individuals with ASD (Floress, Zoder-Martell, &

Schaub, 2017; LaMarca, Gevirtz, Lincoln, & Pineda, 2018; Pineda, Friedrich, & LaMarca, 2014; Silverman, Pina, & Viswesvaran, 2008). Therefore, interventions addressing physiological regulation in response to environmental stressors paired with skill building components can prime the child to be more receptive to interventions targeting sleep, attention, and learning.

In order to ascertain the nuances of physiological regulation and behavioral problems in young children with neurodevelopmental disabilities, future work should incorporate behavioral observations alongside physiological measures. Additionally, integrating measures of sensory behaviors and emotion regulation could elicit insight into the interplay of behavioral, physiological, and neurological factors impacting child development. Further, studies in older and higher-functioning samples of ASD have found differences in physiological response when groups are divided into subgroups of high and low anxiety (Panju et al., 2015; Parma et al., 2021). Thus, distinguishing clinical groups by anxiety symptoms might delineate the relationship between high anxiety symptoms and physiological reactivity. Lastly, including clinical interviews of anxiety that target both traditional and atypical presentations in young children are important in order to clarify the role of anxiety in high-risk populations. Overall, a richer understanding of the complex relationship of physiological markers and behavior difficulties in young children with neurodevelopmental disabilities is a critical step in developing and refining appropriate interventions for early childhood.

CHAPTER 5

PREVALENCE AND PREDICTORS OF TYPICAL AND ATYPICAL ANXIETY IN PRESCHOOL CHILDREN WITH AUTISM SPECTRUM DISORDER

Anxiety disorders are the most prevalent and impairing mental health condition during the preschool period with approximately 20% of 3-to-5-year-olds in the general population meeting criteria for an anxiety disorder (Bufferd et al., 2012; Salum, De Sousa, do Rosário, Pine, & Manfro, 2013). While anxiety disorders are widespread in the general population, they are even more prevalent in specific clinical populations such as those with autism spectrum disorder (ASD) (McCauley, Elias, & Lord, 2020). ASD is a highly prevalent neurodevelopmental disorder occurring in 1 in 54 children that is characterized by social communication deficits and the presence of restricted and repetitive behaviors (Maenner et al., 2020). Across the lifespan, individuals with ASD are twice as likely as neurotypical peers to develop an anxiety disorder, with studies reporting 11-84% of individuals with ASD meeting criteria for an anxiety disorder (van Steensel, Bögels, & Perrin, 2011). Comorbid symptoms of anxiety often result in more severe deficits in adaptive behaviors that reduce quality of life and impact functioning across multiple contexts in individuals with ASD (Lever & Geurts, 2016; Matson & Cervantes, 2014; Wood & Gadow, 2010). Thus, understanding comorbid anxiety in preschool-aged children with ASD can facilitate early and effective intervention in order to improve quality of life throughout the lifespan of individuals with ASD.

Elevated anxiety rates have been well established in school-aged children through adulthood in ASD. Yet, the detection of anxiety in preschool-aged children with ASD remains largely understudied even though roots of anxiety can often be traced to a young age even as early as infancy (Gartstein et al., 2010; Prior, Smart, Sanson, & Oberklaid, 2000; Shephard et al., 2019). Given the significant long-term impact of anxiety disorders and the effectiveness of early intervention in non-ASD preschoolers with early anxiety symptoms, research has increasingly focused on the emergence of anxiety symptoms early in life in an effort to facilitate early identification and treatment. However, there is only one study to date that has included preschool-aged children with ASD and reported the rates and characteristics of anxiety in these young children (Salazar et al., 2015). The majority of the preschool-aged children in this study met diagnostic criteria for an anxiety disorder. Specifically, 66.5% met diagnostic criteria for generalized anxiety disorder (GAD), 52.7% met diagnostic criteria for specific phobia, and 15.1% met diagnostic criteria for social anxiety. Furthermore, there was a high prevalence of cooccurring disorders with 75.6% of children meeting diagnostic criteria for two or more anxiety disorders. The sample, however, included children outside the preschool age (up to 9 years old), so conclusions about prevalence of anxiety in the preschool developmental period, when early intervention may be most effective, cannot be firmly drawn. Furthermore, this study used a clinical-diagnostic measure designed for neurotypical preschoolers, and therefore did not take into account the potentially atypical presentation of anxiety symptoms in ASD. Thus, a current limitation in the field is the lack of diagnostic studies in preschool-aged children using measures specifically normed for detecting anxiety in ASD.

There are several challenges to measuring and diagnosing anxiety in children with ASD that have led to inconsistent findings and a wide reported prevalence range (11-84%). Anxiety is particularly difficult to accurately measure in preschool children given several developmental barriers such as limited speech, lack of socio-emotional insight, and lower cognitive capacity which reduce validity of self-report (Kerns et al., 2014; Skwerer et al., 2019). This has led to a reliance on parent-reported measures for diagnosing anxiety in preschool-aged children. Yet, sole reliance on parent-reported measures does not fully address the barriers of limited language, insight, and cognition. For example, one study using parent-reported measures found that young children with higher language abilities were more likely to be diagnosed with an anxiety disorder, suggesting that children with language delays might face additional challenges to receiving a valid anxiety diagnosis due to measurement limitations (Salazar et al., 2015). Further, a recent study of minimally verbal school-aged children with ASD found that approximately 45% of children met criteria for a specific phobia, whereas generalized anxiety disorder (GAD) was only found in around 3% of the sample (Skwerer et al., 2019). One hypothesis for the unanticipated low rate of GAD is that the lower cognitive and language skills in the sample precluded the ability for the participants to express worries, which is a core feature of GAD. This implies that GAD might not be an appropriate diagnosis in preschool children with ASD using measures developed for neurotypical children. Thus, the use of assessments developed for children with ASD can facilitate the challenging task of measuring and distinguishing anxiety from overlapping developmental difficulties in preschoolers with ASD.

Children with ASD often have other comorbid behaviors like sensory aversions and intellectual disability (ID) which can mimic or overshadow features of anxiety presenting a challenge for a clear differential diagnosis (Kerns et al., 2015; Van Steensel, Bögels, & Wood, 2013). Children with ASD show a range of intellectual abilities with ID occurring in approximately one-third of children with ASD (Baio et al., 2018) but many individuals with ASD and ID are excluded from studies given the difficulty with self-report and a lack of sensitivity of measures to distinguish overlapping features of ID and anxiety. Studies that have included individuals with ASD and ID demonstrate mixed results with some suggesting that anxiety is higher in individuals with ASD without ID (Hallett, V., Lecavalier, L., Sukhodolsky, D. G., Cipriano, N., Aman, M. G., McCracken, J. T., ... & Sikich, 2013; Salazar et al., 2015), while other studies show equal or higher rates in individuals with ASD and ID (Ezell et al., 2018; van Steensel, Bögels, & Perrin, 2011). Salazar et al (2015) found significantly higher rates of overall anxiety in children with an IQ greater than 70, but no differences across specific anxiety disorders. A more recent study used the ADIS/ASA in a sample of children (ages 9-13) with ASD and 30% of the sample included individuals with ID (Kerns et al., 2020). The results indicated elevated rates of anxiety in children with ASD, with 69% of the ASD group meeting criteria for at least one anxiety disorder. Children with comorbid ASD and ID were significantly more likely to meet criteria for specific phobias than children without ID. The developmental trend of concrete fears (phobias) to abstract worries (generalized anxiety) was consistent for mental age as has been demonstrated for chronological age in neurotypical populations (Beesdo, Knappe, & Pine, 2011; Hallett, V., Lecavalier, L., Sukhodolsky, D. G., Cipriano, N., Aman, M. G., McCracken, J. T., ... & Sikich, 2013;

Sukhodolsky et al., 2008). Importantly, this study also assessed the rates of anxiety for those who met criteria for disorders beyond typical phobias, given the high prevalence of specific fears for individuals with ID. Assessing the presence of anxiety excluding typical phobias is of particular interest during the preschool age-range, as typical phobias, such as fear of the dark or thunder, are developmentally typical for children between ages 4-6 (Evans, Gray, & Leckman, 1999) and identifying children who meet criteria beyond developmentally appropriate fears might serve more clinical utility.

Another challenge unique to detecting anxiety in ASD is the presence of atypical or idiosyncratic anxiety. Emerging evidence suggests that anxiety in ASD can present both traditionally (e.g., worry about performance, fear of animals) and atypically (e.g., fear of change, idiosyncratic phobias), which can be difficult to detect in measures developed for the general population (Kerns et al., 2014). For example, fear of beards or toilets are not captured on many typical anxiety measures. Further, traditional social anxiety requires the social awareness necessary to feel social evaluation. Individuals with ASD often have deficits in social awareness, and thus social fear may not be captured on anxiety measures designed for neurotypical individuals. In order to capture atypical and ambiguous features of anxiety in ASD, an ASD addendum was added to the Anxiety Disorders Interview Schedule- Child/Parent, Parent Version (*ADIS/ASA*) (Kerns, Renno, Kendall, Wood, & Storch, 2017). In a sample of children aged 8-13 with ASD, the *ADIS/ASA* reliably captured both typical (k = 0.67-0.91) and atypical (k = 0.77-0.90) features of anxiety. Using a measure designed to characterize ambiguous features of anxiety in ASD enables the nuances of anxiety in ASD to be explored in order to understand how anxiety may present in a unique manner in ASD. Further, the *ADIS/ASA*

includes discriminant items to help delineate core ASD symptomology from anxiety symptoms. For instance, general sensory sensitivities are differentiated from overall sensory aversions to determine the presence of idiosyncratic phobia. To date, no study has used an ASD-specific diagnostic anxiety measure in young children with ASD. Further, this measure has not been validated in an exclusively preschool-aged and low-functioning sample, as it was originally designed for school-aged children and adolescents.

