UCLA
UCLA Electronic Theses and Dissertations

Title

Variability in Autism Symptom Severity: Investigating the Role of Diurnal Cortisol and Daily Stress in Children with High-Functioning Autism

Permalink

https://escholarship.org/uc/item/8qq2s7nr

Author

Renno, Patricia Ann

Publication Date

2014

Peer reviewed|Thesis/dissertation

eScholarship.org Powered by the California Digital Library
University of California

UNIVERSITY OF CALIFORNIA

Los Angeles

Variability in Autism Symptom Severity: Investigating the Role of Diurnal Cortisol and Daily Stress in Children with High-Functioning Autism

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Education

by

Patricia Ann Renno

2014

ABSTRACT OF THE DISSERTATION

Variability in Autism Symptom Severity: Investigating the Role of Diurnal Cortisol and Daily Stress in Children with High-Functioning Autism

by

Patricia Ann Renno

Doctor of Philosophy in Education

University of California, Los Angeles, 2014

Professor Jeffrey J. Wood, Chair

The literature indicates increased rates of anxiety disorders in children with high-functioning autism; however, little research has investigated the determinants and consequences of anxiety in autism spectrum disorder (ASD). Wood and Gadow (2010) have proposed a model in which daily stressors contribute to increased mood dysregulation and anxiety, which then exacerbates clinically impairing ASD symptoms. This study investigated the relation between diurnal cortisol levels and measures of stressors, anxiety, and ASD-symptom severity in 43 youth, aged 7-14, with high-functioning autism. Diurnal salivary cortisol samples as well as parent- and clinician- reports of stressors, anxiety and ASD-symptom severity were collected. Parent-report measures were also collected one-year later. Results from multilevel modeling suggest diurnal cortisol samples in youth with ASD follow the same daily pattern established in

the typically developing population and increased daily cortisol is related to greater ASD-related daily stressors ($t = 2.34$, $p < .05$). Additionally, results from path analysis suggest anxiety partially mediates the relation between ASD-related stressors and ASD-symptom severity. Lastly, results from a multilevel analysis using longitudinal data indicate preliminary support for the mediation model. Because ASD is a prevalent, disabling condition it is of considerable importance to determine factors that are associated with greater symptom severity and functional impairment. Findings from this study establish a relation between physiological response and subjective reports of stressors and anxiety in youth with high-functioning autism and suggest that increased stressors contribute to greater anxiety and ASD symptom severity.

The dissertation of Patricia Ann Renno is approved.

Sandra Graham

Connie Kasari

Nim L. Delafield

Jeffrey J. Wood, Committee Chair

University of California, Los Angeles

2014

For my parents.

TABLE OF CONTENTS

Chapter	Page
1. Introduction	1
2. Method	15
3. Analysis	23
4. Results	29
5. Discussion	36
6. Tables	48
7. Figures	62
8. References	64

LIST OF TABLES

Table	Page
Table 1. Participant Demographics	48
Table 2. Correlations among study measures at intake for total sample (N =43), for subsample that did not complete follow-up (n =19), and for subsample that did complete follow-up (n =24)	49
Table 3. Cross-time Simple Correlations of Same Study Measure at Intake and Follow-up	51
Table 4. Means and Standard Deviations for Study Measures at Intake Time point	52
Table 5. Primary Mixed Model with Fixed Effects of Diurnal Cortisol Pattern	53
Table 6. Correlation Matrix of Study Measures for Aim 2 Testing	54
Table 7. Alternative Model 1: Standardized beta coefficients for pathways in alternative model in which stressors mediate the relation between anxiety and ASD-symptom severity	55
Table 8. Alternative Model 2: Standardized beta coefficients for pathways in alternative unsaturated and saturated models in which stressors mediate the relation between ASD-symptom severity and anxiety.	56
Table 9. Means and Standard Deviations for Study Variables at Follow-up Time Point	57
Table 10. Correlation Matrix of Study Variables at 1-year Follow-up Time Point	58
Table 11. Fixed-Effects Regression Models: Testing the Effects of Change in Stressors (SSS) over Time on Change in Anxiety over Time	59
Table 12. Fixed-Effects Regression Models: Testing the Effects of Change in Anxiety (CASI-4R and MASC-P, respectively) over Time on Change in ASD Symptom Severity over Time	60
Table 13. Fixed-Effects Regression Models: Testing the Effects of Change in Stressors over Time on Change in ASD Symptom Severity over Time	61

LIST OF FIGURES

Figure	Page
Figure 1. Saturated Mediation Model using the SSS, PARS, and SRS variables with standardized beta coefficients	62
Figure 2. Saturated Mediation Model using the SSS, CASI-4R, and SRS variables with standardized beta coefficients	63

VITA

2008	B.A., Psychology, History University of California, Los Angeles Los Angeles, CA
2008-2009	Paraprofessional The Willows Community School
2009-2014	UCLA Graduate Student Researcher, Graduate School of Education and Information Sciences
2011	UCLA Graduate Summer Research Mentorship Fellow
2011-2012	Pilot Grant Award Recipient from the UCLA Center for Autism Research and Treatment (CART); P.I.: Lindsey Sterling, Ph.D., Grant co-author: Patricia Renno
2012	UCLA Graduate Summer Research Mentorship Fellow
2012	Robert Levine Scholarship Fellow
2012	UCLA Graduate Mentorship Award Fellow
2013-2014	Teaching Assistant UCLA Psychology Department

SELECTED PUBLICATIONS AND PRESENTATIONS

Renno, P., Sterling, L., & Wood, J. J. (May 2014). Diurnal Cortisol and Daily Stress in Youth with Autism Spectrum Disorder. Poster presentation presented at 2014 International Meeting for Autism Research. Atlanta, GA.

Ehrenreich-May, J., Storch, E. A., Queen, A. H., Hernandez Rodriguez, J., Ghilain, C. C., Alessandri, M., Lewin, A. B., Arnold, E. B., Murphy, T. K., Lin, C. E., Fujii, C., Renno, P., Piacentini, J. C., Laugeson, E., Wood, J. J. (2014). An open trial of cognitive behavioral therapy for anxiety disorders in early adolescents with autism spectrum disorders. *Focus on Autism and Other Developmental Disabilities.* Advance online publication. doi: 10.1177/1088357614533381

Wood, J. J., Ehrenreich-May, J., Alessandri, M., Fujii, C., Renno, P., Laugeson, E., Piacentini, J. C., De Nadai, A. S., Arnold, E., Lewin, A. B., Murphy, T. K., Storch, E. A. (2014). Cognitive behavioral therapy for early adolescents with autism spectrum disorders and clinical anxiety: a randomized controlled trial. *Behavior Therapy.* Advance online publication. doi: 10.1016/j.beth.2014.01.002

Wood, J. J., Fujii, C. Renno, P., & Van Dyke, M. (2014). Impact of cognitive behavioral therapy on observed autism symptom severity during school recess: a preliminary randomized, controlled trial. *Journal of Autism and Developmental Disabilities.* Advance online publication. doi: 10.1007/s10803-014-2097-7

Sterling, L., Renno, P., Storch, E. A., Ehrenreich-May, J., Lewin, A. B., Arnold, E. B., Lin, C. E., & Wood, J. J. (2014). Validity of the RCADS in youth with autism spectrum disorder. *Autism*. Advance online publication. doi: 10.1177/136236361313510066

Renno, P. & Wood, J. J. (2013). Discriminant and convergent validity of the anxiety construct in children with autism spectrum disorders. *Journal of Autism and Developmental Disabilities, 43,* 2135-2146. doi: 10.1007/s10803-013-1767-1

Fujii, C., Renno, P., McLeod, B., Lin, C. E., Decker, K., Zielinski, K., & Wood, J. J. (2013). Intensive cognitive behavioral therapy for anxiety disorders in school-aged children with autism: a preliminary comparison with treatment-as-usual. *School Mental Health, 5*, 25-37.

Sterling, L., Renno, P., Dawson, G., Wood, J. J. (2013, May). The utility of psychophysiological approaches in differentiating anxiety from core autism symptoms. Talk presented at the International Meeting for Autism Research, San Sebastian, Spain.

Renno, P. & Wood, J. J. (2013, May). MTMM analysis of the anxiety construct in children with ASD. Poster presented at the International Meeting for Autism Research, San Sebastian, Spain.

Renno, P., Lin, C. E., & Wood, J. J. (2013) "Separation Anxiety" In F. Volkmar (Ed.), *Encyclopedia of Autism Spectrum Disorders* (pp. 2817-2823). New York, NY, US: Springer Science + Business Media.

Renno, P., Sterling, L., & Wood, J. J. (2013, January). Stress and Anxiety in Children with ASD. Poster Session presented at the University of California Center for Special Education, Disabilities, & Developmental Risk Annual Research Conference, Santa Barbara, CA.

Storch, E. A., Larson, M. J., Ehrenreich-May, J., Arnold, E. B., Jones, A. M**.,** Renno, P., Fujii, C., Lewin, A. B., Mutch, J., Murphy, T.K. & Wood, J. J. (2012). Peer victimization in youth with autism spectrum disorders and co-occuring anxiety: relations with psychopathology and loneliness. *Journal of Developmental and Physical Disabilities, 24*(6), 575-590.

Wood, J.J., Fujii, C., & Renno, P. (2011). Cognitive Behavioral Treatment and related Mental Health Services for Individuals with Higher Functioning Autism. In B. Reichow, P. Doehring, D.V. Cicchetti & F. R. Volkmar (Eds.), *Evidenced-Based Practices and Treatment for Children with Autism* (pp.197-230). New York, NY, US: Springer Science + Business Media.

Wood, J. J., Drahota, A., Sze, K., Van Dyke, M., Decker, K., Fujii, C., Bahng, C., Renno, P., Hwang, W., & Spiker, M. (2009). Brief report: effects of cognitive behavioral therapy on parent reports of autism symptoms in school-age children with high-functioning autism. *Journal of Autism and Developmental Disorders, 39*, 1609-1612.

CHAPTER ONE: INTRODUCTION

Autism spectrum disorder (ASD) is a developmental disorder characterized by deficits in communication, social interaction, and restricted/repetitive behaviors. ASD is estimated to affect 1 in 50 youth in the United States (Blumberg, Bramlett, Kogan, Schieve, & Jones, 2013). Although people with ASD experience impairments in these three core areas, symptom severity is highly variable among people with ASD, even among those with high-functioning autism. In addition to the core symptom areas, people with ASD are also at increased risk for co-occurring psychiatric conditions such as attention deficit hyperactivity disorder (ADHD), depression, and anxiety (Leyfer et al., 2006).

Anxiety and ASD

Clinical anxiety disorders are common in people with ASD and increased anxiety has been related to greater ASD symptom severity. While anxiety disorders affect about 10% of typically developing elementary-aged children, rates are significantly higher in children with ASD. Estimates from the literature suggest that between 30% and 84% of children with ASD are also diagnosed with a co-occurring anxiety disorder (de Bruin, Ferdinand, Meester, de Nijs, & Verheij, 2007; Simonoff et al., 2008; Sukhodolsky et al., 2008). Muris, Steerneman, Merckelbach, Holdrinet, and Meesters (1998) found that in a sample of children with ASD, 84% had at least one concurrent anxiety disorder. Other studies have noted the high prevalence of anxiety in children with high-functioning autism, Asperger's syndrome, and pervasive developmental disorder-not otherwise specified (PDD-NOS), making it one of the most frequent conditions in children with ASD (Green, Gilchrist, Burton, & Cox, 2000; Simonoff et al., 2008; Sukhodolsky et al., 2008). Greater anxiety is also related to greater autism symptom severity.

Several large studies of children with ASD have found strong linkages between higher anxiety and greater severity of repetitive behaviors (e.g., Spiker, Lin, Van Dyke, & Wood, 2012; Sukhodolsky et al., 2008), sensory symptoms (Ben-Sasson et al., 2008), and total ASD symptoms even when controlling for intellectual impairment, social maladjustment, and speech impairment (Kelly, Garnett, Attwood, & Peterson, 2008). Furthermore, parents rate anxiety as one of the greatest problems for their children with ASD (Mills & Wing, 2005).

Although anxiety disorders seem to occur in the ASD population more frequently than in the typically developing population and potentially worsen ASD-related impairments, researchers are still unsure of the relation between anxiety disorders and ASD. Common to all psychiatric symptoms in the ASD population, controversy exists over whether "true" psychiatric syndromes occur in the ASD population or if they are part of the ASD diathesis or predisposition (Gadow et al., 2006; Lecavalier, Gadow, DeVincent, & Edwards, 2009; Muris et al., 1998; Wood & Gadow, 2010). Preliminary research in the literature suggests that psychiatric symptoms in children with ASD may reflect clinical syndromes similar to those that occur in typically developing children. Confirmatory factor analysis conducted by Lecavalier and colleagues (2009) supported the construct validity of ADHD, oppositional defiant disorder, generalized anxiety disorder, and depression in the ASD population, based on parent and teacher measures. Furthermore, a multi-trait multi-method analysis conducted by Renno and Wood (2013) suggested anxiety is a separate construct from ASD, and that anxiety experienced by children with ASD may reflect anxiety syndromes that occur in typically developing children. Neurobiological research has also leant support to the hypothesis that anxiety symptoms in people with ASD actually reflect the mood state of anxiety (Gadow et al., 2010a; Gadow, Roohi, DeVincent, Kirsch, & Hatchwell, 2010b; Kleinhans et al., 2010).

While various studies have reported on the prevalence of anxiety in children with ASD and reported a link between increased anxiety and ASD symptom severity, few studies have examined the relation between anxiety and ASD. Wood and Gadow (2010) suggest three possible roles of anxiety in ASD. The first possibility is that anxiety is a downstream consequence of ASD. In this scenario, ASD-related symptoms could result in humiliating experiences with peers leading to increased anxiety and avoidance of peers. For example, poor perspective taking could result in an adolescent with ASD disclosing an immature interest to peers, which might lead to a traumatic experience resulting in rejection from the peer group. This experience would therefore lead to increased anxiety and avoidance of peers. A second possibility is that anxiety is a mediator or moderator of ASD symptom severity. As a mediator or moderator, children with ASD and anxiety would fare worse on ASD symptoms than children with just ASD and ASD symptoms such as social skill deficits and repetitive behaviors might be exacerbated by anxiety. The third possible relation between anxiety and ASD is that anxiety is a proxy of core ASD symptoms. This would suggest that underlying anxious behaviors are solely manifestations of ASD rather than underlying mood dysregulation. This relation would occur if there were a lack of discrimination between anxiety and ASD symptoms and if anxious behaviors are actually driven by neurological processes related to ASD and not mood dysregulation.

Stress and ASD

Daily stress may play an important role in understanding the relation between anxiety and ASD symptom severity in children with ASD. In the typically developing population, chronic daily stressors are related to the development of anxiety disorders (Allen, Rapee, & Sandberg, 2008). In typically developing children, chronic daily stressors (e.g., conflict with peers,

academic problems) are associated with increased negative affectivity (Schneiders et al., 2006), a general, non-specific risk factor for anxiety disorders (Muris & Ollendick, 2005; Tortella-Feliu, Balle, & Albert, 2010). In addition to contributing to increased negative affect, under conditions of heightened negative mood, daily stressors can also become specific foci of fear and anxiety for children (Weems, 2008). Research indicates that in typically developing children with a general vulnerability to anxiety, high levels of daily stressors predict increases in anxiety symptoms over time (Brozina & Abela, 2005).

