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Effects Of Cortisol Stress Response Patterns On Autism Related Behaviors In Young Adult Males With Fragile X Syndrome And Autism Spectrum Disorders

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EFFECTS OF CORTISOL STRESS RESPONSE PATTERNS ON AUTISM RELATED BEHAVIORS IN YOUNG ADULT MALES WITH FRAGILE X SYNDROME AND AUTISM SPECTRUM DISORDERS

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ABSTRACT

Fragile X syndrome (FXS) and autism spectrum disorders (ASD) are characterized by deficits in social interactions (reduced eye contact, topic preoccupation, and social withdrawal) and maladaptive behaviors of anxiety, social withdrawal, and restricted interests (Kau, Tierney, Bukelis, Stump, Kates, Trescher et al., 2004). Prior research with individuals who have FXS has indicated that problem behaviors (i.e. social withdrawal and inattention) were correlated with abnormal activation of the hypothalamus-pituitary-adrenal axis (HPAA; Hessl, et. al., 2002); however, very few studies have investigated physiological patterns and associations with social dysfunction and restricted and repetitive behaviors for individuals with idiopathic ASD. The present study investigated the role of cortisol stress response physiological indices on directly observed social affect deficits, repetitive behaviors and overall autistic behaviors within adolescent to young adult males with FXS compared to idiopathic ASD. The study involved two related analyses: 1) examination of mean levels and modulation of salivary cortisol levels in response to two days of assessments and 2) the investigation of the relationship of social affect deficits, restricted and repetitive behaviors and overall autistic behaviors to salivary cortisol stress responses. Adolescent males with FXS with and without ASD demonstrated elevated pre-assessment cortisol levels on Day 1; however, persistent elevation in cortisol stress response at the start of Day 2 was only found in adolescent males with ASD (idiopathic and with FXS). The greatest modulation of cortisol stress response was found in adolescent males with FXS-O on Day 1 with

exact opposite findings on Day 2 with adolescent males with iASD demonstrating the greatest modulation of cortisol stress response. Lastly, lack of modulation of cortisol stress response on Day 2 was predictive of greater social affect deficits in adolescent males with iASD. Adolescent males with iASD and FXS with ASD have distinct neuroendrocrine profiles that differentiate them from FXS alone. Additionally, increased physiological reactivity during testing sessions was associated with greater restricted/repetitive behaviors regardless of genetic or diagnostic status. The results also support a complex interplay of maladaptive cortisol modulation and association with social affect deficits in young adult males with idiopathic ASD.

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CHAPTER 1

INTRODUCTION

Over the past few decades autism spectrum disorder (ASD) has emerged as a pervasive neurodevelopmental disorder characterized by deficits in social communication/interaction and restricted interests and repetitive behaviors across settings (American Psychiatric Association, 2013). ASD presents early in childhood and affects approximately 1 in 42 males in the United States (Centers for Disease Control and Prevention, 2014). Additionally, more than half of individuals with ASD also have intellectual disabilities (ID). Only 10% of individuals with ASD establish independence in adulthood, highlighting the magnitude of research on traits to promote positive adult outcomes as a significant public health priority (Bailey, Raspa, Holiday, Bishop, & Olmsted, 2009; Hartley et al., 2011). The high prevalence of ASD with ID, heterogeneity in ASD, and ultimate taxing of economic, societal and familial care systems illustrate the need to understand pathophysiological mechanisms that may improve the identification and treatment for this disorder.

Several studies have hypothesized that abnormal arousal modulation underlies behavioral attributes seen in ASD (Gabriels et al., 2013; Klusek, Roberts, & Losh, 2015); however, evidence is mixed likely due to varying behavioral phenotypes, intellectual abilities and inclusion of females within samples (Bitsika, Sharpley, Agnew, & Andronicos, 2015; Zinke, Fries, Kliegel, Kirschbaum, & Dettenborn, 2010). One pathophysiological mechanism and biomarker for measuring abnormal arousal

modulation of the hypothalamic-pituitary-adrenal axis (HPAA) is through salivary cortisol. Cortisol is secreted by the HPAA in response to stress and provides a pathophysiological mechanism to understand behaviors related to ASD. Patterns of arousal dysregulation through cortisol response may represent stress vulnerability resulting in the manifestation of core behavioral attributes. The relationship between arousal dysregulation as measured by cortisol and ASD behaviors has not been examined in idiopathic ASD populations with ID. The study of the relationship between pathophysiological mechanisms and core ASD behaviors in these and syndromic populations is important given the negative impact of the severity of these behaviors on the quality of life of individuals with ASD.