There are also a variety of individual factors that may increase risk for or resilience against anxiety in ASD, including ASD symptom severity, and biological sex. ASD symptoms and anxiety features often overlap in that social avoidance, rigid behaviors, sensory aversions, and repetitive behaviors are shared features in both disorders (Kerns et al., 2015). Even neurotypical children with anxiety disorders show more current and lifetime ASD symptoms than children without anxiety disorders, suggesting that anxiety and ASD symptoms might have a shared etiology (Van Steensel, Bögels, & Wood, 2013). Given the overlap of features, ASD symptom severity has been previously examined as a risk for developing anxiety in ASD. Across the lifespan in individuals with ASD, several studies found no relationship between ASD severity and anxiety (Renno & Wood, 2013; Sukhodolsky et al., 2008), while others suggest that ASD symptoms were negatively related to anxiety symptoms (Muris, Steerneman, Merckelbach, Holdrinet, & Meesters, 1998; Wood & Gadow, 2010). The only study of preschool children with ASD found that agoraphobia and night terrors were more common in children with higher ASD symptom severity (Salazar et al., 2015). One complication in studying ASD severity is that IQ and ASD severity are often related, and

thus studies that examine ASD severity without accounting for IQ might be capturing cognitive contributions to anxiety as opposed to isolating the effects of ASD severity on anxiety.

Sex is another potential risk factor for anxiety in preschool children with ASD. In the general population, the lifetime prevalence of anxiety disorders is 1.5-2 times more common in females than males (Mclean, Asnanni, Litz, & Hofmann, 2012), yet, this can vary based on specific disorders and age of the sample. In a neurotypical preschool sample, females were more likely to meet criteria for separation anxiety but not for any other anxiety disorders (Franz et al., 2014). Less is known about the rates of anxiety disorders in females with ASD, however, as they are often excluded from studies given the heterogeneity within ASD and the higher prevalence of ASD in males than females. One longitudinal study of mood disorders in children and adolescents with ASD found that females showed a greater increase in anxiety disorders over time but did not show overall differences from males in the rate of anxiety disorders (Gotham, Brunwasser, & Lord, 2015). Another study also found no significant sex differences for anxiety disorders in a 5- to 9-year-old sample of children with ASD (Salazar et al., 2015), suggesting that the sex effects seen in typically developing samples may not extend to children with ASD. To date, anxiety in female preschoolers with ASD has not been studied.

5.1 PRESENT STUDY

It is critical to characterize comorbid anxiety in children with ASD because it is associated with an increased risk for severe behavioral problems (Christensen et al., 2016; White et al., 2014). Despite the high association of anxiety with ASD and clear

impairment caused by anxiety in ASD, only one study has assessed the prevalence of anxiety in preschool children with ASD and no study has used an ASD-specific measure to assess anxiety in preschool-aged children with ASD. The present study addresses several gaps in the literature, and it is the first to study a sample of low functioning children with ASD using and autism-specific measure of anxiety. Our specific aims are to 1) compare the rate of anxiety disorders (both typical and atypical) in a sample of preschool-aged children with ASD and ID compared to age-matched neurotypical controls; 2) assess if ASD symptom severity, intellectual ability, and sex are risk factors for anxiety disorders; and 3) compare the results of the ADIS/ASA to brief parent-screeners for anxiety and behavioral difficulties.

The core hypothesis of this paper is that preschool-aged children with ASD experience heightened levels of typical and atypical forms of anxiety and exhibit multiple anxiety disorders compared to neurotypical controls. It is anticipated that preschool children with ASD with higher ASD severity will show more atypical anxiety disorders, while females and individuals with higher cognitive ability will show more typical anxiety disorders. It is expected that current measures of anxiety will be highly correlated with typical anxiety disorders on the ADIS/ASA, but not necessarily atypical anxiety features.

CHAPTER 6

METHODS

6.1 PARTICIPANTS

Participants for this study were drawn from an ongoing NIMH study (1R01MH107573-01A1; PI: Roberts) that is focused on the emergence of anxiety symptoms in children aged 3-5 years old with intellectual disabilities. In the present study, 46 children (ASD, n = 28 TD, n = 18) between 48-72 months were included. Approximately 72% of the children in the ASD sample showed significant developmental delays (Mullen ELC < 70). Children were excluded if they were premature (gestational age < 37 weeks), had a history of seizures, or had any known genetic disorders according to parent report. The ASD sample was confirmed to have ASD through a Clinical Best Estimate (CBE) review process (Roberts et al., 2020a). A CBE diagnosis was determined after the assessment by at least 3 members of the research staff who were research reliable on the ADOS-2, one of whom is a licensed psychologist. The ADIS/ADA interviews were completed by research reliably trained team members as detailed below. Individuals in the typically developing (TD) group had no family history of ASD nor known diagnosis of ASD, which was confirmed during the CBE process. Inclusion of a TD comparison group was used for validating the ADIS/ASA in a preschool-aged sample and provided a reference for developmental norms of anxiety symptoms in preschool-aged children. Both males and females were included in the study, given the lack of knowledge of sex effects for anxiety in preschool-aged children.

6.2 MEASURES

Anxiety Diagnosis and Severity. The Anxiety Disorders Interview Schedule for Autism Spectrum and Developmental Disorders: Parent Version (ADIS/ASA) is a well-validated DSM-5 diagnostic measure developed for the differentiation and diagnosis of anxiety disorders in ASD (C. Kerns et al., 2017). The ADIS/ASA protocol was developed by Kerns and colleagues (2017) to capture typical and atypical anxiety presentations in children aged 8-13. The protocol consists of the original ADIS-parent interview with an addendum specific to ASD. Research shows an inter-rater reliability (.89-.99) and 2-week retest reliability (.88-1.00) for the ADIS/ASA. The measure includes Specific Phobia, Separation Anxiety, Social Phobia, and/or Generalized Anxiety disorders based upon the DSM-5 criteria. The ASA addendum assesses for atypical anxiety presentations often seen specifically in ASD like Idiosyncratic Phobias, Social Fearfulness, and Negative Reactions to Change. For the present study, in addition to assessing the prevalence of specific anxiety disorders, group comparisons were tested for 1) Any Anxiety, or meeting criteria for one or more anxiety disorder, 2) Anxiety Excluding Typical Phobias, or any anxiety diagnosis no including typical phobias, 3) Typical Anxiety, or anxiety disorders in the DSM-5, 4) Atypical Anxiety, or ADIS/ASA specific anxiety additions, and 4) Multiple Anxiety Disorders, or meeting criteria for more than one anxiety diagnosis. Each diagnosis has a clinician assigned severity rating from 0 to 8, which allows for a symptom severity and functional impairment index that provides a dimensional symptomatology of DSM-5 defined anxiety. Graduate students who completed research reliable training conducted the ADIS/ASA interviews. Research reliability training consists of attending in-person training, followed by scoring three reliable interviews

independently at 80% reliability. Additionally, three reliability phone calls with the first author and second author (Connor Kerns, PhD) were completed after each interview was scored. Next, each interviewee completed an ADIS/ASA interview that is scored for reliability by Dr. Kerns. Site reliability was conducted on 20% of the interviews (95% Overall Agreement; Diagnostic $\kappa = .81$).

Convergent and Discriminant Validity. Two measures were used for convergent validity and one measure for divergent validity. The Child Behavior Checklist 1.5-5, Anxiety Problems Subscale (CBCL/1.5-5) – Parent Form (Achenbach & Rescorla, 2001) is a 113-item, parent-report questionnaire of emotional and problem behaviors for children aged 1.5-5 years of age. The CBCL has established reliability and validity in clinical populations, with extensive normative data. Further, the CBCL has acceptable internal consistency in school-aged children with ASD (Duarte, Bordin, de Oliveira, & Bird, 2003; J Clin Child Adolesc Psychol. Mazefsky et al., 2011; Pandolfi et al., 2009). The CBCL Anxiety Problems subscale (CBCL-AP) will be used to assess the convergent validity of the ADIS/ASA in this sample. To assess discriminant validity of the ADIS/ASA, the CBCL subscales of Attention and Aggression Problems will be used. The CBCL-AP is a DSM-oriented scale that measures features of typical anxiety disorders like Generalized Anxiety Disorder, Specific Phobia, Social Anxiety, and Separation Anxiety. Raw scores (0-12) will be used for analysis, with higher scores reflecting greater problem behavior.

The Preschool Anxiety Scale (PAS) is a preschool adaptation to the Spence Children's Anxiety Scale (SCAS; Spence, Rapee, McDonald, & Ingram, 2001) designed to measure anxiety symptoms in children aged 2.5-6.5 years of age. The parent-report

measure provides a severity score (ranging 0-4 per item) for Generalized Anxiety, Separation Anxiety, Obsessive Compulsive Disorders, Physical Injury Fear, and a Total Anxiety score. The Total Anxiety score (ranging 0-112) is a continuous severity score and will be used for convergent validity analysis in the present study.

Developmental Level. The Mullen Scales of Early Learning (MSEL) is a standardized developmental measure that assesses the cognitive and motor abilities from birth to 68 months across 5 subscales of development—expressive language, receptive language, gross motor, fine motor, and visual reception. The Early Learning Composite (ELC) is an overall composite score of developmental abilities, excluding gross motor skills. The ELC has a mean score of 100 with a standard deviation of 15. Evidence shows that standard scores can be limited in a low functioning sample due to ceiling effects. Therefore, standard scores were used to characterize the sample, while raw scores were used to assess the relationship between anxiety and cognitive abilities. The MSEL shows good internal consistency for each subscale (0.75 to .08), and test-retest reliability (.70-.80; Mullen, 1995).