Several daily stressors may be particularly relevant in ASD. Many people with ASD encounter daily stressors (Gillott & Standen, 2007) that are specific to having an ASD. ASD symptoms could potentially generate stress for affected individuals when symptom expression is in conflict with social expectations or demands or when ASD symptoms cause punishing reactions from others (e.g., Bellini, 2004; 2006; Gillott & Standen, 2007; Goodwin, Groden, Velicer, & Diller, 2007). Potential sources of stress for children and youth with ASD suggested by Wood and Gadow (2010) include: (1) frequent demands from teachers and others to conform and engage in assigned activities rather than in preferred routines and special interests; (2) trouble understanding the perspectives of others, making daily social interactions unpredictable and at times overwhelming; (3) sensory sensitivities to sound, touch, or light; and (4) teasing and peer rejection related to the social, communicative, and behavioral features of ASD.

Despite the likelihood that people with ASD are particularly vulnerable to daily stress, few studies have examined stress and how it affects people with ASD. Gillot and Standen (2007) conducted a study examining stress and anxiety in 34 adults (14 with intellectual disability and ASD and 20 controls with intellectual disability) and found based on clinician report, that adults with ASD experienced significantly more daily stress than adults in the control group.

Additionally, in the adults with ASD, greater anxiety was related to more stress in the areas of ability to cope with change, anticipation, sensory stimuli, and unpleasant events. This suggests anxiety in people with ASD may further impair their ability to cope with daily stressors. A qualitative study by Muller, Schuler, and Yates (2008) examining adults, aged 18-62, with ASD also found adults with ASD experienced significant stress. People with ASD reported that initiating social interactions and finding and sustaining intimate relationships were major sources of stress and anxiety for themselves. Additionally, in a qualitative study conducted by Stoner, Angell, House, and Block (2007), four sets of parents of children aged 6-8, reported that daily transitions were particularly challenging and stressful for their children and could result in their child engaging in stereotypical or aggressive behaviors. No studies have examined daily stress in children with high-functioning autism and it has been cited as an overlooked problem in these youngsters (Gillot, Furniss, & Walter, 2001; Kim, Szatmari, Bryson, Streiner, & Wilson, 2000; Muris et al., 1998). Further, while one study found a relation between stressors and increased anxiety in adults with ASD (Gillott & Standen, 2007), research has yet to investigate this potential relation in children with ASD.

Model of Psychosocial Predictors of Anxiety and Associated Impairment in ASD

A recent model of the role of stressors in the development of anxiety in children with ASD holds that ASD-specific stressors account for a portion of the heightened anxiety exhibited by affected children (Wood & Gadow, 2010). As noted above, ASD is associated with a number of typical stressful experiences (e.g., peer rejection) that can promote anxiety and avoidant behavior (e.g., avoiding peer interaction). In turn, heightened anxiety has the potential of increasing the severity of certain ASD symptoms, such as speech and language deficits (e.g., dysfluency), maladaptive coping strategies contributing to rigidity and repetitive behaviors, as

well as social disengagement and isolation. Figure 1 illustrates a hypothetical model of the role of stress and anxiety in ASD, which will be tested in the proposed study.

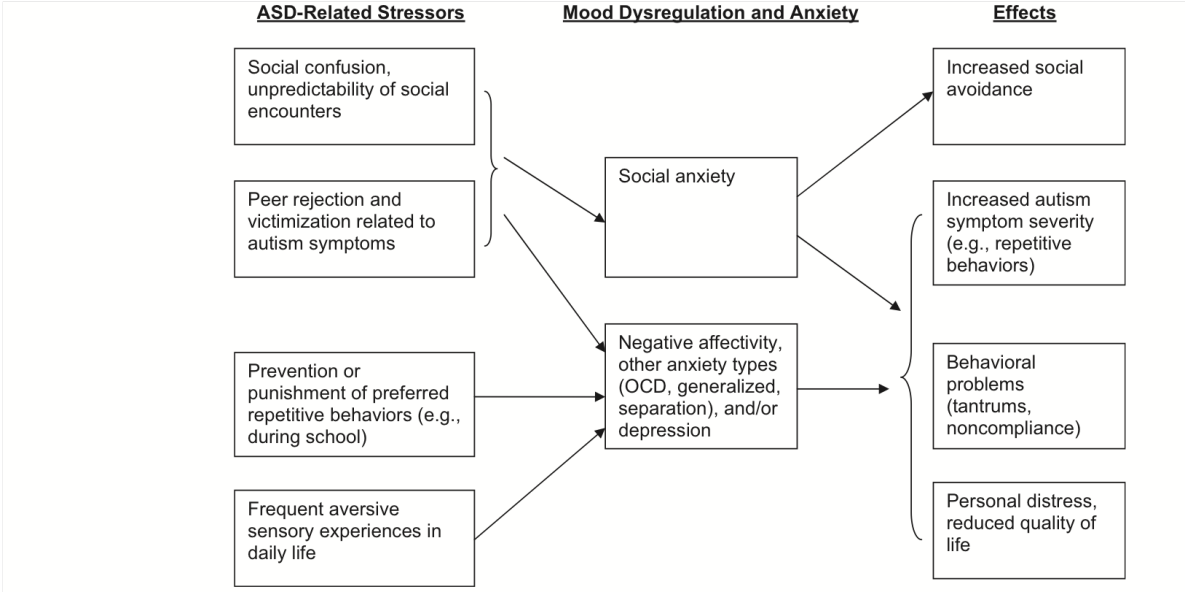


Figure 1. Hypothetical model of clinical anxiety in autism spectrum disorders.

As displayed in the model, ASD stressors related to social encounters may directly contribute to social anxiety and overall negative affectivity. Social interactions are often unpredictable and require a variety of cognitive skills (e.g., perspective taking, turn taking, emotion recognition). These demands may contribute to increased anxiety when children are expected to integrate the complex set of skills needed to interact with peers and adults. Peer rejection and victimization due to ASD symptoms (e.g., immature interests, disruptive repetitive behaviors) likely also contribute to increased social anxiety and negative affectivity. Increased social anxiety as a result of these stressors could contribute to more severe manifestations of ASD symptoms (e.g. social avoidance, repetitive behaviors, behavioral problems). Thirdly, children with ASD are often prevented from engaging in preferred repetitive behaviors, which could be an additional stressor. Repetitive behaviors such as flapping, talking about a preferred topic, or spinning an object are often discouraged at school or at home which could cause a child

significant stress. This stress could lead to increased negative mood, if they are unable to engage in these preferred activities, and contribute to increased generalized anxiety, OCD, and separation anxiety. The result of increased negative mood and anxiety as a result of not being able to engage in these preferred repetitive behaviors could be increased behavioral outbursts, increased ASD symptom severity, and personal distress. Lastly, children with ASD often have sensory sensitivities such as aversions to sounds, textures, lights, and smells which can contribute to anxiety surrounding these sensory experiences. For example, a child with a noise sensitivity may develop anxiety towards hearing the fire alarm at school, resulting in avoidance, distress, and behavioral outbursts at school.

Psychophysiology of Stress

In humans, the hypothalamic-pituitary-adrenal axis (HPA) system is activated in response to stress (Herman & Cullinan, 1997). Cortisol is the main glucocorticoid secreted in response to activation of the HPA system. Typically, cortisol levels follow a diurnal cycle of secretion with highest levels in the morning and a gradual decrease throughout the day. Cortisol levels peak in the morning after a rapid two-fold increase that occurs within 30 minutes of waking up, known as the cortisol awakening response (CAR). Cortisol is also released throughout the day in response to specific stressors. Noninvasive salivary measures of cortisol have been deemed a valid, reliable and noninvasive measure of HPA activity in the typically developing population (Kirschbaum & Hellhammer, 1994).

In typically developing youth, dysregulation of the HPA system has been associated with emotional and behavioral disorders (Gunnar, 2001). Higher levels of cortisol in typically developing children have been associated with internalizing behaviors, such as social anxiety and social withdrawal (Granger, Weisz, & Kauneckis, 1994). Additionally, elevated cortisol levels in

the evening, resulting in a flattened diurnal slope were found in children with posttraumatic stress disorder and a history of trauma (Carrion et al., 2002). Higher levels of cortisol in the evening and greater diurnal cortisol secretion were also found in a longitudinal study of youth, aged 8-16, with depression in comparison to a control group (Goodyer, Park, & Herbert, 2001).

Additionally, it has been suggested that the cortisol awakening response (CAR), a distinct measure of HPA activity, is related to the individual's anticipation for the day (Fries, Dettenborn, & Kirschbaum, 2009). An increased CAR is associated with greater worrying, social stress, and chronic stress (Schlotz, Hellhammer, Schulz, & Stone, 2004; Wust, Federenko, Hellhammer, & Kirschbaum, 2000). Research has shown that people with anxiety disorders (particularly those with panic disorder/agoraphobia) also have an increased CAR, when compared to people with remitted anxiety disorders and healthy control participants (Vreeburg et al., 2010). Conversely, a decreased CAR was found in people with posttraumatic stress disorder (de Kloet et al., 2007; Neylan et al., 2005; Rohleder, Joksimovic, Wolf, & Kirschbaum, 2004; Wessa, Rohleder, Kirschbaum, & Flor, 2006). Further, Fries and colleagues (2009) suggest that while chronic stress may initially be associated with an increased CAR, a blunted CAR may occur for those who are chronically stressed for an extended period of time.

HPA Activity and ASD

The current literature illustrates mixed findings in regard to HPA functioning in children with ASD. Most studies have compared the cortisol levels of children with and without ASD in response to acute stressful laboratory tasks (Richdale & Prior, 1992; Jansen, Gispen-de Wied, van der Gaag, & van Engeland, 2003; Corbett, Mendoza, Wegelin, & Levine, 2006; Corbett, Schupp, & Lanni, 2012; Lopata, Volker, Putnam, Thomeer, & Nida, 2008). Varied findings have emerged, with some studies demonstrating increased cortisol in response to a stressful

laboratory tasks (Corbett et al., 2006; Corbett et al., 2012), and others reporting more complex findings in which cortisol response varied depending on experimental design (Lopata et al., 2008). Corbett and colleagues (2012) examined cortisol responses to children with ASD and typically developing controls to two laboratory social stress paradigms. Results indicated that the paradigms significantly increased cortisol levels for both the ASD and TD groups; however, the ASD group maintained elevated cortisol levels throughout the stressors, whereas cortisol levels decreased in the typically developing controls. Further, researchers found greater within-group variability in the ASD group when compared to the controls. These findings suggest, that children with ASD experience increased and prolonged stress in response to social stressors; however, that there was still a lot of variability within the group.

A few studies have examined diurnal levels of cortisol by collecting saliva samples throughout the day. Some studies have found similar diurnal levels of cortisol in ASD samples as in typically developing samples; however, others have found increased variability and dysregulation in the ASD sample in comparison to a typically developing sample (Corbett, Mendoza, Wegelin, Carmean, & Levine; 2008). Corbett, Constantine, Hendren, Rocke, and Ozonoff's (2009) findings on diurnal cortisol values and parent-reported child stress found significant differences between ASD and typically developing samples. Their findings suggested a flattened slope of cortisol secretion in the ASD sample, with lower levels of cortisol in the morning and higher levels of cortisol in the evening in comparison to the typically developing sample. Brosnan, Turner-Cobb, Munro-Naan, and Jessop (2009) also examined diurnal cortisol levels for males with Asperger's syndrome and typically developing controls, aged 11 to 16. They found a significant main effect for the decline in cortisol throughout the day (7am and 8pm timepoints) and no significant difference between groups, suggesting intact diurnal cortisol

rhythms in children with ASD. While these studies provide some initial understanding of diurnal patterns of cortisol in youth with ASD, their findings are inconsistent with each other warranting further research to clarify patterns of cortisol secretion in youth with ASD. Additionally, many of the studies on HPA activity in children with ASD are limited by small samples sizes and findings are difficult to interpret due to different participant samples among the studies and heterogeneous samples within single studies (e.g., participants with varying cognitive abilities, varying severities of ASD).

Two studies have examined the cortisol awakening response (CAR) in youth with ASD, with incongruent findings. Brosnan and colleagues (2009) also collected salivary cortisol to measure the CAR for 20 males with Asperger's syndrome and 18 typically developing controls, aged 11-16. While the typically developing males showed a significant increase in cortisol levels within the first 30 minutes of waking up, the increase in males with Asperger's syndrome was not significant. Further, 72% of the typically developing males had an increase in cortisol in the morning in contrast to just 60% of males with ASD showing any increase. These findings are in contrast to those by Zinke, Fries, Kliegel, Kirschbaum, and Dettenborn (2010), who found no significant difference in the CAR between typically developing children and children with ASD. Zinke and colleagues collected saliva samples for 15 children with ASD and 25 typically developing controls, aged 6-12. Their results indicated that 80% of children with ASD and 88% of typically developing children showed some increase in cortisol within the first 30 minutes of waking. Additionally, while there was a significant main effect for time, indicating that cortisol levels at time 2 were significantly higher than time 1 for both groups, there was no main effect for group, suggesting no difference in the CAR between children with and without ASD. The conflicting findings of these two studies may be due to differences in sample characteristics and

methodologies; however, complicate general conclusions that can be drawn considering the CAR in ASD samples and indicate further research. Additionally, no research has examined the relation between the CAR and daily stressors and anxiety in youth with ASD.

Establishing the relation between daily stressors and cortisol expression could elucidate the physiology underlying stress and anxiety in ASD. Further, elucidating the relation between cortisol and stressors in youth with ASD has the potential to serve as an objective marker of distress in a population marked by limitations in the ability to report emotional state (Hill, Berthoz, & Frith, 2004). Additionally, despite the documented linkage between ASD and clinical anxiety, studies have not clarified the determinants of this anxiety and related impairment. Very little is known about the development of anxiety in youth with ASD and why it is so prevalent in this population. Studies in typically developing children suggest daily stressors are related to the development of anxiety disorders (Allen, Rapee, & Sandberg, 2008; Craske, 1999; Muris, 2006). This study will examine daily stressors and how they relate to anxiety and increased impairment in youth with ASD. Because stress may lead to heightened anxiety and aggravation of core ASD symptoms, there are significant public health implications regarding the role of stress and anxiety in autism. Furthermore, testing the model proposed above, thereby demonstrating the relation between daily stressors, anxiety and exacerbation of ASD symptoms, provides a rubric for intervention strategies. If an association between daily stressors, anxiety and exacerbation of ASD symptoms is confirmed, treatments might target coping strategies to diminish the impact of such stressors; cortisol may provide an objective marker of the presence and impact of these factors. Evidence suggests that cognitive-behavioral treatment (CBT) is effective when anxiety and related stressors are properly identified, and that this intervention may ultimately lead to improvement in global ASD symptom severity (Wood et al., 2009). Co-occurring anxiety

ultimately could prove to be a target of treatment for some youth with ASD as part of an overall intervention strategy for reducing core symptom severity and impairment.

In addition, the greater the symptom severity and impairment exhibited by children with ASD during middle childhood and adolescence, the poorer the adaptive outcomes in adulthood tend to be (e.g., Cederlund, Hagberg, Billstedt, Gillberg, & Gillberg, 2008; Helt et al., 2008). The majority of youth with ASD continue to have substantial impairment into adulthood (e.g., no close friendships, limited employment). However, youth with fewer ASD symptoms who meet criteria for Asperger's syndrome or pervasive developmental disorder, not otherwise specified (PDD-NOS), have better overall prognoses than children and youth with higher levels of ASD symptoms, who meet full DSM-IV criteria for autistic disorder (Howlin, 2003). Szatmari and colleagues (2009) found that while outcomes for youth with ASD were generally poor, youth with less severe symptoms in adolescence (e.g. Asperger syndrome) had substantially better outcomes in social relationships and daily living skills. Therefore, identifying the causes of factors that are linked with increased ASD symptom severity, such as clinical anxiety, may offer new avenues for prevention and intervention programs aimed at reducing ASD symptom severity and related impairment.