1.1 CORTISOL AS INDEX OF PATHOPHYSIOLOGICAL FUNCTIONING

Although the etiology of ASD is not well understood evidence supports a genetic basis that is heterogeneous and complex with gene-gene and gene-environment interactions leading to the common behavioral phenotype associated with ASD (Abrahams & Geschwind, 2008). The study of pathophysiological mechanisms helps establish biological correlates of core behaviors related to ASD. Physiological factors modulating stress are known to play an important role in social development and have broad associations with pathology. Biomarkers of stress response illustrating allostatic compensatory mechanisms which allow individuals to adapt to stress stimuli serve as indices for stress vulnerability. A number of neurobiological mechanisms have been postulated to account for social and emotional dysfunction in ASD. One such mechanism is abnormal activation and functioning of the HPAA in the peripheral nervous system (Bitsika et al., 2015; Gabriels et al., 2013; Hollocks, Howlin, Papadopoulos, Khondoker,

& Simonoff, 2014). The HPAA stress response systems are measured through secretion of cortisol (Foley & Kirschbaum, 2010). The HPAA involves complex feedback mechanisms with the hypothalamus, pituitary, hippocampus, and frontal cortex in order to regulate stress (Foley & Kirschbaum, 2010). The secretion of cortisol by the HPAA in response to stress is adaptive; yet, chronic abnormal activation can lead to adverse effects such as immune suppression and interference with memory and learning abilities (McEwen, et. al., 1997; Sapolsky, 2000). Cortisol salivary markers of stress complement behavioral assessments and allow for more comprehensive examination of the presentation and underlying mechanisms related to observable behaviors. The inclusion of biomarkers in intellectual disabilities can be particularly relevant given self-report and parental-reports are limited in these populations. Further identification of physiological patterns and the role of the HPAA stress response system on influencing core ASD behaviors in idiopathic and syndromic groups may inform targets for assessment and interventions.

1.2 PATHOPHYSIOLOGICAL FUNCTIONING IN IDIOPATHIC ASD

Hutt and colleagues (1964) first proposed that individuals with ASD may experience physiological hyperarousal that is associated with avoidance of social interactions and novel stimuli and presence of stereotypies. Furthermore, ASD has been described as a disorder accompanied by increased arousal, stress and sensory sensitivity (Corbett, Schupp, Levine, & Mendoza, 2009). However, evidence of physiological dysregulation in idiopathic ASD is varied with hypo-responsiveness to environmental social stressors and hyper-responsiveness to nonevaluative, social stressors such as medical procedures (Taylor & Corbett, 2014). For example, previous research has not

found differences of cortisol awakening response in children with high functioning ASD when compared to typically developing controls (Zinke et al., 2010). Yet, diurnal rhythms of cortisol among individuals with ASD have demonstrated significantly greater variability than typically developing peers as well as elevation in cortisol levels following exposure to novel stimuli (Corbett, Mendoza, Wegelin, Carmean, & Levine, 2007; Corbett et al., 2009). Clear dysregulation of the diurnal rhythm in ASD has been characterized by gradual decrease of cortisol levels over the course of sampling in the morning followed by elevated levels in the evening (Corbett et al., 2007; Corbett et al., 2009). Results suggest that chronic stress and situations characterized by unstable social relationships in childhood have resulted in lower cortisol responsivity or hypocortisolism in ASD (Corbett et al., 2009). Additional research indicated that individuals with ASD who engaged in frequent repetitive or self-injurious behaviors have evinced higher as well as lower cortisol levels than those who engage in little to no repetitive or stereotyped behaviors, demonstrating conflicting results (Bitsika et al., 2015; Gabriels et al., 2013; Lydon et al., 2015). Furthermore, relationships between cortisol and repetitive stereotypies appear to be present in childhood, but dissipate in adolescence, possibly resulting from reduction of these behaviors as individuals progress into adulthood (Bitsika et al., 2015). Hypo-responsiveness to acute stress paradigms, such as the Trier Social Stress Test for Children, with high functioning individuals with ASD demonstrating the absence of physiological response or less reactivity relative to same age peers (Taylor & Corbett, 2014). Yet, opposite patterns have been demonstrated in response to nonsocial, threatening situations with heightened physiological responses to medical procedures(Taylor & Corbett, 2014). In all, mixed evidence exists of positive as

well as negative associations between cortisol stress response patterns and core traits of ASD; illustrating a complex interplay between physiological mechanisms, stress and behaviors. To date, no study has investigated the effect of cortisol stress response on social interaction deficits, restricted interests/repetitive behaviors and overall core behavioral attributes associated with ASD. Additionally, none of these studies used the Autism Diagnostic Observation Schedule as an outcome measure to capture these severity of these behaviors in individuals with idiopathic ASD. Further delineation is needed to provide a comprehensive account of the interaction among HPAA regulatory subsystem and these behavior in idiopathic ASD.