Autism Severity and Diagnosis. The Autism Diagnostic Observation Schedule- 2nd Edition (ADOS-2) is a semi-structured, standardized assessment of communication, social interaction, play, and restricted and repetitive behaviors. The ADOS-2 has five modules administered based upon chronological age and expressive language abilities. The ADOS-2 Calibrated Comparison Score (CSS) is a 1-10 severity score reflecting the overall social communication impairments along with the presence of restrictive, repetitive behaviors compared to children with similar chronological age and expressive language level. The ADOS-2 was administered and scored by graduate-level

professionals who have completed research reliability training. Site reliability was conducted on 20% of the ADOS-2 administrations (83.3% inter-rater reliability).

CHAPTER 7

RESULTS

7.1 ANALYTIC PLAN

For the preliminary analysis, descriptive statistics were conducted to characterize the sample for ASD severity, sex, cognitive ability, and age. T-test analysis were then used to compare the TD and ASD groups on these factors. Then, overall prevalence rates were reported for the presence (ADIS/ASA CSR≥4) or absence of any anxiety disorder, anxiety disorders excluding typical phobias, multiple anxiety disorders, and specific anxiety disorders. Chi-square analyses were used to compare the rates of any anxiety disorder, anxiety excluding typical phobias, typical and atypical anxiety disorders, and multiple anxiety disorders between the ASD and TD groups (Table 2). For the second phase of analysis, bivariate correlations were used within each group to assess the relationship between anxiety and ASD severity and cognitive ability. In order to assess the relationship between sex and anxiety, Fisher's exact tests were used since both variables are binary. Third, correlations were used to assess for convergent and divergent validity. For convergent validity, the relationship between meeting criteria for any anxiety disorder (all categories collapsed) and typical anxiety disorders on the ADIS/ASA and the CBCL-AP and the Spence total score were assessed within the TD group and the ASD group separately. For divergent validity, the relationship between meeting criteria for any anxiety on the ADIS/ASA and the CBCL ADHD and Aggression

subscales were evaluated within each group with the expectation that these scales are not closely related.

7.2 PRELIMINARY ANALYSIS

T-tests were run to compare the TD and ASD groups for autism severity (ADOS-2 CSS), cognitive ability (Mullen ELC), age and sex. As expected, the ASD sample showed significantly higher ADOS-2 CSS scores ($t(1, 36) = 8.53$, $p < 0.001$) and lower Mullen ELC scores ($t(1, 36) = -7.31$, $p < 0.001$) than the TD group. No significant group differences were found for age ($t(1, 44) = -1.58$, $p = .122$). A Fisher's exact test indicated that the distribution of sex was not significantly different across groups ($p = .296$) (Table 7.1).

Table 7.1 Means of Independent Variables by Group

	ASD Severity*	Sex	Mullen ELC*	Age
	Mean (sd)	*M(F)*	*Mean (sd)*	*Mean (sd)*
ASD	6.7 (1.7)	23 (5)	60.4 (16.3)	61.5 (3.5)
TD	1.9 (1.5)	12 (6)	102.9 (18.3)	64.1 (7.7)

*Significant at 0.01

7.3 PREVALENCE ANALYSIS

Rates Any Anxiety Disorder (Including and Excluding Typical Phobias). The ASD group showed that 64% of the ASD sample met criteria for anxiety, whereas 44% of the TD preschool sample met criteria for any anxiety disorder. Chi-square tests of independence were used to compare the rate of meeting criteria for any anxiety disorder between the ASD and TD groups. Results showed that the ASD group was not significantly more likely to meet criteria for an anxiety disorder ($X^2(1, N = 46) = 1.76$ $p = 0.185$) compared to the TD group. Excluding typical phobias, 42.9% of the ASD group continued to meet criteria for anxiety while only 16.7% of the TD group met criteria for anxiety. Results of the chi-square test showed that the ASD sample was significantly more likely to have an anxiety disorder compared to the TD sample when excluding typical phobias ($X^2(1, N = 46) = 4.28$, $p = 0.039$), with a medium effect size ($w = -.31$). Specifically, the ASD sample is 2.8 times more likely to meet criteria for an anxiety disorder when looking beyond typical phobias than the TD sample.

Rates of Typical and Atypical Anxiety. Chi-square tests were run to examine the likelihood of a typical anxiety diagnosis or of an atypical anxiety diagnosis for the ASD group contrasted to the TD group. Results indicate that 50% of the ASD sample met criteria for a typical anxiety disorder compared to 33.3% of the TD sample. No significant differences were seen for the rate of typical anxiety ($X^2(1, N = 46) = 1.24$, $p = 0.266$). For atypical anxiety, the ASD group (42.9%) was significantly more likely to meet criteria for an atypical anxiety disorder compared to the TD group (11.1%; $X^2(1, N = 46) = 5.22$, $p = 0.022$, with a medium effect size ($w = -.34$). Specifically, the ASD

group was 3.9 times more likely to meet criteria for an atypical anxiety disorder than the TD sample.

Rates of Multiple Anxiety Disorders. Approximately 28.6% of the ASD sample met criteria for more than one anxiety disorders compared to approximately 0% of the TD group. A Fisher's Exact test was used to compare the rate of multiple anxiety disorders, given that the sample size for those who met for multiple anxiety disorders was less than 5 for the TD sample. The ASD sample was significantly more likely to meet criteria for multiple anxiety disorders compared to the TD sample ($p = 0.007$) with a medium to large effect size ($w = -.40$).

Table 7.2 Percentage of Anxiety Disorder Diagnoses by Group

	ASD	TD
Any Anxiety	64.3	44.4
Anxiety without Typical Phobia	42.9*	16.7
Typical Anxiety	50.0	33.3
Separation Anxiety	17.9	0
Typical Specific Phobia	46.4	27.8
Social Anxiety Disorder	3.6	5.6
Atypical Anxiety	42.9*	11.1
Idiosyncratic Phobia	28.6	5.6
Other Social Fear	10.7	0
Special Interest Fear	14.3	5.6
Fear of Change	7.1	0
Multiple Anxiety Disorders	28.6*	0

Note: No participant met criteria for GAD. Specific anxiety disorders were too small to compare between groups.

*Significant at $p < 0.05$

7.4 CORRELATES OF ANXIETY DISORDERS

Bivariate correlations were then used to assess the relationship of anxiety to ASD severity and cognitive ability, by group. Moderate, negative correlations were found within the TD group between any anxiety disorder and ELC (r = -.49, p = .093), particularly those who met criteria for anxiety beyond typical phobias (r = -.51, p = .078). In the ASD group, a moderate, positive correlation was found between autism severity and typical anxiety disorders (r = .40, p = .051). ELC was not significantly correlated with any anxiety variables for the ASD group. In order to assess the relationship between sex and anxiety, Fisher's exact tests were used since both variables are binary and the sample of females is less than 10. In the TD sample, females were not more likely to meet criteria for an anxiety disorder than males (p = 0.638), although the samples were small. In the ASD group, using Fisher's exact test, males were more likely than females to meet criteria for an anxiety disorder (p = .041). Gender was a significant protective factor for females in the ASD sample, but no differences were seen the TD sample (see Table 7.3).

Table 7.3 Relationship Between Anxiety and ASD Severity, Cognitive Level, and Gender

		ADOS-2 CSS	**ELC**	**Gender**
ASD	Any Anxiety	$r = 0.39$	$r = -0.22$	$X^2 = 5.20$*
	Anxiety Excluding Phobias	$r = 0.14$	$r = -0.01$	$X^2 = 1.71$
	Typical Anxiety	$r = 0.40$	$r = -0.26$	$X^2 = 2.19$
	Atypical Anxiety	$r = 0.22$	$r = 0.05$	$X^2 = 1.30$
TD	Any Anxiety	$r = 0.43$	$r = -0.49$	$X^2 = 0.45$*
	Anxiety Excluding Phobias	$r = 0.26$	$r = -0.51$	-
	Typical Anxiety	$r = 0.34$	$r = -0.27$	-
	Atypical Anxiety	$r = 0.16$	$r = -0.29$	$X^2 = 1.26$

*Significant at $p < 0.05$

7.5 CONVERGENT AND DIVERGENT VALIDITY

Convergent and divergent validity were assessed through correlation analysis using "any anxiety" for both typical and atypical anxiety and for typical anxiety alone. For convergent validity, the relationship between the ADIS/ASA and the CBCL-AP and the Spence total score were assessed within each group. Within the TD group, meeting criteria for any anxiety disorder, typical and atypical combined, was not significantly related to the Spence total score (r =.27, p = .360), whereas the CBCL-AP showed a correlation with meeting criteria for any anxiety with a medium effect size (r =.53, p = .093). When looking specifically at typical anxiety disorders, the CBCL-AP was highly correlated within the TD sample (r =.77, p = .006). For the ASD group, meeting criteria for any anxiety disorder (typical or atypical) or typical anxiety alone was not correlated with either the CBCL-AP or the Spence total score ($p > 0.05$). Of note, only 1 participant was rated in the elevated range for the Spence parent-reported measure, which could explain the lack of correlation across groups.

For divergent validity, the relationship between the ADIS/ASA and the CBCL ADHD and Aggression subscales were evaluated within each group. Results indicated that meeting criteria for any anxiety disorder was not significantly related to the CBCL Aggression subscale (r =.32, p = .333) or the CBCL ADHD subscale (r =.20, p = .559) for the TD group. Similarly, no significant correlations were found between any anxiety on the ADIS/ASA and the CBCL Aggression subscale (r = -.28, p = .239) or the CBCL ADHD subscale (r = -.05, p = .832) for the ASD group. Of note, the samples included in the present study were small, and thus, it is important to interpret validity with caution.