Due to the high percentages of anxiety disorders in children with high-functioning autism, it is important to understand the role of anxiety in these youngsters. Children with high-functioning autism likely experience high amounts of daily stress, which could influence the development of anxiety disorders and increased ASD symptom severity. This study examined the proposed meditational model between daily stressors, anxiety, and ASD symptoms. Measures of ASD symptoms, anxiety, and stress were collected for 43 participants, aged 7-14. In addition

to clinician, parent, and child interviews and questionnaires, this study collected salivary cortisol as a physiological measure of stress.

Summary of Research Aims, Questions, & Hypotheses

1. Aim 1: Establish a relation between salivary cortisol levels and parent-reports of ASD-related daily stressors and stressful life events.

a) Question 1a: Are parent-reports of ASD-related daily stressors and stressful life events associated with the cortisol awakening response (CAR)?

- *Hypothesis 1a:* Greater parent-reported ASD-related daily stressors and stressful life events will be associated with a greater CAR.

b) Question 1b: Are parent-reports of ASD-related daily stressors and stressful life events associated with cortisol's diurnal slope?

- *Hypothesis 1b:* Greater parent-reported ASD-related daily stressors and stressful life events will be associated with a flattened diurnal slope.

c) Question 1c: Are parent-reports of ASD-related daily stressors and stressful life events associated with cortisol levels in the afternoon?

- *Hypothesis 1c:* Greater parent-reported ASD-related daily stressors and stressful life events will be associated with higher cortisol levels in the afternoon.

d) Question 1d: Are parent-reports of ASD-related daily stressors and stressful life events associated with cortisol levels in the evening?

- *Hypothesis 1d:* Greater parent-reported ASD-related daily stressors and stressful life events will be associated with higher cortisol levels in the evening.

2. Aim 2: Test a cross-sectional version of the Wood and Gadow (2010) model examining the relations among stressors, anxiety, and the severity of ASD symptomatology.

a) Question 2: Does anxiety mediate the relation between stressors and ASD symptom severity?

- *Hypothesis 2:* More stressors will be associated with higher anxiety, which in turn will be associated with greater ASD symptom severity.

3. Aim 3: Use 1-year follow-up data to examine the relation between change in stressors, anxiety, and ASD symptom severity

a) Question 3a: Are changes in stressors over time related to changes in anxiety over time?

- *Hypothesis 3a:* Change in stressors over time will predict change in anxiety over time.

b) Question 3b: Are changes in anxiety over time related to changes in ASD symptom severity over time?

- *Hypothesis 3b:* Change in anxiety over time will predict change in ASD symptom severity over time.

c) Question 3c: Are changes in stressors over time related to changes in anxiety over time?

- *Hypothesis 3c:* Change in stressors over time will predict change in ASD symptom severity over time.

CHAPTER 2: METHOD

Participants

The sample included 43 youth (35 males, 8 females, aged 7-14 years (M=10.02 SD=1.88) and their primary parent. The sample was 65% Caucasian, 20% Mixed, 7% African-American, and 7% Latino/Hispanic. Table 1 presents additional descriptive information. All participants had a diagnosis of autism spectrum disorder (ASD). ASD diagnosis was based on (1) the Autism Diagnostic Interview-Revised (ADI-R; Le Couteur, Lord, & Rutter, 2003); (2) the Autism Diagnostic Observation Scale, Second Edition (ADOS-2; Lord et al., 2012); (3) current clinical judgment; and (4) scoring in the clinical range on the Social Responsiveness Scale (Constantino, 2002). Three participants did not meet the threshold for ASD based on the ADOS-2, however, were included in the study based on meeting criteria for an ASD on the ADI-R, SRS, and current clinical judgment. Additionally, participants had an IQ > 70 (M=104.61, SD=15.37), based on the Wechsler Intelligence Scale for Children-IV (WISC-IV; Wechsler, 2003).

Participants were recruited through the Center for Autism Research and Treatment at the University of California, Los Angeles for a study that was examining the psychophysiology of different emotions in youth with ASD. Participants were referred to the study by a medical center-based autism clinic, parent support groups, and regional centers. The UCLA Institutional Review Board approved the study and participants were assented and consented accordingly.

Measures

ASD Measures

Autism Diagnostic Interview – Revised (*ADI-R;* Le Couteur et al., 2003). The ADI-R is a standardized parent interview with well-established psychometric properties in ASD samples

(Lecavalier et al., 2006). It is aimed at obtaining detailed descriptions of child behaviors associated with the diagnostic criteria for ASD. The focus of the interview is on the three main areas affected by ASD: reciprocal social interaction, communication and language, and repetitive, restricted and stereotyped behaviors.

Autism Diagnostic Observation Schedule, Second Edition (*ADOS-2;* Lord et al., 2012). The ADOS-2 is a semi-structured child interview assessing the child's level of social and communication functioning. It has well-established psychometric properties in ASD samples (Lord et al. 2012). The interviewer provides a variety of social presses to elicit certain behaviors relevant to the diagnosis of ASD. The current study used Module 3, which is designed for children and youth who are verbally fluent. In addition to its use as a diagnostic measure, severity scores were used as a measure of autism symptom severity. A bullying variable was also created and used in this study based on the child's response to the clinician's question asking the child if they had been teased or bullied in the past. Depending on the child's response the variable was coded yes or no.

Social Responsiveness Scale (*SRS;* Constantino & Gruber, 2002). The SRS is a standardized, 65-item, 4-point Likert style parent-report form assessing children's ASD-specific characteristics such as social communication deficits and repetitive behaviors. The SRS has demonstrated robust reliability and validity (Constantino & Gruber, 2005). The SRS raw total was used as a measure of autism symptom severity at intake and follow-up time points. The alphas for the SRS total were .91 at intake and .94 at follow-up.

Anxiety Measures

Pediatric Anxiety Rating Scale (*PARS-C/P;* RUPP, 2002). The PARS-C/P is a clinician-rated interview done separately with child and parent that assesses childhood anxiety symptoms

associated with social phobia, separation anxiety disorder, generalized anxiety disorder, specific phobia, and obsessive compulsive disorder. The interview consists of 50 symptom checklist items and 7 clinician-rated severity items. The PARS has favorable psychometric properties in the typically developing population (RUPP, 2002) with preliminary evidence of acceptable psychometric properties in ASD (Storch et al., 2012). The PARS was administered at the intake assessment and the 5-item severity total score (RUPP, 2002; Storch et al., 2012) was used as a measure of anxiety severity.

Multidimensional Anxiety Scale for Children –Parent version (*MASC-P;* March, 1998). The MASC-P is a 39-item, 4-point Likert style measure in which parents rate their child's level of anxiety. The MASC-P is composed of four subscales (physical symptoms, social anxiety symptoms, harm/avoidance, and separation/panic). The MASC has robust psychometric properties (March et al., 1997) in typically developing samples and evidence of acceptable psychometric properties in ASD samples (White, Schry, & Maddox, 2012). The MASC-P was collected at intake and follow-up and the total score was used as a measure of anxiety severity. Alphas on the MASC-P were .83 at intake and .88 at follow-up.

Child & Adolescent Symptom Inventory-4R (*CASI-4R*; Gadow & Sprafkin, 2005). The CASI-4R is a standardized behavior rating scale for DSM-IV psychiatric disorders in children and youth. It is a 105-item measure that assesses the presence of psychiatric symptoms, severity of symptoms, and related impairment. This study used the generalized anxiety disorder, social phobia, separation anxiety disorder, obsessive-compulsive disorder, and specific phobia sections. The CASI-4R has strong psychometric properties in typically developing samples (Sprafkin, Gadow, Salisbury, Schneider, & Loney, 2002) and has been used in ASD samples (e.g., Sukhodolsky et al., 2008). It was collected at intake and follow-up and the 20-item anxiety total

(Sukhodolsky et al., 2008) was used as a measure of anxiety severity. The alpha level for the CASI-4R 20-item anxiety total at intake was .87 and at follow-up was .91. Additionally, this measure provided information on whether the child was receiving any special education services at school.

Stress Schedule Survey (*SSS;* Groden et al., 2001). The SSS is a parent-report measure that was specifically developed for people with developmental disabilities. It measures 8 dimensions of stress: changes, anticipation, unpleasant events, positive events, sensory/personal contact, food-related activity, social/environmental interaction, and ritual-related stress. It has two supplementary subscales, fears and life events, which are not included in the overall total. Psychometric studies suggest it is a reliable and valid measure of stress in people with ASD (Groden et al., 2001). It was collected at intake and follow-up and the total was used as a measure of ASD-related daily stressors. The alpha on the SSS total at intake was .81, and the alpha on the SSS total at follow-up was .95. A parent-report measure of stressors was chosen for this study due to the preliminary nature of the research and general issues with self-report in ASD. Additionally, there is no child/self-report of daily stressors for youth with ASD. Further, using a parent-report measure of daily stressors was consistent with previous studies in the literature that have relied on parent-reports. Limitations to using a parent-report of daily stressors are further considered in the Discussion section.

Coddington Life Event Record (LER; Coddington, 1972). The LER is a 35-item parent-report that records which of 35 stressful events occurred in the last year (e.g. parent death, loss of job of parent, moved to a new house). It has demonstrated test-retest reliability and convergent and predictive validity in typically developing samples (Blount et al., 2008) and was collected at intake and follow-up as a measure of stress.

Salivary Cortisol Collection (Hanranhan, McCarthy, Kleiber, Lutgendorf, & Tsalikian, 2006). Salivary measures of cortisol have been deemed a valid, reliable and noninvasive measure of HPA activity in the typically developing population (Kirschbaum & Hellhammer, 1994). Saliva was collected at four time-points throughout the day for three consecutive days, resulting in a total of 12 samples for each participant. Saliva was collected as soon as the child woke up, 30 minutes after waking, between 3pm and 5pm, and 30 minutes before bedtime (more information provided below). Four aspects of diurnal cortisol secretion were examined: (1) the cortisol awakening response, (2) the slope from T2 to T4, (3) afternoon and evening levels of cortisol and (4) within-person variability between days.

Saliva Collection Diary To ensure collection fidelity, parents were given a Saliva Collection Diary with sections to complete at each collection time point. In the diary, parents recorded the date and time of the collection, as well as, if the child was experiencing any health problems, accidently ate something, or took any medications prior to the collection. They also reported on the child's sleeping patterns and any times during the day when the child got upset.

Procedure

Research staff contacted interested parents that had called the study phone and conducted a phone screen to determine initial eligibility for the study. Study staff screened for child's age, prior ASD diagnosis, IQ, and verbal ability. If the child met basic inclusion criteria based on the phone screen, families were invited to participate in the study. The study was conducted over two appointments. The purpose of the first appointment was to determine whether the child met inclusion/exclusion criteria based on ASD and cognitive assessments. If a child was referred to the study by another research study at the University of California, Los Angeles that had recently administered the ADOS, ADI-R, and WISC and the parent consented to obtaining those score

reports, families did not have to complete the first appointment. If participants met ASD and IQ criteria, they participated in the second appointment. At the second appointment, participants completed a startle challenge (results reported elsewhere), paper/pencil questionnaires, and a childhood anxiety disorder interview (PARS). Parents were also trained in the salivary cortisol collection protocol. They received the necessary supplies to complete the saliva collection at home. Participants received a one hundred-dollar honorarium for participating in the study.

Cortisol Collection Protocol: Cortisol levels were obtained through noninvasive saliva sampling. Salivary measures of cortisol have been deemed a valid, reliable and noninvasive measure of HPA activity in the typically developing population (Kirschbaum & Hellhammer 1994). Salivary methods of cortisol collection have been developed in recent years that minimize discomfort and are easy to complete at home. Saliva was collected at four time-points during the day for three consecutive school days, resulting in a total of 12 samples for each participant. Saliva was collected as soon as the child woke up, 30 minutes after waking, between 3pm and 5pm, and 30 minutes before bedtime.

Parents were trained in collecting home samples of their child's saliva at their lab visit. This study used the current standard procedure for collecting saliva (Hanranhan et al., 2006). At each collection time-point, youth were asked to gently chew on a cotton swab for 1-2 minutes, saturate it with saliva, and then place in a pre-labeled microtube. Parents were also asked to complete the home diary, which recorded the time of each collection and any significant daily events that may have impacted cortisol level (e.g., illness). Participants were asked not to eat, drink, or brush their teeth 30 minutes prior to collecting saliva. Saliva was stored in the participant's refrigerator until the 3-day home collection was complete; then, samples were mailed to the UCLA laboratory and kept in a freezer set at -20 degrees Celsius until assayed.

To ensure fidelity and participant compliance with collection times, this study used electronic time stamps for 20% (n = 7) of the sample (i.e., parents were asked to stamp the diary at each time point with the electronic time stamp, which recorded the time the saliva was actually collected). The participants given the timestamps were randomly selected. Compliance with the cortisol collection schedule was excellent. Ninety-five percent of the collection times were stamped using the electronic time stamp. Additionally, 98% of the saliva samples were taken within 10 minutes of the time specified on the protocol and 85% of the saliva samples were taken within 5 minutes of the time specified on the protocol.

Samples were assayed in a psychophysiological laboratory at UCLA. On the day of analysis, samples were brought to room temperature and centrifuged for 15 minutes at approximately 3,000 RPM to separate the saliva from the swab and any other sediments in the sample. Samples were then assayed using the Salimetrics Salivary Cortisol Enzyme Immunoassay Kits, which have been designed and validated for the measurement of salivary cortisol. Salivary cortisol levels are highly correlated with plasma cortisol levels (r=.90, $p<0.0001$; Kirschbaum & Hellhammer, 1994) and thus an accurate measure of the body's cortisol concentration at the time of collection. All samples from each participant were assayed in the same batch to reduce variability. Additionally, all samples were tested in duplicate to ensure accurate values. The intra-assay coefficients of variation ranged from 4-7% and the interassay coefficients of variation ranged from 3-11%.

Approximately one year from the date of their intake appointment, families were contacted and mailed questionnaires for the 1-year follow-up portion of the study. The 1-year follow-up questionnaires assessed the child's current levels of stressors, anxiety, and ASD symptoms (i.e. the Sress Schedule Survey, Life Events Record, Multidimensional Anxiety Scale

for Children-parent version, Child and Adolescent Symptom Inventory-4R, and Social Responsiveness Scale). Parents completed the questionnaires and returned them by mail to UCLA.

CHAPTER THREE: ANALYSIS

The first aim of this study was to determine if there was a relation between diurnal cortisol levels and stressors in youth with ASD. I predicted the relation between cortisol levels and stressors in youth with ASD would mirror the pattern established in the typical literature: a greater number of daily stressors would be associated with (1) an altered cortisol awakening response (CAR), such that there would be an increased CAR in those who report more daily stressors; (2) a flatter diurnal slope between collection times two and four; (3) higher levels of cortisol in the afternoon due to the cumulative effect of stressors experienced throughout the day and (4) higher levels of cortisol in the evening due to the cumulative effect of stressors experienced throughout the day. The computer software, ReaderFit, fit data points from the cortisol assay to a curve, which modeled the subject's diurnal cycle. Data were checked for outliers by using established ranges of values for children in the literature (Gunnar, 2001; e.g. <0.01 μg/dl or >1.0 μg/dl are outliers). For data points out of the typical range, diaries were reviewed to find a possible explanation for the unusual value (e.g. participant ate food prior to collection).

Seventy-nine percent of participants completed and returned the saliva samples (n = 34). This percentage return rate is similar to the average response rate of 80% that is reported in the literature (Adam, 2009; Rosmalen et al., 2005). One participant did not return the saliva collection diary with their samples and thus their samples were not included in the cortisol analyses. Of the participants who returned their samples, 96.7% of the samples were returned. Participants, on average, returned 11.6 of the requested 12 samples. Five percent of the samples were taken more than 5 minutes off the specified time of collection, but not more than 10

minutes. Additionally, 5% of samples were taken more than 10 minutes off of the specified time. Since the cortisol awakening response is dependent on accurate timing in the morning, T1 samples that were taken more than 5 minutes past the child waking up were not included in analyses (15 T1 samples were not used). For the three other time points throughout the day, samples were not included in analyses if they were collected more than 10 minutes past the specified time (14 T2, T3, and T4 samples were not used).

