University of South Carolina

Scholar Commons

2015

The Role of Effortful Control And Cortisol In The Emergence of ADHD, ASD, And Anxiety In Boys With FXS

Marissa L. Robinson
University of South Carolina

Follow this and additional works at: https://scholarcommons.sc.edu/etd

Part of the School Psychology Commons

THE ROLE OF EFFORTFUL CONTROL AND CORTISOL IN THE EMERGENCE OF
ADHD, ASD, AND ANXIETY IN BOYS WITH FXS

by

Marissa L. Robinson

Bachelor of Science
North Greenville University, 2010

Master of Arts
University of South Carolina, 2012

_____

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

2015

Accepted by:

Jane E. Roberts, Major Professor

Lindsay M. McCary, Committee Member

Svetlana Shinkareva, Committee Member

Fred Greer, Committee Member

Lacy Ford, Senior Vice Provost and Dean of Graduate Studies

ABSTRACT

ADHD, ASD, and anxiety are three of the most common co-occurring disorders in children with FXS leading to increased social, academic, and behavioral difficulties (Bailey, Raspa, Olmsted, & Holiday, 2008). Early identification and treatment of these co-occurring mental health disorders is essential to promote optimal outcomes; therefore, the study of early precursors and underlying mechanisms of these disorders within a developmental framework is warranted. The current dissertation includes two sequential and related studies investigating impaired effortful control as a precursor and HPA axis dysfunction (measured through salivary cortisol) as an underlying mechanism to the emergence of ADHD, ASD, and anxiety in young boys with FXS. Results indicate that boys with FXS exhibit lower levels of effortful control and higher levels of cortisol compared to typically developing controls; however, no significant relationship between effortful control and cortisol was found. Additionally, significantly higher levels of ASD symptoms were found in the FXS group. Due to significant variability in individual ADHD, ASD, and anxiety trajectories across FXS and typically developing young boys, no specific trend in developmental trajectories was found. Impaired effortful control did not significantly relate to ADHD, ASD, or anxiety symptoms; however, increased cortisol levels were found to relate to increased anxiety symptoms. Future research is necessary to better understand the development of these disorders within FXS throughout childhood to assist with early detection and treatment efforts.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CHAPTER 1

INTRODUCTION

Fragile X Syndrome (FXS) is the most common inherited cause of intellectual

disability affecting as many as 1 in 2,500 males (Fernandez-Carvajal *et al.* 2009;

Hagerman, 2008). An expanded number of CGG trinucleotide repeats at the *FMR1* gene

(Xq27.3) on the X chromosome causes FXS. The consequence of expanded CGG repeats

is a reduction of the expression of the fragile X mental retardation protein (FMRP),

leading to atypical brain development (Crawford, Acuna, & Sherman, 2001). The full

mutation of fragile X occurs when the expansions of CGG reach 200 or more while the

FX premutation occurs with 55-199 CGG repeats (Yu et al., 1991). Cognitive impairment

is one of the core features of FXS as IQ scores typically fall in the moderate range of

intellectual disabilities (IQ scores between 45 and 60; e.g. Loesch et al., 2002; Hall et al.,

2008; Hooper et al., 2008). Gender differences exist within the full mutation as females

typically experience less severe impairment compared to males due to the second X

chromosome available to produce the FMRP protein. Diagnoses can be made via pre- or

post-natal genetic testing; however, in the absence of a family history, FXS is not

typically diagnosed in boys until 37 months (Bailey, Raspa, Bishop, & Holiday, 2009).

While intellectual disabilities are almost hallmark in fragile X syndrome, nearly

80% of individuals with FXS have one or more co-occurring condition (Bailey, Raspa,

Olmstead, and Holiday, 2008). Attention-deficit hyperactivity disorder (ADHD; 84%),

autism spectrum disorder (ASD; 46%), and anxiety (70%) are three of the most

commonly identified comorbid conditions within FXS (Bailey, Raspa, Olmstead, and

Holiday, 2008; Cordeiro, Ballinger, Hagerman, & Hessl, 2011). Individuals with FXS

and other co-occurring conditions tend to have poorer outcomes including greater

behavior problems, more restrictive school placements, lower levels of personal

independence, reduced language skills, and poorer social skills (e.g. Faraone, Biederman,

& Zimmerman, 2005; Handen, Janosky, & McAuliffe, 1997; Bailey et al., 2000; Hatton

et al., 2002; Kau et al, 2004). Additionally, many individuals with FXS experience sub-

threshold symptoms of ADHD, ASD, and anxiety and experience some of these negative

outcomes due to the symptom manifestations (Bailey et al., 2008). For example, more

than 80% of children with FXS have been treated for attention problems, 90% of children

with FXS exhibit one or more symptoms of ASD, and 56-70% of children with FXS

receive treatment for anxiety problems (Bailey et al., 2008; Clifford et al., 2007). Due to

the greater negative outcomes associated with comorbidities in FXS as well as sub-

threshold symptom manifestations, there is a greater need for early identification and

further research on the study of symptom severity to facilitate treatment efforts.

In children without FXS, ADHD typically emerges before the age of 5 with an

average diagnosis age of 7 (CDC, 2010) while ASD typically emerges between the ages

of 2 and 3 years with an average diagnosis age of 4 (Lord et al., 2006; CDC, 2012).

Anxiety disorders typically emerge during middle childhood before the age of 12

(Beesbo, Knappe, & Pine, 2009). While children with FXS exhibit higher rates of these

comorbid disorders, it is not known when comorbid conditions emerge in children with

FXS. In order to better understand the emergence of these disorders, a study of the

2

precursors and underlying physiological mechanisms of these co-occurring disorders is needed.

One such potential precursor is effortful control, which is broadly defined as the ability to regulate responses. Deficits in effortful control exist in ADHD, ASD, and anxiety in typically developing populations (Nigg, 2006; Konstantareas & Stewart, 2006; Calkins & Fox, 2002). Physiological functioning, specifically the activity of the hypothalamic-pituitary-adrenal (HPA) axis, which is responsible for managing biological stress in humans, has been linked to the regulation of effortful control in typically developing children (Watamura, Donzella, Ketes, & Gunnar, 2004) and may serve as an underlying mechanism to the emergence of comorbid conditions in FXS.

While effortful control deficits and HPA axis dysfunction are associated with ADHD, ASD, and anxiety in typically developing populations, the role of effortful control and HPA axis functioning in the emergence of ADHD, ASD, and anxiety is not well understood in typically developing children as well as children with FXS. Our initial work showed low levels of effortful control in boys with FXS that did not improve over time unlike typically developing boys that showed increased levels of effortful control over time (Robinson et al., in preparation). This dissertation includes two follow-up studies that examine how effortful control and HPA axis dysfunction relates to comorbid disorders within FXS. More specifically, the first study investigates the relationship between effortful control and HPA axis functioning, measured by salivary cortisol, in boys with FXS compared to boys with typical development. The subsequent study examines how effortful control and cortisol predict the emergence of ADHD, ASD, and anxiety symptoms in boys with FXS compared to typically developing boys.

The Neuroconstructivist Theory

Existing work suggests that ADHD, ASD, and anxiety within FXS are best understood when behavioral and physiological factors are integrated within a developmental framework (Cornish et al., 2004). The neuroconstructivistic framework recognizes the role of genetics on brain development resulting in behavior and cognition that may change with development (Cornish et al., 2004; see Figure 1.1). This theoretical approach will be applied to the current studies to examine the potential precursors, mechanisms, and developmental course of ADHD, ASD, and anxiety in boys with FXS compared to boys with typical development.

Emergence of ADHD, ASD, and Anxiety in FXS

ADHD, ASD, and anxiety are three of the most commonly co-occurring conditions in children with FXS (Bailey et al., 2008). Due to the high comorbidities, early identification is essential to prevent or minimize these secondary conditions to promote optimal outcomes. The study of precursors (i.e. effortful control) and underlying mechanisms (i.e. cortisol) will help build an understanding of the emergence of these co-occurring disorders as well as the developmental pathways within FXS.

ADHD. Attention-deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder characterized by a pattern of symptoms of inattention and/or hyperactivity and impulsivity present across multiple settings (American Psychiatric Association, 2013). Symptoms of ADHD may include failure to pay close attention to details, difficulty organizing tasks and activities, excessive talking, fidgeting, or an inability to remain seated at appropriate times. Three subtypes of ADHD exist based on symptom composition: predominately inattentive presentation, predominately hyperactive-

4

impulsive presentation, or combined presentation (inattention and hyperactive-impulsive symptoms). ADHD is the most prevalent childhood mental health diagnosis affecting over 6% of all children with even higher rates in clinical groups (CDC, 2013; American Psychiatric Association, 2013). Between 41% and 93% of children with FXS meet criteria on parent rating checklist for ADHD (see Sullivan et al., 2006 for review). Of the subtypes of ADHD, children with FXS ages 7 to 13 years meet criteria for the inattentive presentation more than hyperactive-impulsive presentation or the combined presentation (Sullivan et al., 2006).

There are several mechanisms that may affect the development of ADHD within FXS including age and intellectual level. At early ages, children with FXS display attention difficulties along with hyperactivity. As time progresses, the attention difficulties persist while hyperactivity decreases (Wheeler et al., 2014; Hagerman, 2002). Intellectual level may also play a large role in the emergence of comorbid FXS and ADHD; however, compared to other groups with intellectual disabilities including Down syndrome and idiopathic intellectual disability, boys with FXS exhibit higher rates of inattention, restlessness, distractibility, and impulsivity, suggesting that symptoms of ADHD are not limited to intellectual impairments (Turk, 1998).

Co-occurring ADHD and FXS may result in severe social, academic, and adaptive behavior difficulties. Specifically, children with intellectual disabilities and ADHD experience greater restrictive school placements, increased school suspensions, and higher rates of inpatient psychiatric treatments compared to children with intellectual disabilities without ADHD (Faraone, Biederman, & Zimmerman, 2005; Handen, Janosky, & McAuliffe, 1997). As children with FXS and ADHD enter adulthood, they

are at increased risk for educational, mental health, and legal problems (Pearson et al., 2003) suggesting the need for identification in childhood and treatment throughout the lifespan. In terms of treatment, stimulant medications used to target ADHD symptoms have been shown to increase academic, attention, and physiological regulation skills in boys with FXS (Roberts et al., 2011). While children with FXS and symptoms of ADHD may encounter increased negative experiences compared to children with FXS without symptoms of ADHD, positive effects are associated with targeted treatments. Therefore, a better understanding of the precursors and underlying mechanisms of ADHD can assist in the identification and treatment of ADHD symptoms in FXS.

ASD. Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social communication and the presence of restricted, repetitive patterns of behavior, interest, or activities (American Psychiatric Association, 2013). Social communication impairments may include deficits in social-emotional reciprocity, deficits in nonverbal communication, or deficits in developing, maintaining, or understanding relationships. Restricted and repetitive patterns of behavior, interest, or activities may consist of motor stereotypies, echolalia, lining up toys, difficulties with transitions, highly fixated interests, or hyper- or hypo-responsiveness to sensory input. ASD affects 1 in 68 children and 1 in 42 boys (CDC, 2015). A strong genetic correlate is evident within ASD as 10% of all children with ASD have a genetic or chromosomal condition including FXS (CDC, 2012). Ninety percent of males with FXS exhibit one or more symptom consistent with ASD and 60-75% of males and 20-45% of females with FXS meet criteria for ASD (Clifford et al., 2007; Hall, Burns, Lightbody, & Reiss, 2008; Harris et al., 2008; Klusek et al., 2014).

The most common symptoms of ASD displayed by children with FXS include language delays, poor eye contact, eye-gaze aversion, repetitive motor movements, atypical sensory responses, social avoidance, hyperarousal, impulsivity, and attentional impairments (Hatton et al., 2006; Hessl et al., 2001; Kaufmann et al., 2004). Children with co-occurring FXS and ASD perform more similarly to children with idiopathic ASD than children with FXS-only suggesting the impact of ASD symptoms in children with FXS (Rogers et al., 2001). Co-occurring FXS and ASD leads to greater negative outcomes including lower levels of adaptive behavior, poorer developmental and intellectual abilities, greater problem behavior, delayed or reduced language skills, and lower social competence compared to individuals with FXS without ASD and children with idiopathic ASD (Cohen, 1995; Bailey et al., 2000; Rogers et al., 2001; Hatton et al., 2002; Kau et al, 2004; Kaufman, 2004).

Due to increased rates of ASD associated with increased impairment, the risk factors and underlying mechanisms of ASD continue to be studied. Intellectual level has been shown to predict ASD; however, over 60% of children with ASD do not have an intellectual disability; suggesting that IQ plays a limited role in the emergence of ASD (Charman et al., 2011; CDC, 2012). Autism symptoms persist across childhood (Elmose et al., 2013); however, few studies have examined the stability of ASD symptoms within FXS. Stability in the diagnostic classification (ASD versus no ASD) has been established within FXS (Hatton et al., 2006; Hernandez et al., 2009); however, ASD symptom severity has been found to increase over time (Hatton et al., 2006) and decrease slightly as social skills improved (Hernandez et al., 2009). These studies illustrate the stability of ASD diagnoses (ASD versus no ASD), yet they do not clearly explain developmental

changes in ASD symptom severity that may occur within FXS (Hatton et al., 2006; Hernandez et al., 2009).

        Anxiety. Anxiety disorders are characterized by excessive and unrealistic worry about routine tasks or events, or to a specific object or ritual (American Psychiatric Association, 2013). Anxiety can be difficult to diagnosis in childhood as children experience developmentally appropriate fears and worries; therefore, anxiety disorders are identified when the fears and worries become excessive and/or unrealistic and interfere with daily functioning (Creswell, Waite, & Copper, 2014). Many anxiety disorders emerge during childhood with separation anxiety, generalized anxiety disorder, and social phobia being the most common (see Table 1.1 for description; American Psychiatric Association, 2013; Beesbo, Knappe, & Pine, 2011) Due to developmental changes in childhood fears and worries, anxiety symptoms differ across ages (Weems & Costa, 2005). Separation anxiety disorder is most prevalent in early childhood, typically emerging before age 5 and becoming rare in adolescence while generalized anxiety typically emerges around age 8 and increases in prevalence into adulthood (Copeland, Angold, Shanahan, & Costello, 2015; Weems & Costa, 2005). Social phobia is most prevalent in adolescents due to increased social demands and fears of failure and criticism (Weems & Costa, 2005). Significant homotypic and heterotypic continuity has been found in anxiety symptoms across childhood as young children with an anxiety disorders continue to meet criteria for an anxiety disorder at later ages; however, the specific anxiety disorder may change with age (e.g. separation anxiety disorder at age 5 to generalized anxiety disorder in adolescence; e.g. Bufferd et al., 2012; Gregory et al., 2007). Anxiety disorders in childhood are also linked to increased rates of anxiety and

depression in adulthood (Copeland et al., 2015; Woodward & Fergusson, 2001). Additionally, most adults with anxiety disorders experienced clinical levels of anxiety in childhood (Woodward & Fergusson, 2001).

About 12% of children from non-clinical community samples meet criteria for an anxiety disorder at least once in their lifetime (Costello et al., 2011). Rates of anxiety are even higher in FXS as 86% of males (aged 5 to 26) and 76% of females meet diagnostic criteria for one or more anxiety disorder (Cordeiro, Ballinger, Hagerman, & Hessl, 2011). Based on these high prevalence rates within FXS, anxiety disorders are the second most commonly reported co-occurring disorder within FXS (Bailey et al., 2008; Cordeiro et al., 2011). Many individuals with FXS who do not meet diagnostic criteria for an anxiety disorder report features of anxiety disorders suggesting increased rates of anxiety symptoms within FXS and the continuum of symptoms associated with FXS (Cordeiro et al., 2011). Anxiety and social withdrawal have been shown to be core features of the FXS behavioral phenotype based on both parent and teacher reports (Sullivan et al., 2007). Anxiety symptoms within FXS most often manifest as avoidance behaviors, specific phobias, and compulsions (Sullivan et al., 2007); however, symptoms of generalized anxiety occur in higher functioning and older individuals with FXS (Kaufman, 2009). Social anxiety has also been shown to be a prominent symptom in individuals with FXS and may be a key feature in the co-occurrence of FXS and ASD (Kaufmann, Capone, Clarke, & Budimirovic, 2008; Simonoff et al., 2008).

The study of precursors and underlying mechanism of anxiety within FXS is important to assist in the identification of anxiety within FXS in order to promote treatment. There are several mechanisms and precursors that may impact the

development of anxiety within FXS including age, effortful control, and HPA functioning. Anxiety disorders are highly heritable and have been found to persist across the lifespan (Bittner et al., 2007; Ferdinand, Dieleman, Ormel, & Verhulst, 2007). However, developmental changes in anxiety symptoms also exist. For example, separation anxiety decreases with age (Costello et al., 2011). Within FXS, anxiety symptoms show relative stability over time (Wheeler et al., 2014); however, age related changes in mechanisms related to anxiety exist in children with FXS as hyporesponsiveness is associated with infancy in FXS while hyperresponsiveness is associated with early childhood ages (Roberts, Tonnsen, Robinson, & Shinkareva, 2012). Due to evident developmental changes in anxiety symptoms in the general population as well as age related changes in mechanisms related to anxiety within FXS, the developmental trajectories of anxiety within FXS need further investigation.