CHAPTER 8

DISCUSSION

Autism spectrum disorder is a highly prevalent and complex disorder with up to 80% of children with ASD also meeting criteria for anxiety (Maenner et al., 2020; White et al., 2009). Children with ASD and anxiety are more likely to have adaptive impairments, increased social difficulties, and a lower quality of life as they age (C. M. Kerns et al., 2015; Lever & Geurts, 2016; Smith, Ollendick, & White, 2019; van Steensel, Bögels, & Dirksen, 2012). Thus, detecting anxiety symptoms and providing targeted treatment early in childhood has the potential to impact long-term developmental trajectories for individuals with ASD.

Anxiety in young children with ASD and low cognitive abilities, however, has not been well studied and this population is often excluded from research given barriers to measurement and challenges associated with a lack of clear behavioral symptoms during the preschool age (South, Rodgers, & Van Hecke, 2017). The present study utilized a clinician administered interview with an addendum designed to capture the nuanced presentation of anxiety in ASD to provide a clearer picture of the early manifestations of anxiety in children with ASD the majority of whom (i.e., 72%) also have cognitive impairment. Results indicated that 64% of preschool children with ASD met criteria for an anxiety disorder with important variance associated with the manner of classifying anxiety disorders and with individual differences as outlined below.

8.1 PREVALENCE OF ANXIETY DISORDERS

In the ASD group, 64% of the preschool children met criteria for either a typical or atypical anxiety disorder including specific phobias that are fairly common during this developmental period (e.g., 28% of our TD sample met criteria for a specific phobia). The rates of any anxiety disorder in our preschool sample saturated with cognitive impairment is fairly similar to rates of 69% reported by Kerns et al. (2020) using the ADIS/ASA addendum in a sample of children with ASD and varied intellectual abilities aged 9-13 years old. Since specific phobias are so common in preschool children, some studies have examined the rates of anxiety excluding phobias to understand which children meet criteria beyond common developmental fears. When excluding specific phobias, 43% of those with ASD met criteria for any anxiety disorder which was significantly greater than the rate of 17% observed in the TD group. These results continued to parallel the findings of the Kerns et al. (2020) study, which showed that approximately 36% of school-aged children with ID met criteria for anxiety excluding phobias. Given the limited work examining anxiety in children with ID, these two studies show consistent and steady rates of combined typical and atypical anxiety across preschool and late childhood for children with ASD and intellectual disabilities.

The rate of DSM-specified, typical anxiety disorders in the ASD group (50%) was also consistent with previously reported ranges of 42-55% (de Bruin, Ferdinand, Meester, de Nijs, & Verheij, 2007; Kerns et al., 2020; White, Oswald, Ollendick, & Scahill, 2009), although this sample was younger (M = 61.5 months) and had lower cognitive functioning (M = 60.4 Mullen ELC) than in previous studies. Importantly, in studies using the ADIS/ASA children in the ASD groups met criteria for atypical phobias, which

are often noted qualitatively but rarely documented systematically through anxiety measures (Magiati, Ozsivadjian, & Kerns, 2017). Studies that have included atypical presentations of anxiety, including the current study, have found that rates of anxiety are closer to two-thirds (den Houting, Adams, Roberts, & Keen, 2018; Kerns et al., 2020; Kerns et al., 2014) of the ASD samples compared to the previously reported rates of DSM-specific anxiety disorders (42-55%; de Bruin, Ferdinand, Meester, de Nijs, & Verheij, 2007; Kerns et al., 2020; White, Oswald, Ollendick, & Scahill, 2009). DSM-specific anxiety measures are not calibrated to sensitively capture atypical anxiety presentations, suggesting that previously reported rates of anxiety in ASD might underrepresent anxiety as in manifests in young and low functioning populations. Thus, using measures that assess for atypical presentations of anxiety are vital for accurately identifying anxiety in those with ID and ASD, which is approximately one-third of the ASD population (Baio et al., 2018).

The ASD group was significantly more likely to meet for multiple anxiety disorders than the TD group (29% vs. 0% respectively), which is consistent with previous studies (Kerns et al., 2020; Kerns et al., 2014). Many studies simply report “at least one” anxiety disorder for children with ASD and do not report specific results for multiple anxiety disorders (see van Steensel et al., 2011; see White et al., 2009), even though evidence suggests that anxiety disorders are highly co-occurring (Sherbourne et al., 2010). The functional impacts of meeting criteria for more than one anxiety disorder in children with ASD are largely unknown. It is possible that children with multiple anxiety disorders experience compounding effects over time of increased impairment, comorbid internalizing problems, and familial stress, as is seen in typical populations (Beesdo et al.,

2011; Sherbourne et al., 2010), but longitudinal studies examining the impacts of multiple anxiety disorders for children with ASD are lacking. The pattern of children with ASD meeting criteria for multiple anxiety disorders could reflect a general vulnerability to anxiety disorders in individuals with ASD rather than a vulnerability for a specific disorder or it could indicate that multiple disorders early in life transition into specific disorders later in life. Future work is needed to determine the impacts of multiple anxiety disorders as well as the trajectory and persistence of these disorders over development.

In terms of specific anxiety disorders, 53% of the ASD group met criteria for either typical phobias (46.4%) or idiosyncratic phobias (28.6%). Previous research using the ADIS/ASA has also found that specific phobias compose approximately half of anxiety disorders in youth with ASD (Kerns et al., 2020; Kerns et al., 2014). These studies, along with the present study, affirm that specific phobias, including idiosyncratic fears, are the most common presentation of anxiety in children with young chronological or mental age, whereas more abstract fears and worries tend to be more prevalent in older and higher functioning children, as no children in the present study met criteria for GAD. This follows the developmental trajectory of anxiety in typical populations, which typically begins with specific fears and separation anxiety and generalizes to worries and fears of judgment with cognitive ability and age (Beesdo et al., 2011; Hallett, V., Lecavalier, L., Sukhodolsky, D. G., Cipriano, N., Aman, M. G., McCracken, J. T., ... & Sikich, 2013; Sukhodolsky et al., 2008). This work has shown that a developmental shift occurs across time where the rates of generalized anxiety increase, and the rate of phobias decrease (Kerns et al., 2014; Sukhodolsky et al., 2008). One hypothesis is that young

children with cognitive impairment are unable to express generalized anxiety (abstract cognitions), and instead, parents are more likely to report specific fear behaviors (concrete cognitions) that they observe behaviorally. Thus, in children with low chronological or mental age, the prevalence of anxiety disorders beyond typical fears can provide more clinical utility for understanding which children are at the highest risk for long-term difficulties with anxiety. Future studies utilizing longitudinal data are needed to understand the trajectory of developmentally appropriate fears and to determine if preschool children who meet criteria for typical phobias continue to show features of anxiety as they age.

8.2 CORRELATES OF ANXIETY DISORDERS

In addition to reporting elevated rates of anxiety, correlates of anxiety were examined to determine potential risk factors for anxiety early in life. The ASD group did not have a relationship between anxiety and cognitive ability despite the majority having an intellectual impairment. In contrast, the TD group showed a relationship in which lower cognitive ability was associated with increased presence of anxiety disorders. The lack of relationship between cognitive ability and anxiety in the ASD group may be due to the restricted IQ range in this sample. Previous studies have shown mixed results for the relationship between cognitive ability and anxiety symptoms in ASD, with some research suggesting that language ability is a limiting factor in measuring anxiety in low functioning populations (Kerns et al., 2020; Lecavalier et al., 2014; White et al., 2014). Importantly, the decrease in anxiety as cognitive ability increased in the TD sample could reflect a developmental shift of "growing out of" typical preschool fears. For instance, as neurotypical children develop over time, they are less likely to have fears of the dark or

of insects (typical phobias) because they can cognitively process and rationalize that the threat level is low. However, the cognitive effect was not present in the ASD group, suggesting that they may not develop out of typical fears at the same rate as their TD peers, potentially because of their lower mental age. This is supported by studies of older children and adolescents with ASD who show significantly higher rates of phobias, suggesting divergent trajectories of anxiety between children with ASD and TD (Kerns et al., 2014, 2020) Longitudinal work is essential to assess the stability of meeting criteria for anxiety, particularly typical phobias, in preschool children over time.

In terms of the effect of ASD symptom severity, there was a relationship of greater ASD severity associated with increased typical anxiety disorders for the ASD group. In a study by Kerns et al. 2014, more typical anxiety disorders and increased ASD severity were related to atypical anxiety presentations, although in an older and high functioning population (Kerns et al., 2014). The ASD group in the present study is purposefully focused on a low functioning subtype of ASD, and thus, assessing the relationship of ASD severity and anxiety might be more informative with a sample of varied characterizations. Further, some studies suggest that specific ASD symptoms like sensory over-responsivity (Green & Ben-Sasson, 2010) and restricted and repetitive behaviors (Spiker, Lin, Van Dyke, & Wood, 2012) are related to increased anxiety, and thus, pulling apart specific ASD features might delineate the relationship between ASD severity and anxiety.