A series of models were run in SAS to determine the best fit for the subject, day, and time hierarchical structure of the cortisol data. Ultimately, a mixed model with fixed effects using compound symmetry fit the data best. In this structure, the cortisol concentrations were nested within the individual. After the initial model was fit, the overall diurnal pattern of cortisol secretion was analyzed in relation to measures of stress and anxiety. Data was then tested using mixed modeling in SPSS to check for significant predictors of the cortisol awakening response (T2 to T1), slope of cortisol from T2 to T4, afternoon cortisol levels, and evening cortisol levels. In this set of analyses, study questionnaires and interviews (e.g., the SSS, PARS) were the independent variables and cortisol values were the dependent variables. Level-1 (daily) variables were the cortisol concentrations, while Level-2 (individual) variables were the parent- and clinician-report questionnaires. Thirdly, a series of simple correlations were run to test the associations among the variables. Given the structure of the data and the possibility that cortisol levels are predictive of anxiety levels and ASD-symptom severity, I did not want to assume the independent variable and dependent variables. Unlike in the previous analyses in which parent-report measures of stressors, anxiety, and ASD-symptom severity predicted cortisol levels, these analyses were run without any assumption of the independent variable and dependent variable, to assess the strength of the relation between cortisol levels and anxiety and ASD-symptom

severity. A series of simple correlations were run in SPSS in which the averages of each of the cortisol variables taken over the 3 collection days (cortisol awakening response, diurnal slope, afternoon cortisol levels, and evening cortisol levels) were correlated with anxiety symptoms (Multidimensional Anxiety Scale for Children-parent version, Child and Adolescent Symptom Inventory-4R, and Pediatric Anxiety Rating Scale) and ASD-symptom severity (Social Responsiveness Scale and Autism Diagnostic Observation Scale).

The second aim of this study was to examine the relations among ASD-related stressors, anxiety, and severity of ASD symptomatology. This aim tested a cross-sectional version of the Wood and Gadow (2010) model hypothesizing that daily stressors increase anxiety, which in turn exacerbates ASD symptom severity. I predicted that parent reports of greater ASD-related stressors (Stress Schedule Survey) and stressful life events (Life Events Record) would be linked with higher levels of clinical anxiety based on diagnostician and parent reports (Pediatric Anxiety Rating Scale, Multidimensional Anxiety Scale for Children-parent version, Child and Adolescent Symptom Inventory-4R) and greater ASD symptom severity based on diagnostician and parent report (Autism Diagnostic Observation Schedule, Social Responsiveness Scale). Initial analyses examined the simple correlations between (1) stressors (Stress Schedule Survey, Life Event Record) and anxiety (Pediatric Anxiety Rating Scale, Multidimensional Anxiety Scale for Children, Child and Adolescent Symptom Inventory-4R); (2) anxiety (Pediatric Anxiety Rating Scale, Multidimensional Anxiety Scale for Children, Child and Adolescent Symptom Inventory-4R) and ASD symptom severity (Autism Diagnostic Observation Scale, Social Responsiveness Scale); and (3) stressors (Stress Schedule Survey, Life Event Record) and ASD symptom severity (Autism Diagnostic Observation Scale, Social Responsiveness Scale).

Path analysis using regression in EQS 6.2 was then employed to test a model of indirect effects. There was 2% missing data for the Stress Schedule Survey, 5% missing data for the Life Event Record, 5% missing data for the Multidimensional Anxiety Scale for Children-parent version, 21% missing data for the Child and Adolescent Symptom Inventory-4R, 14% missing data for the Pediatric Anxiety Scale for Children, 2% missing data for the Social Responsiveness Scale, and 26% missing data for the Autism Diagnostic Observation Scale. The Pediatric Anxiety Rating Scale was not collected for the first 6 participants and the Child and Adolescent Symptom Inventory-4R were not collected for the first 8 participants. Additionally, 10 participants had previous Autism Diagnostic Observation Scale (ADOS) testing that was used to determine eligibility and therefore another ADOS was not administered for this study. However, because this study used the algorithm score as a current measure of ASD severity, we did not use the ADOS scores for participants with previous testing in this study because they might not accurately reflected the child's current levels of ASD symptom severity. Missing data was imputed using Maximized Likelihood estimation in EQS. Based on the initial findings from the correlation matrix, a series of mediation models were tested. The three main pathways tested were: (1) stressors predicting anxiety; (2) anxiety predicting ASD symptom severity; and (3) stressors predicting ASD symptom severity while controlling for anxiety. Path models were tested using regression with one variable per position. Path analysis in EQS 6.2 allowed for simultaneous modeling of regression pathways in the models. Unsaturated models, in which there are a total of two pathways (from the independent variable to the mediator and from the mediator to the dependent variable) were examined for significant regression coefficients. If the two pathways were significant, a saturated model in which there were three pathways was tested (pathways from the independent variable to the mediator, from the mediator to the dependent

variable and from the independent variable to the dependent variable). Due to the sample size, the intervening variable model was tested using a joint significance test in which support for the model is denoted by significant alpha and beta paths (links between stressors and anxiety and anxiety and ASD severity, respectively; e.g., MacKinnon, Lockwood, Hoffman, West, & Sheets, 2002). Additionally, fit indices were checked and alternative models in which the 3 constructs were placed in different positions within the model were examined.

The third aim of this study was to test a longitudinal version of the Wood & Gadow (2010) model, using 1-year follow-up data. The longitudinal version of the model used parent-report measures of stressors, anxiety, and ASD collected 1-year from the initial visit at UCLA to examine if change in stressors over time predicted change in anxiety levels and ASD symptom severity a year later. A series of mixed models with fixed effects were conducted in SPSS to examine if change in each of these variables predicted change in the other variables. Variables were group mean centered so that at each time point each participant's score for the measure was centered around their individual mean at both time points (Raudenbush & Bryk, 2002).

Of the 43 total participants, 34 participants were eligible to complete the 1-year follow-up questionnaires within the study's timeline. Seventy percent of the eligible participants returned the questionnaires *(n* = 24). A series of analyses were run to determine if the subsample of participants that completed the follow-up time point represented the full gamut of the original sample. First, independent samples t-tests were conducted for study variables to determine if there were any differences between groups at intake for the participants that completed the 1-year follow-up assessment in comparison to those that did not. There were no significant differences between groups on the Stress Schedule Survey total, $t(40) = 1.05$, $p > .05$, the Life Event Record $t(39) = -1.04$, $p > .05$, the Multidimensional Anxiety Scale for Children total $t(39)$

= .60, $p > .05$, the Child and Adolescent Symptom Inventory-4R 20-item anxiety total $t(32) = 1.98$, $p > .05$, the Pediatric Anxiety Rating Scale 5-item total $t(35) = .87$, $p > .05$, the Social Responsiveness Scale total, $t(40) = -.43$, $p > .05$, and the Autism Diagnostic Observation Scale algorithm total, $t(30) = -.77$, $p > .05$, at the intake time point. Secondly, correlations at the intake assessment of study measures were compared among the subsamples of participants that did and did not complete the follow-up. Correlations are reported in Table 2. Overall correlations among study measures at intake were similar for participants that completed follow-up and participants that did not complete follow-up. While most correlations were similar for both groups, differences between groups were found on the Life Event Record. For the subsample with follow-up data, the Life Event Record was significantly correlated with the MASC-P ($r = .61$, $p < .01$, the CASI-4R ($r = .68$, $p < .01$), and the PARS ($r = .44$, $p < .05$), while it was not significantly correlated with these measures for the subsample without follow-up data. Thirdly, paired samples t-tests were run to determine if there were changes over time in the means of study measures. Paired samples t-tests indicated there was not a significant difference in means at intake and follow-up for the Life Event Record, Multidimensional Anxiety Scale for Children-parent version, and Child and Adolescent Symptom Inventory-4R. Scores on the Stress Schedule Survey were significantly higher at the intake time point than the follow-up time point, $t(23) = 2.46$, $p = .02$, and scores on the Social Responsiveness Scale were also significantly higher at the intake time point than at the follow-up time point, $t(23) = 3.10$, $p = .01$. Fourthly, cross-time simple correlations were examined to determine the degree of relation between study measures at intake and follow-up time points. These correlations are displayed in Table 3. Each measure was significantly correlated with itself at intake and follow-up, with the exception of the Life Event Record ($r = .22$, $p > .05$).

CHAPTER FOUR: RESULTS

Aim 1: Establish a relation between salivary cortisol levels and parent-reports of ASD-related daily stressors and stressful life events.

Means and standard deviations for study questionnaires and cortisol variables are presented in Table 4. Data was fit in SAS using a mixed model with fixed effects. Cortisol values were nested within each individual. Analyses were run to characterize the overall pattern of diurnal cortisol in youth with ASD. Table 5 displays results from this primary model. The slope of diurnal cortisol from T1 (waking-up) to T2 (30 minutes after waking-up) was significant (t = 1.97, p = .05). Additionally, the slope of diurnal cortisol from T2 (30 minutes after waking-up) to T3 (afternoon) was significant (t = -2.02, p < .05). The slope of diurnal cortisol from T3 (afternoon) to T4 (evening) was not significant (t = 69, p > .05). Further total stressors on the parent-report of daily stressors (Stress Schedule Survey, SSS) significantly predicted higher overall cortisol levels throughout the day irrespective of time point (t = 2.34, p < .05). Age was not a significant predictor of overall levels of cortisol (t = .26, p > .05). Additionally, the T1 to T2 slope, T2 to T3 slope, and T3 to T4 slope were not significantly related to total stressors on the SSS (t = -1.05, p > .05; t = -.029, p > .05; t = .91, p > .05, respectively).

Mixed Models with Fixed Effects in SPSS

Follow up analyses were run using mixed models with fixed effects in SPSS to determine if stressors, anxiety symptoms, and ASD symptoms simultaneously predicted cortisol values. The first series of models examined the relation between the CAR and these other variables. CAR was significantly related to the SSS fears subscale, with an increased CAR predicting decreased fear-related stressors (B = -.018, t(28) = -2.53, p < .05). Age also significantly

predicted CAR, with an increased CAR associated with older participants (B = .032, t(28) = 2.28, p < .05). Additionally the number of months the child had been in school at the time of the assessment was significantly related to CAR (B = -.019, t(26) = -2.63, p < .05). A decreased CAR was associated with more months in school. Lastly, CAR was marginally related to the SSS unpleasant events subscale, with greater unpleasant events stressors associated with an increased CAR (B = .008, t(30) = 1.80, p < .10).

A series of mixed models with fixed effects were also run with the cortisol diurnal slope from T2 to T4 as the dependent variable. Greater stressors on the SSS sensory subscale significantly predicted a flattened slope (B = -.001, t(33) = -2.21, p < .05). Greater stressors on the SSS social subscale also significantly predicted a flattened slope (B = -.002, t(33) = -2.32, p < .05). Months in school at the time of the assessment significantly predicted the diurnal slope, with fewer months in school predicting a flatter slope (B = .001, t(33) = 2.08, p < .05). Additionally, greater stressors on the SSS unpleasant events subscale marginally predicted a flattened slope (B = -.001, t(33) = -1.93, p < .10). Finally, greater ASD-symptom severity on the Social Responsiveness Scale (SRS) total marginally predicted a flatter slope from T2 to T4 (B = -.0002, t(32) = -1.89, p < .10).

A series of mixed models with fixed effects were also run in SPSS to examine predictors of afternoon and evening cortisol levels. Higher scores on the SSS social subscale significantly predicted greater cortisol levels in the afternoon (B = .009, t(32) = 2.67, p < .05). More stressors related to fears on the SSS fears subscale significantly predicted lower cortisol levels in the afternoon (B = -.005, t(32) = -2.85, p < .01). Greater number of hours of sleep at night also significantly predicted lower afternoon cortisol levels (B = -.020, t(33) = -2.44, p < .05). Greater anxiety on the PARS 5-item total significantly predicted higher afternoon cortisol levels (B =

.003, $t(31) = 2.22$, $p < .05$). Greater ASD-symptom severity on the SRS total was also significantly related to higher afternoon cortisol levels ($B = .001$, $t(31) = 2.79$, $p < .01$). Lastly, fewer months in school was marginally related to higher afternoon cortisol levels ($B = -.004$, $t(33) = -1.91$, $p < .10$).

A fourth series of mixed models with fixed effects was analyzed in SPSS to determine significant predictors of evening cortisol levels. Not receiving school services significantly predicted higher levels of cortisol in the evening ($B = .055$, $t(23) = 2.33$, $p < .05$).

Simple Correlations in SPSS

A series of simple correlations were examined among average cortisol values over the 3 days and parent- and clinician-report measures of anxiety and ASD-symptom severity. The average cortisol awakening response (CAR) was not significantly correlated with the Multidimensional Anxiety Scale for Children-parent version (MASC-P; $r = -.08$, $p > .05$), the Pediatric Anxiety Rating Scale (PARS; $r = .17$, $p > .05$), the Child and Adolescent Symptom Inventory-4R (CASI-4R; $r = -.10$, $p > .05$), the Social Responsiveness Scale (SRS; $r = .16$, $p > .05$), or the Autism Diagnostic Observation Scale severity score (ADOS; $r = -.01$, $p > .05$). The diurnal slope was not significantly correlated with MASC-P ($r = .07$, $p > .05$), the PARS ($r = -.18$, $p > .05$), the CASI-4R ($r = .01$, $p > .05$), the SRS ($r = -.26$, $p > .05$), or the ADOS severity score ($r = .01$, $p > .05$). Afternoon cortisol levels were significantly correlated with the SRS ($r = .41$, $p < .05$) and marginally correlated with the PARS ($r = .30$, $p = .10$). They were not significantly correlated with the MASC-P ($r = -.05$, $p > .05$), the CASI-4R ($r = -.00$, $p > .05$), or the ADOS ($r = .29$, $p > .05$). Evening cortisol levels were not significantly correlated with the MASC-P ($r = -.14$, $p > .05$), the PARS ($r = -.06$, $p > .05$), the CASI ($r = -.20$, $p > .05$), the SRS ($r = -.11$, $p > .05$), or the ADOS ($r = .14$, $p > .05$).

Aim 2: Test a cross-sectional version of the Wood and Gadow (2010) model examining the relations among stressors, anxiety, and the severity of ASD symptomatology.

Means, standard deviations, and intercorrelations for variables related to Aim 2 are presented in Table 6. As shown in Table 6, many of the correlations among stress, anxiety, and ASD symptom severity variables were significant. There was a statistically significant relation between the parent-report of ASD-related stressors (Stress Schedule Survey) and the parent-report measures of anxiety (Multidimensional Anxiety Scale for Children-parent version, $r = .45$, $p < .01$ and Child and Adolescent Symptom Inventory-4R, $r = .54$, $p < .01$) as well as the clinician-report measure of anxiety (Pediatric Anxiety Rating Scale, $r = .52$, $p < .01$). The Life Event Record was only significantly correlated with the Child and Adolescent Symptom Inventory-4R ($r = .37$, $p < .05$). The parent- and clinician-report anxiety measures, Multidimensional Anxiety Scale for Children-parent version, Pediatric Anxiety Rating Scale, and Child and Adolescent Symptom Inventory-4R, were all significantly correlated with the parent-report measure of ASD-symptom severity (Social Responsiveness Scale; $r = .38$, $p < .05$, $r = .54$, $p < .01$, $r = .52$, $p < .01$, respectively). None of the anxiety variables were significantly correlated with the ADOS severity score. Lastly, the parent-report measure of ASD-related stressors (Stress Schedule Survey) was significantly correlated with the parent-report measure of ASD symptom severity (Social Responsiveness Scale; $r = .50$, $p < .01$).