1.3 AUTISM SPECTRUM DISORDERS IN FRAGILE X SYNDROME

Fragile X Syndrome (FXS) is the most common known single gene cause of ASD with up to 90% of males demonstrating symptoms associated with an ASD (Cohen et al., 2005; Thurman, McDuffie, Kover, Hagerman, & Abbeduto, 2015). FXS is caused by a trinucleotide expansion of Cytosine Guanine Guanine (CGG) polymorphism in the 5' untranslated regulatory region of the Fragile X Mental Retardation-1 (FMR1) gene on the X chromosome (Kaufmann & Reiss, 1999). Individuals with the presence of more than 200 CGG repeats are said to have the full mutation, which results in hypermethylation of the FMR1 promoter and silences the transcription of the FMR1 gene. Full mutation FXS affects approximately one in 4,000 males in the general population (Crawford, Acuña, & Sherman, 2001). Additionally, RNA transcriptional silencing from hypermethylation of the FMR1 gene results in diminished or absent production of the Fragile X Mental Retardation Protein (FMRP). FMRP is thought to regulate protein synthesis at synaptic sites of neurons and is essential for normal brain development (Schneider, Hagerman, &

Hessl, 2009). As a result, full mutation FXS in males is characterized by mild to moderate intellectual disabilities and a host of behavioral attributes such as attention problems, hyperactivity, anxiety, and autism spectrum disorders (Bailey, Raspa, Olmsted, & Holiday, 2008; Cordeiro, Ballinger, Hagerman, & Hessl, 2011; Cornish, Cole, Longhi, Karmiloff-Smith, & Scerif, 2013; Roberts et al., 2009).

Previous studies using the Autism Diagnostic Observation Schedule (ADOS) or the Autism Diagnostic Interview-Revised (ADI-R) indicate prevalence estimates with 67% to 74% of males with FXS meeting criteria for an ASD (Clifford, Dissanayake, Bui, Huggins, Taylor, & Loesch, 2007; Hall, Lightbody, & Reiss, 2008). Subsequent research has focused on differentiating ASD in FXS from other related disorders. These findings suggest that sustained poor eye contact and social avoidance throughout interactions, social indifference, reduced nonverbal behavior, and social withdrawal appear to be distinctly specific to individuals with FXS and ASD (fxASD) (Kau et al., 2004; Kaufmann et al., 2004; Budimirovic et al., 2006; Roberts et al., 2007; Hessl, Glaser, Dyer-Friedman, & Reiss, 2006). Additionally, robust findings in several previous studies illustrate a negative relationship between IQ and ASD symptomatology in FXS with lower IQ scores associated with greater severity of ASD behaviors on a variety of measures (Bailey, Hatton, Skinner, & Mesibov, 2001; Hall, Lightbody, Hirt, Rezvani, & Reiss, 2010; Thurman, McDuffie, Kover, Hagerman, & Abbeduto, 2015b). Furthermore, reduced FMRP is thought to disrupt autism susceptibility genes, lowering the threshold of interacting genetic, physiological and environmental factors that result in ASD within FXS. Thus, FXS provides a more straightforward genetic context for deciphering the

development and trajectories of core behavioral features associated with ASD in comparison to multifactorial, idiopathic populations (Thurman et al., 2015).

1.4 PATHOPHYSIOLOGICAL FUNCTIONING IN FRAGILE X SYNDROME

Elevated levels of baseline or pre-social cortisol have been found in children who have FXS (David Hessl, Glaser, Dyer-Friedman, & Reiss, 2006; Wisbeck et al., 2000). Furthermore, suppressed cortisol regulation accounted for a significant amount of variance in maladaptive behaviors, such as withdrawal, and social and attention deficits, of children with FXS when compared to unaffected, typically developing siblings (Hessl et al., 2002; Hessl et al., 2006). Thus, elevated baseline/pre-social and suppressed regulation of HPAA responses of cortisol are correlated with social and emotional dysfunction in FXS with the most frequent impairments presenting as extreme shyness, social withdrawal, social anxiety and ASD (Roberts, Clarke, Alcorn, Carter, Long, & Kaufmann, 2009). Of particular note, Hall and colleagues (2008) measured salivary cortisol at four approximate time points during one day of evaluation including pre-breakfast (8 am), pre-ADOS-G (3 pm), pre-dinner (5 pm) and pre-bedtime (9 pm). Interestingly, a negative relationship was found between ADOS-G total score and pre-ADOS cortisol levels in males with FXS. This indicates that males with lower cortisol levels prior to ADOS-G administration demonstrated greater observable autistic behaviors (Hall, Lightbody, & Reiss, 2008). These studies indicate that abnormal activation of the HPAA may predict severity of autistic behaviors in FXS and differ across environmental settings of stress.

1.5 CURRENT STUDY AIMS

The purpose of this study is to examine the pattern of physiological stress responses within FXS (with (fxASD) and without ASD (FXS-O)) and idiopathic ASD (iASD) and how these physiological indicators mediate autistic related behaviors within these populations. Behavioral phenotypes of social gaze avoidance, patterns of withdrawal, social and nonverbal communication deficits are also prevalent in individuals with idiopathic ASD; however, no study to our knowledge has yet examined the differences in pathophysiological and environmental mechanisms related to stress in individuals with idiopathic ASD. Direct between-group comparisons of physiological patterns within individuals with FXS and idiopathic ASD are needed to assess similarities and differences in symptomatology. Additionally, results highlighting the role of autism symptomatology measured continuously will capture the effect of physiological processes on dimensional aspects of behaviors. This will be of particular utility in FXS due to significant behavioral presentations associated with ASD, which influences increased rates of ASD diagnoses in this population. Knowledge regarding the neuroendocrine and physiological basis of FXS and strong phenotypic overlap with ASD allow for differentiation of specific phenotypes associated with FMR1 genetic variability. Prior research has investigated HPAA responses, modulation of stress levels, and relationships with physiological mechanisms and behavioral phenotypes in males with FXS, but no comparisons exist in the literature that include males with idiopathic ASD. Although review of literature is illustrating subtle differences in neurobiological mechanisms underlying behaviors associated with ASD in FXS and idiopathic populations (Abbeduto, McDuffie, & Thurman, 2014). This research study aims to