Lastly, the ASD group showed that males were more likely to meet criteria for an anxiety disorder than females. In previous studies of gender and anxiety in individuals with ASD, the findings are more mixed and limited, as many studies have predominantly

male samples (Mandy et al., 2012; Solomon, Miller, Taylor, Hinshaw, & Carter, 2012). A recent study found that for preschool children with ASD, males have higher rates of internalizing behavior compared to females on the CBCL (Prosperi et al., 2020). Consistent with Prosperi et al. 2020, the present study found that males with ASD are at a higher risk for anxiety than females, suggesting a potential protective factor from comorbid anxiety young females with ASD. Given the limited research on anxiety in females with ASD, it is also possible that females show a different set of symptoms or behaviors related to anxiety than males, that are not captures via typical parent-report measures. While this is the first study to evaluate cognitive ability, ASD severity, and sex as correlates of anxiety in a low functioning sample of preschool children with ASD, the groups were small for these analyses and should be viewed as preliminary data to guide future work.

8.4 VALIDITY OF ADIS/ASA IN PRESCHOOL CHILDREN

Although anxiety is common and contributes to functional impairments for children with ASD, distinguishing comorbid anxiety is challenging because of overlapping features and inconsistency across measures. The present study included a sample of young children with ASD and cognitive impairment, a sample often excluded from studies due to measurement challenges. Further, the ADIS/ASA has yet to be used in a young, low functioning sample, and thus, validity analyses were conducted using common anxiety and behavioral screening measures for young children. While the sample for validity analysis was small, the TD group followed expected trends of a medium effect size between meeting criteria for any anxiety disorder and the CBCL-AP and a large effect size for meeting criteria for typical anxiety disorders and the CBCL-

AP. The ASD sample, however, did not show a relationship between meeting for anxiety on the ADIS/ASA and anxiety on either parent-screening measure, perhaps reflecting the arduous task of interpreting internalizing problems from external actions. Additionally, the ASD group met criteria for atypical anxiety disorders, suggesting that brief parent screeners are not equipped or designed to capture atypical presentations of anxiety commonly seen in ASD. This highlights a barrier to using screening measures for validating a clinician interview in this population in that the measurement challenges for these screening tools that justify using a clinician interview, still exist. Thus, the argument for the need of alternative means for assessing anxiety in young children with neurodevelopmental disabilities (i.e., clinician interview, observational assessments, physiological components) is faced with the challenge of using potentially invalid or skewed measures to attempt to validate new ways of measurement. Therefore, it is important to use multiple sources of information, and perhaps place more weight on clinical judgement and direct observation, in young children with multiple developmental and behavioral challenges.

8.4 LIMITATIONS

The present study was the first to use the ADIS/ASA in a young sample of children with ASD and cognitive impairment but was limited in several aspects. First, although the ADIS/ASA utilizes clinical judgment, the interviews were still restricted to parent perspective in this low developmental group. Given the language and socio-emotional delays in this population, incorporating behavioral and physiological measures of anxiety alongside parent perspective is needed to create a comprehensive picture of anxiety in young children with ASD and low cognitive abilities. Further, longitudinal

data would provide insight into the stability of anxiety diagnoses and the developmental trajectories of children who present with elevated anxiety symptoms in early life, a limitation of this cross-sectional design. While the present study is novel in the age range, cognitive ability, and the clinical interview used, it is limited in size which produces less power for analysis and reduced generalizability of the results. Thus, the results should be viewed as a preliminary understanding into this specific population and used as a steppingstone to more comprehensive, longitudinal, and wide scale studies of anxiety in young children with ASD and cognitive delays.

8.5 CONCLUSION

The present study was the first to use a standardized, diagnostic anxiety interview that accounts for ASD in young children with ASD and ID and aimed to magnify our understanding of diagnosing anxiety in young children given that anxiety can exacerbate social and communication deficits, decrease adaptive skills, and contribute to a litany of secondary problems including sleep difficulties, parental stress, self-injurious behaviors, and depression (Kerns et al., 2015; see Zaboski & Storch, 2018). Our findings indicate that anxiety disorders are highly prevalent and emerge early in individuals with ASD and ID, with over half of the children with ASD meeting criteria for an anxiety disorder. Differential profiles of anxiety distinguished the clinical sample in that the children with ASD and ID showed significantly higher rates of anxiety excluding phobias, multiple anxiety disorders, and atypical anxiety disorders. Further, consistent with previous studies of children with ASD and ID, specific phobias were the most common type of anxiety disorders, whereas the abstract fears and worries that compose GAD were not observed. Given the high rate of specific phobias and the lack of understanding regarding

the developmental resolution of these across time in young children with ASD, developing and implementing treatment for anxiety that focuses on specific fears is probably highly relevant and appropriate for children with young mental or chronological age. Future research into the developmental trajectories of preschool anxiety as well as adding physiological mechanisms and behavioral observations would strengthen our knowledge of anxiety and its impacts on children with ASD. Understanding anxiety in ASD from multiple aspects (parent-report, behavioral observations, physiological measures) and at a young age allows for the tailoring of early interventions to decrease the long-term impairments that co-morbid anxiety can cause.

REFERENCES

Abbeduto, L., Thurman, A. J., McDuffie, A., Klusek, J., Feigles, R. T., Ted Brown, W., … Roberts, J. E. (2019). ASD Comorbidity in Fragile X Syndrome: Symptom Profile and Predictors of Symptom Severity in Adolescent and Young Adult Males. *Journal of Autism and Developmental Disorders*, *49*(3), 960–977. https://doi.org/10.1007/s10803-018-3796-2

Achenbach, T.M., & Rescorla, L. A. (2001). Manual for the ASEBA School-Age Forms and Profiles. *Journal of Child Neurology*, *40*(4), 791–799. https://doi.org/10.1016/S1474-4422(03)00585-4

Akshoomoff, N. (2006). Use of the Mullen Scales of Early Learning for the assessment of young children with Autism Spectrum Disorders. *Child Neuropsychology*, *12*(4–5), 269–277. https://doi.org/10.1080/09297040500473714

American Psychological Association (APA). (2013). Diagnostic and statistical manual of mental disorders: DSM-V. In *DSM-V*.

Bailey, D. B., Hatton, D. D., Skinner, M., & Mesibov, G. (2001). Autistic Behavior, FMR1 Protein, and Developmental Trajectories in Young Males with Fragile X Syndrome. *Journal of Autism and Developmental Disorders*. https://doi.org/10.1023/A:1010747131386

Baio, J., Wiggins, L., Christensen, D. L., Maenner, M. J., Daniels, J., Warren, Z., … Dowling, N. F. (2018). Prevalence of Autism Spectrum Disorder Among Children

Aged 8 Years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2014. *MMWR Surveill Summ*, *67*(6).

Bakker, M. J., Tijssen-De Koning, M. A. J., Van Der Meer, J. N., Koelman, J. H. T. M., & Boer, F. (2009). Increased whole-body auditory startle reflex and autonomic reactivity in children with anxiety disorders. *Journal of Psychiatry and Neuroscience*, *34*(4), 314–322.

Beauchaine, T. P. (2001). Vagal tone, development, and Gray's motivational theory: Toward an integrated model of autonomie nervous system functioning in psychopathology. *Development and Psychopathology*. https://doi.org/10.1017/S0954579401002012

Beesdo, K., Knappe, S., & Pine, D. S. (2011). Anxiety and anxiety disorders in children and adolescents: Developmental issues and implications for DSM-V. *Psychiatric Clinics of North America*, *32*(3), 483–524. https://doi.org/10.1016/j.psc.2009.06.002.Anxiety

Boccia, M. L., & Roberts, J. E. (2000). Behavior and autonomic nervous system function assessed via heart period measures: The case of hyperarousal in boys with fragile X syndrome. *Behavior Research Methods, Instruments, and Computers*. https://doi.org/10.3758/BF03200783

Bufferd, S., Dougherty, L. R., Ph, D., Carlson, G. A., Rose, S., Klein, D. N., & Ph, D. (2012). *Psychiatric Disorders in Preschoolers: Continuity From Ages 3 to 6*. (November), 1157–1164.

Caravella, K. E., & Roberts, J. E. (2017). Adaptive skill trajectories in infants with fragile X syndrome contrasted to typical controls and infants at high risk for autism. *Research in Autism Spectrum Disorders*. https://doi.org/10.1016/j.rasd.2017.05.002

Carruthers, S., Kent, R., Hollocks, M. J., & Simonoff, E. (2020). Brief Report : Testing the Psychometric Properties of the Spence Children ' s Anxiety Scale (SCAS) and the Screen for Child Anxiety Related Emotional Disorders (SCARED) in Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders*, *50*(7), 2625–2632. https://doi.org/10.1007/s10803-018-3774-8

Christensen, D. L., Baio, J., Braun, K. V. N., Bilder, D., Charles, J., Constantino, J. N., … Yeargin-Allsopp, M. (2016). Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2012. *Morbidity and Mortality Weekly Report. Surveillance Summaries*, *65*(3), 1–23. https://doi.org/10.15585/mmwr.ss6503a1

Cohen, I. L. (1995). A Theoretical Anaylsis of the Role of Hyperarousal in the Learning and Behavior of Fragile X Males. *Mental Retardation and Developmental Disabiliites Research Reviews*, 286–291.