Anxiety disorders can be difficult to clinically diagnose in children with FXS as the diagnostic process often relies on verbal expression of symptoms, which may be lacking in children with intellectual impairments (Bailey et al., 2008). Many treatments and services for anxiety are linked to clinical diagnoses; therefore, some children with FXS may not receive the appropriate services needed if appropriate diagnoses are not made. Thus, children with FXS are not only at increased risk for displaying anxiety symptoms and developing anxiety disorders, but they are also at increased risk for not receiving targeted interventions (Bailey et al., 2008).

Impaired Effortful Control: Potential Precursor to ADHD, ASD, and Anxiety

Children with FXS exhibit impairments in effortful control that may serve as a precursor to ADHD, ASD, and anxiety. One conceptualization of effortful control is

through temperament, which is defined as individual differences in self-regulation, motor reactivity, and emotional reactivity (Rothbart & Derryberry, 1981; Rothbart & Bates, 1998). The study of temperament allows for the investigation of constitutionally based biological and behavioral factors that have been associated with mental health outcomes (Rothbart & Derryberry, 1981; Rothbart & Bates, 1998). A number of definitions and theories regarding temperament exist; however, the work of Mary Rothbart has the most solid empirical basis to date.

Temperament factors are relatively stable over time yet influenced by development, heredity, and experience allowing for the study of early states as well as developmental changes (Rothbart & Derryberry, 1981; Rothbart & Bates, 1998). Effortful control reflects one's ability to suppress or inhibit a dominant response to perform a subdominant response (Posner & Rothbart, 2000). Effortful control consists of two major components: inhibitory control, which reflects one's ability to inhibit behavioral responses, and attentional control, which reflects one's ability to focus and shift attention when needed (Muris & Ollendick, 2005). Effortful control emerges during the toddlerhood and early preschool years and continues to develop through childhood and adolescence in children with typical development (Posner & Rothbart, 2000).

Effortful control impacts both behavioral and emotional development and, when impaired, it can lead to increased risk for negative outcomes. In typically developing children effortful control has been shown to be a risk factor for ADHD and anxiety (e.g. Murray & Kochanska, 2002; Nigg, 2006; Eisenberg et al., 2001) as low levels of effortful control relate to increased ADHD and anxiety symptoms. For example, in typically developing children (boys and girls) followed longitudinally from 2 years to 5 years of

age, lower levels of effortful control related to increased parent rated measures of attention problems at age 4 (Murray & Kochanska, 2002). Effortful control has also been shown to be the only temperament construct to differentiate children with ASD from typically developing children ages 3 to 10 as children with ASD exhibit reduced levels of effortful control (Konstantareas & Stewart, 2006). Additionally, in a study of boys ages 10 to 15 years comparing levels of effortful control in three groups: boys with ADHD, boys with ASD, and typically developing boys, children in the clinical groups (ADHD/ ASD) scored significantly lower than typically developing children on measures of effortful control further supporting the link between effortful control deficits and the symptom manifestation of ADHD and ASD (Samyn, Roeyers, & Bijttebier, 2011). The relationship between effortful control and anxiety symptoms has limited empirical support compared to symptoms of ADHD and ASD. In a longitudinal study of typically developing children ages 5 to 9, lower levels of effortful control related to increased internalizing symptoms at ages 5 and 7 but not at age 9 (Eisenberg, Sadovsky, et al., 2005). Additionally, adolescents with anxiety disorders displayed lower levels of effortful control compared to controls (Vervoort et al., 2011). Effortful control served as a protective factor for the adolescents with anxiety as higher levels of effortful control related to decreased internalizing symptoms (Vervoort et al., 2011). While the relationship between impaired effortful control and anxiety symptoms has been established in adolescents, very little research has investigated the link between effortful control and anxiety in young children. Overall, reduced levels of effortful control may serve as a risk factor for ADHD, ASD, and anxiety in children.

The study of effortful control in FXS is relatively new. A factor analytic study of the temperament factors of the Child Behavior Questionnaire (i.e. negative affectivity, surgency/ extraversion, and effortful control; Rothbart, Ahadi, Hershey, & Fisher, 2001) revealed a factor structure within FXS similar to the original Rothbart factor structure in typically developing children supporting the application of Rothbart's factor structure in the current studies (Roberts, Tonnsen, Robinson, McQuillin, & Hatton, 2013). Children with FXS typically display lower levels of effortful control across ages compared to typically developing children and show no changes in levels of effortful control over time unlike typically developing children who show a steady increase across ages (Robinson et al., in preparation; Posner & Rothbart, 2000). Additionally, greater autism symptoms have been found to relate to lower levels effortful control in young boys with FXS (Robinson et al., in preparation). The relationship between effortful control and ADHD and anxiety symptoms in children with FXS has not been examined to date. Overall, children with FXS exhibit impairments in effortful control across ages, which may serve as a precursor for mental health disorders as is observed in children with typical development. However, effortful control has not been examined as a potential precursor to ADHD, ASD, and ADHD symptoms in children with FXS.

Physiological Functioning: Potential Mechanism in ADHD, ASD, and Anxiety

The relationship between physiological functioning and behavior is complex and multifaceted. Theoretical advances, like the neuroconstructivist model, have facilitated this work in FXS with recognition of the multiple etiological mechanisms for disorders including genetic, neurobiological, and environmental factors that operate in a transactional manner over time. There are numerous reasons to incorporate physiological

processes in research of typically developing individuals and those with neurodevelopmental disabilities like FXS. First, physiological data may help identify underlying relevant physiological mechanisms in the absence of clear overt behavioral symptoms. Second, physiological measures can provide a measure of response that may not otherwise be available which is particularly relevant in very young children or those with limited language or cognitive function. Third, physiological markers can document biological vulnerability to later-emerging symptoms or disorders both within and across etiologically-specific groups. Finally, physiological markers may reflect individual differences that could affect treatment response.

While a number of physiological systems are implicated in the regulation of effortful control, the hypothalamic- pituitary-adrenal (HPA) axis has been a focus given its role in managing biological stress responses in humans. As part of the neuroendocrine system, the HPA axis plays a critical role in an organism's ability to adapt to biopsychosocial challenges (McEwen, 2004). When stressed, the HPA axis stimulates the hypothalamus to secrete corticotrophin-releasing hormone (CRH), which causes the pituitary to release ACTH that ultimately stimulates the adrenal gland to secrete the hormone cortisol. Cortisol is used to measure stress and is found in plasma but can also be reliably measured in saliva. The activity of the HPA axis system is part of normal coping and stress regulation (Gunnar, 1987); however, HPA axis dysfunction, including both under- and over-activity, leads to negative effects on brain development resulting in learning, memory, and attention deficits (see Sandi & Pinelo-Nava, 2007 for review). This suggests an inverse U-shaped relationship between cortisol levels and cognitive

functioning exists such that significantly high and significantly low levels of cortisol lead to impairments. Optimal performance is linked with moderate levels of cortisol.

HPA axis dysfunction is common in many clinical groups including those with ADHD, ASD, and anxiety disorders. Specifically, children with ADHD alone tend to exhibit reduced levels of cortisol compared to typically developing children in the morning, at bedtime, and during stressful events (Isaksson, Nilsson, Nyberg Hogmark, & Lindblad, 2012; Ma et al., 2011; Isaksson, Nilsson, Lindbald, 2013). Children with ASD also display HPA axis dysfunction. In a study evaluating stress regulation during psychosocial stress situations, typically developing children showed significant decreases in stress over time while children with ASD exhibited increased cortisol levels following the stress situation suggesting impairments in stress regulatory abilities for children with ASD (Corbett, Schupp, & Lanni, 2012). However, when not encountering a stressful situation, children with ASD tend to exhibit reduced levels of cortisol compared to typically developing children (Ćurin, Terzić, Petković, et. al, 2003). High baseline cortisol levels (see Costello et al., 2011 for review) have been shown to serve as risk factors for the development of anxiety. Overall, anxiety disorders are typically associated with chronically high levels of cortisol (Costello et al., 2011) while children with ADHD and ASD typically exhibit reduced levels of cortisol (e.g. Ma, Chen, Chen, Liu, & Wang, 2011; Ćurin, Terzić, Petković, et. al, 2003). Due to the known link between physiological functioning and mental health disorders, HPA axis activity may operate as an underlying mechanism of ADHD, ASD, and anxiety suggesting that over- or under- activity of the HPA axis may lead to impairments in behavior regulation consistent with symptoms of ADHD, ASD, or anxiety.

HPA axis dysfunction is evident in children with FXS. Specifically, boys with FXS tend to have chronically high levels of cortisol compared to typically developing children suggesting increased arousal and stress reactivity (Wisbeck et al. 2000; Hessl et al., 2002; Hessl, Glaser, Dyer-Friedman, & Reiss, 2006; Hessl, Rivera, & Reiss, 2004). These high levels of cortisol have also been linked to symptoms of ADHD and anxiety within FXS in some studies (Hessl et al., 2002) but not others (Hessl, Glaser, Dyer-Friedman, & Reiss, 2006). Conversely, reduced levels of cortisol have been found to relate to ASD specific symptoms in boys with FXS (Hall et al., 2008). Compared to typically developing children and boys with FXS and no ASD, boys with co-occurring FXS and ASD are less able to regulate their response to a stressful event which was associated with elevated initial levels that may have constrained reactivity (Roberts et al., 2009). These results suggest impairments in normal HPA axis functioning in children with FXS. These elevated or decreased releases of cortisol may serve as an underlying mechanism of effortful control impairments leading to the emergence of ADHD, ASD, or anxiety.

There is both theoretical and empirical evidence to link effortful control and HPA axis function given that they both represent aspects of regulation. Children with typical development with higher levels of effortful control produced lower levels of baseline cortisol when controlling for age in typically developing children ages 12 to 36 months (Watamura, Donzella, Ketes, & Gunnar, 2004). Additionally, higher levels of cortisol during a challenging situation relate to lower levels of effortful control in children of typical development suggesting that increased HPA axis functioning may serve as an underlying mechanism of effortful control with high levels signaling poor physiological

regulation (Dettling, Parker, Lane, Sebanc, & Gunnar, 2000). While the relationship between cortisol and effortful control has been established in typically developing samples, this link has yet to be studied within FXS supporting the need for this study focused on such factors.

Current Studies

ADHD, ASD, and anxiety are complex mental health disorders that require the integration of behavioral and physiological factors to fully understand their emergence and development over time. Due to the increased rates of ADHD, ASD, and anxiety disorders within FXS and even higher rates of symptoms of these disorders, this single-gene disorder provides an ideal model to study precursors and underlying mechanisms of these disorders. Using a neuroconstructivistic approach, the following sequential studies used a dimensional approach to assess the precursors and underlying mechanisms of ADHD, ASD, and anxiety symptoms within FXS. The neuroconstructivistic approach highlights the bi-directional nature of multiple factors suggesting that precursors to these mental health disorders interact with one another over time. Therefore, the following sequential studies aimed to examine the role of development in the emergence and stability of ADHD, ASD, and anxiety symptoms. The first study examined whether physiological dysfunction, as measured by cortisol, serves an underlying mechanism of effortful control impairments in children with FXS compared to typically developing children. The subsequent study examined how impaired effortful control and physiological dysfunction relate to the emergence of ADHD, ASD, and anxiety in boys with FXS compared to typically developing boys.

Study 1: What is the relationship between effortful control and cortisol in young boys with FXS compared to typically developing boys controlling for chronological age and developmental level?

It is hypothesized that boys with FXS will exhibit lower levels of effortful control and increased cortisol levels compared to typically developing boys. A negative relationship between effortful control and cortisol is anticipated for both groups.

Study 2: Does effortful control and cortisol predict the emergence of ADHD, ASD, or anxiety symptoms in young boys with FXS compared to typically developing boys while controlling for chronological age and developmental level?

It is hypothesized that children with FXS will exhibit higher levels of ADHD, ASD, and anxiety symptoms with relative stability across ages. Lower levels of effortful control are hypothesized to predict higher levels of ADHD, ASD, and anxiety symptoms in both groups. Higher levels of cortisol are expected to relate to increased levels of anxiety symptoms. Lower levels of cortisol are expected to relate to increased levels of ADHD and ASD symptoms.

The current dissertation included two sequential studies that overlap but are not identical. Due to differences in sample sizes and measures used, the methods and results are discussed for each study separately. While these studies have slight differences, they are interrelated and impact one another.

Table 1.1. Description of Anxiety Disorders in Childhood

Anxiety Disorder	Description
Separation Anxiety Disorder	excessive worry about separation from a major attachment figure
Generalized Anxiety Disorder	excessive and uncontrolled worry across multiple situations and activities
Social Phobia	intense and persistent fear of social or public situations

(American Psychiatric Association, 2013)

Table 1.2. Labels and definitions of scales comprising effortful control.

	Definition	ECBQ*	CBQ*
Attention Focusing	Ability to sustain attention on a task or object.	X	X
Attentional Shifting	Capacity to shift attention from on task or object to another.	X	
Inhibitory Control	Ability to suppress actions or responses.	X	X
Low-Intensity Pleasure	Enjoyment or pleasure resulting from stimuli of low intensity, rate, complexity, novelty, or incongruity.	X	X
Perceptual Sensitivity	Ability to detect slight, low-intensity stimuli from the environment.		X
Cuddliness	Desire for closeness and pleasure with others.	X	
Smiling and Laughter	Positive affect related to changes in stimulus intensity, rate, complexity, and incongruity		X

Based on factor analytic studies (Putnam et al., 2006; Rothbart et al., 2001)
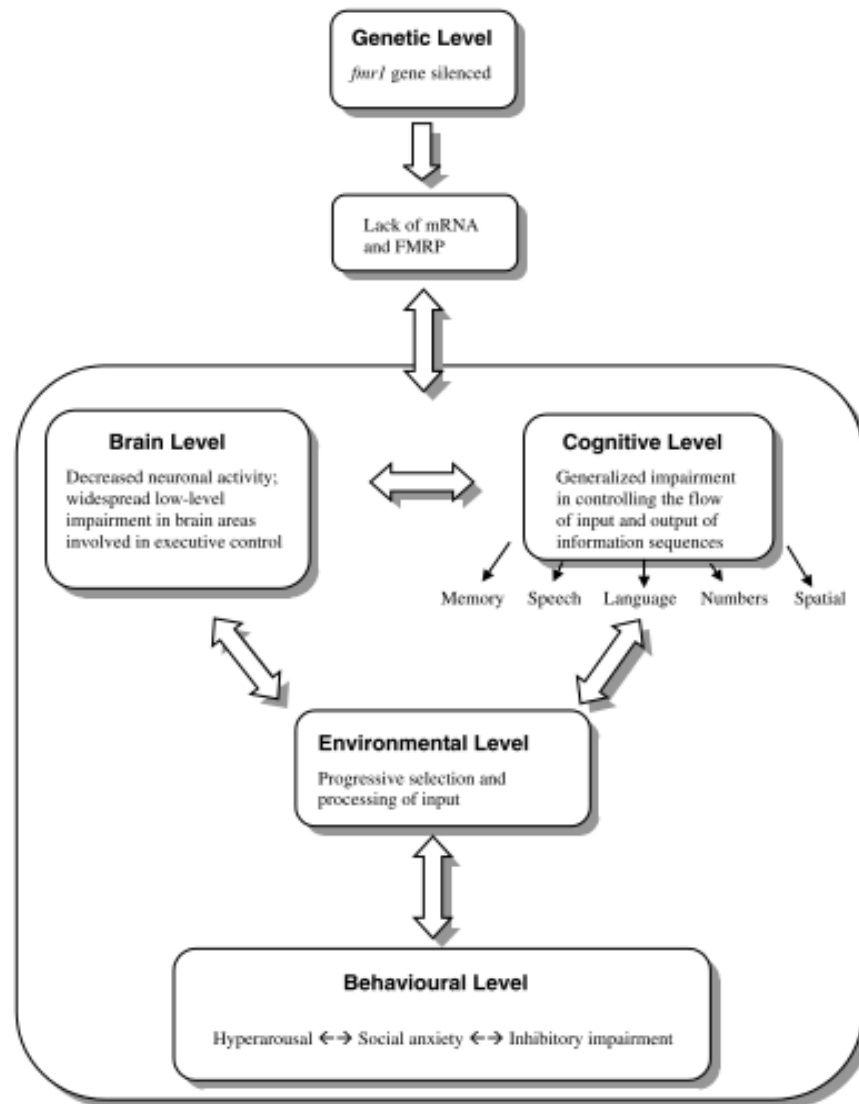
Figure 1.1. The Neuroconstructivistic Approach

CHAPTER 2

STUDY 1

2.1 METHODS

Participants

Participants for the current study included 46 males with FXS and 17 typically

developing males (TD). Participants were drawn from two existing datasets. The first is

an extant dataset of a series of longitudinal studies on the early development of children

with FXS from the University of North Carolina. The second is from an ongoing study at

the University of South Carolina focusing the early emergence of ASD in young children

with FXS. For both the UNC and USC studies, recruitment of participants took place

through research registries, mailings, and listserve emails. Criteria for the current study

included the participants' first assessment after the age of 17 months with data from a

parent-rated temperament questionnaire, parent interview, and salivary samples. This

early assessment time was chosen due to the study goals of examining the emergence of

behaviors. Typically developing children were identified as those without any suspected

or identified delays or disabilities as well as no history of FXS. These children also

scored within two standard deviations of the mean on a measure of developmental level.