address: 1) Do cortisol stress response patterns (pre-assessment or change) differ between idiopathic ASD and FXS with and without ASD on Days 1 and 2 of evaluation and 2) do cortisol stress response levels predict social affect deficits, restricted/repetitive behaviors, or overall autistic behaviors in males with idiopathic ASD and FXS with and without ASD?

The overarching purpose of the present study is to examine similarities and differences in HPAA stress response patterns in adolescents with FXS (with and without ASD) compared to idiopathic ASD and how these neuroendocrine determinants predict autistic behaviors. It is hypothesized that males with FXS (with and without ASD) will be differentiated from males with idiopathic ASD by their elevated pre-assessment cortisol levels and reduced change cortisol rates (subtracting pre-assessment from post-assessment cortisol levels). Furthermore, it is hypothesized that elevated pre-assessment cortisol levels and decreased HPAA regulation (change) of stress responses will be strongly associated with autism related behaviors, such as greater social affect deficits and presence of repetitive behaviors in males with FXS, unlike idiopathic ASD, demonstrating differences in physiological pathways.

CHAPTER 2

METHODS

2.1 PARTICIPANTS

Subjects included 69 adolescent males participating in a larger longitudinal, cross-site study on language and factors that impact the transition into adulthood for males with fragile X syndrome (FXS) and idiopathic ASD (iASD). Males with FXS had the full mutation of the *FMR1* gene (> 200 CGG repeats) which was confirmed through genetic reports within the study. Idiopathic autism status was confirmed through educational and diagnostic reports as well as genetic testing records which ruled out known genetic disorders associated with autism, such as FXS. All participants were between 15 to 22 years old, verbally communicative (minimum combination of at least 3 words), spoke English as their primary language, and lived with their biological parents during enrollment in the longitudinal study. Despite attempts to match groups on nonverbal intellectual abilities, participants with iASD had slightly higher nonverbal IQs, particularly when compared to fxASD participants; see Table 2.1 below.

Adolescent males with FXS and iASD were recruited through various agencies including the South Carolina Department of Disabilities and Special Needs (SC DDSN), the Research Participant Registry Core of the Carolina Institute for Developmental Disabilities (CIDD) at the University of North Carolina at Chapel Hill (UNC), local South Carolina parent support groups, and a previous participant pool out of the University of California at the Davis M.I.N.D. Institute. Institutional review boards at

USC-Columbia and U.C. Davis approved all study protocols. All data consisted of findings from each participant's first time of evaluation regardless of site location, as part of the longitudinal, cross-site study. Each family was compensated for travel expenses and provided $50 for their participation.

2.2 MEASURES

Characterization of Autism

ADI-R. The Autism Diagnostic Observation Schedule-Second Edition (ADOS-2; (Lord et al., 2012) and ADI-R were administered to confirm and classify autism spectrum disorders in adolescent males with FXS and iASD (fxASD, FXS-Only, iASD). Only participants who met diagnostic criteria for an autism spectrum disorder on both the ADOS-2 and ADI-R were classified as having an autism spectrum disorder (fxASD or iASD). The Autism Diagnostic Interview-Revised (Lord, Rutter, & Le Couteur, 1994) is a standardized, semi-structured parent interview that is comprised of 93 items which capture a child's communication abnormalities, social interaction difficulties, presence of restricted and repetitive behaviors and developmental delays. Each item is rated on a scale from 0 (within normal limits or absence of abnormal behaviors) to 3 (severely delayed or presence of abnormal behaviors). On the first day of their son's visit, the ADI-R was administered and scored live by examiners who completed standard research reliability (i.e., training with an independent ADI-R trainer). Participants met autism spectrum criteria if they scored at or above cutoffs in four domains including communication, social interaction, restricted and repetitive behaviors, and developmental delays and concerns.

ADOS-2. The ADOS-2 consists of a series of semi-structured interview and play opportunities between an examiner and a participant, allowing for the observation of developmentally appropriate and inappropriate responses to these social exchanges. All examiners completed research reliability in order to standardize administration and scoring (i.e., reliable with an independent ADOS trainer). All administrations were scored live by the examiners, then reliability was completed by one of the research reliable staff who scored the administration from video and was blind to diagnostic group. Interrater reliability was conducted as participants were randomly selected for percent agreement, which was found to be moderately strong at 79% from the MIND and 71% from USC on the algorithm items. The ADOS-2 (Lord et al., 2012) was used as a continuous measure of social affect deficits, restricted and repetitive behaviors, and overall autistic traits (Gotham, Pickles, Lord, 2009). The ADOS-2 has also been reliably used as an outcome measure with children who have ASD (Aldred, Green, & Adams, 2004; Charman et al., 2005) and a measure of autism symptomatology in individuals with syndromic and idiopathic ASD (Hustyi, Hall, & Reiss, 2014; Oakes, Kover, & Abbeduto, 2012; Thurman et al., 2015b). Raw scores for social affect deficits, repetitive behaviors, and overall autistic behaviors were used as dependent variables in analyses.