Cohen, S., Masyn, K., Mastergeorge, A., & Hessl, D. (2015). Psychophysiological Responses to Emotional Stimuli in Children and Adolescents with Autism and Fragile X Syndrome. *Journal of Clinical Child & Adolescent Psychology*, *44*(2), 250–263. https://doi.org/10.1080/15374416.2013.843462

Corbett, B. A., Muscatello, R. A., & Baldinger, C. (2019). Comparing stress and arousal systems in response to different social contexts in children with ASD. *Biological*

Psychology, *140*(December 2018), 119–130. https://doi.org/10.1016/j.biopsycho.2018.12.010

Cordeiro, L., Ballinger, E., Hagerman, R., & Hessl, D. (2011). Clinical assessment of DSM-IV anxiety disorders in fragile X syndrome: prevalence and characterization. *Journal of Neurodevelopmental Disorders*, *3*(1), 57–67. https://doi.org/10.1007/s11689-010-9067-y

Davis, M. (2006). Neural systems involved in fear and anxiety measured with fear-potentiated startle. *American Psychologist*, *61*(8), 741–756. https://doi.org/10.1037/0003-066X.61.8.741

Dawson, G., & Burner, K. (2011). Behavioral interventions in children and adolescents with autism spectrum disorder: A review of recent findings. *Current Opinion in Pediatrics*. https://doi.org/10.1097/MOP.0b013e32834cf082

de Bruin, E. I., Ferdinand, R. F., Meester, S., de Nijs, P. F. A., & Verheij, F. (2007). High Rates of Psychiatric Co-Morbidity in PDD-NOS. [Article]. *Journal of Autism & Developmental Disorders*, *37*(5), 877–886.

den Houting, J., Adams, D., Roberts, J., & Keen, D. (2018). Exploring anxiety symptomatology in school-aged autistic children using an autism-specific assessment. *Research in Autism Spectrum Disorders*, *50*(November 2017), 73–82. https://doi.org/10.1016/j.rasd.2018.03.005

Dijkhuis, R. R., Ziermans, T., van Rijn, S., Staal, W., & Swaab, H. (2019). Emotional Arousal During Social Stress in Young Adults With Autism: Insights From Heart Rate, Heart Rate Variability and Self-Report. *Journal of Autism and Developmental Disorders*, *49*(6), 2524–2535. https://doi.org/10.1007/s10803-019-04000-5

Edmiston, E. K., Jones, R. M., & Corbett, B. A. (2016). Physiological Response to Social Evaluative Threat in Adolescents with Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders*, *46*(9), 2992–3005. https://doi.org/10.1007/s10803-016-2842-1

Evans, D. W., Gray, F. L., & Leckman, J. F. (1999). The rituals, fears and phobias of young children: Insights from development, psychopathology and neurobiology. *Child Psychiatry and Human Development*, *29*(4), 261–276. https://doi.org/10.1023/A:1021392931450

Ezell, J., Hogan, A., Fairchild, A., Hills, K., Klusek, J., Abbeduto, L., & Roberts, J. (2018). Prevalence and Predictors of Anxiety Disorders in Adolescent and Adult Males with Autism Spectrum Disorder and Fragile X Syndrome. *Journal of Autism and Developmental Disorders*, *0*(0), 0. https://doi.org/10.1007/s10803-018-3804-6

Floress, M. T., Zoder-Martell, K., & Schaub, R. (2017). Social skills plus relaxation training with a child with ASD in the schools. *Research in Developmental Disabilities*, *71*(October), 200–213. https://doi.org/10.1016/j.ridd.2017.10.012

Franz, L., Angold, A., Copeland, W., Costello, J., Towe-Goodman, N., & Egger, H. (2014). Preschool Anxiety Disorders in Pediatric Primary Care: Prevalence and Comorbidity. *J Am Acad Child Adolesc Psychiatry.*, *52*(12), 1294–1303. https://doi.org/10.1016/j.jaac.2013.09.008.Preschool

Freeman, R. L., Horner, R. H., & Reichle, J. (1999). Relation between heart rate and problem behaviors. *American Journal on Mental Retardation*. https://doi.org/10.1352/0895-8017(1999)104<0330:RBHRAP>2.0.CO;2

Garber, K. B., Visootsak, J., & Warren, S. T. (2008). Fragile X syndrome. *European Journal of Human Genetics*, *16*(6), 666–672. https://doi.org/10.1038/ejhg.2008.61

Gartstein, M. A., Bridgett, D. J., Rothbart, M. K., Robertson, C., Iddins, E., Ramsay, K., & Schlect, S. (2010). A latent growth examination of fear development in infancy: contributions of maternal depression and the risk for toddler anxiety. *Developmental Psychology*, *46*(3), 651–668. https://doi.org/10.1037/a0018898

Geisler, F. C. M., Kubiak, T., Siewert, K., & Weber, H. (2013). Cardiac vagal tone is associated with social engagement and self-regulation. *Biological Psychology*. https://doi.org/10.1016/j.biopsycho.2013.02.013

Gorka, S. M., Nelson, B. D., Sarapas, C., Campbell, M., Lewis, G. F., Bishop, J. R., … Shankman, S. A. (2013). Relation Between Respiratory Sinus Arrythymia and Startle Response During Predictable and Unpredictable Threat. *Journal of Psychophysiology*, *27*(2), 95–104. https://doi.org/10.1027/0269-8803/a000091

Gotham, K., Brunwasser, S. M., & Lord, C. (2015). Depressive and Anxiety Symptom Trajectories From School-Age Through Young Adulthood in Samples With Autism Spectrum Disorder and Developmental Delay. *J Am Acad Child Adolesc Psychiatry.*, *54*(5), 369–376. https://doi.org/0.1016/j.jaac.2015.02.005

Green, S. A., & Ben-Sasson, A. (2010). Anxiety disorders and sensory over-responsivity in children with autism spectrum disorders: Is there a causal relationship? *Journal of Autism and Developmental Disorders*, *40*(12), 1495–1504. https://doi.org/10.1007/s10803-010-1007-x

Hahn, L. J., Brady, N. C., Warren, S. F., & Fleming, K. K. (2015). Do Children With Fragile X Syndrome Show Declines or Plateaus in Adaptive Behavior? *American*

Journal on Intellectual and Developmental Disabilities. https://doi.org/10.1352/1944-7558-120.5.412

Hallett, V., Lecavalier, L., Sukhodolsky, D. G., Cipriano, N., Aman, M. G., McCracken, J. T., ... & Sikich, L. (2013). Exploring the manifestations of anxiety in children with autism spectrum disorders. *Journal of Autism and Developmental Disorders*, *43*(10), 2341–2352. https://doi.org/10.1007/s10803-013-1775-1.Exploring

Hernandez, R. N., Feinberg, R. L., Vaurio, R., Passanante, N. M., Thompson, R. E., & Kaufmann, W. E. (2009). Autism spectrum disorder in fragile X syndrome: A longitudinal evaluation. *American Journal of Medical Genetics, Part A*, *149*(6), 1125–1137. https://doi.org/10.1002/ajmg.a.32848

Hogan, A. L., Caravella, K. E., Ezell, J., Rague, L., Hills, K., & Roberts, J. E. (2017). Autism Spectrum Disorder Symptoms in Infants with Fragile X Syndrome: A Prospective Case Series. *Journal of Autism and Developmental Disorders*. https://doi.org/10.1007/s10803-017-3081-9

Hollocks, M. J., Howlin, P., Papadopoulos, A. S., Khondoker, M., & Simonoff, E. (2014). Differences in HPA-axis and heart rate responsiveness to psychosocial stress in children with autism spectrum disorders with and without co-morbid anxiety. *Psychoneuroendocrinology*, *46*, 32–45. https://doi.org/10.1016/j.psyneuen.2014.04.004

Kaufmann, W. E., Cortell, R., Kau, A. S. M., Bukelis, I., Tierney, E., Gray, R. M., … Stanard, P. (2004). Autism spectrum disorder in fragile X syndrome: Communication, social interaction, and specific behaviors. *American Journal of Medical Genetics*. https://doi.org/10.1002/ajmg.a.30229

Kerns, C., Kendall, P. C., Berry, L., Souders, M. C., Franklin, M. E., Schultz, R. T., … Herrington, J. (2014). Traditional and atypical presentations of anxiety in youth with autism spectrum disorder. *Journal of Autism and Developmental Disorders*, *44*(11), 2851–2861. https://doi.org/10.1007/s10803-014-2141-7

Kerns, C. M., Kendall, P. C., Zickgraf, H., Franklin, M. E., Miller, J., & Herrington, J. (2015). Not to Be Overshadowed or Overlooked: Functional Impairments Associated With Comorbid Anxiety Disorders in Youth With ASD. *Behavior Therapy*, *46*(1), 29–39. https://doi.org/10.1016/j.beth.2014.03.005

Kerns, C., Renno, P., Kendall, P. C., Wood, J. J., & Storch, E. A. (2017). Anxiety Disorders Interview Schedule–Autism Addendum: Reliability and Validity in Children With Autism Spectrum Disorder. *Journal of Clinical Child and Adolescent Psychology*, *46*(1), 88–100. https://doi.org/10.1080/15374416.2016.1233501

Kerns, C., Winder-Patel, B., Iosif, A. M., Nordahl, C. W., Heath, B., Solomon, M., & Amaral, D. G. (2020). Clinically Significant Anxiety in Children with Autism Spectrum Disorder and Varied Intellectual Functioning. *Journal of Clinical Child and Adolescent Psychology : The Official Journal for the Society of Clinical Child and Adolescent Psychology, American Psychological Association, Division 53*, 1–16. https://doi.org/10.1080/15374416.2019.1703712

Kleberg, J. L., Högström, J., Nord, M., Bölte, S., Serlachius, E., & Falck-Ytter, T. (2017). Autistic Traits and Symptoms of Social Anxiety are Differentially Related to Attention to Others' Eyes in Social Anxiety Disorder. *Journal of Autism and Developmental Disorders*, *47*(12), 3814–3821. https://doi.org/10.1007/s10803-016-2978-z

Klusek, J., Martin, G. E., & Losh, M. (2014). Consistency between research and clinical diagnoses of autism among boys and girls with fragile X syndrome. *Journal of Intellectual Disability Research*. https://doi.org/10.1111/jir.12121