Based on the correlation analysis, a series of models were tested for mediation. If variables were not significantly related in the correlation matrix, they were not used to test for mediation. Three sets of models composed of the variables that were significantly related to each other were tested. The general model being tested was that anxiety mediated the relation between stressors and ASD symptom severity. Since the Life Event Record (LER) and ADOS severity scores were

largely not significantly related to other variables in the correlation matrix, the Stress Schedule Survey (SSS) total was used as a measure of stressors and the Social Responsiveness Scale (SRS) was used as a measure of ASD symptom severity in all three models. For the first set of models the Multidimensional Anxiety Scale for Children-parent version (MASC-P) total was used for the anxiety variable, for the second set of models the Pediatric Anxiety Rating Scale (PARS) was used for the anxiety variable and for third set of models the Child and Adolescent Symptom Inventory-4R (CASI-4R) was used for the anxiety model. To test for mediation, unsaturated and saturated models were examined using path analysis in EQS 6.2 (Bentler, 2006).

Unsaturated models were first tested to see if there was a significant effect of the independent variable (stressors) on the mediator (anxiety) and if there was a significant effect of the mediator on the dependent variable (ASD symptom severity) while controlling for the independent variable (stressors). For the first set of models using the Multidimensional Anxiety Scale for Children-parent version (MASC-P), the MASC-P did not significantly predict the Social Responsiveness Scale (SRS; outcome variable), so that model was not tested further (t = 1.43, $p > .05$). For the second set of models using the Pediatric Anxiety Rating Scale (PARS), the stressors (SSS) did significantly predict anxiety (PARS; $t = 2.31$, $p < .05$) and anxiety (PARS) did significantly predict ASD symptom severity (SRS) while controlling for stressors (SSS; $t = 2.99$, $p < .05$). For the third set of models using the Child and Adolescent Symptom Inventory-4R (CASI-4R), stressors (SSS) did significantly predict anxiety (CASI-4R; $t = 2.36$, $p < .05$) and anxiety (CASI-4R) did significantly predict ASD symptom severity (SRS) while controlling for stressors (SSS; $t = 2.63$, $p < .05$).

Saturated models with the PARS and CASI-4R variables were then examined. The saturated models with the standardized regression coefficients and statistical significance are

shown in Figures 1 and 2. In Figure 1, the pathways between (1) the stressors (SSS) and anxiety (PARS) and (2) anxiety (PARS) and ASD symptom severity (SRS) were statistically significant ($t = 3.78$, $p < .05$ and $t = 2.53$, $p < .05$, respectively); while (3) the path from stressors (SSS) to ASD symptom severity (SRS) was not statistically significant ($t = 1.94$, $p > .05$). Additionally, in Figure 2, the pathways between (1) stressors (SSS) and anxiety (CASI-4R) and (2) anxiety (CASI-4R) and ASD symptom severity (SRS) were statistically significant ($t = 3.74$, $p < .05$ and $t = 2.21$, $p < .05$, respectively); while (3) the path from stressors (SSS) to ASD symptom severity (SSS) was not statistically significant ($t = 1.94$, $p > .05$).

Additionally, goodness of fit indices were checked for the three sets of models. In the saturated models, model fit was perfect and therefore not tested. In the unsaturated models, the Comparative Fit Indices (CFI) ranged from .89 to .95 and the Normed Fit Indices ranged from .88 to .99. The R^2s for the unsaturated models ranged from .22 to .28 and the R^2s for the saturated models ranged from .28 to .37.

Alternative models were also tested to further investigate the relation among stressors, anxiety, and ASD-symptom severity using the same variables (SSS, MASC-P, CASI-4R, and PARS, and SRS). The first set of alternative models examined if stressors mediated the relation between anxiety and ASD-symptom severity. The second set of alternative models examined if stressors mediated the relation between ASD symptom severity and anxiety. Standardized beta coefficients for the alternative models are presented in Tables 7 and 8. Based on analysis of pathways among the variables in the unsaturated and saturated models none of these models met criteria to suggest partial mediation.

Aim 3: Use 1-year follow-up data to examine the relation between change in stressors, anxiety, and ASD symptom severity

To examine the relation between change in stressors, anxiety, and ASD symptom severity over time, data was examined using mixed models with fixed effects in SPSS. Data was organized in long form with each participant having two lines of data to represent the two time points. Table 9 presents a summary of means and standard deviations for study measures at follow-up. Table 10 displays the correlations among study variables at the follow-up time point. A series of regressions were run to examine (1) if change in stressors was related to change in anxiety; (2) if change in anxiety was related to change in ASD symptom severity; and (3) if change in stressors was related to change in ASD symptom severity. Regression coefficients reflect the change in the independent variable (i.e., slope from T1 to T2) on the change in the dependent variable (i.e., slope from T1 to T2).

Tables 11-13 present a summary of the fixed effects regression used to test if changes in daily stressors and anxiety were related to changes in ASD symptom severity over time. Change in stressors significantly predicted change in anxiety on the MASC-P and CASI-4R ($t = 3.70$, $p < .01$, $t = 5.08$, $p < .01$, respectively). Additionally, change in anxiety on the MASC-P and CASI-4R over time predicted change in ASD symptom severity over time ($t = 2.50$, $p < .05$, $t = 3.82$, $p < .01$, respectively). Thirdly, change in daily stressors over time significantly predicted change in ASD symptom severity over time ($t = 3.55$, $p < .01$). Lastly, controlling for change in stressors, change in anxiety on the CASI-4R predicted change in ASD symptom severity over time ($t = 2.00$, $p = .05$).

CHAPTER FIVE: DISCUSSION

This study aimed to investigate the potential relation between diurnal cortisol levels and stressors in youth, aged 7-14 with high-functioning autism. Additionally, it aimed to examine a hypothetical model in which increased ASD-related stressors contribute to increased anxiety, which then exacerbates ASD symptom severity. Findings suggest some relation between diurnal cortisol levels and a parent-report measure of the child's severity of ASD-related daily stressors. Additionally, significant relations among ASD-related stressors, anxiety symptoms, and ASD symptom severity suggest anxiety may partially mediate the relation between stressors and increased ASD symptom severity. Lastly, changes in stressors, anxiety symptoms, and ASD symptom severity were significantly related to each other over the span of one year.

Results from this study suggest the pattern of diurnal cortisol secretion in youth with ASD is similar to the established pattern in typically developing populations. Findings from this study showed there was a significant increase in cortisol within the first 30 minutes of waking up. Additionally, there was a significant decline from the morning to the afternoon. There was no significant difference between afternoon and evening levels of cortisol. These findings are consistent with an intact diurnal cortisol pattern in youth with ASD that follows the established pattern of cortisol secretion in the literature, in which cortisol levels peak in the morning and then gradually decrease to lower levels in the afternoon and evening. These findings are similar to those of Corbett and colleagues (2006) and Brosnan and colleagues (2009) that found a significant decline in cortisol levels from morning to evening in youth with ASD. However, unlike Brosnan and colleagues (2009) who did not find evidence of the CAR in the morning, our sample did show a significant increase in cortisol within 30 minutes of waking up. Our findings

of a significant CAR were similar to those of Zinke et al (2010), who also found a significant CAR in children with ASD. Reasons for these discrepant findings could be that our sample's age range was more aligned with Zinke et al. (2010), whereas Brosnan et al. (2009) had a slightly older age range in for their sample. Additionally, the current study had a larger sample size than Brosnan et al. (2009). While the increase in cortisol in the morning was significant in this study, there was substantial variability between- and within-participants, with some participants showing increases and decreases in cortisol in the morning depending on the day. Further research is needed to better understand possible differences between participants that showed a consistent increase in CAR and those that showed no increase or a decline in the morning.

Additionally, this study suggests that diurnal cortisol may be a useful psychophysiological measure of stress in youth with ASD. Greater ASD-related daily stressors reported by the parent significantly predicted higher levels of diurnal cortisol. Furthermore, findings from mixed model analyses suggest a flatter diurnal slope from peak to trough was related to greater sensory stressors, social stressors, and fewer months since the beginning of the school year. These findings support our hypothesis that a flatter diurnal cortisol slope is associated with greater stressors. They contribute to previous research conducted by Corbett and colleagues (2009) that found that children with ASD have a flatter diurnal slope than typically developing controls. Moreover, the relation between a flatter slope and greater stressors is similar to findings in the literature on typically developing children in which a flatter diurnal cortisol slope has been associated with internalizing disorders (Carrion et al., 2002; Goodyer, Park, & Herbert, 2001). Higher afternoon levels of cortisol were also related to greater social stressors and fewer months in school, supporting our hypothesis that greater stress would be associated with increased afternoon cortisol levels. These findings were similar to Corbett and

colleagues (2009) who found that evening cortisol measurements (which are usually similar to afternoon readings) were related to greater stress. These findings together suggest that higher levels of cortisol in the afternoon and evening may be related to increased stress, due to the cumulative effect of stressors experienced throughout the day.

The only findings that did not support the general pattern that an increased CAR and greater afternoon cortisol levels were not related to increased daily stressors were with the SSS fears subscale. Children's stress reactions towards fears were significantly associated with a diminished CAR and lower afternoon levels. While it is unclear why this pattern of results occurred, since all other associations were in the other direction, it is possible that it is related to the psychometrics of the fear subscale. In the SSS, the fears subscale is not one of the main areas of daily stressors and is not included in the total. Additionally, in psychometric testing conducted by the authors of the measure they found that the fears subscale had a very skewed distribution and seemed to reflect characteristics of the person rather than daily stressors (Groden et al., 2001). Additionally, the subscale is composed of 6 very specific fears and had relatively low internal consistency in this study (alpha = .55). Some of these factors may have contributed to this unexpected finding.

Interestingly, some measures of anxiety and ASD-symptom severity were significantly related to diurnal cortisol. Greater anxiety on the PARS was related to greater cortisol in the afternoon in both the simple correlational and the multilevel model analyses. This is similar to findings in previous research that have shown youth with internalizing disorders, such as PTSD and depression have increased levels of cortisol in the afternoon (Carrion et al., 2002; Goodyer, Park, & Herbert, 2001). In addition, greater ASD symptom severity was also related to a flatter cortisol slope and higher levels of cortisol in the afternoon. This is the first study to report this

association. It's possible that greater dysregulation of this system is related to greater severity of ASD. Lastly, receiving special education services at school was related to lower evening cortisol levels. This suggests that receiving school services may alleviate some of the stress associated with attending school, resulting in lower cortisol levels in the evening.

Further, some of the variables controlled for in cortisol analyses were significantly related to cortisol variables and should be considered in future research. Greater age of the participant was associated with an increased CAR. Because the CAR is generally seen as adaptive and is related to a person's anticipation and preparation for the upcoming demands and challenge of the day (Fries et al., 2009), this finding may suggest that as children with ASD grow older their HPA system may become more regulated. However, depending on the size of the increase, it could also be an indication that older youth are experiencing more daily stressors, resulting in an increased CAR in the morning. An increased CAR in typically developing samples has been associated with greater social stress and chronic stress (Schlotz et al., 2004; Wust et al., 2000). Age was not significantly related to diurnal slope, afternoon/evening cortisol levels, or overall cortisol levels throughout the day. Additionally, fewer hours of sleep at night were related to higher afternoon cortisol levels suggesting sleep is an important factor to consider when measuring cortisol. Children with ASD often have irregular sleep that may be influencing irregular diurnal cycles of cortisol secretion.

Findings from this study also indicate anxiety may partially mediate the relation between ASD-related stressors and greater ASD symptom severity. These results lend support to the hypothesized meditational model proposed by Wood and Gadow (2010), in which increased daily stress as a result of having an ASD contributes to greater anxiety, which then exacerbates core ASD symptoms. These findings are similar to other findings in studies of typically

developing children in which greater daily stress is related to the development of anxiety (Allen, Rapee, & Sandberg, 2008). Additionally, other studies have reported on the relation between higher anxiety and greater ASD severity (Spiker, Lin, Van Dyke, & Wood, 2012; Sukhodolsky et al., 2008). This is the first study to examine a potential meditation model to test the relation between these three variables in an ASD sample. In addition to meeting criteria for a statistical indirect effect (Mackinnon et al., 2002), a series of alternative models was also analyzed to determine if they better explained the relations among these variables. None of the alternative models that were tested met criteria for mediation. Future studies should replicate these findings to provide additional evidence of this preliminary relation.

Thirdly, the longitudinal data provides further evidence for the relation among these variables. Change in stressors over one year predicted change in anxiety levels. Further, change in anxiety levels over one year predicted change in ASD symptom severity. Change in stressors over one year also predicted change in ASD symptom severity. Lastly, while controlling for change in stressors, change in anxiety also predicted change in ASD symptom severity. These findings were robust and suggest strong relations among these variables. There are few studies currently in the literature examining the impact of stressors on anxiety and ASD symptom severity in ASD samples, despite the fact that rates of anxiety in children with ASD are so high. These findings mirror the findings in the typically developing literature in which increased stressors are related to the development of increased anxiety. Additionally, they suggest greater anxiety dynamically contributes to greater ASD symptom severity. These findings are relevant to clinical research and suggest potential areas to intervene. Results from this study suggest that stressors and anxiety symptoms lead to further ASD related impairment. Interventions targeting stressors and anxiety could lead to global improvement in ASD symptoms (Wood et al., 2009).

On a speculative note, the potential pathways through which increased stress might produce increased anxiety and, resultantly, increased ASD severity are considered briefly. For example, a daily stressor that could contribute to increased anxiety and ASD-symptom severity is playing with other children on the schoolyard. The unpredictability of these social encounters could be particularly stressful if a child is not sure if they will be accepted or rejected by their group of friends. It could increase anxiety about maintaining those friendships, and worries about somehow upsetting the friends. Additionally, this could lead to heightened anxiety about approaching other peers to play with for fear that it would upset their other friends. The heightened anxiety could lead to greater repetitive behaviors during play, avoidance of other peers, and isolation if set friends decide not to play with the child for the day. Another hypothetical example to illustrate the potential pathways might be if a child has a sensory sensitivity to smells and has to eat lunch in the school cafeteria. This daily stressor could contribute to anxiety and worry about having to go the cafeteria for lunch. The increased anxiety around this experience could then contribute to increased isolation in the cafeteria, if the child is occupied with worry about the smell. Additionally, the child may engage in more repetitive behaviors, such as spinning small objects, as a coping mechanism to deal with the increased anxiety.

Limitations

There were several limitations to this study. One limitation was that the study relied on parent-report of stressors and did not include a child-report of stressors. It would have been informative to include self-report measures to better understand the child's experience of stress and anxiety. In a study by Bitsika, Sharpley, Sweeney, and McFarlane (2014), researchers did find a significant relation between child-reports of anxiety and cortisol levels and not parent-

report of anxiety and cortisol levels. This suggests that youth with ASD may be able to report levels of anxiety that correspond with their physiological state. Furthermore, this study did not include a teacher-report of the child's daily stressors. Children are at school for a large portion of the day and the teacher's perspective on the child's daily stressors would have been interesting to examine, especially in regard to stressors related to relations with peers and schoolwork. While our daily stressor questionnaire (SSS) did not include many questions related to school-related stressors, making parent-report the most appropriate method, this type of information on school-related stressors would be very interesting to collect from the teacher in the future.

Additionally, there was a large age range in this sample. While wide age ranges are common for clinical samples due to recruitment concerns, it is likely that younger children experience different stressors than older children. In addition, some research suggests that puberty influences HPA activity and cortisol secretion. While this study did statistically control for the effect of age as a proxy of development, the findings of this study are limited by not having an indicator of pubertal status. Studies of HPA activity are increasingly using parent-report questionnaires of child's pubertal status to create homogeneous samples of pre- or post-pubescent samples.