Cognitive Measure

Leiter-R. Nonverbal intellectual ability (IQ) was examined using the growth composite scores of the Leiter International Performance Scale- Revised (Leiter-R; Roid & Miller, 1997). The Leiter-R is a brief, standardized measure of nonverbal intellectual ability for individuals ranging from 2 to 21 years of age. Overall, the Leiter-R was normed on a large, representative sample of adolescents and young adults with inclusion

of 123 individuals (63.4% male) with mild to severe cognitive delays, yielding high rates of reliability for the brief IQ screener for individuals from 11 to 20 years of age (r=0.89; Roid & Miller, 1997). The Visual Reception Battery subtests of Figure Ground, Form Completion, Sequential Order, and Repeated Patterns have shown consistent internal consistency reliability (α=0.65-0.86). Nonverbal mental age growth composite scores from the Leiter-R Brief IQ were used to illustrate levels of intellectual ability in males with iASD and FXS. Growth composite scores provided an estimate of absolute performance on an equal-interval scale unlike raw or age equivalent scores. Growth composite scores are also less susceptible to flooring effects than standard scores when measuring intellectual ability within individuals with cognitive disabilities.

Cortisol Stress Response to Social Challenges

To study potential Hypothalamic-Pituitary-Adrenal Axis (HPAA) abnormalities and its relationship to behaviors in iASD and FXS (with and without ASD), two samples of salivary cortisol were taken for each participant on both days to characterize the groups with attempts to match social testing stress. The first cortisol sample, "pre-assessment cortisol," was taken within 15 minutes of the onset of the evaluation to demonstrate pre-social challenge cortisol levels. The second cortisol sample, "post-assessment cortisol," was taken at the end of all evaluations to depict cortisol levels after social challenges during the day. Pre-assessment cortisol was subtracted from post-assessment cortisol levels which served as a measurement of stress regulation to assist with interpretation of the participants' physiological response to social challenges (ie. testing throughout the day).

Cortisol samples were collected using a salivette (oral cotton swab), which soaked in a participant's mouth for approximately 1 minute. Participants were asked to avoid consuming citric acid and dairy products for at least 60 minutes prior to sampling in order to reduce contamination and provide valid identification of physiological measurements. All saliva samples were taken around the same time of day (pre-assessment: 9:00am on Days 1 and 2, post-assessment: 2:30pm on Day 1 and 12:00pm on Day 2), then stored at -20 °C until analysis. Cortisol levels were determined employing a competitive solid phase time-resolved fluorescence immunoassay with flouromeric end point detection (DELFIA) using radioimmunoassay (Hessl et al., 2002). The intra-assay coefficient of variation was between 4.0 to 6.7%, and the corresponding inter-assay coefficients of variation were between 7.1 to 9.0%. Cortisol levels were measured in micrograms/deciliters, data were log transformed for normality, and pre-assessment, post-assessment and change cortisol levels were calculated from each day of direct assessment.

2.3 PROCEDURES

Participants were tested in a quiet room in the laboratory at the University of South Carolina or the University of California at the Davis M.I.N.D. Institute (UC Davis). Testing sessions for the larger, parent study included two consecutive days of evaluation that lasted approximately 5 hours on the first day and 3 hours on the second day. All assessments were videotaped with a Panasonic AVCCAM AG-AC90 camcorder. This study includes selected measures from the overall testing evaluation in which none of the assessments were designed to be stress inducing.

2.4 DATA ANALYSIS

The primary goal of this study is to investigate neuroendocrine determinants of stress response patterns and how these predict social deficits, repetitive behaviors/restricted interests and overall autistic behaviors in adolescent males with FXS (with and without ASD) and idiopathic ASD. All analyses were conducted using SPSS or R version 3.0.3 (R Core Team 2013). Data were first analyzed for violations of assumptions including homogeneity of variance, normality of residuals, and multicollinearity. Cortisol samples were log transformed due to positive skewed distribution in order to normalize the data to perform linear regression analyses. Missing data cases (approximately 8%) were listwise deleted. Missing data was the result of insufficient saliva to measure cortisol stress responses, exclusion of 1 administration and scoring of the direct measure of autistic behaviors and lack of administration of a subtest for 1 measure of intelligence. A Repeated Measure ANOVA was used to examine the question of do cortisol physiological stress response patterns (pre-assessment and change) differ in males with FXS-Only, fxASD and iASD across testing days. Secondly, multiple regression analyses were then conducted to examine the predictability of autistic related behaviors (social affect deficits, repetitive behaviors/restricted interests, and overall autistic behaviors) within each diagnostic group. Follow up complementary analyses were conducted to investigate the impact of autism behaviors on a continuous dimension rather than segmenting groups categorically based on each aim.