Klusek, J., Roberts, J. E., & Losh, M. (2015). Cardiac autonomic regulation in autism and Fragile X syndrome: A review. *Psychological Bulletin*, *141*(1), 141–175. https://doi.org/10.1037/a0038237

Kootz, J. P., Marinelli, B., & Cohen, D. J. (1982). Modulation of response to environmental stimulation in autistic children. *Journal of Autism and Developmental Disorders*. https://doi.org/10.1007/BF01531308

Kushki, A., Brian, J., Dupuis, A., & Anagnostou, E. (2014). Functional autonomic nervous system profile in children with autism spectrum disorder. *Molecular Autism*, *5*(1), 1–10. https://doi.org/10.1186/2040-2392-5-39

LaMarca, K., Gevirtz, R., Lincoln, A. J., & Pineda, J. A. (2018). Facilitating Neurofeedback in Children with Autism and Intellectual Impairments Using TAGteach. *Journal of Autism and Developmental Disorders*, *48*(6), 2090–2100. https://doi.org/10.1007/s10803-018-3466-4

Lecavalier, L., Wood, J. J., Halladay, A. K., Jones, N. E., Aman, M. G., Cook, E. H., … Scahill, L. (2014). Measuring anxiety as a treatment endpoint in youth with autism spectrum disorder. *Journal of Autism and Developmental Disorders*, *44*(5), 1128–1143. https://doi.org/10.1007/s10803-013-1974-9

Lever, A. G., & Geurts, H. M. (2016). Psychiatric Co-occurring Symptoms and Disorders in Young , Middle-Aged , and Older Adults with Autism Spectrum Disorder.

Journal of Autism and Developmental Disorders, *46*(6), 1916–1930. https://doi.org/10.1007/s10803-016-2722-8

Levine, J. C., Fleming, R., Piedmont, J. I., Cain, S. M., & Chen, W. J. (2016). Heart rate variability and generalized anxiety disorder during laboratory-induced worry and aversive imagery. *Journal of Affective Disorders*, *205*, 207–215. https://doi.org/10.1016/j.jad.2016.07.019

Lord, C., Risi, S., & Pickles, A. (2004). Trajectory of language development in autistic spectrum disorders. In *Developmental Language Disorders: From Phenotypes to Etiologies*. https://doi.org/10.4324/9781410610881

Lord, C., Rutter, M., DiLavore, P. C., Risi, S., Gotham, K., & Bishop, S. L. (2012). *Autism diagnostic observation schedule: ADOS-2*. Western Psychological Services Los Angeles, CA.

Lydon, S., Healy, O., Reed, P., Mulhern, T., Hughes, B. M., & Goodwin, M. S. (2016). A systematic review of physiological reactivity to stimuli in autism. *Developmental Neurorehabilitation*, *19*(6), 335–355. https://doi.org/10.3109/17518423.2014.971975

Maenner, M. J., Shaw, K. A., Baio, J., Washington, A., Patrick, M., DiRienzo, M., … Dietz, P. M. (2020). Prevalence of autism spectrum disorder among children aged 8 Years-Autism and developmental disabilities monitoring network, 11 Sites, United States, 2016. *MMWR Surveillance Summaries*. https://doi.org/10.15585/MMWR.SS6904A1

Magiati, I., Ozsivadjian, A., & Kerns, C. M. (2017). Phenomenology and Presentation of Anxiety in Autism Spectrum Disorder. *Anxiety in Children and Adolescents with*

Autism Spectrum Disorder: Evidence-Based Assessment and Treatment, 33–54. https://doi.org/10.1016/B978-0-12-805122-1.00003-X

Mandy, W., Chilvers, R., Chowdhury, U., Salter, G., Seigal, A., & Skuse, D. (2012). Sex differences in autism spectrum disorder: Evidence from a large sample of children and adolescents. *Journal of Autism and Developmental Disorders*, *42*(7), 1304–1313. https://doi.org/10.1007/s10803-011-1356-0

Matson, J. L., & Cervantes, P. E. (2014). Research in Developmental Disabilities Review article Commonly studied comorbid psychopathologies among persons with autism spectrum disorder. *Research in Developmental Disabilities*, *35*(5), 952–962. https://doi.org/10.1016/j.ridd.2014.02.012

McCauley, J. B., Elias, R., & Lord, C. (2020). Trajectories of co-occurring psychopathology symptoms in autism from late childhood to adulthood. *Development and Psychopathology*, *32*(4), 1287–1302. https://doi.org/DOI: 10.1017/S0954579420000826

Mclean, C. P., Asnanni, A., Litz, B., & Hofmann, S. G. (2012). Gender differences in anxiety disorders: prevalence, course of illness, comorbidity and burden of illness. *J Psychiatr Res.*, *45*(8), 1027–1035. https://doi.org/10.1016/j.jpsychires.2011.03.006.Gender

Mian, N. D., Carter, A. S., Pine, D. S., Wakschlag, L. S., & Briggs-Gowan, M. J. (2015). Development of a novel observational measure for anxiety in young children: The Anxiety Dimensional Observation Scale. *Journal of Child Psychology and Psychiatry*, *56*(9), 1017–1025. https://doi.org/10.1111/jcpp.12407

Moskowitz, L. J., Mulder, E., Walsh, C. E., McLaughlin, D. M., Zarcone, J. R., Proudfit, G. H., & Carr, E. G. (2013). A multimethod assessment of anxiety and problem behavior in children with autism spectrum disorders and intellectual disability. *American Journal on Intellectual and Developmental Disabilities*, *118*(6), 419–434. https://doi.org/10.1352/1944.7558.118.6.419

Moskowitz, L. J., Walsh, C. E., Mulder, E., McLaughlin, D. M., Hajcak, G., Carr, E. G., & Zarcone, J. R. (2017). Intervention for Anxiety and Problem Behavior in Children with Autism Spectrum Disorder and Intellectual Disability. *Journal of Autism and Developmental Disorders*, *47*(12), 3930–3948. https://doi.org/10.1007/s10803-017-3070-z

Mullen, E. (1995). Mullen scales of early learning. Circle Pines, MN: American Guidance Service. *Inc.(21)*.

Muris, P., Steerneman, P., Merckelbach, H., Holdrinet, I., & Meesters, C. (1998). Comorbid Anxiety Symptoms in Children with Pervasive Developmental Disorders. *Journal of Anxiety Disorders*, *12*(4), 387–393. https://doi.org/10.1016/s0887

Oostra, B. A., & Willemsen, R. (2003). A fragile balance: FMR1 expression levels. *Human Molecular Genetics*, *12*(suppl 2), R249–R257. https://doi.org/10.1093/hmg/ddg298

Paniccia, M., Paniccia, D., Thomas, S., Taha, T., & Reed, N. (2017). Clinical and non-clinical depression and anxiety in young people: A scoping review on heart rate variability. *Autonomic Neuroscience: Basic and Clinical*, *208*(August), 1–14. https://doi.org/10.1016/j.autneu.2017.08.008

Panju, S., Brian, J., Dupuis, A., Anagnostou, E., & Kushki, A. (2015). Atypical sympathetic arousal in children with autism spectrum disorder and its association with anxiety symptomatology. *Molecular Autism*, *6*(1), 1–10. https://doi.org/10.1186/s13229-015-0057-5

Parma, V., Cellini, N., Guy, L., McVey, A., Rump, K., Worley, J., … Herrington, J. (2021). Profiles of Autonomic Activity in Autism Spectrum Disorder with and without Anxiety. *Journal of Autism and Developmental Disorders*, (0123456789). https://doi.org/10.31234/osf.io/jkcyr

Patriquin, M. A., Hartwig, E. M., Friedman, B. H., Porges, S. W., & Scarpa, A. (2019). Autonomic response in autism spectrum disorder: Relationship to social and cognitive functioning. *Biological Psychology*, *145*(August 2018), 185–197. https://doi.org/10.1016/j.biopsycho.2019.05.004

Patriquin, M. A., Scarpa, A., Friedman, B. H., & Porges, S. W. (2013). Respiratory sinus arrhythmia: A marker for positive social functioning and receptive language skills in children with autism spectrum disorders. *Developmental Psychobiology*.

Pineda, J. A., Friedrich, E. V. C., & LaMarca, K. (2014). Neurorehabilitation of social dysfunctions: A model-based neurofeedback approach for low and high-functioning autism. *Frontiers in Neuroengineering*, *7*(AUG), 1–6. https://doi.org/10.3389/fneng.2014.00029

Porges, S. W. (1992). Vagal tone: a physiologic marker of stress vulnerability. *Pediatrics*, *90*(3 Pt 2), 498–504. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1513615

Porges, W. (1995). Cardiac vagal tone: A physiological index of stress. *Neuroscience & Biobehavioral Reviews*, (2), 225–233.