Another limitation of this study was that it focused on high-functioning youth with ASD only. This study only included youth with ASD and an IQ > 70 which characterizes approximately 62% of the ASD population (Center for Disease Control and Prevention, 2012). Due to the preliminary nature of this study only children with IQ > 70 were included to ensure that the children had the cognitive ability to complete a self-report measure regarding anxiety. Additionally, due to the wide range of symptom severity across the ASD spectrum, it seemed that children with IQ > 70 might experience different types of stressors and anxiety than children

with IQ < 70. Thus to create a more homogenous sample, IQ was used. Now that a link between cortisol and parent reported measures of stress have been established it would be informative to translate these findings and investigate the stress and cortisol patterns in children with ASD and IQ < 70, who may have even greater difficulties communicating their emotional states.

This study also did not have a typically developing comparison group. While a comparison group would have been informative, data from typically developing children was not needed for the main aims of this study. In this study, the focus was on the impact of stress in children with ASD specifically. The link between stress and anxiety has already been established for typically developing children. While it would be informative to know if these factors collectively influence the severity of autism-like symptoms in typically developing children, this is a less-informative analysis than identifying sources of increased autism severity in children with ASD, for whom lessened symptomatology is a predictor of better long-term prognosis. It would still be advantageous in future research to have a control group to use as a benchmark for comparison with the cortisol patterns of children with ASD, primarily to check for the expected differences between groups based on previous research (e.g. Corbett et al., 2006).

Future Directions

This study contributes to the growing literature on stress and anxiety in youth with autism spectrum disorder. This was one of the first studies to show that cortisol, a psychophysiological measure of stress, was related to parent-reported measures of stress in youth with autism. Additional research on the relation between psychophysiological measures of stress and anxiety is warranted. Future research incorporating a child self-report of stress would be informative to investigate if child-reported levels of stress also relate to diurnal cortisol levels. While self-reports are often not collected for children with ASD due to communication difficulties and

insight and emotion recognition deficits, it would still be interesting to collect child self-reports of stress to gather the child's perspective on the stress they experience in response to common daily stressors.

Further, while this study used the Stress Schedule Survey (SSS; Groden et al., 2001), a measure that was designed specifically for persons with developmental disabilities, it would be useful for future research to develop a new questionnaire that quantifies stress reactions to daily stressors specifically for youth with high-functioning autism. While the SSS contained many informative areas of stress for youth with high-functioning autism, a new measure that incorporated more questions about stressors related to peer relations, victimization and bullying, and school would be especially interesting to investigate in youth with high-functioning autism. In addition, since parents are not with their child for most of the day while their child is in school, it would be interesting to obtain the teachers' perspectives on youths' reactions to stressors.

Moreover, while this study indicates that daily stressors are related to increased anxiety and autism severity, more research is also needed on other determinants of anxiety in youth with ASD. Compromised executive functioning skills may also contribute to increased anxiety in youth with ASD. In typically developing youth, impairments in executive functioning have been linked to increased risk for anxiety disorders (Eisenberg et al., 2001; Muris, De Jong, & Engelen, 2004) and in a longitudinal study conducted by Feng and colleagues (2008), children with low attentional control were more likely to develop anxiety. Additionally, in a study by Muris and colleagues (2004), attentional control interacted with neuroticism, such that children high in neuroticism were more likely to develop internalizing disorders if they were also low in attentional control. Low attentional control can lead to maladaptive approaches to regulating

negative emotion including ruminative thinking and difficulty shifting focus from distressing stimuli (Derryberry & Reed, 2002). Deficits in executive functioning skills are common in youth with ASD and in particular, impairments in working memory (Pennington & Ozonoff, 1996), cognitive flexibility (Geurts et al., 2004), planning (Geurts et al., 2004), fluency (Goldberg et al., 2005), and effortful control (Corbett et al., 2009) have been frequently reported in individuals with ASD. While some studies have reported generalized impairment in executive functioning skills in children with ASD (Geurts et al., 2004; Goldberg et al., 2005), other studies have demonstrated that although executive functioning deficits are common in ASD, they are not universal to the disorder (Happe et al., 2006; Liss et al., 2001), with significant variability within the population. Thus, it is possible that executive functioning deficits in some individuals with ASD may contribute to the increased rates of anxiety disorders in these youth. However, this hypothesis has yet to be tested in research on individuals with ASD.

Additionally, further research should examine the trajectories of anxiety symptoms in persons with ASD. While clinical experience suggests that some anxiety symptoms may increase in adolescence for youth with ASD as they may encounter more daily stressors and become increasingly aware of their differences, no research has verified this. Additionally it is not known if anxiety symptoms for adolescents with ASD persist into adulthood or diminish on their own. Also, research has not yet clarified other additional consequences to anxiety such as an increased risk for depression in persons with ASD. While this study showed that in the short time span of a year increased stressors and anxiety corresponded to greater ASD symptom severity, it is unclear whether that relation continues into adulthood and what might be additional consequences. Future research should utilize longitudinal designs to determine the patterns of anxiety in children, adolescents, and adults and how they are related to overall ASD impairment and rates

of psychopathology. Further, rates of anxiety are higher in typically developing girls than boys and it would be it would be interesting to examine gender differences in ASD samples.

Recently many funding agencies have advocated the collection of biomarkers, such as cortisol, to further our research on psychological processes (Adam & Kumari, 2009). Future research should continue to incorporate cortisol measurement in addition to other psychophysiological measures in their studies. In typically developing youth, researchers are beginning to consider if diurnal cortisol levels can be used as a predictor and preventative measure of adolescent depression (Adam, Sutton, Doane, & Mineka, 2008). In ASD, much more research is needed to characterize the diurnal patterns of cortisol in youth with ASD, but it's possible that cortisol data in ASD could be used similarly in the future. Additionally, incorporating cortisol measurement in anxiety and depression treatment studies in ASD could serve as an objective measure of treatment response and could potentially provide interesting information on the effects of treatment.

Due to the high rates of anxiety disorders in children with autism spectrum disorder, it is important to understand the psychosocial and psychoneuroendocrine factors that contribute to increased anxiety in these youngsters. Findings from this study add to the development of research aimed at understanding the determinants and consequences of anxiety for youth with ASD. Additionally, this study provides novel information on arousal/regulatory systems underlying stress and anxiety in youth with ASD. Establishing a relation between cortisol patterns and stress in youth with ASD is a preliminary step in this process. Furthermore, findings from the models of indirect effects among daily stressors, anxiety and ASD symptoms may provide guidance for intervention strategies. Stressors and anxiety could prove to be important

targets of treatment for some youth with ASD as part of an overall intervention strategy for reducing core symptom severity and impairment (cf. Storch et al., 2013; Wood et al., 2009).

Table 1

Participant Demographics

Characteristics	Number of Participants (%)
Total N	43 (100%)
Child sex (male)	35 (81%)
Child age	*M*=10.02 (*SD*=1.88)
Child Full Scale IQ	*M*=104.61 (*SD*=15.37; *n*=38)
Child ethnic background	
Caucasian	28 (65%)
Latino / Latina	3 (7%)
African American	3 (7%)
Multiracial	9 (21%)
Parent graduated from college	24 (63%; *n*=38)
Parent married/remarried	29 (76%; *n*=38)
Family Income of greater than $40,000 per year	28 (80%; *n*=35)

Table 2

Correlations among study measures at intake for total sample (N =43), for subsample that did not complete follow-up (n =19), and for subsample that did complete follow-up (n =24)

	Total (*N*=43)	Without Follow-up (*n*=19)	With Follow-up (*n*=24)
Stress Schedule Survey			
LER	-.03	-.23	.22
MASC-P	.45**	.39	.49*
CASI-4R	.54**	.66**	.41
PARS	.52**	.45	.56**
SRS	.50**	.58*	.47*
ADOS algorithm	-.17	-.16	-.10
Life Event Record			
MASC-P	.31	-.00	.61**
CASI-4R	.37*	.08	.68**
PARS	.17	-.08	.44*
SRS	-.05	-.41	.30
ADOS algorithm	-.28	-.33	-.29
Multidimensional Anxiety Scale for Children-parent report			
CASI-4R	.73**	.79**	.70**
PARS	.54**	.53	.54**
SRS	.38*	.22	.51*

ADOS	-.13	-.03	-.17
Child and Adolescent Symptom Inventory-4R			
PARS	.60**	.62*	.60**
SRS	.52**	.43	.60**
ADOS	-.21	-.08	-.18
Pediatric Anxiety Rating Scale			
SRS	.54**	.46	.64**
ADOS	.09	.34	-.07
Social Responsiveness Scale			
ADOS	.14	.44	-.07

Note: SSS Stress Schedule Survey (parent-report), Life Event Record (parent-report), MASC-P Multidimensional Anxiety Scale for Children (parent-report), PARS Pediatric Anxiety Rating Scale (clinician-report), CASI-4R Child Adolescent Symptom Inventory-4R (parent-report), SRS Social Responsiveness Scale (parent-report), ADOS Autism Diagnostic Observation Scale (clinician-report),

** $p < .05$, ** $p < .01$*

Table 3

Cross-time Simple Correlations of Same Study Measure at Intake and Follow-up

Measure	r (n = 24)
Stress Schedule Survey	.68**
Life Event Record	.22
Multidimensional Anxiety Scale for Children-parent report	.81**
Child and Adolescent Symptom Inventory-4R	.58**
Social Responsiveness Scale	.66**

* $p < .05$, ** $p < .01$

Table 4

Means and Standard Deviations for Study Measures at Intake Time point

	Intake	
Measure	Mean (SD)	Range
Stressors		
Stress Schedule Survey total score	120.93 (25.75)	63 – 187
LER total score	4.71 (2.43)	0 - 11
Anxiety		
MASC-P total score	56.32 (14.48)	24 - 85
CASI-4R 20-item score	17.00 (9.57)	3 – 42
PARS-5 item total	15.03 (4.38)	5 - 23
Autism Symptom Severity		
SRS total score	99.60 (22.34)	59 – 143
ADOS severity score	10.53 (3.75)	4 - 21
Cortisol measures		
Cortisol Awakening Response	.08980 (.16944)	-.219 - .621
Diurnal slope from T2 to T4	-.02435 (.01311)	-.053 - .010
Afternoon level	.10364 (.05657)	.038- .354
Evening level	.06592 (.06361)	.012 - .352

Table 5

Primary Mixed Model with Fixed Effects Characterizing Diurnal Pattern of Cortisol Secretion

	Cortisol Concentration	
Variable	Estimate	SE
Fixed Effects		
Intercept	-.188	.175
Slope from T1 to T2	.190*	.097
Slope from T2 to T3	-.326*	.162
Slope from T3 to T4	.105	.154
SSS total	.003*	.001
Age	.001	.006
T1 to T2 Slope*Stress total	-.001	.001
T2 to T3 Slope*Stress total	>-.000	.001
T3 to T4 Slope*Stress total	.001	.001

* $p < .05$

Table 6

Correlation Matrix of Study Measures for Aim 2 Testing

Measure	1.	2.	3.	4.	5.	6.	7.
1. SSS total score	1						
2. LER total score	-.03	1					
3. MASC-P total score	.45**	.31	1				
4. PARS 5-item score	.52**	.17	.54**	1			
5. CASI 20-item score	.54**	.37*	.73**	.60**	1		
6. SRS total score	.50**	-.05	.38*	.54**	.52**	1	
7. ADOS algorithm total	-.17	-.28	-.13	.09	-.21	.14	1
Mean	120.93	4.71	56.32	15.03	17.00	99.60	10.53
SD	25.75	2.43	14.48	4.38	9.57	22.34	3.75

Note: SSS Stress Schedule Survey, LER Life Event Record, MASC-P Multidimensional Anxiety Scale-parent version, PARS Pediatric Anxiety Rating Scale, CASI Child and Adolescent Symptom Inventory-4 Revised, SRS Social Responsiveness Scale, SD Standard deviation

$*p < .05$, $**p < .01$

Table 7

Alternative Model 1: Standardized beta coefficients for pathways in alternative model in which stressors mediate the relation between anxiety and ASD-symptom severity.

	Unsaturated Model		Saturated Model		
	Path A	Path B	Path A	Path B	Path C
Model 1: SSS mediating the relation between the MASC-P and SRS	.19	.46*	.45*	.44*	.18
Model 2: SSS mediating the relation between the PARS and SRS	.32*	.41*	.52*	.30	.39*
Model 3: SSS mediating the relation between the CASI-4R and SRS	.37*	.34*	.54*	.32	.35*

*Note: To suggest partial mediation, in the unsaturated models pathways A and B need to be significant. Additionally, in the saturated models pathways A and B need to be significant while pathway C should not be significant. SSS Stress Schedule Survey (parent-report), MASC-P Multidimensional Anxiety Scale for Children (parent-report), PARS Pediatric Anxiety Rating Scale (clinician-report), CASI-4R Child Adolescent Symptom Inventory-4R (parent-report), SRS Social Responsiveness Scale (parent-report), * $p < .05$*

Table 8

Alternative Model 2: Standardized beta coefficients for pathways in alternative unsaturated and saturated models in which stressors mediate the relation between ASD-symptom severity and anxiety.

	Unsaturated Model		Saturated Model		
	Path A	Path B	Path A	Path B	Path C
Model 1: SSS mediating the relation between the SRS and MASC-P	.21	.36*	.50*	.35*	.35
Model 2: SSS mediating the relation between the SRS and the PARS	.41*	.37*	.50*	.34*	.38*
Model 3: SSS mediating the relation between the SRS and the CASI-4R	.37*	.40*	.50*	.37*	.35*

*Note: To suggest partial mediation, in the unsaturated models pathways A and B need to be significant. Additionally, in the saturated models pathways A and B need to be significant while pathway C should not be significant. SSS Stress Schedule Survey (parent-report), MASC-P Multidimensional Anxiety Scale for Children (parent-report), PARS Pediatric Anxiety Rating Scale (clinician-report), CASI-4R Child Adolescent Symptom Inventory-4R (parent-report), SRS Social Responsiveness Scale (parent-report), * $p < .05$*

Table 9

Means and Standard Deviations for Study Measures at Follow-up Time point

	Follow-up
Measure	Mean (SD)
Stress Schedule Survey total score	111.79 (34.66)
LER total score	3.83 (2.20)
MASC-P total score	57.96 (16.16)
CASI-4R 20-item score	14.67 (10.29)
SRS total score	85.66 (26.19)

Table 10

Correlation matrix of study measures at 1-year Follow-up Time Point

	1.	2.	3.	4.	5.
1. SSS total score	1				
2. LER total score	.30	1			
3. MASC-P total score	.46*	.27	1		
4. CASI 20-item score	.49*	.00	.78**	1	
5. SRS total score	.66**	.14	.56**	.67**	1
Mean	111.79	3.83	57.96	14.67	85.67
SD	34.66	2.20	16.16	10.26	26.19

Note: SSS Stress Schedule Survey, LER Life Event Record, MASC-P Multidimensional Anxiety Scale-parent version, CASI Child and Adolescent Symptom Inventory-4 Revised, SRS Social Responsiveness Scale, SD Standard deviation

$*p < .05$, $**p < .01$

Table 11

Fixed-Effects Regression Models: Testing the Effects of Change in Stressors (SSS) over Time on Change in Anxiety over Time

Variable	B	SE B	T
CASI-4R 20 item anxiety score	.22	.04	5.08**
MASC-P total score	.18	.05	3.70**

* $p < .05$, ** $p < .01$

Table 12

Fixed-Effects Regression Models: Testing the Effects of Change in Anxiety (CASI-4R and MASC-P, respectively) over Time on Change in ASD Symptom Severity over Time

Variable	B	SE B	T
ASD Symptom Severity (Independent variable: CASI-4R)	1.15	.30	3.82**
ASD Symptom Severity (Independent variable: MASC-P)	.72	.29	2.5*