The typically developing group was matched with the FXS participant group on a broad

age range. Only males were included in the current study due to greater heterogeneity in

the phenotype of females with FXS. Participants with FXS ranged in age from 18 months

to 7 years (M=4.21, SD=1.64) while TD participants ranged in age from 18 months to 4

years (M=2.72, SD=1.64). See Table 2.1 for an overview of the data.

Measures

       Effortful Control. Parent-rated temperament questionnaires measuring aspects of

reactivity and self-regulation were collected at each assessment. To best examine

temperament longitudinally across childhood, multiple temperament scales were

developed to appropriately account for developmental changes. Due to the longitudinal

nature of the current study, two temperament scales were used including the Early

Childhood Behavior Questionnaire (ECBQ; Putnam, Gartstein, & Rothbart, 2006) and

the Child Behavior Questionnaire (CBQ; Rothbart et al., 2001). The ECBQ is used for

children ages 16 to 35 months while the CBQ is used for children ages 3 to 7 years.

       Factor analytic studies exist for the scales and the following three temperament

constructs are consistently reported: effortful control, surgency/extraversion, and negative

affectivity (Putnam et al., 2006; Putnam, Rothbart, & Garstein, 2008; Rothbart et al.,

2001).   The current study focused on effortful control and computed this construct using

the scales with the highest factor loadings per published findings. While the ECBQ, and

CBQ are theoretically unified, item and scale content overlap but are not identical given

the change in behavioral expression associated with developmental factors (e.g., attention

focusing in toddler aged children is expressed differently than attention focusing for

children in middle childhood). Thus, there are minor differences in the factor composition

of effortful control across scales reflecting this developmental effect (see Table 1.2 for

review). For the ECBQ, effortful control is comprised of attention focusing, attentional

shifting, inhibitory control, low-intensity pleasure, and cuddliness (see Table 1.2 for scale

definitions; Putnam, Garstein, & Rothbart, 2006), while the CBQ effortful control composite is comprised of attention focusing, inhibitory control, low-intensity pleasure, perceptual sensitivity, and smiling and laughter (Rothbart et al., 2001).

These temperament scales have been extensively evaluated and show strong convergent and discriminate validity, inter-rater reliability, internal reliability, and reliability across ages (Putnam, Rothbart, & Garsten, 2008). Effortful control in early childhood predicts effortful control in later childhood suggesting strong homotypic continuity (Putnam, Rothbart, & Garstein, 2008). Consistent with previous work, the effortful control scale was computed by taking the mean of the standardized temperament scales (see Table 1.2). For the current study, internal reliability estimates of effortful control ranged from .54 to .84. For the FXS group, effortful control scores ranged from -2.24 to 1.19 (M=-0.25, SD=0.9). For the TD group, effortful control scores ranged from -0.77-2.33 (M=0.67, SD=0.93).

Salivary Cortisol. Salivary samples were collected two times during each assessment to measure cortisol. The current study used only the first salivary sample termed "baseline cortisol," which was collected within 15 minutes of the start of the assessment to represent pre-assessment cortisol levels. The second salivary sample termed "reactivity" was not included in the current study for two reasons. First, the current study aimed to explore the role of HPA axis functioning when not undergoing a stressful situation. Secondly, the reactivity sample was missing for many of the participants in the current study significantly limiting the sample size. The salivary samples were collected using a Salivette (Salimetrics, LLC 2005), which resembles an oral cotton swab that the participant saturated in his mouth for at least one minute.

24

Participants were asked to avoid consumption of food or liquid (except water) 30 minutes before the sample. Assessment times were scheduled to start and end at the approximate same time each day and the collection time was recorded to control for diurnal variation in cortisol levels. The saliva samples were processed using the Salimetrics' Salivary Cortisol Enzyme Immunoassay kit (EIA; Salimetrics LLC, 2005). Serum and saliva cortisol are positively correlated using the Salimetrics EIA (r=.91, p<.0001; Salimetrics LLC, 2005). The mean inter-assay coefficient of variation for the current sample was 6.53 (Range=5.40- 8.11%) Each sample was assayed twice and the correlations among the assays was >.95. Cortisol levels are reported in micrograms/deciliter. Across all observations, cortisol levels varied from 0.04 to 1.79 (M=0.28, SD=0.3) for the FXS group and 0.04 to 0.52 (M=0.11, SD=0.11) for the TD group.

Developmental Level. The Vineland Adaptive Behavior Scale (VABS; Sparrow, Balla, Cicchetti, & Doll, 1984; VABS- 2; Sparrow, Cicchetti, & Balla, 2005) is a semi-structured interview that measures adaptive behavior skills in 4 domains: communication, socialization, daily living skills, and motor skills. Mothers completed the interview of their child's current abilities at each assessment. Domain and adaptive behavior composite (ABC) standard scores were calculated. For the current study, two versions of the Vineland were used due to the release of an updated version during the data collection period. The VABS has been extensively used with typically developing and clinical populations. Test-retest reliability estimates vary around .80 suggesting stability over time. Uniformality among raters has also been found as inter-rater reliability estimates range from .6 to .7. In terms of validity, the VABS has been shown to correlate well with other measures of adaptive behavior including Adaptive Behavior Inventory for Children

(ABIC; .58) and AAMD Adaptive Behavior (.40 to .70). Overall, the VABS is a robust well-supported measure of developmental skills for children. The VABS is commonly used as a measure of development in children with FXS as it can be very difficult to obtain valid intelligence scores (Rogers et al., 2001; Hatton et al., 2006; Kaufman et al., 2004). This scale is also widely used with children with ASD and has been shown to positively correlate with measures of cognitive ability and mental age in children with ASD supporting the use of this scale in the current study (Wells, Condillac, Perry, & Factor, 2009; Freeman, Del'Homme, Guthrie, & Zhang, 1999). In the current sample, ABC scores ranged from 36 to 119 for the FXS group (M=60.39, SD=13.62) while the total scores for the TD group ranged from 85 to 114 (M=100.59, SD=8.62). These ABC scores were found to highly correlate (r=0.91) with a concurrent and direct assessment of developmental skills measured by the Early Learning Composite score of the Mullen Early Scales (Mullen, 1995).

Procedures

The effortful control and salivary cortisol measures were all completed within a larger assessment battery. Interested parents were provided with study information over the phone and through email. All children who met the inclusion/exclusion criteria were enrolled in the study. Informed consent was completed for each participant by the parent and the individual assessments were conducted within the family home. The parent rated scales were completed within two weeks before or after the in-person assessment. Following each assessment, families were given a small stipend and a summary report of their child's developmental skills. All data were scored and double-checked at 100%. The data were then double-entered and verified for 20% accuracy.

Data Analysis

All analyses were performed using the R Project for Statistical Computing and the NLME package (version 2.14.2; R Foundation for Statistical Computing, 2012). To address the hypotheses, analyses were conducted through a series of steps. First, descriptive analyses and correlations were conducted. Second, initial analyses of covariance (ANCOVA) and linear regression models were built to examine potential predictors. The final step included determining the models with the best fit. Diagnostic analyses of the data were performed to examine the key assumptions of ANCOVA and linear regression. Plots of residual and error terms supported the independence and normality of residuals, homoscedasticity of variance, and homogeneity of regression slopes. No influential data points were found, including outliers. Due to a skewed distribution, cortisol was transformed using a log base 10 transformation.

Analyses of covariance (ANCOVA) were conducted to compare mean levels of effortful control and cortisol across the FXS and TD groups controlling for age. Developmental level was tested as a covariate but was not independent of group membership so it was not included in the ANCOVA. Multiple linear regression models were conducted to assess the relationship between effortful control and cortisol. The final model included cortisol predicting effortful control while controlling for age, developmental level, and group membership. , $Y=B_0+ B_1(Age)+ B_2(Developmental Level)+ B_3(Group)+ \beta_{B4}(Cortisol)+ e_i$, Interaction effects were tested examining the role of group membership, age, and developmental level with cortisol.; however, due to decreased model fit and non-significant findings, these interaction effects were not included in the final model.

27

2.2 RESULTS

ANCOVA results revealed that when controlling for age, boys with FXS exhibited significantly lower levels of effortful control (M=-0.25, SD=0.9) compared to typically developing boys (M=0.67, SD=0 .93, $F_{(2,60)}$=12.11, p<.0001). Boys with FXS exhibited significantly higher levels of cortisol (M=0.28, SD=0.3) compared to boys with typical development (M= 0.11, SD= 0.11, $F_{(2,60)}$=14.85, p=.0003) when controlling for age. Figure 2.1 illustrates this comparison.

The results (Table 2.2) of the linear regression model examining the relationship between cortisol and effortful control indicated that when controlling for age, developmental level, and group membership, cortisol levels did not significantly predict effortful control (B=0.22, p=0.53). Increasing age (B=0.24, p=0.01) and developmental level (B=0.03, p=.005) did significantly predict higher levels of effortful control. Due to the null finding, a post-hoc power analysis using R was conducted and indicated a 11.56% chance of detecting a small effect (.02), a 64.73% chance of detecting medium effect (.15) and a 96.66% chance of detecting a large effect (.35) based on the sample size (n=63), model predictors (df=4), and significant level (p=.05). These results suggest that sufficient power was available to detect a medium and large effect.

Table 2.1. Study 1 Descriptives

	FXS (n=46)		TD (n=17)	
	M (SD)	Range	M (SD)	Range
Age (years)	4.21 (1.64)	1.47 – 7.81	2.76 (0.78)	1.54 – 4.15
Developmental Level	60.39 (13.62)	36 – 119	100.59 (8.62)	85 – 114
Effortful Control	-0.25 (0.9)	-2.24 – 1.19	0.67 (0.93)	-0.77 – 2.33
Cortisol	0.28 (0.3)	0.04 -1.79	0.11 (0.11)	0.04 – 0.52

Developmental Level= Vineland Adaptive Behavior Composite Score

Table 2.2. Linear Regression Results, Outcome: Effortful Control

Variable	B	SE	t-value	p	F	df	p	R^2
Intercept	-3.01	1.15	-2.61	0.01	7.02	4, 58	0.0001	0.30
Age (years)	0.24	0.08	2.87	0.005				
Developmental Level	0.03	0.01	3.29	0.001				
Group	-0.06	0.44	-0.14	0.89				
Cortisol	0.22	0.34	0.64	0.53				

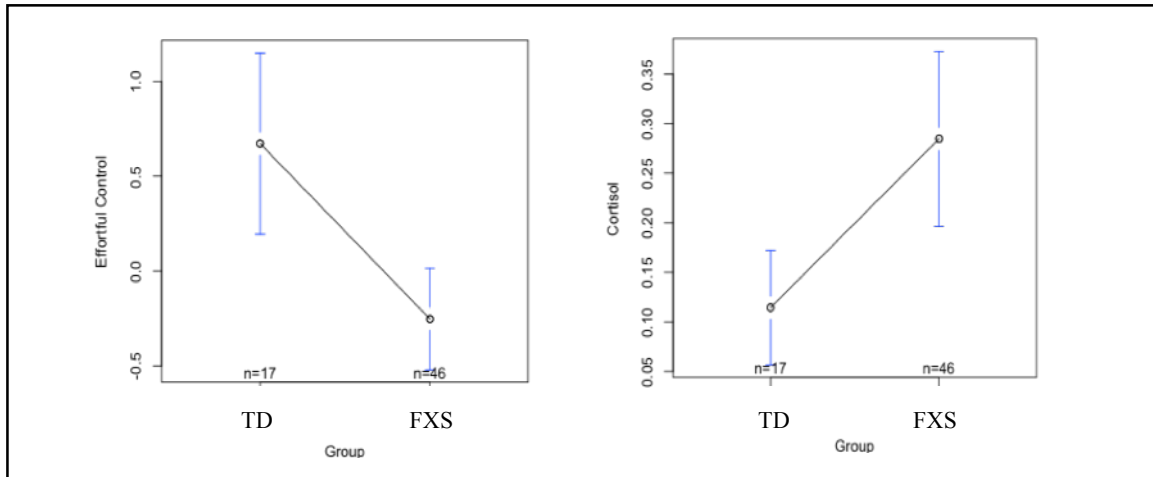Note: n= 63; Developmental Level= Vineland Adaptive Behavior Composite Score

Figure 2.1. Group comparisons of mean levels of effortful control and cortisol.

CHAPTER 3

STUDY 2

3.1 METHODS

Participants

Participants for the current study included 60 males with FXS assessed between 1 and 3 times and 34 males with typical development (TD) assessed between 1 and 2 times with a one-year interval between all assessments. Participants were drawn from two existing datasets. The first is an extant dataset of two longitudinal studies on the early development of children with FXS from the University of North Carolina. The second is from an ongoing study at the University of South Carolina focusing the early emergence of ASD in young children with FXS. For both the UNC and USC studies, recruitment of participants took place through research registries, mailings, and listserve emails.

The current study included participants with FXS and of typical development with data from a parent-rated temperament questionnaire, a parent-rated behavior questionnaire, salivary samples, a parent interview of developmental disabilities, and observer ratings of ASD symptoms. Typically developing children were identified as those without any suspected or identified delays or disabilities as well as no history of FXS. These children also scored within two standard deviations of the mean on a measure of developmental level. Typically developing boys were matched within a broad chronological age range (1.5- 5 years). Only males were included in the current study due to greater heterogeneity in the phenotype of females in females with FXS.

Participants with FXS were assessed between 1 and 3 times totaling 118 observations (Time 1: N= 60, Time 2: N=42, Time 3: N=16) while the TD participants were assessed one to two times yielding 39 total observations (Time 1: N=34, Time 2: N=5). See Table 3.1 for an overview of the observations. Across all observations, participants with FXS ranged in age from 18 months to 5.9 years (M=4.14, SD= 1.09) while the typically developing group ranged in age from 23 months to 4.5 years (M=3.12, SD= 0.81). Table 3.2 reports the characteristics of the participants at the first observation by group.

Measures

ADHD and Anxiety Symptoms. Symptoms of ADHD and anxiety were assessed using the Child Behavior Checklist (CBCL; Achenbach, 1991; Achenbach & Rescorla, 2001). The CBCL is a parent-rated measure of adaptive and maladaptive behaviors. When completing the scale, parents were asked to think about their child's behavior over the past 6 months. This widely used measure assesses several areas of behavioral and emotional functioning in children age 1.5 to 18 years. The version for ages 1.5 to 5 years was used in the current study to best assess the emergence of ADHD and anxiety symptoms. A large normative sample was collected for the CBCL. Strong reliability and validity has been documented with estimates of test-retest reliability at $r=.95$ (Achenbach and Rescorla 2001; Achenbach et al. 2003). The CBCL is frequently used with children with FXS (Tonnsen et al., 2013; Hatton et al., 2002; Kau et al., 2004). For the current study, two specific scales were drawn from both forms that assess the diagnostic criteria of ADHD and anxiety disorders based on the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (American Psychiatric Association, 1994). High internal reliability

estimates of .85 for the ADHD problems scale and .77 for the anxiety problems scale have been reported (Nakamura, Ebesutani, Bernstein, & Chorpita, 2008).

For the DSM-specific scales, raw scores are converted to T-scores that rank in average range (≤65), borderline clinical range (66-69), or clinically significant range (≥70). The average T-score for ADHD symptoms was for the FXS group 59.18 (SD=6.96, range=50-76) and 52.38 (SD=3.57, range=50-64) for the TD group. In the FXS group, 22.5% percent (n=18) of the participants had elevated ADHD scores at one or more assessment and no participants in the TD group had ADHD T-scores above 65. The average T-score for anxiety symptoms was 55.55 (SD=7.83, range= 50-86) for the FXS group and 52.69 (SD=5.43, range=50-73) for the TD group. Seven FXS participants (8.75%) had anxiety T-scores above 65 at one or more assessment while two participants (0.06%) in the TD group had elevated anxiety scores.

The publisher recommends using raw scores in research as the T-scores are truncated at 50 (Achenbach, 1991) resulting in a floor effect. For the current sample, the average raw score for ADHD symptoms was 7.33 for FXS group (SD=2.71, range= 0-16) and 4.15 for the TD group (SD=2.21, range=1-9). For children with FXS, the average raw score for anxiety 3.78 (SD=2.44, range= 0-15) and 2.59 for the TD group (SD=2.44, range=0-10). Raw scores were standardized for all analyses.

ASD Symptoms. The *Childhood Autism Rating Scale* (CARS; Schopler, Reichler, DeVellis, & Daly, 1980) is an examiner rating scale of behavioral symptoms of ASD. Children are rated in 15 areas related to ASD characteristics including social behaviors, activity level, adaptation, and communication. A composite score of 15 to 60 is obtained from the sum of the items yielding a measure of symptom severity and ASD

diagnoses are consistent with scores of 30 or above. The CARS was completed by examiners following each assessment based on behaviors observed as well as on parent reported behaviors. The CARS is a well-established measure with extensive reliability and validity evidence (Schopler, Reichler, DeVellis, & Daly, 1980). The measure has high internal consistency ratings above .90, inter-rater reliability estimates around .71, test-retest reliabilities ranging from .90 at one year apart to .77 at two years. Furthermore, strong sensitivity and specificity values of .88 or higher support the accuracy of the instrument in identifying individuals with and without ASDs. Although not an ASD diagnostic measure, the CARS is often used in research settings to assess ASD behaviors in children with FXS (Hatton et al., 2006; Shanahan, Roberts, Hatton, Reznick, & Goldsmith, 2008). The CARS is standardized on children ages 2 and up. Due to the age restriction, observations before 24 months were removed from the ASD symptoms analyses.