Table 2.1 *Participant Demographics for Each Diagnostic Group*

	iASD (*n*=15)	fxASD (*n*=33)	FXS-O (*n*=21)
Chronological Age in Years *M (SD)*	17.9 (2.9)	17.8 (2.2)	18.6 (2.7)
Nonverbal IQ Growth Score[1] *M (SD)*	481.3 (12.8)[a]	459.9 (13.6)[b]	467.2 (10.3)[b]
Nonverbal Brief IQ[1] *M (SD)*	59.1 (23.1)[a]	38.3 (4.5)[b]	40.9 (6.5)[b]
Race %			
Caucasian	80.0	84.8	85.7
African American	13.3	3.0	4.8
Asian	0	3.0	4.8
Multiracial	6.7	6.0	4.8
Other	0	3.2	0
Ethnicity %			
Non-Hispanic	93.3	90.9	90.5
Hispanic	6.7	9.1	4.8
Not specify	0	0	4.8

Note. [1]Leiter International Performance Scale- Revised. iASD= idiopathic autism spectrum disorder; fxASD = fragile X syndrome and autism spectrum diagnosis; FXS-O = fragile X syndrome only. Means in the same row with different superscripts differ significantly at $p < .05$.

CHAPTER 3

RESULTS

Means and standard deviations for pre-assessment and change raw cortisol levels (ug/dL) are presented in Table 3.1. Means and standard deviations of social affect, repetitive behaviors/restricted interests, and overall autistic behaviors as captured by the ADOS-2 are presented in Table 3.2. Repeated Measure ANOVAs were used to examine cortisol stress response patterns (pre-assessment and change) across Day 1 and 2 of assessments. Multivariate linear regression analyses were employed in order to examine the interaction of cortisol stress response (pre-assessment and change) and diagnostic group as predictors of directly observed social deficits, repetitive behaviors/restricted interests, and overall autistic behaviors after controlling for nonverbal IQ.

3.1 CORTISOL STRESS RESPONSE PATTERNS IN YOUNG ADULT MALES WITH iASD AND FXS

Pre-Assessment Cortisol Stress Response. A Repeated Measure ANOVA was conducted to examine the effect of day and diagnostic group (iASD, fxASD and FXS-O) on pre-assessment cortisol stress response patterns. The model was not statistically significant when comparing pre-assessment cortisol stress response patterns across days, $F(1, 67)=.46$, $p=.50$. Additionally, diagnostic group did not significantly predict changes in cortisol stress response, $F(2, 66)=1.57$, $p=.22$. However, the interaction of day and diagnostic group resulted in a statistically significant model, $F(2, 66)=5.57$, $p=.006$,

η_p^2=.144. Post hoc Repeated Measures ANOVA analyses found that adolescent males with iASD had significantly lower rates of pre-assessment cortisol stress response on Day 1 than fxASD or FXS-O males. On Day 2 adolescent males with FXS-O were approaching significant differences in pre-assessment cortisol levels when compared to adolescent males with iASD and fxASD. Complementary analyses illustrated that autism severity and nonverbal mental age did not predict pre-assessment cortisol on the 1st or 2nd day of evaluation (p=.40 and p=.79). See Figure 3.1 and 3.2 for visual representations of pre-assessment cortisol between diagnostic groups on Days 1 and 2 and Table 3.3 for complete Repeated Measures ANOVA findings.

Change Cortisol Stress Response. Analyses were conducted to examine the effect of day and diagnostic group on change cortisol stress response patterns. The model was not significant when comparing change cortisol (baseline minus post-assessment) patterns across days, $F(1, 67)=.09$, $p=.77$. However, the interaction of diagnostic group and day on cortisol stress response patterns resulted in a significant model, $F(2, 66)=7.23$, $p=.001$, $\eta_p^2=.18$. Post hoc Repeated Measures ANOVA analyses found that FXS-O adolescent males had significantly greater cortisol regulatory responses on Day 1 than iASD and fxASD. On Day 2, adolescent males with FXS-O and fxASD had significantly lower change cortisol responses than iASD males. See Figures 3.1 and 3.2 for visual representation of change cortisol between diagnostic groups on Days 1 and 2 and Table 3.4 for complete Repeated Measures ANOVA findings. Complementary analyses demonstrated that autism severity was approaching significance for predicting change cortisol on day 1 (B=.03, $p=.08$), but not day 2 (B=.02, $p=.44$). Greater autism severity predicted increased cortisol stress response from pre- to post-assessment levels following

evaluation on day 1 only. Investigation of these effects based on genetic status found no such relationship in participants with FXS. However, within males with iASD, greater autism severity again was approaching significance for predicting increased cortisol stress response from pre- to post-assessment levels on day 1 (B=.14, p=.08).