* $p < .05$, ** $p < .01$

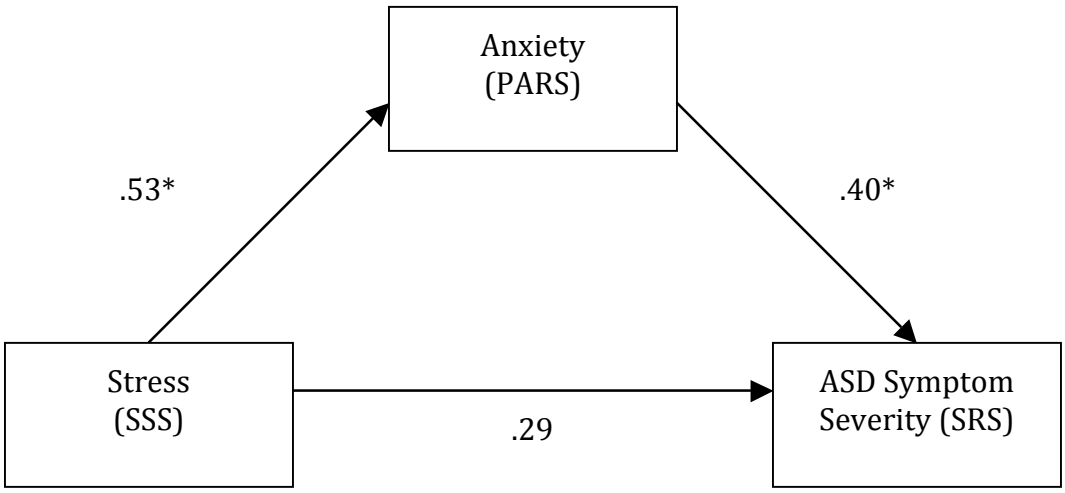
Table 13

Fixed-Effects Regression Models: Testing the Effects of Change in Stressors over Time on Change in ASD Symptom Severity over Time

Variable	B	SE B	T
Stress Schedule Survey Total	.37	.10	3.55**

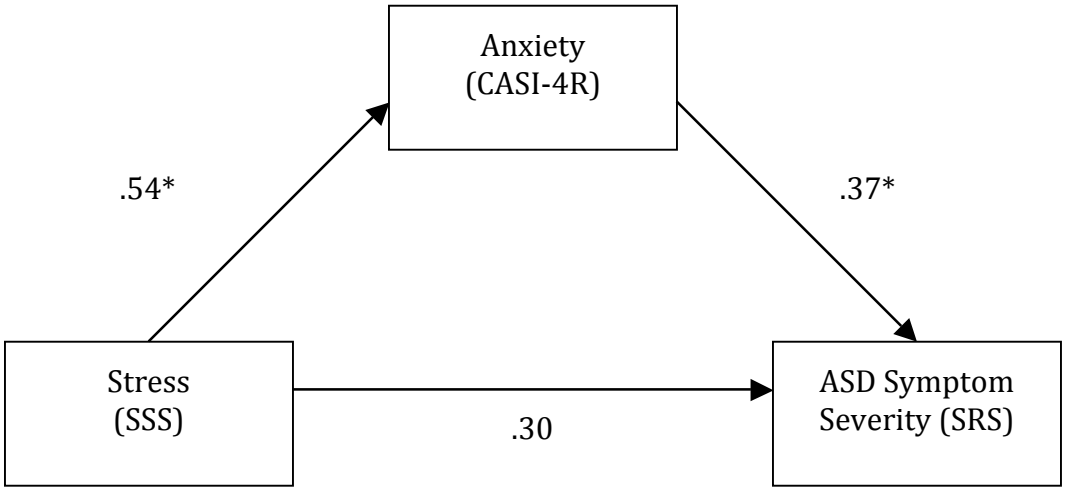
* $p < .05$, ** $p < .01$

Figure 1. Saturated Mediation Model using the SSS, PARS, and SRS variables with standardized beta coefficients



Note: SSS Stress Schedule Survey, PARS Pediatric Anxiety Rating Scale, SRS Social Responsiveness Scale * $p < .05$

Figure 2. Saturated Mediation Model using the SSS, CASI-4R, and SRS variables with standardized beta coefficients



Note: SSS Stress Schedule Survey, CASI-4R Child and Adolescent Symptom Inventory-4R, SRS Social Responsiveness Scale, * $p < .05$

References

Adam, E. K. (2006). Transactions among adolescent trait and state emotion and diurnal and momentary cortisol activity in naturalistic settings. *Psychoneuroendocrinology, 31*, 664-679. doi:10.1016/j.psyneuen.2006.01.010

Adam, E. K., & Kumari, M. (2009). Assessing salivary cortisol in large-scale, epidemiological research. *Psychoneuroendocrinology, 34*, 1423-1436. doi:10.1016/j.psyneuen.2009.06.011

Adam, E. K., Sutton, J. M., Doane, L. D., & Mineka, S. (2008). Incorporating hypothalamic-pituitary-adrenal axis measures into preventive interventions for adolescent depression: Are we there yet? *Development and Psychopathology, 20*, 975-1001. doi:10.1017/S0954579408000461

Allen, J. L., Rapee, R. M., & Sandberg, S. (2008). Severe life events and chronic adversities as antecedents to anxiety in children: a matched control study. *Journal of Abnormal Child Psychology, 36*, 1047-1056. doi:10.1007/s10802-008-9240-x

Bellini, S. (2004). Social skill deficits and anxiety in high-functioning adolescents with autism spectrum disorders. *Focus on Autism & Other Developmental Disabilities, 19,* 78-86. doi:10.1177/10883576040190020201

Bellini, S. (2006). The development of social anxiety in adolescents with autism spectrum disorders. *Focus on Autism & Other Developmental Disabilities, 21*(3), 138-145. doi:10.1177/10883576060210030201

Ben-Sasson, A., Cermak, S.A., Orsmond, G.I., Tager-Flusberg, H., Kadlec, M.B., & Carter, A.S. (2008). Sensory clusters of toddlers with autism spectrum disorders: differences in

affective symptoms. *Journal of Child Psychology and Psychiatry, 49*, 817-825. doi:10.1111/j.1469-7610.2008.01899.x

Bitsika, V., Sharpley, C. F., Sweeney, J. A., & McFarlane, J. R. (2014). HPA and SAM axis responses as correlated of self- vs parental ratings of anxiety in boys with autistic disorder. *Physiology & Behavior, 127*, 1-7. doi:10.1016/j.physbeh.2013.12.011

Blount, R. L., Simons, L. E., Devine, K. A., Jaaniste, T., Cohen, L. L., Chambers, C. T., & Hayutin, L. G. (2008). Evidence-based assessment of coping and stress in pediatric psychology. *Journal of Pediatric Psychology, 33*(9), 1021-1045. doi:10.1093/jpepsy/jsm071

Blumberg, S. J., Bramlett, M. D., Kogan, M. D., Schieve, L. A., & Jones, J. R. (2013). Changes in prevalence of parent-reported autism spectrum disorder in school-aged U.S. children: 2007 to 2011-2012. *National Health Statistics Report, 65*, 1-7.

Brosnan, M., Turner-Cobb, J., Munro-Naan, Z., & Jessop, D. (2009). Absence of a normal cortisol awakening response (CAR) in adolescent males with Asperger syndrome (AS). *Psychoneuroendocrinology*, *34*(7), 1095-1100. doi:10.1016/j.psyneuen.2009.02.011

Brozina, K. & Abela, J. R. (2005). Behavioural inhibition, anxious symptoms, and depressive symptoms: A short-term prospective examination of a diathesis-stress model. *Behaviour Research and Therapy, 44*, 1337-1346. doi: 10.1016/j.brat.2005.09.010

Carrion, V. G., Weems, C. F., Ray, R. D., Glasser, B., Hessl, D., & Reiss, A. L. (2002). Diurnal salivary cortisol in pediatric posttraumatic stress disorder. *Biological Psychiatry, 51,* 575–582. doi:10.1016/S0006-3223(01)01310-5

Cederlund, M., Hagberg, B., Billstedt, E., Gillberg, I., & Gillberg, C. (2008). Asperger syndrome and autism: a comparative longitudinal follow-up study more than 5 years after original

diagnosis. *Journal of Autism and Developmental Disorders, 38*, 72–85. doi:10.1007/s10803-007-0364-6

Centers for Disease Control and Prevention (2012). Prevalence of autism spectrum disorders—autism and developmental disabilities monitoring network, 14 sites, United States, 2008. *Morbidity and Mortality Weekly Report. Surveillance Summaries, 61*(3), 1-19.

Chang, Y., Quan, J., & Wood, J. J. (2012). Effects of anxiety disorder severity on social functioning in children with autism spectrum disorders. *Journal of Developmental and Physical Disabilities, 24,* 235-245. doi:10.1007/s10882-012-9268-2

Coddington, R. (1972). The significance of life events as etiologic factors in the disease of children: II. A study of a normal population. *Journal of Psychosomatic Research, 16,* 205–213. doi:10.1016/0022-3999(72)90045-1

Cohen, I. L., Schmidt-Lackner, S., Romanczyk, R., & Sudhalter, V. (2003). The PDD Behavior Inventory: a rating scale for assessing response to intervention in children with PDD. *Journal of Autism and Developmental Disorders, 33*, 31-45. doi:10.1023/A:1022226403878

Cohen, I. L. & Sudhalter, V. (2005). The PDD Behavior Inventory. Lutz, FL: Psychological Assessment Resources, Inc.

Constantino, J. N. (2002). The Social Responsiveness Scale. Los Angeles: Western Psychological Services.

Constantino, J. N., & Gruber, C. P. (2005). *The social responsiveness scale (SRS) manual.* Los Angeles: Western Psychological Services.

Corbett, B. A., Constantine, L. J., Hendren, R., Rocke, D., & Ozonoff, S. (2009). Examining

executive functioning in children with autism spectrum disorder, attention deficit hyperactivity disorder and typical development. *Psychiatry Research, 166*, 210-222. doi:10.1016/j.psychres.2008.02.005

Corbett, B. A., Mendoza, S., Wegelin, J. A., Carmean, V., & Levine, S. (2008). Variable cortisol circadian rhythms in children with autism and anticipatory stress. *Journal of Psychiatry & Neuroscience, 33*(3), 227-234.

Corbett, B. A., Mendoza, S., Wegelin, J. A., & Levine, S. (2006). Cortisol circadian rhythms and response to stress in children with autism. *Psychoneuroendocrinology, 31*, 59-68. doi:10.1016/j.psyneuen.2005.05.011

Corbett, B. A., Schupp, C. W., & Lanni, K. E. (2012). Comparing biobehavioral profiles across two social stress paradigms in children with and without autism spectrum disorders. *Molecular Autism*, *3*(13), 1-10. doi:10.1186/2040-2392-3-13

Craske, M. G. (1999). *Anxiety disorders: Psychological approaches to theory and treatment.* Boulder. CO: Westview Press.

de Bruin, E. I., Ferdinand, R. F., Meester, S., de Nijs, P. F., & Verheij, F. (2007). High rates of psychiatric co-morbidity in PDD-NOS. *Journal of Autism and Developmental Disorders, 37*, 877-886. doi:10.1007/s10803-006-0215-x

de Kloet, C. S., Vermetten, E., Heijnen, C. J., Geuze, E., Lentjes, E. G., & Westenberg, H. G. (2007). Enhanced cortisol suppression in response to dexamethasone administration in traumatized veterans with and without posttraumatic stress disorder. *Psychoneuroendocrinology, 32,* 215–226. doi:10.1016/j.psyneuen.2006.12.009

Derryberry, D., & Reed, M. (2002). Anxiety-related attentional biases and their regulation by attentional control. *Journal of Abnormal Psychology, 111*, 225–236. doi:10.1037/0021-

843X.111.2.225

Eisenberg, N., Cumberland, A., Spinrad, T. L., Fabes, R. A., Shepard, S. A., Reiser, M., et al. (2001). The relations of regulation and emotionality to children's externalizing and internalizing problem behavior. *Child Development, 72*(4), 1112-1134. doi:10.1111/1467-8624.00337

EQS 6.2 for windows [computer program]. (2006). Version 6.2. Multivariate Software, Encino

Feng, X. F., Shaw, D. S., & Silk, J. S. (2008). Developmental trajectories of anxiety symptoms among boys across early and middle childhood. *Journal of Abnormal Psychology, 117*(1), 32-47. doi:10.1037/0021-843X.117.1.32

Fries, E., Dettenborn, L., Kirschbaum, C., (2009). The cortisol awakening response (CAR): facts and future directions. *International Journal of Psychophysiology, 72,* 67-73. doi:10.1016/j.ijpsycho.2008.03.014

Gadow, K. D., DeVincent, C. J., Pisarevskaya, V., Olvet, D. M., Xu, W., Mendell, N., … Hatchwell, E. (2010a). Parent-child DRD4 genotype as a potential biomarker for oppositional, anxiety, and repetitive behaviors in children with autism spectrum disorder. *Progress in Neuropsychopharmacology & Biological Psychiatry, 34*(7), 1208-14. doi:10.1016/j.pnpbp.2010.06.019

Gadow, K. D., DeVincent, C. J., & Pomeroy, J. (2006). ADHD symptom subtypes in children with pervasive developmental disorder. *Journal of Developmental Disorders, 36,* 271-283. doi:10.1007/s10803-005-0060-3

Gadow, K. D., Roohi, J., DeVincent, C. J., Kirsch, S., & Hatchwell, E. (2010b). Glutamate transporter gene (SLC1A1) single nucleotide polymorphism (rs301430) and repetitive

behaviors and anxiety in children with autism spectrum disorder. *Journal of Autism and Developmental Disorders, 40*(9), 1139-1145. doi:10.1007/s10803-010-0961-7

Gadow, K. D., & Sprafkin, J. (2005). *Child and Adolescent Symptom Inventory-4R*. Stony Brook, NY: Checkmate Plus.

Geurts, H. M., Verte, S., Oosterlaan, J., Roeyers, H., & Sergeant, J. A. (2004). How specific are executive functioning deficits in attention deficit hyperactivity disorder and autism? *Journal of Child Psychology and Psychiatry, 45*(4), 836–854. doi:10.1111/j.1469-7610.2004.00276.x

Gillott, A., Furniss, F., & Walter, A. (2001). Anxiety in high-functioning children with autism. *Autism, 5*(3), 277-286. doi:10.1177/1362361301005003005

Gillott, A., & Standen, P. J. (2007). Levels of anxiety and sources of stress in adults with autism. *Journal of Intellectual Disabilities, 11*(4), 359-370. doi:10.1177/1744629507083585

Goldberg, M. C., Mostofsky, S. H., Cutting, L. E., Mahone, E. M., Astor, B. C., Denckla, M. B. & Landa, R. J. (2005). Subtle executive impairment in children with autism and children with ADHD. *Journal of Autism and Developmental Disorders, 35*(3), 279-293. doi:10.1007/s10803-005-3291-4

Goodwin, M. S., Groden, J., Velicer, W. F., & Diller, A. (2007). Brief report: Validating the stress survey schedule for persons with autism and other developmental disabilities. *Focus on Autism and Other Developmental Disabilities, 22*(3), 183-189. doi:10.1177/10883576070220030501

Goodyer, I. M., Park, R. J., & Herbert, J. (2001). Psychosocial and endocrine features of chronic first-episode major depression in 8–16 year olds. *Biological Psychiatry*, *50*(5), 351-357. doi:10.1016/S0006-3223(01)01120-9

Granger, D. A., Weisz, J. R., & Kauneckis, D. (1994). Neuroendocrine reactivity, internalizing behavior problems and control-related cognition in clinic-referred children and adolescents. *Journal of Abnormal Psychology, 103*(2), 267-276. doi:10.1037/0021-843X.103.2.267

Green, J., Gilchrist, A., Burton, D., & Cox, A. (2000). Social and psychiatric functioning in adolescents with Asperger syndrome compared with conduct disorder. *Journal of Autism and Developmental Disorders, 30,* 279-293.