In the current sample, CARS scores ranged from 16.5 to 42 for the FXS group (M=27.09, SD=5.47) while the total scores for the TD group ranged from 15 to 29 (M=16.06, SD=2.7). Twenty-six of the FXS participants (32.5%) had scores of 30 or higher at one or more assessment. No participants in the TD group had CARS scores above 29.

Effortful Control. Parent-rated temperament questionnaires measuring aspects of reactivity and self-regulation were collected at each assessment. To best examine temperament longitudinally across childhood, multiple temperament scales were developed to appropriately account for developmental changes. Due to the longitudinal nature of the current study, two temperament scales were used including the Early

Childhood Behavior Questionnaire (ECBQ; Putnam, Gartstein, & Rothbart, 2006) and the Child Behavior Questionnaire (CBQ; Rothbart et al., 2001). The ECBQ is used for children ages 16 to 35 months while the CBQ is used for children ages 3 to 6 years. For all scales, mothers indicated how often their child displayed specific behaviors based on a scale of 1 (never) to 7 (always).

Factor analytic studies exist for the scales and the following three temperament constructs are consistently reported: effortful control, surgency/extraversion, and negative affectivity (Putnam et al., 2006; Putnam, Rothbart, & Garstein, 2008; Rothbart et al., 2001). The current study focused on effortful control and computed this construct using the scales with the highest factor loadings per published findings. While the ECBQ and CBQ are theoretically unified, item and scale content overlap but are not identical given the change in behavioral expression associated with developmental factors (e.g., attention focusing in toddler aged children is expressed differently than attention focusing for children in middle childhood). Thus, there are minor differences in the factor composition of effortful control across scales reflecting this developmental effect (see Table 1.2 for review). For the ECBQ, effortful control is comprised of attention focusing, attentional shifting, inhibitory control, low-intensity pleasure, and cuddliness (see Table 1.2 for scale definitions; Putnam, Garstein, & Rothbart, 2006), while for the CBQ, effortful control composite is comprised of attention focusing, inhibitory control, low-intensity pleasure, perceptual sensitivity, and smiling and laughter (Rothbart et al., 2001).

These temperament scales have been extensively evaluated and show strong convergent and discriminate validity, inter-rater reliability, internal reliability, and reliability across ages (Putnam, Rothbart, & Garsten, 2008). Effortful control in early

childhood predicts effortful control in later childhood suggesting strong homotypic continuity (Putnam, Rothbart, & Garstein, 2008). Consistent with previous work, effortful control scale was computed by taking the mean of the standardized temperament scales (see Table 2). For the current study, internal reliability estimates of effortful control ranged from .70 to .87. Across all observations, levels of effortful control varied from -2.74 to 2.16 (M=-0.22, SD= 0.96) for the FXS group and -0.97 to 2.35 (M=0.68, SD=0.8) for the TD group.

Salivary Cortisol. Salivary samples were collected two times during each assessment to measure cortisol. The current study used the first salivary sample termed "baseline cortisol," which was collected within 15 minutes of the start of the assessment to represent pre-assessment cortisol levels. The second salivary sample termed "reactivity" was not included in the current study for two reasons. First, the current study wanted to explore the role of HPA axis functioning when not undergoing a stressful situation. Secondly, the reactivity sample was missing for many of the participants in the current study significantly limiting the sample size and power. The salivary samples were collected using a Salivette (Salimetrics, LLC 2005), which resembles an oral cotton swab that the participant saturated in his mouth for at least one minute. Participants were asked to avoid consumption of food or liquid (except water) 30 minutes before the sample. Assessment times were scheduled to start and end at the approximate same time each day and the collection time was recorded to control for diurnal variation in cortisol levels. The saliva samples were processed using the Salimetrics' Salivary Cortisol Enzyme Immunoassay kit (EIA; Salimetrics LLC, 2005). Serum and saliva cortisol are positively correlated using the Salimetrics EIA (r=.91, p<.0001; Salimetrics LLC, 2005).

The mean inter-assay coefficient of variation for the current sample was 6.53 (range=5.40- 8.11%). Each sample was assayed twice and the correlations among the assays was >.95. Cortisol levels are reported in micrograms/deciliter. Cortisol levels were included for the first time point for a subset of the sample (FXS N: 25; TD N: 21) due to missing data at subsequent assessment times. Cortisol levels varied from 0.04 to 1.75 (M=0.33, SD=0.34) for the FXS group and 0.04 to 0.52 (M=0.14, SD=0.13) for the TD group.

Developmental Level. The Vineland Adaptive Behavior Scale (VABS; Sparrow, Balla, Cicchetti, & Doll, 1984; VABS- 2; Sparrow, Cicchetti, & Balla, 2005) is a semi-structured interview that measures adaptive behavior skills in 4 domains: communication, socialization, daily living skills, and motor skills. Mothers completed the interview of their child's current abilities at each assessment. Domain and adaptive behavior composite (ABC) standard scores were calculated. For the current study, two versions of the Vineland were used due to the release of an updated version during the data collection period. The VABS has been extensively used with typically developing and clinical populations. Test-retest reliability estimates vary around .80 suggesting stability over time. Uniformality among raters has also been found as inter-rater reliability estimates range from .6 to .7. In terms of validity, the VABS has been shown to correlate well with other measures of adaptive behavior including Adaptive Behavior Inventory for Children (ABIC; .58) and AAMD Adaptive Behavior (.40 to .70). Overall, the VABS is a robust well-supported measure of developmental skills for children. The VABS is commonly used as a measure of development in children with FXS as it can be very difficult to obtain valid intelligence scores (Rogers et al., 2001; Hatton et al., 2006; Kaufman et al.,

2004). This scale is also widely used with children with ASD and has been shown to positively correlate with measures of cognitive ability and mental age in children with ASD supporting the use of this scale in the current study (Wells, Condillac, Perry, & Factor, 2009; Freeman, Del'Homme, Guthrie, & Zhang, 1999). In the current sample, ABC scores ranged from 33 to 85 for the FXS group (M=58.25, SD=9.26) while the total scores for the TD group ranged from 85 to 118 (M=100, SD=9.14). These ABC scores were found to highly correlate (r=0.84) with a concurrent and direct assessment of developmental skills measured by the Early Learning Composite score of the Mullen Early Scales (Mullen, 1995).

Procedures

The parent rated temperament and behavior scales, developmental measures, examiner rating, and salivary samples were all completed within a larger assessment battery. Interested parents were provided with study information over the phone and through email. All children who met the inclusion/exclusion criteria were enrolled in the study. Informed consent was completed for each participant by the parent and the individual assessments were conducted within the family home. The parent rated scales were completed within two weeks before or after the in-person assessment. Following each assessment, families were given a small stipend and a summary report of their child's developmental skills. All data were scored and double-checked at 100%. The data were then double-entered and verified for 20% accuracy.

Data Analysis

The primary goal of this study was to compare the trajectories of ADHD, ASD, and anxiety symptoms in boys FXS compared to typically developing children and to

examine the unique roles that effortful control and cortisol play in these trajectories. Multilevel modeling (MLM) was used to analyze participants' trajectories using the R Project for Statistical Computing and the NLME package (version 2.14.2; R Foundation for Statistical Computing, 2012). MLM is a strong method for conceptualizing individual change over time because it uses a single analysis of repeated measures data patterns (Raudenbush & Bryk, 2002). MLM uses a two-level hierarchical model of within- (Level 1) and between- (Level 2) subject variances to model change and continuity over time. The Level 1 includes the intercept, which models the individual's status at the mean age, and slope, which models the individual's growth rate. Level 2 describes differences between individuals in these parameters. MLM accommodates for samples with differing group sizes and timing of assessments (Raudenbush & Bryk, 2002). MLM was used for all analyses examining the growth trajectories of the outcome measures (ADHD, ASD, and anxiety symptoms) as well as the relationship between effortful control and the outcome measures.

Diagnostic analyses of the data were conducted to examine the key assumptions of hierarchical linear modeling. Plots of residual and error terms examined the independence and normality of residuals at Level 1 as well as homoscedasticity of variance. The data was examined for influential data points including outliers and none were found.

The CBCL ADHD- and anxiety-raw scores were standardized to make interpretation meaningful. Age in years and developmental level were centered at the mean for MLM analyses so that the intercept could be interpreted as the predicted level of the outcome (ADHD, ASD, and anxiety symptoms) at the mean age/developmental

level for the sample, this effectively controls for differences in age and development level across participants.

To assess the primary hypotheses, a series of multilevel unconditional and conditional models were built testing the effect of effortful control (level 2 predictor) on the initial level and change of ADHD, ASD, and anxiety symptoms (dependent variables). Two separate analyses were conducted for each of the dependent variables (ADHD, ASD, and anxiety symptoms) examining the independent role of effortful control. Before assessing the role of effortful control on the initial level and change in the dependent variable, unconditional models were fit to estimate the level and change in the dependent variable over time illustrated in the following equation illustrating the within-subjects effects.

Level 1 (within–subjects): $Y_{ti} = \pi_{0i} + \pi_{1i} Age_{ti} + e_{ti}$

Level 2 (between-subjects): $\pi_{0i} = \beta_{00} + r_{0i}$

$$\pi_{1i} = \beta_{10} + r_{1i}$$

Y_{ti} represents the dependent variable (ADHD, ASD, and anxiety symptoms) for time t within individual i. π_{0i} and π_{1i} represent the intercept and slope, respectively, for individual i. e_{ti} represents the random error for model. This basic growth model was used as a foundation for several models to address the hypotheses.

To test the hypothesis that children with FXS will exhibit higher levels of ADHD, ASD, and anxiety symptoms with relative stability across ages, group membership was added as a fixed effect to the growth model while controlling for developmental level. Age varied randomly to model the growth trajectory. The basic growth model with covariates included:

Level 1 (Within-Subjects)	$Y_{ti} = \pi_{0i} + \pi_{1i}\,\text{Age} + \pi_{2i}\,Developmental\ Level + \pi_{3i}\,Group + e_{ti}$

$$\pi_{0i} = \beta_{00} + r_{0i}$$

Level 2 (Between-Subjects)	$\pi_{1i} = \beta_{10} + r_{ii}$
	$\pi_{2i} = \beta_{20}$
	$\pi_{3i} = \beta_{30}$

To examine whether the level and change in the dependent variable will vary as a function of effortful control, this predictor was added to the model as a Level 1 effect. The random effects of effortful control was tested to allow for variability with-in individuals; however, it was not included in the final model as they were not significant and did not increase to the model fit.

The final models for each outcome (ADHD, ASD, and Anxiety symptoms) included the following:

Level 1 (Within-Subjects)	$Y_{ti} = \pi_{0i} + \pi_{1i}\,\text{Age} + \pi_{2i}\,Developmental\ Level + \pi_{3i}\,Group + \pi_{4i}\,Effortful\ Control + e_{ti}$

$$\pi_{0i} = \beta_{00} + r_{0i}$$
$$\pi_{1i} = \beta_{10} + r_{1i}$$

Level 2 (Between-Subjects)	$\pi_{2i} = \beta_{20}$
	$\pi_{3i} = \beta_{30}$
	$\pi_{4i} = \beta_{40}$

The intercept coefficient (π_{0i}) measures the overall level in the outcome as a function of age (π_{11}), developmental level (π_{2i}), group (π_{3i}), and effortful control (π_{4i}). β estimates measure the fixed effects *while r* estimates measure the random effect of age.

Due to the fact that only the first time point was available for the participants with cortisol data, multi-level modeling could not be used. Multiple linear regression models were, instead, conducted to assess the relationship between cortisol and the outcome

measures (ADHD, ASD, and anxiety symptoms) while controlling for age, developmental level, and group membership.

Diagnostic analyses of the data were conducted to examine the key assumptions of linear regression. Plots of residual and error terms examined the independence and normality of residuals as well as homoscedasticity of variance. The data was examined for influential data points including outliers and none were found. Due to a skewed distribution, cortisol was transformed using a log base 10 transformation.

To examine the relationship between cortisol levels and ADHD, ASD, and anxiety symptoms, three separate multiple linear regression models were built. The final model included cortisol predicting the outcome (ADHD, ASD, and anxiety symptoms) while controlling for age, developmental level, and group membership, $Y = B_0 + B_1(Age) + B_2(Developmental\ Level) + B_3(Group) + \beta_{B4}(Cortisol) + e_i$. Interaction effects of cortisol with age and group were tested; however, due to decreased model fit and non-significant findings, these interaction effects were not included in the final model.

Additionally, other variables were tested as confounding factors in the MLM and linear regression analyses. Specifically, the form of the Rothbart measure used to measure effortful control was tested and did relate to ASD outcome measures. This will be discussed as a limitation of the current study. A dummy coded variable of missing cortisol was coded and determined not to be a confounding factor.

3.2 RESULTS

ADHD Symptoms

When examining the hypothesis that the FXS group will exhibit higher levels of ADHD symptoms with relative stability across ages, a series of multi-level models were

built controlling for developmental level with age and group membership as predictors. Results of the basic growth model (see Table 3.3- Model 1) indicated no significant differences in mean levels of ADHD symptoms across the sample (β_{00} =-0.14, p=0.58). However, the intercept of the slope is significant (r_{0i}=0.64, p<.0001) indicating significant variability in ADHD trajectories. Figure 3.1 illustrates the variability in individual trajectories for each group. The fixed effect of age was significant indicating that mean levels of ADHD symptoms increase as age increases (β_{10}=0.08, p=0.09). The random slope of age (r_{1i}=0.08, p=.80) is not significant. Developmental level had a negative effect on ADHD symptoms such that as developmental level increased, ADHD symptoms decreased (β_{20}=-0.01, p=0.04). The fixed effect of group membership was not significant indicating that mean levels of ADHD symptoms did not differ across the groups (β_{30}=0.21, p=0.51). These results suggest no specific trend in ADHD symptoms between groups. Figure 3.2 compares the flat trajectories of ADHD symptoms by group. To examine the hypotheses of lower levels of effortful control predicting higher levels of ADHD symptoms in both groups effortful control was added as a predictor to the basic growth model. Effortful control was not found to significantly predict mean levels of ADHD symptoms (β_{40}=-0.07, p=0.26; Table 3.3 -Model 2).

The results (Table 3.4) of the linear regression model examining the relationship between cortisol and ADHD symptoms indicated that when controlling for age, developmental level, and group membership, cortisol levels did not significantly predict ADHD symptoms (B=0.20, p=0.62). Increasing age (B=0.26, p=0.04) did significantly predict higher levels of ADHD symptoms.

ASD Symptoms

To examine the hypothesis that the FXS group will exhibit higher levels of ASD symptoms with relative stability across ages, a multi-level model was built controlling for developmental level with age and group as predictors (Table 3.5-Model 1). Results of the basic growth model indicated that when controlling for developmental level, there were significant differences in mean levels of ASD symptoms (β_{00} =19.76, p=0.0000) across the sample. Additionally, significant variability in individual trajectories was found (r_{0i}=3.42, p<.0001; See Figure 3.3). While the fixed effect of age was not significant (β_{10}=-0.08, p=0.82), the random effect of age reached significance indicating that variability in age among individuals predicted variability in ASD trajectories (r_{1i}=2.15, p=.002). While developmental level was used a covariate, a significant fixed effect was found indicating that lower developmental levels related to increased mean levels of ASD symptoms (β_{20} =-0.13, p=0.001). Similarly, group membership significantly predicted differences in ASD symptoms (β_{30}=6.14, p=0.007) indicating that the FXS group displayed higher mean levels of ASD symptoms. Figure 3.3 illustrates the variability in individual trajectories across ages for each group. Figure 3.4 displays the mean differences and growth trajectories in ASD symptoms across groups. To examine the hypotheses of lower levels of effortful control predicting higher levels of ASD symptoms in both groups effortful control was added as a predictor to the basic growth model (see Table 10). Effortful control was not found to significantly predict mean levels of ASD symptoms (β_{40}=-0.64, p=0.09; Table 3.5-Model 2).

The results (Table 3.6) of the linear regression model examining the relationship between cortisol and ASD symptoms indicated that when controlling for age,

developmental level, and group membership, cortisol levels did not significantly predict ASD symptoms (B=-1.80, p=0.37). Similar to the multi-level modeling results, lower levels of developmental level related to higher levels of ASD symptoms (B=-0.22, p=0.01).

Anxiety Symptoms

To examine the hypothesis that the FXS group will exhibit higher levels of anxiety symptoms with relative stability across ages, a multi-level model was built controlling for developmental level with age and group as predictors (Table 3.7- Model 1). Results of the basic growth model indicated that when controlling for developmental level, there were no significant differences in mean levels of anxiety symptoms (β_{00}=-0.23, p=0.45) across the sample; however, the random slope of the intercept was significant demonstrating variability in anxiety slopes across the sample (r_{0i}=0.74, p<.0001; see Figure 3.5). The fixed effect of age did not reach significance indicating that age did not related to mean levels of anxiety symptoms (β_{00}=-0.03, p=0.62). Similarly, the growth rate or the random slope of age was not significant indicating no variability in trends in anxiety trajectories across ages (r_{1i}=0.000004, p=.99). Mean levels of anxiety symptoms did not differ across groups (β_{30}=0.30, p=.44). Figure 3.6 illustrates the flat trajectories of anxiety symptoms across groups. To examine the hypotheses of lower levels of effortful control predicting higher levels of anxiety symptoms in both groups effortful control was added as a predictor to the basic growth model (see Table 3.7- Model 2). Effortful control was not found to significantly predict mean levels of anxiety symptoms (β_{40}=-0.04, p=0.62).