Prior, M., Smart, D., Sanson, A., & Oberklaid, F. (2000). Does shy-inhibited temperament in childhood lead to anxiety problems in adolescence? *Journal of the American Academy of Child and Adolescent Psychiatry*, *39*(4), 461–468. https://doi.org/10.1097/00004583-200004000-00015

Prosperi, M., Turi, M., Guerrera, S., Napoli, E., Tancredi, R., Igliozzi, R., … Vicari, S. (2020). Sex Differences in Autism Spectrum Disorder: An Investigation on Core Symptoms and Psychiatric Comorbidity in Preschoolers. *Frontiers in Integrative Neuroscience*, *14*, 594082. https://doi.org/10.3389/fnint.2020.594082

Renno, P., & Wood, J. J. (2013). Discriminant and Convergent Validity of the Anxiety Construct in Children with Autism Spectrum Disorders. *Journal of Autism and Developmental Disorders*, *43*(9), 2135–2146. https://doi.org/10.1007/s10803-013-1767-1

Roberts, J. E., Bradshaw, J., Will, E., Hogan, A. L., McQuillin, S., & Hills, K. (2020a). Emergence and rate of autism in fragile X syndrome across the first years of life. *Development and Psychopathology*, *32*(4), 1335–1352. https://doi.org/10.1017/S0954579420000942

Roberts, J. E., Bradshaw, J., Will, E., Hogan, A. L., McQuillin, S., & Hills, K. (2020b). Emergence and rate of autism in fragile X syndrome across the first years of life. *Development and Psychopathology*, *32*(4), 1335–1352. https://doi.org/10.1017/S0954579420000942

Roberts, J. E., J Long, A. C., McCary, L. M., Quady, A. N., Rose, B. S., Widrick, D., … Roberts, J. (2013). *Cardiovascular and Behavioral Response to Auditory Stimuli in*

Boys With Fragile X Syndrome. *38*(3), 276–284. https://doi.org/10.1093/jpepsy/jss114

Roberts, J. E., Tonnsen, B., Robinson, A., & Shinkareva, S. V. (2012). Heart activity and autistic behavior in infants and toddlers with fragile X syndrome. *American Journal on Intellectual and Developmental Disabilities*, *117*(2), 90–102. https://doi.org/10.1352/1944-7558-117.2.90

Rogers, S J. (1996). Brief report: early intervention in autism. *Journal of Autism and Developmental Disorders*, *26*(2), 243–246. https://doi.org/10.1007/BF02172020

Rogers, Sally J, & Vismara, L. A. (2008). Evidence-based comprehensive treatments for early autism. *Journal of Clinical Child and Adolescent Psychology : The Official Journal for the Society of Clinical Child and Adolescent Psychology, American Psychological Association, Division 53*, *37*(1), 8–38.

Salazar, F., Baird, G., Chandler, S., Tseng, E., O'sullivan, T., Howlin, P., … Simonoff, E. (2015). Co-occurring Psychiatric Disorders in Preschool and Elementary School-Aged Children with Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders*, *45*(8), 2283–2294. https://doi.org/10.1007/s10803-015-2361-5

Salum, G. A., De Sousa, D. A., do Rosário, M. C., Pine, D. S., & Manfro, G. G. (2013). Pediatric anxiety disorders: From neuroscience to evidence-based clinical practice. *Revista Brasileira de Psiquiatria*, *35*(SUPPL. 1), 3–21. https://doi.org/10.1590/1516-4446-2013-S108

Schmitz, J., Krämer, M., Tuschen-Caffier, B., Heinrichs, N., & Blechert, J. (2011). Restricted autonomic flexibility in children with social phobia. *Journal of Child*

Psychology and Psychiatry and Allied Disciplines, *52*(11), 1203–1211. https://doi.org/10.1111/j.1469-7610.2011.02417.x

Shephard, E., Bedford, R., Milosavljevic, B., Gliga, T., Jones, E. J. H., Pickles, A., … Charman, T. (2019). Early developmental pathways to childhood symptoms of attention-deficit hyperactivity disorder, anxiety and autism spectrum disorder. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *60*(9), 963–974. https://doi.org/10.1111/jcpp.12947

Sherbourne, C. D., Sullivan, G., Craske, M. G., Roy-Byrne, P., Golinelli, D., Rose, R. D., … Stein, M. (2010). *The Effects of Multiple Anxiety Disorders on Patient Functioning and Well-Being*. https://doi.org/10.7249/RB9525

Silverman, W. K., Pina, A. A., & Viswesvaran, C. (2008). Evidence-based psychosocial treatments for phobic and anxiety disorders in children and adolescents. *Journal of Clinical Child and Adolescent Psychology*. https://doi.org/10.1080/15374410701817907

Skwerer, D. P., Joseph, R. M., Eggleston, B., Meyer, S. R., & Tager-flusberg, H. (2019). *Prevalence and Correlates of Psychiatric Symptoms in Minimally Verbal Children and Adolescents With ASD*. *10*(February), 1–11. https://doi.org/10.3389/fpsyt.2019.00043

Smith, I. C., Ollendick, T. H., & White, S. W. (2019). Anxiety moderates the influence of ASD severity on quality of life in adults with ASD. *Research in Autism Spectrum Disorders*, *62*(June 2018), 39–47. https://doi.org/10.1016/j.rasd.2019.03.001

Solomon, M., Miller, M., Taylor, S. L., Hinshaw, S. P., & Carter, C. S. (2012). Autism symptoms and internalizing psychopathology in girls and boys with autism spectrum

disorders. *Journal of Autism and Developmental Disorders*, *42*(1), 48–59. https://doi.org/10.1007/s10803-011-1215-z

South, M., Rodgers, J., & Van Hecke, A. (2017). Anxiety and ASD: Current Progress and Ongoing Challenges. *Journal of Autism and Developmental Disorders*, *47*(12), 3679–3681. https://doi.org/10.1007/s10803-017-3322-y

Spence, S. H., Rapee, R., McDonald, C., & Ingram, M. (2001). The structure of anxiety symptoms among preschoolers. *Behaviour Research and Therapy*, *39*(11), 1293–1316. https://doi.org/10.1016/S0005-7967(00)00098-X

Spiker, M. A., Lin, C. E., Van Dyke, M., & Wood, J. J. (2012). Restricted interests and anxiety in children with autism. *Autism : The International Journal of Research and Practice*, *16*(3), 306–320. https://doi.org/10.1177/1362361311401763

Stephens, R. L., Langworthy, B., Short, S. J., Goldman, B. D., Girault, J. B., Fine, J. P., … Gilmore, J. H. (2018). Verbal and nonverbal predictors of executive function in early childhood. *Journal of Cognition and Development*, *19*(2), 182–200. https://doi.org/10.1080/15248372.2018.1439493

Sukhodolsky, D. G., Scahill, L., Gadow, K. D., Arnold, L. E., Aman, M. G., McDougle, C. J., … Vitiello, B. (2008). Parent-rated anxiety symptoms in children with pervasive developmental disorders: Frequency and association with core autism symptoms and cognitive functioning. *Journal of Abnormal Child Psychology*, *36*(1), 117–128. https://doi.org/10.1007/s10802-007-9165-9

Thayer, J. F., Friedman, B. H., & Borkovec, T. D. (1996). Autonomic characteristics of generalized anxiety disorder and worry. *Biological Psychiatry*, *39*(4), 255–266. Retrieved from

http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L26083149%5Cnhttp://dx.doi.org/10.1016/0006-3223(95)00136-0

Tonnsen, B. L., Malone, P. S., Hatton, D. D., & Roberts, J. E. (2013). Early negative affect predicts anxiety, not autism, in preschool boys with fragile X syndrome. *Journal of Abnormal Child Psychology*, *41*(2), 267–280. https://doi.org/10.1007/s10802-012-9671-2

van Steensel, F. J. A., Bögels, S. M., & Perrin, S. (2011). Anxiety Disorders in Children and Adolescents with Autistic Spectrum Disorders: A Meta-Analysis. *Clinical Child and Family Psychology Review*, *14*(3), 302–317. https://doi.org/10.1007/s10567-011-0097-0

Van Steensel, F. J. A., Bögels, S. M., & Wood, J. J. (2013). Autism spectrum traits in children with anxiety disorders. *Journal of Autism and Developmental Disorders*, *43*(2), 361–370. https://doi.org/10.1007/s10803-012-1575-z

van Steensel, F. J. a, Bögels, S. M., & Dirksen, C. D. (2012). Anxiety and quality of life: clinically anxious children with and without autism spectrum disorders compared. *The Journal of Child Psychology and Psychiatry*, *41*(6), 731–738. https://doi.org/10.1080/15374416.2012.698725

White, S. W., Mazefsky, C. A., Dichter, G. S., Chiu, P. H., Richey, J. A., & Ollendick, T. (2014). Social-cognitive, physiological, and neural mechanisms underlying emotion regulation impairments: understanding anxiety in autism spectrum disorder. *International Journal of Developmental Neuroscience*, *39*(540), 22–36.

White, S. W., Oswald, D., Ollendick, T., & Scahill, L. (2009). Anxiety in children and adolescents with autism spectrum disorders. *Clinical Psychology Review*, *29*(3), 216–229. https://doi.org/10.1016/j.cpr.2009.01.003

Wigham, S., Rodgers, J., South, M., McConachie, H., & Freeston, M. (2015). The Interplay Between Sensory Processing Abnormalities, Intolerance of Uncertainty, Anxiety and Restricted and Repetitive Behaviours in Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders*, *45*(4), 943–952.

Wood, J. J., & Gadow, K. D. (2010). Exploring the Nature and Function of Anxiety in Youth with Autism Spectrum Disorders. *Clinical Psychology: Science and Practice*, *17*(4), 281–292. https://doi.org/10.1111/j.1468-2850.2010.01220.x

Zaboski, B. A., & Storch, E. A. (2018). Comorbid autism spectrum disorder and anxiety disorders: A brief review. *Future Neurology*, *13*(1), 31–37. https://doi.org/10.2217/fnl-2017-0030

Zainal, H., Magiati, I., Tan, J. W. L., Sung, M., Fung, D. S. S., & Howlin, P. (2014). A preliminary investigation of the Spence children's anxiety parent scale as a screening tool for anxiety in young people with autism spectrum disorders. *Journal of Autism and Developmental Disorders*, *44*(8), 1982–1994. https://doi.org/10.1007/s10803-014-2075