Green, S. (2011). *SOR and anxiety in youth with autism: commonalities in neurobiology and treatment response* [Grant F31 proposal to the National Institute of Mental Health].

Groden, J., Diller, A., Bausman, M., Velicer, W., Norman, G., & Cautela, J. (2001). The development of a Stress Survey Schedule for persons with autism and other developmental disabilities. *Journal of Autism and Developmental Disorders, 31*, 207–217. doi:10.1023/A:1010755300436

Gunnar, M. R. (2001) The role of glucocorticoids in anxiety disorders: A critical analysis. In: M. W. Vasey & M. R. Dadds (Eds.), *The developmental psychopathology of anxiety* (pp. 143-159). New York, NY: Oxford University Press.

Hanranhan, K., McCarthy, A.M., Kleiber, C., Lutgendorf, S., & Tsalikian, E. (2006). Strategies for salivary cortisol collection and analysis in research with children. *Applied Nursing Research, 19*, 95–101. doi:10.1016/j.apnr.2006.02.001

Happe, F., Booth, R., Charlton, R., & Hughes, C., (2006). Executive function deficits in autism spectrum disorders and attention-deficit/ hyperactivity disorder: examining profiles across domains and ages. *Brain and Cognition, 61*(1), 25–39. doi: 10.1016/j.bandc.2006.03.004

Helt, M., Kelley, E., Kinsbourne, M., Pandey, J., Boorstein, H., Herbert, M., & Fein, D. (2008).

Can children with autism recover? If so, how? *Neuropsychology Review, 18,* 339-366. doi:10.1007/s11065-008-9075-9

Herman, J. P., & Cullinan, W. E., (1997). Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocorticol axis. *Trends in Neuroscience, 20*(2), 78-84. doi:10.1016/S0166-2236(96)10069-2

Hill, E., Berthoz, S., & Frith, U. (2004). Brief report: cognitive processing of own emotions in individuals with autistic spectrum disorder and in their relatives. *Journal of Autism and Developmental Disorders, 34,* 229-235. doi:10.1023/B:JADD.0000022613.41399.14

Howlin, P. (2003). Outcome in high-functioning adults with autism with and without early language delays: Implications for the differentiation between autism and Asperger syndrome. *Journal of Autism and Developmental Disorders, 33*, 3–13. doi:10.1023/A:1022270118899

Jansen, L. M., Gispen-de Wied, C. C., van der Gaag, R. J., & van Engeland, H. (2003). Differentiation between autism and multiple complex developmental disorder in response to psychosocial stress. *Neuropsychopharmacology, 28*(3), 582–590. doi:10.1038/sj.npp.1300046

Kelly, A. B., Garnett, M. S., Attwood, T., & Peterson, C. (2008). Autism spectrum symptomatology in children: The impact of family and peer relationships. *Journal of Abnormal Child Psychology, 36*(7), 1069-1081. doi:10.1007/s10802-008-9234-8

Kim, J. A., Szatmari, P., Bryson, S. E., Streiner, D. L. & Wilson, F. J. (2000). The prevalence of anxiety and mood problems among children with autism and asperger syndrome. *Autism, 4*(2), 117–32. doi:10.1177/1362361300004002002

Kirschbaum, C., & Hellhammer, D. H., (1994). Salivary cortisol in psychoneuroendocrine

research: recent developments and applications. *Psychoneuroendocrinology, 19*(4), 313-333. doi:10.1016/0306-4530(94)90013-2

Kleinhans, N. M., Richards, T., Weaver, K., Johnson, l. C., Greenson, J., Dawson, G., & Aylward, E. (2010). Association between amygdala response to emotional faces and social anxiety in autism spectrum disorders. *Neuropsychologia, 48*(12), 3665-70. doi:10.1016/j.neuropsychologia.2010.07.022

Le Couteur, A., Lord, C., & Rutter, M. (2003). *The autism diagnostic interview-revised*. Los Angeles: Western Psychological Services.

Lecavalier, L., Aman, M., McDougle, C. J., McCracken, J., Vitiello, B., Tierney, E., … Kau, A. S. (2006). Validity of the autism diagnostic interview—revised. *American Journal on Mental Retardation*, *111*, 199–215. doi:10.1352/0895-8017(2006)111[199:VOTADI]2.0.CO;2

Lecavalier, L., Gadow, K. D., DeVincent, C. J., & Edwards, M. C. (2009). Validation of DSM-IV model of psychiatric syndromes in children with autism spectrum disorder. *Journal of Autism and Developmental Disorders, 39*, 278-289. doi:10.1007/s10803-008-0622-2

Leyfer, O. T., Folstein, S. E., Bacalman, S., Davis, N. O., Dinh, E., Morgan, J., ... & Lainhart, J. E. (2006). Comorbid psychiatric disorders in children with autism: interview development and rates of disorders. *Journal of Autism & Developmental Disorders, 36*, 849-861. doi:10.1007/s10803-006-0123-0

Liss, M., Fein, D., Allen, D., Dunn, M., Feinstien, C., Morris, R., ... & Rapin, I. (2001). Executive functioning in high-functioning children with autism. *Journal of Psychology and Psychiatry, 42*(2), 261-270. doi: 10.1111/1469-7610.00717

Lopata, C. L., Volker, M. A., Putnam, S. K., Thomeer, M. L., & Nida, R. E. (2008). Effect of

social familiarity on salivary cortisol and self-reports of social anxiety and stress in children with high functioning autism spectrum disorders. *Journal of Autism and Developmental Disorders, 38,* 1866-1877. doi:10.1007/s10803-008-0575-5

Lord, C., Risi, S., Lambrecht, L., Cook, E. H., Leventhal, B. L., DiLavore, P. C., Pickles, A., & Rutter, M. (2000). The autism diagnostic observation schedule—generic: A standard measure of social and communication deficits associated with the spectrum of autism. *Journal of Autism and Developmental Disorders, 30*(3), 205–223. doi:10.1023/A:1005592401947

Lord, C., Rutter, M., DiLavore, P. C., Risi, S., Gothman, K., Bishop, S. L. (2012). *Autism Diagnostic Observation Schedule, Second Edition (ADOS-2).* Torrance, CA: Western Psychological Services.

MacKinnon, D. P., Lockwood, C. M., Hoffman, J. M., West, S. G., & Sheets, V. (2002). A comparison of methods to test mediation and other intervening variable effects. *Psychological Methods, 7,* 83-104. doi:10.1073/1082-989X.7.1.83

March, J. (1998). *Manual for the Multidimensional Anxiety Scale for Children.* Toronto: Multi-Health Systems Inc.

March, J. S., Parker, J. D., Sullivan, K., Stallings, P., & Conners, C. K. (1997). The Multidimensional Anxiety Scale for Children (MASC): factor structure, reliability, and validity. *Journal of American Academy of Child and Adolescent Psychiatry, 36,* 554-565. doi:10.1097/00004583-199704000-00019

Mills, R., & Wing, L. (2005). Researching interventions in ASD and priorities for research: surveying the membership of the NAS. London: National Autistic Society.

Muller, E., Schuler, A., & Yates, G. B. (2008). Social challenges and supports from the

perspective of individuals with Asperger syndrome and other autism spectrum disabilities. *Autism, 12*(2), 173-190. doi:10.1177/1362361307086664

Muris, P. (2006). The pathogenesis of childhood anxiety disorders: considerations from a developmental psychopathology perspective. *International Journal of Behavioral Development, 30*(1), 5-11.

Muris, P., de Jong, P.J., & Engelen, S. (2004). Relationships between neuroticism, attentional control, and anxiety disorders symptoms in non-clinical children. *Personality and Individual Differences, 37*, 789– 797. doi: 10.1016/j.paid.2003.10.007

Muris, P. & Ollendick, T. H. (2005). The role of temperament in the etiology of child psychopathology. *Clinical Child and Family Psychology Review, 8*(4), 271-289. doi: 10.1007/s10567-005-8809-y

Muris, P., Steerneman, P., Merckelbach, H., Holdrinet, I., & Meesters, C. (1998). Comorbid anxiety symptoms in children with pervasive developmental disorders. *Journal of Anxiety Disorders, 12,* 387-393. doi:10.1016/S0887-6185(98)00022-X

Neylan, T. C., Brunet, A., Pole, N., Best, S. R., Metzler, T. J., Yehuda, R., & Marmar, C. R. (2005). PTSD symptoms predict waking salivary cortisol levels in police officers. *Psychoneuroendocrinology, 30*, 373–381. doi:10.1016/j.psyneuen.2004.10.005

Pennington, B., & Ozonoff, S. (1996). Executive functions and developmental psychopathology. *Journal of Child Psychology and Psychiatry, 37*, 51–87. doi:10.1111/j.1469-7610.1996.tb01380.x

Renno, P. & Wood, J. J. (2013). Discriminant and convergent validity for the anxiety construct in children with autism spectrum disorder. *Journal of Autism and Developmental Disorders, 43*(9), 2135-2146. doi:10.1007/s10803-013-1767-1

Research Units on Pediatric Psychopharmacology Anxiety Study Group (RUPP) (2002). The pediatric anxiety rating scale (PARS): development and psychometric properties. *Journal of the American Academy of Child & Adolescent Psychiatry, 41*, 1061-1069. doi:10.1097/00004583-200209000-00006

Raudenbush, S. W., & Bryk, A. S. (2002). Hierarchical linear models: Applications and data analysis methods (2nd ed.). Thousand Oaks, CA: Sage Publications.

Richdale, A. L., & Prior, M. R. (1992). Urinary cortisol circadian rhythm in a group of high-functioning children with autism. *Journal of Autism and Developmental Disorders, 22*(3), 433-447. doi:10.1007/BF01048245

Rohleder, N., Joksimovic, L., Wolf, J. M., & Kirschbaum, C. (2004). Hypocortisolism and increased glucocorticoid sensitivity of pro-inflammatory cytokine production in Bosnian war refugees with posttraumatic stress disorder. *Biological Psychiatry, 55,* 745–751. doi:10.1016/j.biopsych.2003.11.018

Rosmalen, J. G., Oldehinkel, A. J., Ormel, J., de Winter, A. F., Buitelaar, J. K., Verhulst, F. C. (2005). Determinants of salivary cortisol levels in 10-12 year old children: a population-based study of individual differences. *Psychoneuroendocrinology, 30*, 483-495.

Schlotz, W., Hellhammer, J., Schulz, P., & Stone, A. A. (2004). Perceived work overload and chronic worrying predict weekend–weekday differences in the cortisol awakening response. *Psychosomatic Medicine, 66,* 207–214. doi:10.1097/01.psy.0000116715.78238.56

Schneiders, J., Nicolson, N. A., Berkhof, J., Feron, F. J., van Os, J., & deVries, M. W. (2006).

Mood reactivity to daily negative events in early adolescence: relationship to risk for psychopathology. *Developmental Psychology, 42*(3), 543-554. doi: 10.1037/0012-1649.42.3.543

Simonoff, E., Pickles, A., Charman, T., Chandler, S., Loucas, T., & Baird, G. (2008). Psychiatric disorders in children with autism spectrum disorders: prevalence, comorbidity, and associated factors in a population-derived sample. *Journal of the American Academy of Child and Adolescent Psychiatry, 47*, 921-929. doi:10.1097/CHI.0b013e318179964f

Spiker, M. A., Lin, C. E., Van Dyke, M., & Wood, J. J. (2012). Restricted interests and anxiety in children with autism. *Autism, 16*, 306–320. doi:10.1177/1362361311401763

Sprafkin, J., Gadow, K. D., Salisbury, H., Schneider, J., & Loney, J. (2002). Further evidence of reliability and validity of the child symptom inventory-4: parent checklist in clinically referred boys. *Journal of Clinical Child and Adolescent Psychology, 31*(4), 513–524. doi:10.1207/S15374424JCCP3104_10

Stemberger, R. T., Turner, S. M., Beidel, D. C., & Calhoun, K. S. (1995). Social phobia: an analysis of possible developmental factors. *Journal of Abnormal Psychology, 104*(3), 526-531. doi:10.1037/0021-843X.104.3.526

Stoner, J. B., Angell, M. E., House, J. J., & Bock, S. J. (2007). Transitions: perspectives from parents of young children with autism spectrum disorder (ASD). *Journal of Developmental and Physical Disabilities, 1,* 23-39. doi:10.1007/s10882-007-9034-z

Storch, E. A., Wood, J. J., Ehrenreich-May, J., Jones, A. M., Park, J. M., Lewin, A. B., & Murphy, T. K. (2012). Convergent and discriminant validity and reliability of the pediatric anxiety rating scale in youth with autism spectrum disorders. *Journal of Autism and Developmental Disorders, 42*(11), 2374–2382. doi:10.1007/s10803-012-1489-9

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

Szatmari, P., Bryson, S., Duku, E., Vaccarella, L., Zwaigenbaum, L., Bennett, T., & Boyle, M. H. (2009). Similar developmental trajectories in autism and Asperger syndrome: from early childhood to adolescence. *Journal of Child Psychology and Psychiatry, 50*(12), 1459-67. doi:10.1111/j.1469-7610.2009.02123.x

Tortella-Feliu, M., Balle, M., & Albert, S. (2010). Relationships between negative affectivity, emotion regulation, anxiety, and depressive symptoms in adolescents as examined through structural equation modeling. *Journal of Anxiety Disorders, 24*(7), 686-693. doi:10.1016/j.janxdis.2010.04.012

Vreeburg, S. A., Zitman, F. G., van Pelt, J., DeRijk, R. H., Verhagen, J. C., van Dyck, R., ... & Penninx, B. W. (2010). Salivary cortisol levels in persons with and without different anxiety disorders. *Psychosomatic Medicine*, *72*(4), 340-347. doi:10.1097/PSY.0b013e3181d2f0c8

Wechsler, D. (2003). *Wechsler intelligence scale for children* (4th ed.). San Antonio, TX: Psychological Corporation.

Weems, C. F. (2008). Developmental trajectories of childhood anxiety: Identifying continuity and change in anxious emotion. *Developmental Review, 28,* 488-502. doi:10.1016/j.dr.2008.01.001

Wessa, M., Rohleder, N., Kirschbaum, C., & Flor, H. (2006). Altered cortisol awakening

response in posttraumatic stress disorder. *Psychoneuroendocrinology 31*, 209–215. doi:10.1016/j.psyneuen.2005.06.010

White, S. W., Schry, A. R., & Maddox, B. B. (2012). Brief report: the assessment of anxiety in high-functioning adolescents with autism spectrum disorder. *Journal of Autism and Developmental Disorders, 42*, 1138-1145. doi:10.1007/s10803-011-1353-3

Wood, J. J., Drahota, A., Sze, K., Van Dyke, M., Decker, K., Fujii, C., … Spiker, M. (2009). Brief report: Effects of cognitive behavioral therapy on parent-reported autism symptoms in school-age children with high-functioning autism. *Journal of Autism and Developmental Disorders, 39*(11), 1608-1612. doi:10.1007/s10803-009-0791-7

Wood, J. J. & Gadow, K. D. (2010). Exploring the nature and function of anxiety in youth with autism spectrum disorders. *Clinical Psychology: Research and Practice, 17,* 281–292. doi:10.1111/j.1468-2850.2010.01220.x

Wust, S., Federenko, I., Hellhammer, D. H., & Kirschbaum, C. (2000). Genetic factors, perceived chronic stress, and the free cortisol response to awakening. *Psychoneuroendocrinology, 25*, 707–720. doi:10.1016/S0306-4530(00)00021-4

Zinke, K., Fries, E., Kliegel, M., Kirschbaum, C., & Dettenborn, L. (2010). Children with high‐functioning autism show a normal cortisol awakening response (CAR). *Psychoneuroendocrinology, 35*(10), 1578‐1582. doi:10.1016/j.psyneuen.2010.03.009