The results (Table 3.8) of the linear regression model examining the relationship between cortisol and anxiety symptoms indicated that when controlling for age, developmental level, and group membership, cortisol levels did significantly predict anxiety symptoms (B=0.79, p=0.04). More specifically, higher cortisol levels predicted higher levels of anxiety.

Post-hoc Analyses

Post-hoc exploratory descriptive analyses were conducted to examine the comorbidities of elevated symptoms of ADHD, ASD, and anxiety symptoms in the FXS participants at one or more time points. Table 3.9 presents these results. Nine participants (15%) had elevated ADHD and ASD symptoms, 1 participant (1.66%) had elevated ADHD and anxiety symptoms, and 3 participants (5%) had elevated levels of ADHD, ASD, and anxiety symptoms.

Table 3.1. Overview of Observation Totals by Predictor Variables

| Group | Time | Variable | |
		Effortful Control (EC)	Cortisol
FXS	1	60	25
	2	42	0
	3	16	0
	Total	119	25
TD	1	34	21
	2	5	0
	Total	39	21

Table 3.2. Study 2 Descriptives at First Observation

		Group				
		FXS			TD	
Variable	n	M (SD)	Range	n	M (SD)	Range
Age (years)	60	4.01 (1.07)	1.5- 5.76	34	3.07 (0.83)	1.98- 4.5
Developmental Level	60	58.38 (10.95)	33- 85	34	99.53 (9.26)	85-118
ADHD Symptoms[+]	60	0.2 (0.87)	-2.05- 2.38	34	-0.69 (0.72)	-1.73- 0.8
ASD Symptoms	60	27.48 (5.44)	16.5- 42	34	16.21 (2.86)	15-29
Anxiety Symptoms[+]	60	0.02 (0.94)	-1.16- 3.33	34	-0.4 (0.61)	-1.16- 0.91
Effortful Control[+]	60	-0.5 (1.06)	-2.74- 2.06	34	0.6 (0.79)	-0.97- 2.35
Cortisol	25	0.33 (0.34)	0.04- 1.79	21	0.14 (0.13)	0.04- 0.52

Developmental Level=Vineland Adaptive Behavior Composite Score
ADHD/ Anxiety Symptoms= Child Behavior Checklist DSM Standardized Raw Scores
ASD Symptoms= Childhood Autism Rating Scale Total Score
[+]Standardized value

Table 3.3. Linear Model of Growth in ADHD Symptoms

Variable	Model 1			Model 2		
	Estimate	SE	Variance Estimate	Estimate	SE	Variance Estimate
Intercept	-0.14	0.25	0.64*	-0.11	0.25	0.62*
Growth Rate (Age)	0.14**	0.05	0.08	0.15*	0.05	0.05
Developmental Level	-0.01*	0.007		-0.01*	0.007	
Group	0.21	0.32		0.18	0.32	
Effortful Control				-0.07	0.06	
Level-1 error			0.47			0.49
	$\chi2$ model fit improvement[a]=0.06			$\chi2$ model fit improvement= 0.005		

Note: *p<.05, **p<.01, ***p<.001; [a]Model fit compared to unconditional model
Number of observations: 157
Number of groups: 94
Developmental Level= Vineland Adaptive Behavior Composite Score
ADHD Symptoms= Child Behavior Checklist Standardized DSM ADHD Raw Score

Table 3.4. Linear Regression Results of Cortisol Predicting ADHD Symptoms

Variable	*B*	*SE*	t-value	p	F	df	p	*R²*
Intercept	-1.85	1.73	-1.07	0.29	5.41	4, 41	0.001	0.28
Age (years)	0.26	0.12	2.15	0.04				
Developmental Level	0.005	0.02	0.31	0.76				
Group	0.82	0.57	1.43	0.16				
Cortisol	0.20	0.39	0.50	0.62				

Note: n= 46

Developmental Level= Vineland Adaptive Behavior Composite Score

ADHD Symptoms= Child Behavior Checklist Standardized DSM ADHD Raw Score

Table 3.5. Linear Model of Growth in ASD Symptoms

| Variable | Model 1 | | | Model 2 | | |
	Estimate	SE	Variance Estimate	Estimate	SE	Variance Estimate
Intercept	19.76***	1.35	3.42***	19.97***	1.35	3.45***
Growth Rate (Age)	-0.08	0.37	2.15**	0.03	0.37	2.09**
Developmental Level	-0.13**	0.04		-0.12**	0.04	
Group	6.14***	1.76		5.92**	1.76	
Effortful Control				-0.64	0.37	
Level-1 error			1.97			1.95
	χ2 model fit improvement[a]=9.89**			χ2 model fit improvement= 8.90**		

Note: *p<.05, **p<.01, ***p<.001; [a]Model fit compared to unconditional model

Number of observations: 157

Number of groups: 94

Developmental Level= Vineland Adaptive Behavior Composite Score

ASD Symptoms= Childhood Autism Rating Scale Total Score

Table 3.6. Linear Regression Results of Cortisol Predicting ASD Symptoms

Variable	B	SE	t-value	p	F	df	p	R^2
Intercept	39.93	8.85	4.52	.00001	15.9	4, 34	.000000	0.61
Age (years)	-1.01	0.61	-1.64	0.11				
Developmental Level	-0.22	0.08	-2.79	0.01				
Group	2.53	3.09	0.82	0.42				
Cortisol	-1.80	1.97	-0.91	0.37				

Note: n= 39

Developmental Level= Vineland Adaptive Behavior Composite Score

ASD Symptoms= Childhood Autism Rating Scale Total Score

Table 3.7. Linear Model of Growth in Anxiety Symptoms

	Model 1			Model 2		
Variable	Estimate	SE	Variance Estimate	Estimate	SE	Variance Estimate
Intercept	-0.23	0.30	0.74***	-0.22	0.31	0.73***
Growth Rate (Age)	0.03	0.06	.000004	0.04	0.06	.000005
Developmental Level	-0.002	0.009		-0.001	0.009	
Group	0.30	0.40		0.29	0.40	
Effortful Control				-0.04	0.08	
Level-1 error			0.62			0.63
	χ2 model fit improvement[a]=.00000			χ2 model fit improvement= .00000		

Note: *p<.05, **p<.01, ***p<.001; [a]Model fit compared to unconditional model
Number of observations: 157
Number of groups: 94
Developmental Level= Vineland Adaptive Behavior Composite Score
Anxiety Symptoms= Child Behavior Checklist Standardized DSM Anxiety Raw Score

Table 3.8. Linear Regression Results of Cortisol Predicting Anxiety Symptoms

Variable	B	SE	t-value	p	F	df	p	R^2
Intercept	-0.46	1.65	-0.28	0.78	1.36	4, 41	0.26	0.03
Age (years)	0.04	0.11	0.33	0.74				
Developmental Level	0.01	0.01	0.44	0.66				
Group	0.15	0.55	0.27	0.79				
Cortisol	0.79	0.38	2.01	0.04				

Note: n= 39

Developmental Level= Vineland Adaptive Behavior Composite Score

Anxiety Symptoms= Child Behavior Checklist Standardized DSM Anxiety Raw Score

Table 3.9. Descriptives of Group Comorbidities

Groups	n	%`	Age M (SD)	Developmental Level M (SD)	Effortful Control M (SD)
FXS + ADHD + ASD	9	15%	4.31 (0.6)	53.11 (10.2)	-0.38 (1.03)
FXS + ADHD + Anxiety	1	1.66%	5.65	56	-0.73
FXS + ASD + Anxiety	0	0.00%			
FXS + ADHD + ASD + Anxiety	3	5%	3.45 (1.02)	54.67 (6.81)	-0.14 (0.81)

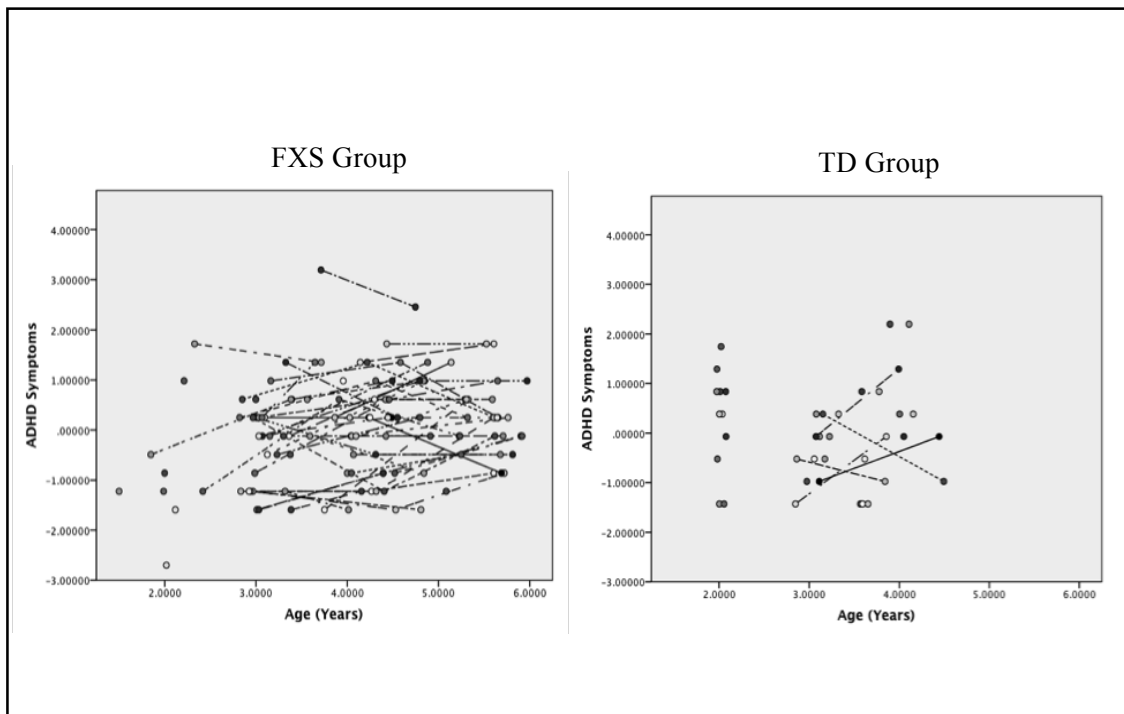Developmental Level= Vineland Adaptive Behavior Composite Score

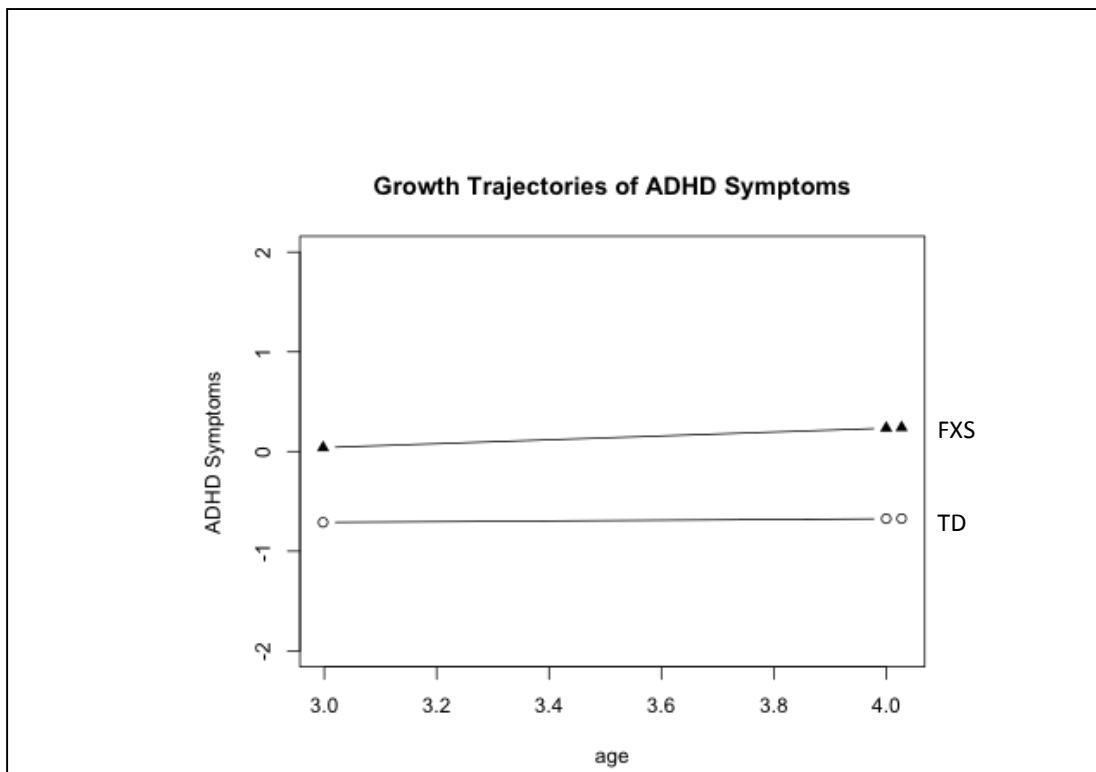Figure 3.1. Individual trajectories of ADHD symptoms

Figure 3.2. Comparison of FXS and TD growth trajectories of ADHD symptoms from ages 2-4 years.
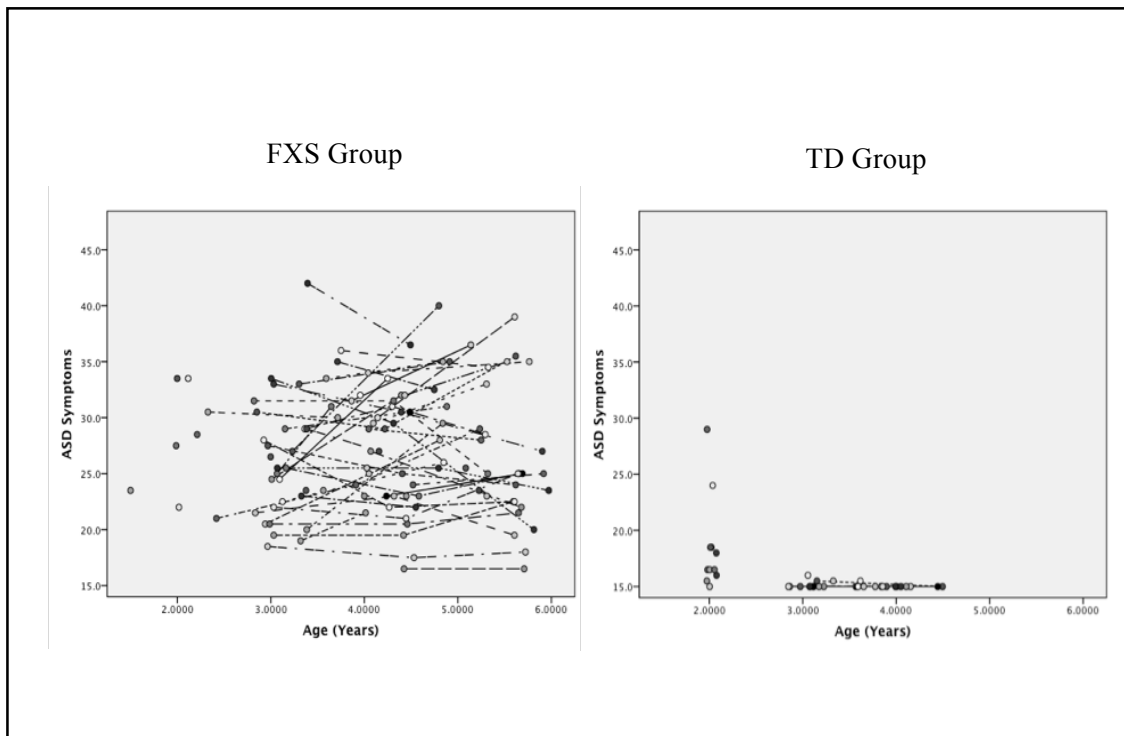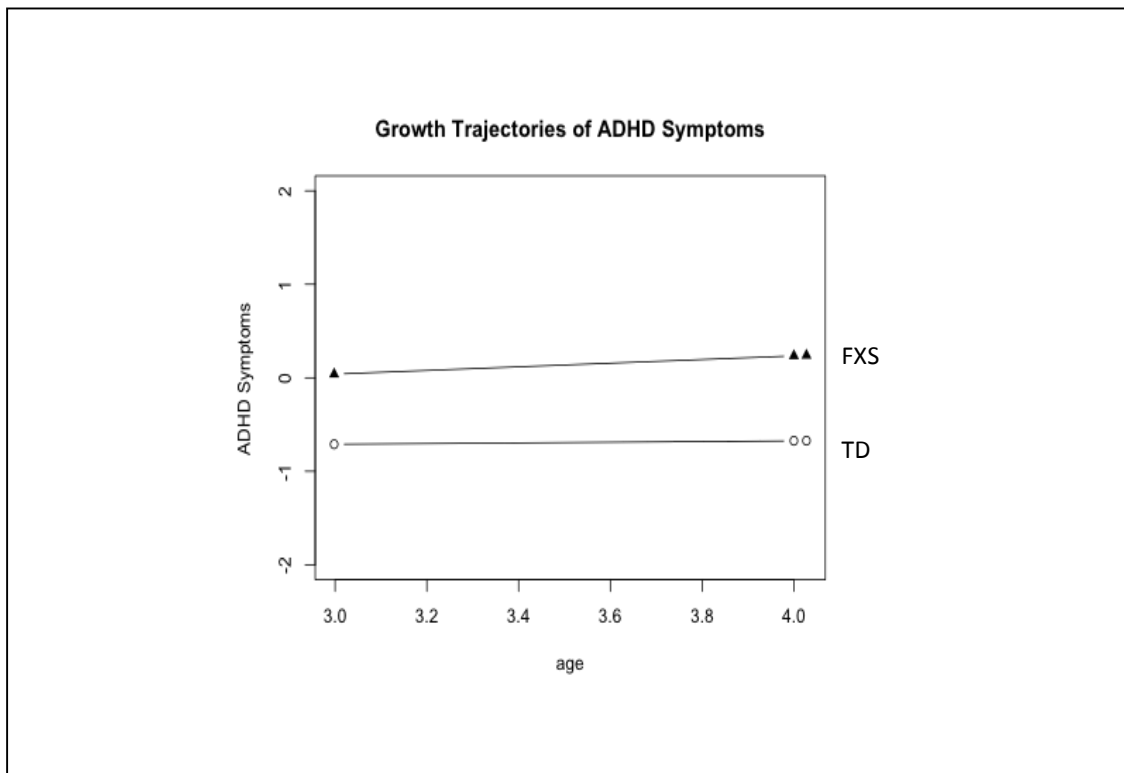
Figure 3.3. Individual trajectories of ASD symptoms