3.2 CORTISOL STRESS RESPONSE PREDICTING ASD RELATED BEHAVIORS IN YOUNG ADULT MALES WITH iASD AND FXS

Pre-Assessment Cortisol Day 1 and Diagnostic Groups. A multivariate effect for the combined dependent variables of social affect deficits, repetitive/restricted behaviors and overall autistic behaviors in respect to the interaction of pre-assessment cortisol on Day 1 and diagnostic group after controlling for nonverbal mental age was not significant: $\lambda = 0.985$, $F(4,62)=.236$, p=.92. All linear regression models were significant when investigating the impact of predictors (diagnostic groups, nonverbal IQ, pre-assessment cortisol on Day 1, and interaction of pre-assessment cortisol and diagnostic groups) on each dependent variable with young adult males with FXS-O set as the reference group: Social Affect: $F(6, 62)=3.66$, p=.004; Restricted/Repetitive Behaviors: $F(6, 62)=3.93$, p=.002; Overall Autistic Behaviors: $F(6, 62)=6.53$, $p<0.001$. Greater nonverbal IQ scores predicted lower social affect deficits (B= -.08, p=.04) and overall ASD behaviors across all groups (B= -.11, p=.02). Pre-assessment cortisol stress response on Day 1 and its interaction with each diagnostic group did not significantly predict any of the dependent variables; see Table 3.5 for complete results. Complementary analyses investigated the effect of pre-assessment cortisol on day 1 and nonverbal mental age on behaviors related to autism after collapsing diagnostic groups together. Similarly to above, only increased nonverbal mental age predicted reduced

social affect deficits (B= -.07, *p*=.04), restricted/repetitive behaviors (B= -.04, *p*=.04) and overall ASD behaviors (B= -.11, *p*=.01).

Pre-assessment Day 2 and Diagnostic Groups. A multivariate effect for the combined dependent variables of social affect deficits, repetitive/restricted behaviors and overall autistic behaviors in respect to the interaction of pre-assessment cortisol on Day 2 and diagnostic group after controlling for nonverbal mental age was also not significant: $\lambda = 0.96$, $F(4,62)=.70$, $p=.59$. All linear regression models were significant when investigating the impact of predictors (diagnostic groups, nonverbal IQ, pre-assessment cortisol on Day 2, and interaction of pre-assessment cortisol and diagnostic groups) on each dependent variable with young adult males with FXS-O set as the reference group: Social Affect Deficits: $F(6,62)=3.69$, $p=.003$. Restricted/Repetitive Behaviors: $F(6,62)=3.74$, $p=.003$. Overall ASD Behaviors: $F(6,62)=6.48$, $p<.001$. Greater nonverbal IQ scores predicted lower social affect deficits (B= -.08, *p*=.049) and overall ASD behaviors (B= -.12, *p*=.01) and was approaching significance for predicting lower restricted, repetitive behaviors (B= -.04, *p*=.07) across all groups. Pre-assessment cortisol stress response on Day 2 and its interaction with each diagnostic group did not predict any of the dependent variables; see Table 3.6 for complete results. Complementary analyses investigated the effect of pre-assessment cortisol on day 2 and nonverbal mental age on behaviors related to autism after collapsing diagnostic groups together. As with previous analyses, only increased nonverbal mental age predicted reduced social affect deficits (B= -.07, *p*=.045), restricted/repetitive behaviors (B= -.04, *p*=.044) and overall ASD behaviors (B= -.11, *p*=.01).

Change Cortisol Day 1 and Diagnostic Groups. A multivariate effect for the combined dependent variables of social affect deficits, repetitive/restricted behaviors and overall autistic behaviors in respect to the interaction of change cortisol on Day 1 and diagnostic group after controlling for nonverbal mental age was also not significant: λ = 0.90, $F(4,62)=1.57$, $p=.19$. All linear regression models were significant when investigating the impact of predictors (diagnostic groups, nonverbal IQ, change cortisol on Day 1, and interaction of change cortisol and diagnostic groups) on each dependent variable with young adult males with FXS-O set as the reference group: Social Affect Deficits: $F(6,62)=3.83$, $p=.003$; Restricted/Repetitive Behaviors: $F(6,62)=4.48$, $p<.001$; Overall ASD Behaviors: $F(6,62)=6.41$, $p<.001$. Greater nonverbal IQ scores were found to predict lower social affect deficits (B= -.08, $p=.04$) and overall ASD behaviors across all groups (B= -.10, $p=.02$). Change cortisol stress response on Day 1 and its interaction with each diagnostic group did not predict any of the dependent variables; see Table 3.7 for complete results. Complementary analyses, which collapsed diagnostic groups, found that change cortisol on day 1 (B=1.45, $p=.06$) and nonverbal mental age were approaching significance for predicting restricted and repetitive behaviors (B= -.03, $p=.06$). Increased cortisol stress response from pre- to post-assessment levels were associated with greater restricted and repetitive behaviors. Meanwhile, greater nonverbal mental age was predictive of reduced restricted and repetitive behaviors. Finally, after dichotomizing participants into those with increasing versus decreasing cortisol stress responses throughout the day, males with increasing cortisol stress response patterns on day 1 were approaching significance for predicting increased restricted and repetitive behaviors (B=1.03, $p=.06$).