Figure 3.4. Comparison of FXS and TD growth trajectories of ASD symptoms from ages 2-4 years.
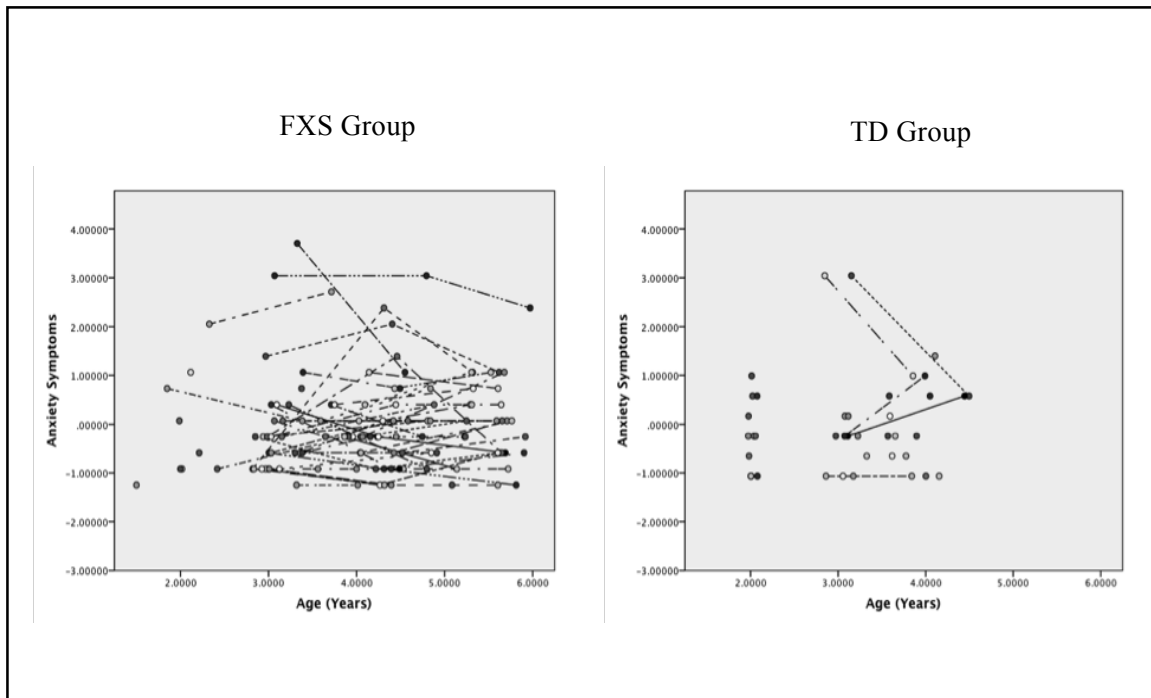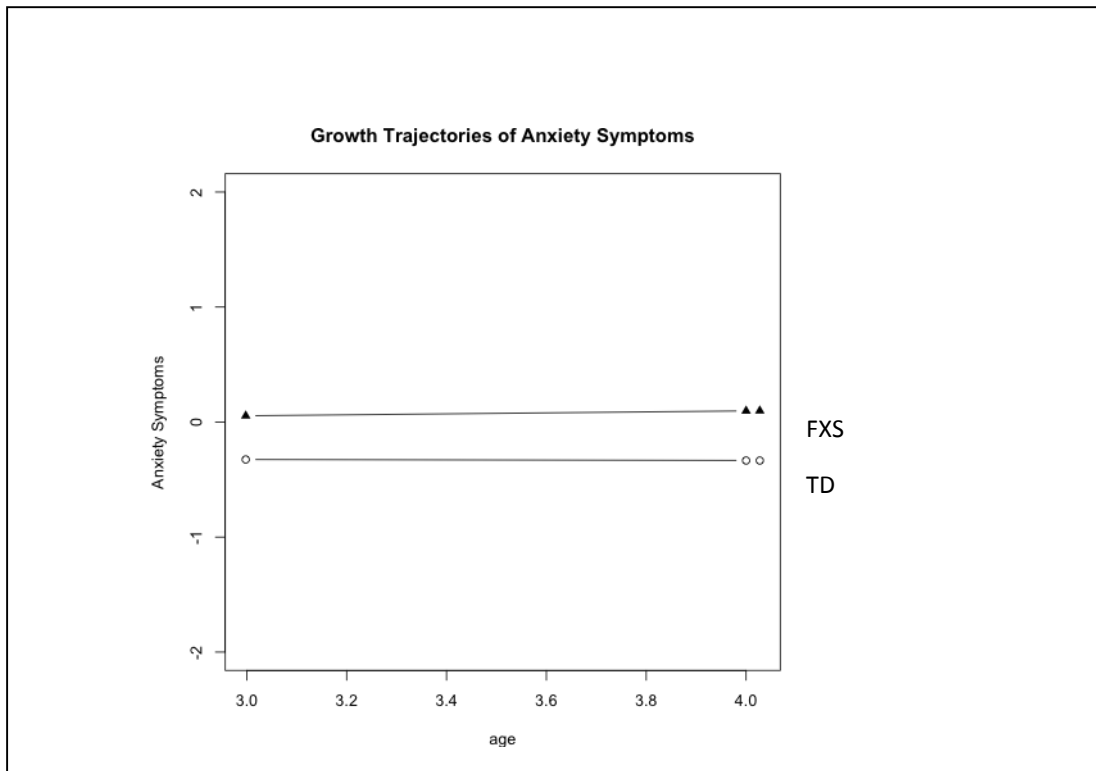
Figure 3.5. Individual Trajectories of Anxiety Symptoms

Figure 3.6. Comparison of FXS and TD growth trajectories of anxiety symptoms from ages 2-4 years.

CHAPTER 4

DISCUSSION

Attention-Deficit Hyperactivity Disorder, ASD, and anxiety are three of the most common mental health disorders in children with higher rates in children with FXS. To facilitate early intervention and treatment efforts across children with FXS and typically developing populations, early identification and a better understanding of the precursors and mechanisms of these disorders is necessary. The neuroconstructivist approach is an ideal theory because this approach recognizes the bi-directional role of genetics, cognition, physiology, and behavior interacting over time (Cornish et al., 2004). The current studies investigated the relationship between impaired effortful control (precursor) and HPA axis dysfunction (underlying mechanism) and their individual impacts on the emergence and stability of ADHD, ASD, and anxiety symptoms in boys with FXS compared to typically developing boys.

Study 1

Consistent with previous literature, the current studies revealed lower levels of effortful control and higher levels of cortisol in boys with FXS compared to boys of typical development (Robinson et al., in preparation; Wisbeck et al. 2000; Hessl et al., 2002; Hessl, Glaser, Dyer-Friedman, & Reiss, 2006; Hessl, Rivera, & Reiss, 2004). While effortful control and cortisol have theoretical and empirical links in young children (Watamura et al., 2004; Dettling et al., 2000), the current studies do not support this relationship in children with FXS. Previous research has shown that a negative

relationship between cortisol levels and parent reported effortful control in typically developing children ages 12 to 36 months with the strongest relationships in children ages 24 months of age (Watamura et al., 2004). Similarly, in a sample of typically developing children ages 3 to 5 years, lower levels of effortful control were related to increased levels of cortisol (Dettling et al., 2000). While the current sample included young children of similar ages, no previous studies have examined the relationship between cortisol and effortful control in clinical samples. Previous findings in typically developing samples and the null finding in FXS may suggest that the link between effortful control and cortisol requires typical brain development that is not seen in children with FXS. However, this is the first study to examine this relationship and future research is needed to replicate these results and to understand why effortful control and cortisol may not be related in children with FXS.

Study 2

ADHD, ASD, Anxiety Symptoms in FXS. We hypothesized that children with FXS would exhibit higher levels of ADHD, ASD, and anxiety symptoms. Group membership did predict increased levels of ASD symptoms as the FXS displayed higher mean levels of ASD across all ages. We did not find this same effect with ADHD and anxiety symptoms. While the current studies focused on symptom severity rather than diagnostic classification to better address the emergence of symptoms, over 32% of the FXS participants had clinically significant ASD symptoms, 22.5% had clinically significant ADHD symptoms, and only 8.75% of the FXS displayed clinically significant anxiety symptoms. Therefore, ASD symptoms were most prevalent in our study and consistent with reported prevalence rates of 29% based on CARS total scores in a broader

age range of boys with FXS (e.g. 18 months to 14 years; Hatton et al., 2006). See Figure 4.1. Our findings support the early emergence of ASD symptoms in young children with FXS.

The prevalence rates of ADHD and anxiety disorders in our sample are much lower than reported rates 41- 93% meeting criteria for ADHD (Sullivan et al., 2006) and 86% of males with FXS meeting criteria for anxiety disorders (Cordeiro et al., 2011). Most previous studies examining prevalence rates of ADHD and anxiety symptoms within FXS included children and adults ages 5 and up; therefore, our study is one of the first to examine these behavioral symptoms in young children with FXS. While our prevalence rates of ADHD and anxiety are far below expectations for children with FXS, our rates exceed prevalence rates of these disorders in preschool-aged children within the general population (see Figure 4.2). For example, a meta-analysis of ADHD diagnoses revealed an average prevalence rate of 10.5% in children ages 3 to 5 years old (Willcutt, 2012), and rates of anxiety disorders in preschoolers have been reported to range from 6.1 to 9.5% (Costello, Egger, and Angold, 2005).

In addition, the increased prevalence rate of ASD symptoms compared to ADHD and anxiety symptoms is consistent with the average age of diagnosis as ASD can be reliability diagnosed at 2 to 3 years old with symptoms often appearing as early as 12 months of age (Ozonoff et al., 2010) while ADHD is typically diagnosed between the ages of 5 and 7 in the general population (CDC, 2010) and anxiety in middle childhood before the age of 12 (Beesbo, Knappe, & Pine, 2009). Our studies used young children ages 18 months to 6 years, which falls most in line with the emergence and identification of ASD. While statistically significant group differences in ADHD and anxiety

symptoms were not found, elevated symptoms were reported and exceed general population prevalence rates during the preschool years highlighting the vulnerability of these young children with FXS.

      Developmental trajectories. A major focus of this longitudinal study was to examine the developmental trajectories of ADHD, ASD, and anxiety symptoms in boys with FXS compared to typically developing children. The current studies indicate significant variability in ADHD, ASD, and anxiety trajectories across FXS and typically developing young boys. More specifically, we did not find specific developmental trajectories for the FXS group due to the variability of symptoms profiles among individuals as some showed increases while others decreased and some remain stable across time. While we hypothesized changes over time in the FXS group, the lack of this finding is consistent with the heterogeneity of the FXS phenotype. Developmental changes may be more apparent when examining the trajectories of symptom severity in more homogeneous groups of children with FXS (i.e. similar developmental levels, diagnosed ADHD +FXS, etc.). Additionally, developmental changes may been seen later in childhood and early adolescence as our study focused on the emergence of these symptom profiles in young children. Due to developmental and cognitive delays in children with FXS, developmental changes may occur later than the typical population, which may be evident when examining a broader age range.

      Effortful control. Despite significantly lower levels of effortful control in boys with FXS compared to boys of typical development, effortful control was not found to significantly predict ADHD, ASD, or anxiety symptoms. The relationships of impaired effortful control with ADHD and ASD symptoms have been shown in previous studies of

young children without FXS of similar ages to our sample (Murray & Kochanska, 2002;

Kochanstantareas & Strewart, 2003); however, no studies have examined the link

between effortful control and anxiety in children under the age of 5 indicating that the

link between effortful control and anxiety symptoms is unknown. Our null finding

linking effortful control with ADHD and ASD symptoms in children with FXS was not

expected due to the significant impairments in effortful control that exist in FXS.

However, the fact that effortful control and these behavioral symptoms are not related is

important, as this is the first study to examine these relationships in FXS. FXS is a

multifaceted genetic disorder that causes changes in brain development (Cornish et al.,

2004); therefore, the lack of the relationship between effortful control and these

behavioral outcomes within FXS is still important to our understanding of the FXS

phenotype.

      Cortisol. Increased cortisol was found to predict increased anxiety symptoms.

Due to the low prevalence rate of elevated anxiety symptoms in our current FXS sample

compared to previously reported prevalence rates, the relationship between cortisol and

anxiety in the current study was unexpected. The elevated cortisol levels in the FXS

group that link to anxiety symptoms shows the robust role of physiological mechanisms

such that HPA axis dysfunction is evident before behavioral symptoms of anxiety. These

findings may facilitate earlier identification and treatment of anxiety in children within

FXS as physiological dysregulation may serve as a precursor to anxiety symptoms and

disorders.

      Cortisol was not found to significantly relate to ADHD or ASD symptoms in the

current study. Previous research in ADHD and ASD groups report decreased cortisol

levels in the clinical groups compared to typically developing children (Ma, Chen, Chen, Liu, & Wang, 2011; Ćurin, Terzić, Petković, et. al, 2003). Conversely, FXS literature suggests overall increased cortisol levels compared to typically developing children (Wisbeck et al. 2000; Hessl et al., 2002; Hessl, Glaser, Dyer-Friedman, & Reiss, 2006; Hessl, Rivera, & Reiss, 2004), which is more similar to clinical groups with anxiety disorders (Costello et al., 2001). Reduced levels of cortisol have been found to relate to ASD symptoms in adolescent boys with FXS (Hall, Lightbody, & Reiss, 2008), but there are mixed findings in the relationship between cortisol levels and ADHD symptoms in boys with FXS (ages 6-17) as one study found a positive relationship between cortisol and ADHD symptoms while another did not find a significant relationship between the two factors (Hessl et al., 2002; Hessl et al., 2002). Therefore, the lack of the relationship between ADHD and ASD symptoms may be due to the fact that our FXS group showed increased levels of cortisol instead of decreased levels that are shown in other clinical groups to relate to ADHD and ASD symptoms. Additionally, previous studies examining cortisol and behavior outcomes included older age groups than used in the current study indicating that our participants may not be showing clinically significant symptoms at young ages.

Limitations

Several limitations need to be noted regarding the current studies. First, the participants included in these studies were drawn from three related and sequential yet independent studies on the early development of FXS. Due to the low incidence rates of FXS, it was important to utilize subjects from all studies; however, two site locations

were included (UNC and USC) and procedures differed slightly, which may result in site effects that could confound some results.

Second, our FXS and typically developing participants were not matched on developmental age. However, a measure of adaptive skills was included and used as a covariate in our analyses to control for differences in developmental level. This proxy measure of developmental level was found to strongly correlate with a direct assessment of developmental or early cognitive skills further supporting its use. While our parent reported measure was found to be reliable measure of developmental level, it did not provide an estimate of mental age. Throughout all analyses, the measure of developmental level was found to relate to effortful control, ADHD symptoms, and ASD symptoms. Significant group differences in developmental level were expected and found between the FXS and TD groups. More specifically, the FXS group displayed significantly lower developmental levels compared to the TD group. Due to the correlation between developmental level and group membership, the use of developmental level as a covariate may have accounted for group differences. Similarly, the effect of effortful control on ADHD, ASD, or anxiety symptoms may have been overshadowed by the effects of developmental level. The use of developmental level as a covariate was necessary to account for developmental delays in the FXS; however, it is not known if additional effects would have been found if developmental level was accounted for in other ways, such as mental age matching.

Third, due to age related changes in cognitive and temperament development, two versions of the Rothbart temperament scales were utilized to take into account age related changes. Two scales based on age (18-36 months, 3-7 years) were included in the

analyses together so that we could examine the behavioral outcomes across ages. If we used only the childhood scale (ages 3-7) we would not have been able to examine the emergence of these behaviors and if we did not include the childhood scale, we would have lost the longitudinal nature of the study. While strong reliability exists for the use of these scales, the use of two scales may pose a measurement issue.

Future Directions

Due to the greater negative outcomes associated with co-occurring conditions within FXS including severe social, academic, and adaptive behavior difficulties (Pearson et al., 2003; Bailey et al., 2008), early identification and treatment is essential. The current studies are one of the first to examine comorbidities within FXS and also one of the first to examine the emergence of these comorbidities by examining behavioral outcomes in young children with FXS. Examining the emergence of these disorders highlights the need for a better understanding of the trajectories of these disorders to better facilitate the timing and specificity of treatment efforts. For example, due to the link between impaired physiological functioning and anxiety, treatment efforts to reduce or prevent the development of anxiety symptoms may focus on stress reduction efforts. Future research may include examining the trajectories of ADHD, ASD, and anxiety symptoms throughout middle childhood and adolescence to see if developmental profiles emerge or if individual variability continues. The individual variability in the growth trajectories of ADHD, ASD, and anxiety across boys with FXS supports the need for individualized treatment plans of all children with FXS.

The current studies utilized parent rating scales and observer ratings of ADHD, ASD, and anxiety symptoms. While these procedures are supported in the literature and

are highly predictive of diagnostic classification, future research should include direct

assessment and diagnostic procedures of ADHD, ASD, and anxiety. More specifically,

our prevalence rates of ASD symptoms is consistent with previous research using the

CARS; however, increased prevalence rates as high as 60-75% of males with FXS have

been shown using direct assessment of ASD specific symptoms such as the Autism

Diagnostic Observation Schedule (Clifford et al., 2007; Hall, Burns, Lightbody, & Reiss,

2008; Harris et al., 2008). No studies to date have used diagnostic measures of ADHD

symptoms in children with FXS as all studies to date have used informant rating scales.

Therefore, the true prevalence rates of ADHD within FXS are not yet known. Future

research may examine the emergence of ADHD, ASD, and anxiety symptoms using

direct assessment to make diagnostic decisions, which may provide more sensitivity in

identifying clinically significant symptoms at early ages. However, the rating scales and

observer ratings are still important as they highlight symptom severity, inform treatment

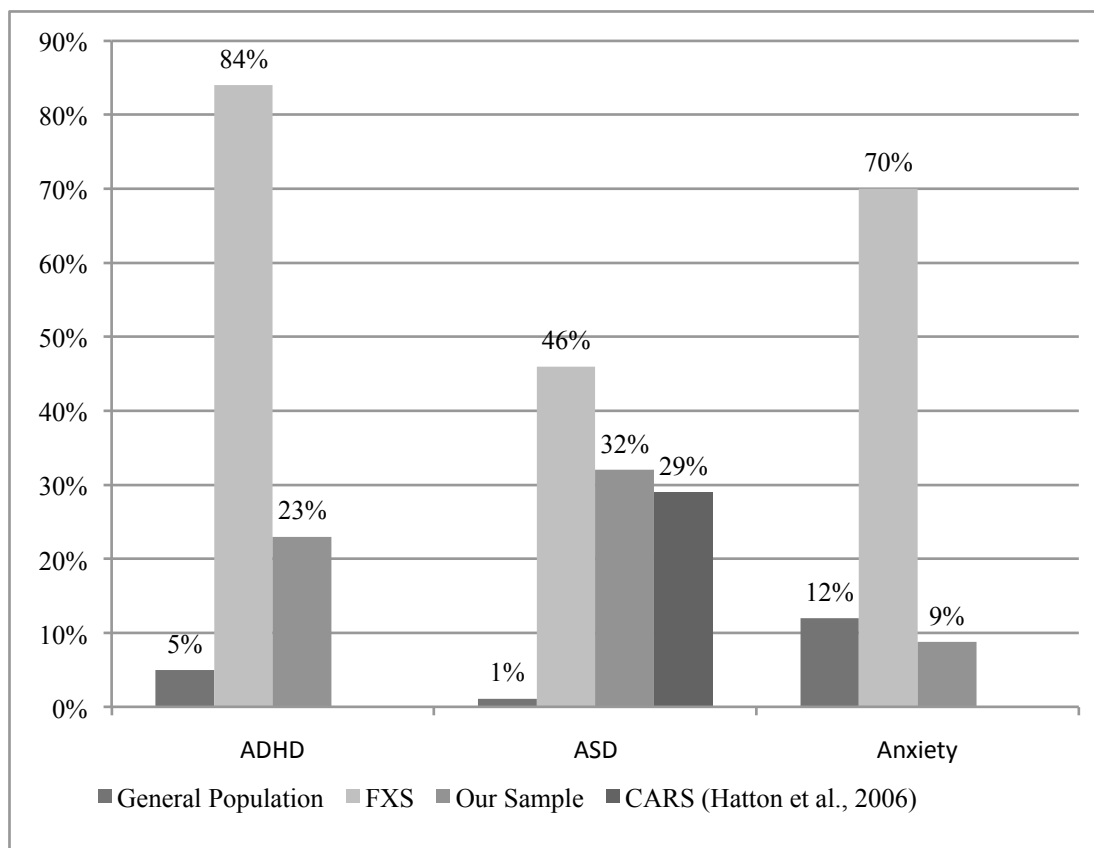efforts, and can be used as screening tools.

Figure 4.1. Comparison of prevalence rates among the general population, FXS populations, and the current FXS sample.
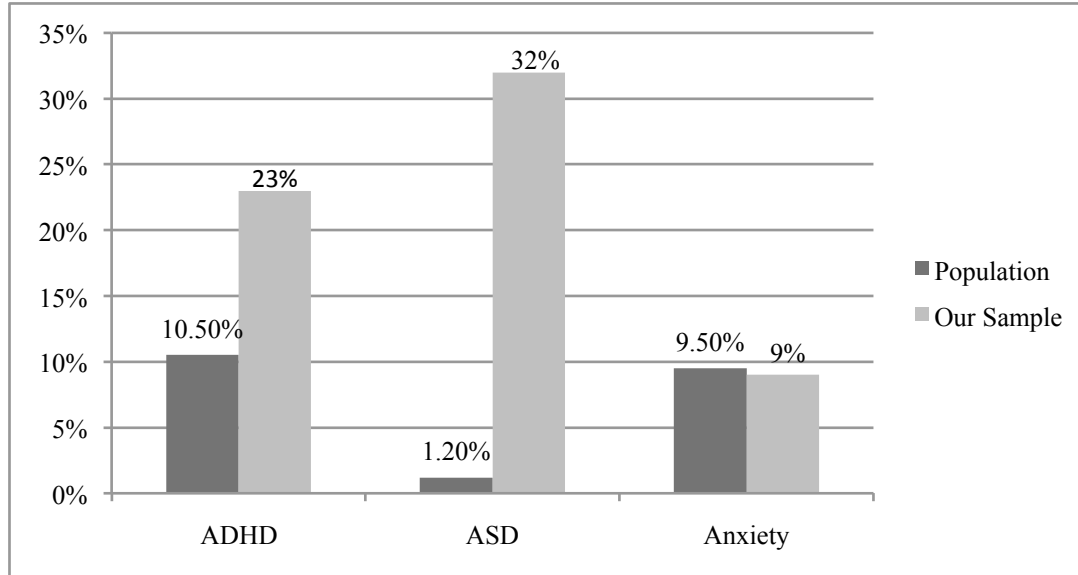
Figure 4.2. Comparison of prevalence rates in preschool-aged children and the current FXS sample.

REFERENCES

Achenbach, T. M. (1991). Manual for the child behavior checklist/4– 18 and 1991
profile. Burlington: University of Vermont Department of Psychiatry.

Achenbach, T. M., & Rescorla, L. A. (2001). Manual for the ASEBA school-age forms &
profiles. Burlington: University of Vermont, Research Center for Children,
Youth, & Families.

Achenbach, T. M., Dumenci, L., & Rescorla, L. A. (2003). DSM- oriented and
empirically based approaches to constructing scales from the same item pools.
Journal of Clinical Child and Adolescent Psychology, 32, 328–340.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental
disorders, 5th edition: DSM-5*. Washington, D.C: American Psychiatric
Association.

Bailey, D. B., Hatton, D. D., Mesibov, G. B., Ament, N., Skinner, M.(2000). Early
development, temperament, and functional impairment in ASD and fragile X
syndrome. *J ASD Dev Disord, 30*, 557–567.

Bailey, D. B., Raspa, M., Olmsted, M., & Holiday, D. B. (2008). Co-occurring conditions
associated with FMR1 gene variations: findings from a national parent survey.
American Journal of Medical Genetics. Part A, *146A*(16), 2060–9.
doi:10.1002/ajmg.a.32439.

Bailey, D. B., Raspa, M., Bishop, E., & Holiday, D. (2009). No change in the age of

    diagnosis for fragile x syndrome: findings from a national parent survey.

    Pediatrics, *124*(2), 527–33. doi:10.1542/peds.2008-2992.

Beesdo, K., Knappe, S., & Pine, D. S. (2009). Anxiety and Anxiety Disorders in Children

    and Adolescents: Developmental Issues and Implications for DSM-V.*The*

    Psychiatric Clinics of North America, *32*(3), 483–524.

    doi:10.1016/j.psc.2009.06.002.

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. doi:10.1016/j.psc.2009.06.002.

Bittner, A., Egger, H. L., Erkanli, A., Jane Costello, E., Foley, D. L., & Angold, A.

    (2007). What do childhood anxiety disorders predict? *Journal of Child*

    Psychology and Psychiatry, and Allied Disciplines, *48*(12), 1174–83.

    doi:10.1111/j.1469-7610.2007.01812.x.

Bufferd, S. J., Dougherty, L. R., Carlson, G. A., Rose, S., & Klein, D. N. (2012). Psychiatric

    disorders in preschoolers: continuity from ages 3 to 6. American Journal of

    Psychiatry, 169(11), 1157-1164. doi:10.1176/appi.ajp.2012.12020268.

Calkins, S. D., & Fox, N. A. (2002). Self-regulatory processes in early personality

    development: A multilevel approach to the study of childhood social withdrawal and

    aggression. *Development and Psychopathology, 14*, 477–498.

Centers for Disease Control and Prevention. (2010). Increasing Prevalence of Parent-

    Reported Attention-Deficit/Hyperactivity Disorder Among Children --- United

    States, 2003 and 2007. *MMWR*, *59*(44, 1439-1443.

Centers for Disease Control and Prevention. (2012). Prevalence of ASDs--ASD and

    Developmental Disabilities Monitoring Network, 14 sites, United States, 2008.

    Morbidity and Mortality Weekly Report. Surveillance Summaries (Washington,

    D.C. : 2002), *61*(3), 1–19.

Centers for Disease Control and Prevention. (2013). Mental health surveillance among

    children--United States, 2005-2011. *Morbidity and Mortality Weekly Report.*

    Surveillance Summaries (Washington, D.C. : 2002), *62 Suppl 2*(2), 1–35.

    Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23677130

Centers for Disease Control and Prevention. (2015). ASDs. Data & Statistics. Retrieved

    from http://www.cdc.gov/ncbddd/ASD/data.html

Charman, T., Pickles, A, Simonoff, E., Chandler, S., Loucas, T., & Baird, G. (2011). IQ

    in children with ASDs: data from the Special Needs and ASD Project (SNAP).

    Psychological Medicine, *41*(3), 619–27. doi:10.1017/S0033291710000991.

Clifford, S., Dissanayake, C., Bui, Q. M., Huggins, R., Taylor, A. K., & Loesch, D. Z.

    (2007). ASD spectrum phenotype in males and females with fragile X full

    mutation and premutation. *Journal of ASD and Developmental Disorders*, *37*(4),

    738–47. doi:10.1007/s10803-006-0205-z.

Cohen, I. (1995). Behavioral profiles of autistic and nonautistic fragile X males. *Dev Brain*

    Dys, 8, 252–269.

Copeland, W. E., Angold, A., Shanahan, L., & Costello, E. J. (2014). Longitudinal patterns

    of anxiety from childhood to adulthood: the great smoky mountains study. Journal of

the *American Academy of Child and Adolescent Psychiatry, 53*(1), 21–33. doi:10.1016/j.jaac.2013.09.017.

Corbett, B. A, Schupp, C. W., & Lanni, K. E. (2012). Comparing biobehavioral profiles across two social stress paradigms in children with and without ASDs. *Molecular ASD*, *3*(1), 13. doi:10.1186/2040-2392-3-13.

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. doi:10.1007/s11689-010-9067-y

Cornish, K. M., Turk, J., Wilding, J., Sudhalter, V., Munir, F., Kooy, F., & Hagerman, R. (2004). Annotation: Deconstructing the attention deficit in fragile X syndrome: a developmental neuropsychological approach. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *45*(6), 1042–53. doi:10.1111/j.1469-7610.2004.t01-1-00297.x

Costello, J. E. , Egger, H., Copeland, W., Erkanli, A., & Angold, A. (2005). The development epidemiology of anxiety disorders: Phenomenology, prevalence and comorbidity. *Child and Adolescent Psychiatric Clinics of North America, 14*, 631–648.

Costello, E. J., Egger, H. L., Copeland, W., Erkanli, A., & Angold, A. (2011). The developmental epidemiology of anxiety disorders: phenomenology, prevalence, and comorbidity. In W. K. Silverman & Andy P. Field (Eds.), *Anxiety Disorders in Children and Adolescents*. New York: Cambridge University Press.

Crawford, D. C., Acuna, J. M., & Sherman, S. L. (2001). FMR1 and the fragile X
syndrome: human genome epidemiology review. *Genet Med, 3*(5), 359-371.

Creswell, C., Waite, P., & Cooper, P. J. (2014). Assessment and management of anxiety
disorders in children and adolescents. Archives of Disease in Childhood, 99, 674–
678. doi:10.1136/archdischild-2013-303768

Ćurin, J. M., Terzić, J., Petković, Z. B., Zekan, L., Terzić, I. M., & Šušnjara, I. M.
(2003). Lower cortisol and higher ACTH levels in individuals with autism.
Journal of Autism and Developmental Disorders, *33*(4), 443–448.
doi:10.1023/A:1025019030121.

Dettling, A. C., Parker, S. W., Lane, S., Sebanc, a., & Gunnar, M. R. (2000). Quality of
care and temperament determine changes in cortisol concentrations over the day
for young children in childcare. *Psychoneuroendocrinology, 25*(8), 819–36.
Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10996476.

Eisenberg N., Cumberland A., Spinrad T. L., Fabes R. A., Shepard S. A., Reiser
M.,…Guthrie, I. K. (2001). The relations of regulation and emotionality to
children's externalizing and internalizing problem behavior. *Child Development
72*, 1112–1134.

Eisenberg, N., Sadovsky, A., Spinrad, T. L., Fabes, R. A., Sandra, H., Valiente, C., …
Shepard, S. A. (2005). The Relations of Problem Behavior Status to Children's
Negative Emotionality, Effortful Control, and Impulsivity: Concurrent Relations
and Prediction of Change. *Developmental Psychology, 41(*1), 193–211.

Elmose, M., Trillingsgaard, A., Jørgensen, M., Nielsen, A., Bruhn, S. S., & Sørensen, E. U. (2013). Follow-up at mid-school age (9-13 years) of children assessed for ASD before the age of four. *Nordic journal of psychiatry*, (0), 1-7.

Faraone, S. V, Biederman, J., & Zimmerman, B. (2005). Correspondence of parent and teacher reports in medication trials. *European Child & Adolescent Psychiatry*, *14*(1), 20–7. doi:10.1007/s00787-005-0415-5.

Ferdinand, R. F., Dieleman, G., Ormel, J., & Verhulst, F. C. (2007). Homotypic versus heterotypic continuity of anxiety symptoms in young adolescents: evidence for distinctions between DSM-IV subtypes. *Journal of Abnormal Child Psychology*, *35*(3), 325–33. doi:10.1007/s10802-006-9093-0.

Fernandez-Carvajal, I., Walichiewicz, P., Xiaosen, X., Pan, R., Hagerman, P. J., & Tassone, F. (2009). Screening for Expanded Alleles of the *FMR1* Gene in Blood Spots from Newborn Males in a Spanish Population. *The Journal of Molecular Diagnostics*, *11*, 324-329.

Freeman, B. J., Del'Homme, M., Guthrie, D., & Zhang, F. (1999). Vineland adaptive behavior scale scores as a function of age and initial IQ in 210 autistic children. *Journal of ASD and developmental disorders, 29*(5), 379-384. doi: 10.1023/A:1023078827457.

Gregory, A. M., Caspi, A., Moffitt, T. E., Koenen, K., Eley, T. C., & Poulton, R. (2007). Juvenile mental health histories of adults with anxiety disorders. American Journal of Psychiatry, 164(2), 301-308.

Gunnar, M.R., 1987. Psychobiological studies of stress and coping: an introduction. *Child Dev 58*, 1403–1407.

Hagerman R. J. (2002). The physical and behavioral phenotype. In Randi Hagerman &
Paul Hagerman (Eds.), *Fragile X syndrome: diagnosis, treatment, and research.
3rd ed*. Baltimore: Johns Hopkins University.

Hagerman, P. J. (2008). The fragile X prevalence paradox. *Journal of Medical Genetics*,
45, 498–499.

Handen, B. L., Janosky, J., & McAuliffe, S. (1997). Long-term follow-up of children
with mental retardation/borderline intellectual functioning and ADHD. *Journal of
Abnormal Child Psychology*, *25*(4), 287–95.

Hall, S. S., Lightbody, A. A. & Reiss, A. L. (2008). Compulsive, self-injurious, and
autistic behavior in children and adolescents with fragile X syndrome. *American
Journal of Mental Retardation : AJMR*, *113*(1), 44–53. doi:10.1352/0895-
8017(2008)113[44:CSAABI]2.0.CO;2.

Harris, S. W., Hessl, D., Goodlin-Jones, B., Ferranti, J., Bacalman, S., Barbato, I., …
Hagerman, R. J. (2008) Autism profiles of males with fragile X syndrome.
American Journal on Mental Retardation, 113, 127–438.

Hatton, D. D., Hooper, S. R., Bailey, D. B., Skinner, M., Sullivan, K., & Wheeler A.
(2002). Problem behavior in boys with fragile X syndrome. *Am J Hum Genet,
108,*105–116.

Hatton, D. D., Sideris, J., Skinner, M., Mankowski, J., Jr, D. B. B., Roberts, J., & Mirrett,
P. (2006). Autistic Behavior in Children With Fragile X Syndrome : Prevalence ,
Stability , and the Impact of FMRP, *1813*, 1804–1813. doi:10.1002/ajmg.a

Hessl, D., Dyer-Friedman, J., Glaser, B., Wisbeck, J., Barajas, R. G., Taylor, A., & Reiss, A. L. (2001). The Influence of Environmental and Genetic Factors on Behavior Problems and Autistic Symptoms in Boys and Girls With Fragile X Syndrome. *Pediatrics*, *108*(5), e88–e88. doi:10.1542/peds.108.5.e88.

Hessl, D., Glaser, B., Dyer-Friedman, J., Blasey, C., Hastie, T., Gunnar, M., & Reiss, A. L. (2002). Cortisol and behavior in fragile X syndrome. *Psychoneuroendocrinology*, *27*(7), 855–72.

Hessl, D., Glaser, B., Dyer-Friedman, J., & Reiss, A. L. (2006). Social behavior and cortisol reactivity in children with fragile X syndrome. *Journal of Child Psychology and Psychiatry, 6*, 602–610. doi:10.1111/j.1469-7610.2005.01556.x.

Hessl, D., Rivera, S. M., & Reiss, A. L. (2004). The neuroanatomy and neuroendocrinology of fragile X syndrome. *Mental Retardation and Developmental Disabilities Research Reviews*, *10*(1), 17–24. doi:10.1002/mrdd.20004.

Hernandez, R. N., Feinberg, R. L., Vaurio, R., Passanante, N. M., Thompson, R. E., & Kaufmann, W. E. (2009). ASD in fragile X syndrome: a longitudinal evaluation. *American Journal of Medical Genetics. Part A*, *149A*(6), 1125–37. doi:10.1002/ajmg.a.32848,

Hooper, S. R., Hatton, D., Sideris, J., Sullivan, K., Hammer, J., Schaaf, J., … Bailey, D. P. (2008). Executive functions in young males with fragile X syndrome in comparison to mental age-matched controls: baseline findings from a longitudinal study. *Neuropsychology*, *22*(1), 36–47. doi:10.1037/0894-4105.22.1.36.

Isaksson, J., Nilsson, K. W., Nyberg, F., Hogmark, Å., & Lindblad, F. (2012). Cortisol

    levels in children with Attention-Deficit/Hyperactivity Disorder. *Journal of*

    Psychiatric Research, 46(11), 1398–1405. doi:10.1016/j.jpsychires.2012.08.021.

Isaksson, J., Nilsson, K. W., & Lindblad, F. (2013). Early psychosocial adversity and

    cortisol levels in children with attention-deficit/hyperactivity disorder. *European*

    Child & Adolescent Psychiatry, 22, 425–432. doi:10.1007/s00787-013-0383-0.

Kau, A. S., Tierney, E., Bukelis, I., Stump, M. H., Kates, W. R., Trescher, W. H. &

    Kaufmann, W. E. (2004). Social behavior profile in young males with fragile X

    syndrome: Characteristics and specificity. *American Journal of Medical Genetics*

    Part A, 126A(1), 9–17. doi: 10.1002/ajmg.a.20218.

Kaufmann, W. E. (2009). Fragile X: Expansion of a genetic disorder. In B. K. Shapiro &

    P. J. Accardo (Eds.), *Neurogenetic syndromes: Behavioral issues and their*

    treatment. Baltimore: Paul H. Brookes Publishing Co.

Kaufmann, W. E., Cortell, R., Kau, A. S., Bukelis, I., Tierney, E.,…Stanard, P. (2004),

    ASD in fragile X syndrome: Communication, social interaction, and specific

    behaviors. American Journal of Medical Genetics Part A, 129A: 225–234.

    doi: 10.1002/ajmg.a.30229.

Kaufmann, W. E., Capone, G. T., Clarke, M., & Budimirovic, D. B. (2008). ASD in

    genetic intellectual disability: Insights into idiopathic ASD. In A. W. Zimmerman

    (Ed.), *Current theories and evidence. ASD*. Totowa, JN: Humana Press.

Klusek, J., Martin, G. E., & Losh, M. (2014). A comparison of pragmatic language in

    boys with autism and fragile X syndrome. *Journal of Speech, Language, and*

    Hearing Research, *57,* 1692-1707.

Konstantareas, M. M., & Stewart, K. (2006). Affect regulation and temperament in

children with ASD. *Journal of ASD and Developmental Disorders*, *36*(2), 143–54.

doi:10.1007/s10803-005-0051-4

Loesch, D. Z., Bui, Q. M., Dissanayake, C., Clifford, S., Gould, E.,…Huggins, R. M.

(2007). Molecular and cognitive predictor of the continuum of autistic behaviours

in fragile X. *Neurosci Biobehav Rev, 31(3). 315-326. doi:*

10.1016/j.neubiorev.2006.09.007.

Lord, C., Risi, S., DiLavore, P. S., Shulman, C., Thurm, A., & Pickles, A. (2006).

Autism from 2 to 9 years of age. *Arch Gen Psychiatry, 63*(6), 694-701.

doi:10.1001/archpsyc.63.6.694.

Ma, L., Chen, Y.-H., Chen, H., Liu, Y.-Y., & Wang, Y.-X. (2011). The function of

hypothalamus-pituitary-adrenal axis in children with ADHD. *Brain Research*,

1368, 159–62. doi:10.1016/j.brainres.2010.10.045.

McEwen, B.S., 2004. Protection and damage from acute and chronic stress: Allostasis

and Allostatic overload and Relevance to the pathophysiology of psychiatric

disorders. Ann. *NY Acad. Sci. 1032*, 1–7.

Mullen, E. M. (1995). *Mullen Scales of Early Learning* (AGS ed.). Circle Pines, MN:

American Guidance Service Inc.

Muris, P., & Ollendick, T. (2005). The role of temperament in the etiology of child

psychopathology. Clinical Child and Family Psychology, 8, 271–289.

Murray, K. T., & Kochanska, G. (2002). Effortful control: factor structure and relation to

externalizing and internalizing behaviors. *Journal of Abnormal Child Psychology*,

30(5), 503–14. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12403153.

Nakamura, B. J., Ebesutani, C., Bernstein, A., & Chorpita, B. F. (2009). A Psychometric

    Analysis of the Child Behavior Checklist DSM-Oriented Scales. *Journal of*

    Psychopathology and Behavioral Assessment, *31*, 178–189. doi:10.1007/s10862-

    008-9119-8.

Nigg, J. T. (2006), Temperament and developmental psychopathology. *Journal of Child*

    Psychology and Psychiatry, 47, 395–422. doi: 10.1111/j.1469-

    7610.2006.01612.x.

Nygren, G., Cederlund, M., Sandberg, E., Gillstedt, F., Arvidsson, T., Carina Gillberg, I.,

    … Gillberg, C. (2012). The prevalence of autism spectrum disorders in toddlers:

    A population study of 2-year-old Swedish children. *Journal of Autism and*

    Developmental Disorders, 42, 1491–1497. doi:10.1007/s10803-011-1391-x

Ozonoff, S., Iosif, A.-M., Baguio, F., Cook, I. C., Hill, M. M., Hutman, T., … Young, G.

    S. (2010). A prospective study of the emergence of early behavioral signs of

    autism. *Journal of the American Academy of Child and Adolescent Psychiatry, 49*,

    256–266. doi:10.1016/j.jaac.2009.11.009.

Pearson, D. A., Santos, C. W., Roache, J. D., Casat, C. D., Loveland, K. A., Lachar, D.,

    … Cleveland, L. A. (2003). Treatment effects of methylphenidate on behavioral

    adjustment in children with mental retardation and ADHD. *Journal of the*

    American Academy of Child and Adolescent Psychiatry, *42*(2), 209–16.

    doi:10.1097/00004583-200302000-00015.

Posner, M. I., & Rothbart, M. K. (2000). Developing mechanisms of self-regulation.

    Development and Psychopathology, 12 , 427-441.

Putnam, S. P., Gartstein, M. A, & Rothbart, M. K. (2006). Measurement of fine-grained

 aspects of toddler temperament: the early childhood behavior questionnaire.

 Infant Behavior & Development, *29*(3), 386–401.

 doi:10.1016/j.infbeh.2006.01.004.

Putnam, S. P., Rothbart, M. K., & Maria, A. (2008). Homotypic and Heterotypic

 Continuity of Fine-grained Temperament during Infancy , Toddlerhood , and

 Early Childhood, *405*, 387–405. doi:10.1002/icd.

Raudenbush, S. W., & Bryk, A. S. (2002). Hierarchical Linear Models: Application and

 Data Analysis Methods (2nd Ed). Thousand Oaks: Sage.

Roberts, J. E., Mankowski, J. B., Sideris, J., Goldman, B. D., Hatton, D. D., Mirrett, P.

 L., … Bailey, D. B. (2009). Trajectories and predictors of the development of

 very young boys with fragile X syndrome. *Journal of Pediatric Psychology*,

 34(8), 827–36. doi:10.1093/jpepsy/jsn129.

Roberts, J. E., Miranda, M., Boccia, M., Janes, H., Tonnsen, B. L., & Hatton, D. D.

 (2011). Treatment effects of stimulant medication in young boys with fragile X

 syndrome. *Journal of Neurodevelopmental Disorders*, *3*(3), 175–84.

 doi:10.1007/s11689-011-9085-4.

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.

 doi:10.1352/1944-7558-117.2.90.

Roberts, J. E., Tonnsen, B. L., Robinson, M., McQuillin, S. D., & Hatton, D. D. (2013). Temperament factor structure in fragile X syndrome: The Children's Behavior Questionnaire. *Research in Developmental Disabilities*, *35*(2), 563–571. doi:10.1016/j.ridd.2013.11.024

Robinson, M. L., Poe, M., Van Horn, M. L., Hatton, D. D., & Roberts, J. E. (in preparation). Developmental trajectories of effortful control in young boys with fragile X syndrome.

Rogers, S. J., Wehner, E., Hagerman, R. J. (2001). The behavioral phenotype in fragile X: Symptoms of ASD in very young children with fragile X syndrome, idiopathic ASD, and other developmental disorder. *J Dev Behav Pediatr, 22*, 409-417.

Rothbart, M. K., Ahadi, S. A., Hershey, K. L., & Fisher, P. (2001). Investigations of Temperament at Three to Seven Years: The Children's Behavior Questionnaire. *Child Development*, *72*(5), 1394–1408. doi:10.1111/1467-8624.00355.

Rothbart, M. K., & Bates, J. E. (1998). Temperament. In N. Eisenberg (Ed.), *Handbook of child psychology* (5th ed., pp. 105-176). New York: Wiley.

Rothbart, M. K., & Derryberry, D. (1981). Development of individual differences in temperament. In M. E. Lamb (Ed.), *Advances in developmental psychology*. Hillsdale, N.J.: Lawrence Erlbaum Associates.

Samyn, V., Roeyers, H., & Bijttebier, P. (2011). Effortful control in typically developing boys and in boys with ADHD or ASD. *Research in Developmental Disabilities*, *32*(2), 483–90. doi:10.1016/j.ridd.2010.12.038.

Sandi, C., & Pinelo-Nava, M. T. (2007). Stress and memory: behavioral effects and neurobiological mechanisms. *Neural Plasticity*, *2007*, 78970. doi:10.1155/2007/78970.

Schopler, E., Reichler, R., & Renner, B. (1988). The Childhood ASD Rating Scale (CARS). Los Angeles: Western Psychological Services.

Shanahan, M., Roberts, J., Hatton, D., Reznick, J., & Goldsmith, H. (2008). Early temperament and negative reactivity in boys with fragile X syndrome. *Journal of Intellectual Disability Research : JIDR*, *52*(10), 842–54. doi:10.1111/j.1365-2788.2008.01074.x.

Simonoff, E., Pickles, A., Charman, T., Chandler, S., Loucas, T., & Baird, G. (2008). Psychiatric disorders in children with ASDs: prevalence, comorbidity, and associated factors in a population-derived sample. *Journal of the American Academy of Child and Adolescent Psychiatry*, *47*(8), 921–9. doi:10.1097/CHI.0b013e318179964f.

Sparrow, S. S., Balla, D. A., Cicchetti, D. V., & Doll, E. A., (1984). *Vineland adaptive behavior scales: Interview edition, survey form manual.* Circle Pines, MN: American Guidance Service.

Sparrow, S. S., Cicchetti, D. V., & Balla, D. A. (2005). Vineland Adaptive Behavior Scales—Second Edition. Circle Pines, MN: AGS.

Sullivan, K., Hatton, D., Hammer, J., Sideris, J., Hooper, S., Ornstein, P., & Bailey, D. B. (2006). ADHD Symptoms in Children With FXS. American Journal of Medical Genetics, *2288*, 2275–2288. doi:10.1002/ajmg.a.

Sullivan, K., Hatton, D. D., Hammer, J., Sideris, J., Hooper, S., Ornstein, P. A, & Bailey, D. B. (2007). Sustained attention and response inhibition in boys with fragile X syndrome: measures of continuous performance. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics : The Official Publication of the International Society of Psychiatric Genetics*, *144B*(4), 517–32. doi:10.1002/ajmg.b.30504.

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), 267–80. doi:10.1007/s10802-012-9671-2.

Turk J. 1998. Fragile X syndrome and attentional deficits. *Journal of Applied Research in Intellectual Disabilities, 11*, 175–191.

Vervoort, L., Wolters, L. H., Hogendoorn, S. M., Prins, P. J., de Haan, E., Boer, F., & Hartman, C. a. (2011). Temperament, attentional processes, and anxiety: diverging links between adolescents with and without anxiety disorders? *Journal of Clinical Child and Adolescent Psychology : The Official Journal for the Society of Clinical Child and Adolescent Psychology, American Psychological Association, Division 53*, *40*(1), 144–55. doi:10.1080/15374416.2011.533412.

Watamura, S. E., Donzella, B., Kertes, D. a, & Gunnar, M. R. (2004). Developmental changes in baseline cortisol activity in early childhood: relations with napping and effortful control. *Developmental Psychobiology, 45*(3), 125–33. doi:10.1002/dev.20026

Weems, C. F., & Costa, N. M. (2005). Developmental differences in the expression of

    childhood anxiety symptoms and fears. *Journal of the American Academy of Child*

    and Adolescent Psychiatry, *44*, 656–663. doi:10.1097/01.chi.0000162583.25829.4b.

Wells, K., Condillac, R., Perry, A., & Factor, D. C. (2009). A comparison of three

    adaptive behavior measures in relation to cognitive level and severity of ASD.

    Journal on Developmental Disabilities, 15(3), 55-63).

Wheeler, A., Raspa, M., Bann, C., Bishop, E., Hessl, D., Sacco, P., & Bailey, D. B.

    (2014). Anxiety, attention problems, hyperactivity, and the Aberrant Behavior

    Checklist in fragile X syndrome. *American Journal of Medical Genetics. Part A*,

    164(1), 141–55. doi:10.1002/ajmg.a.36232

Willcutt, E. G. (2012). The Prevalence of DSM-IV Attention-Deficit/Hyperactivity

    Disorder: A Meta-Analytic Review. *Neurotherapeutics, 9*, 490–499.

    doi:10.1007/s13311-012-0135-8

Wisbeck, J. M., Huffman, L. C., Freund, L., Gunnar, M. R., Davis, E. P., Reiss, A. L.,

    2000. Cortisol and social stressors in children with fragile X: a pilot study. *J Dev*

    Behav Pediatr 21, 278–282.

Woodward, L. J., & Fergusson, D. M. (2001). Life course outcomes of young people with

    anxiety disorders in adolescence. *Journal of the American Academy of Child &*

    *Adolescent Psychiatry, 40(*9), 1086-1093.

Yu, S., Pritchard, M., Kremer, E., Lynch, M., Nancarrow, J.,…Richards, R. J. (1991).

    Fragile X geno- type characterized by an unstable region of DNA. Science, 252,

    1179–1181.