Change Cortisol on Day 2 and Diagnostic Groups. A multivariate effect for the combined dependent variables of social affect deficits, repetitive/restricted behaviors and overall autistic behaviors in respect to the interaction of change cortisol on Day 2 and diagnostic group after controlling for nonverbal mental age was also not significant: λ = 0.89, $F(4,62)=1.90$, $p=.12$. All linear regression models were again significant when investigating the impact of predictors (diagnostic groups, nonverbal IQ, change cortisol on Day 2, and interaction of change cortisol and diagnostic groups) on each dependent variable with young adult males with FXS-O set as the reference group: Social Affect Deficits: $F(6,62)=4.91$, $p<.001$; Restricted/Repetitive Behaviors: $F(6,62)=3.69$, $p=.003$; Overall ASD Behaviors: $F(6,62)=6.62$, $p<.001$. Greater nonverbal IQ scores predicted lower social affect deficits (B= -.07, $p=.049$) and overall ASD behaviors (B= -.11, $p=.01$) and was approaching significance for predicting lower restricted, repetitive behaviors (B= -.04, $p=.069$) across all groups. Additionally, a greater reduction in cortisol stress response on Day 2 predicted lower social affect deficits (B= -10.67, $p=.03$) and was approaching significance for predicting lower overall ASD behaviors (B= -9.68, $p=.10$) in young adults males with iASD only. An interaction between change cortisol stress response and a diagnosis of fxASD or FXS-O was not found; see Table 3.8 for complete results. Complementary analyses were run which collapsed diagnostic groups. Similarly to previous effects, only increased nonverbal mental age accounted for reduced social affect deficits (B= -.07, $p=.03$), restricted/repetitive behaviors (B= -.03, $p= .08$) and overall ASD behaviors (B= -.10, $p=.01$). Change cortisol stress response on day 2 was not predictive of behaviors related to autism even after dichotomizing participants into those with increasing compared to decreasing levels from pre- to post-assessment.

Table 3.1. *Raw Cortisol Stress Response Levels (ug/dL) for Males with iASD and FXS*

Cortisol Levels by Days	Day 1		
	iASD (n=15)	fxASD (n=33)	FXS-O (n=21)
Pre-Assessment			
M (SD)	5.00 (4.15)	8.08 (6.30)	6.61 (6.08)
Post-Assessment			
M (SD)	4.61 (3.96)	5.76 (5.34)	3.22 (1.95)
Change			
M (SD)	0.40 (5.32)	2.31 (6.41)	3.38 (5.70)
		Day 2	
Pre-Assessment			
M (SD)	7.17 (5.03)	8.94 (18.18)	4.95 (3.56)
Post-Assessment			
M (SD)	4.02 (3.96)	6.01 (4.58)	4.22 (2.63)
Change			
M (SD)	-3.16 (3.04)	-2.94 (15.26)	-0.73 (3.45)

Table 3.2. *ASD Raw Scores for Young Adult Males with iASD and FXS*

	iASD (n=15)	fxASD (n=33)	FXS-O (n=21)
Social Affect Deficits			
M (SD)	9.00 (4.44)	9.97 (3.32)	5.81 (4.25)
Repetitive Behaviors			
M (SD)	3.47 (1.77)	3.97 (2.17)	1.81 (1.60)
Overall ASD Behaviors			
M (SD)	12.47 (4.69)	13.94 (4.28)	7.62 (4.74)

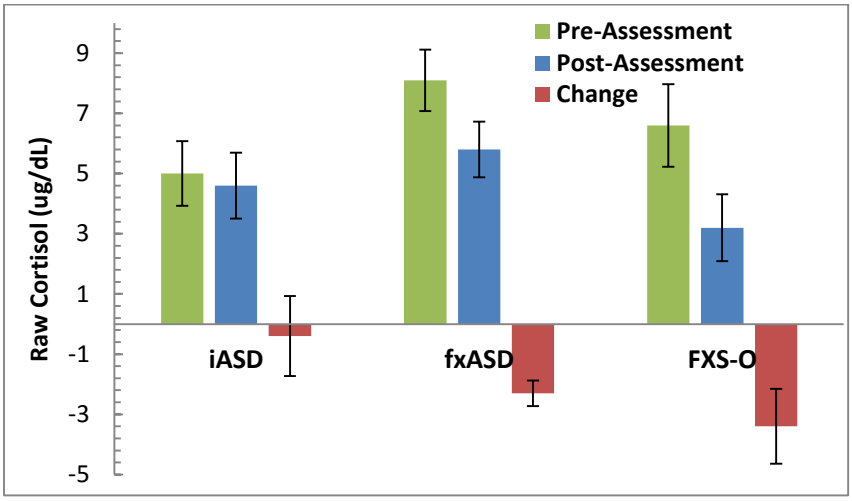

Figure 3.1. Day 1 Pre-Assessment, Post-Assessment and Change Cortisol Stress Response Patterns in iASD and FXS Males

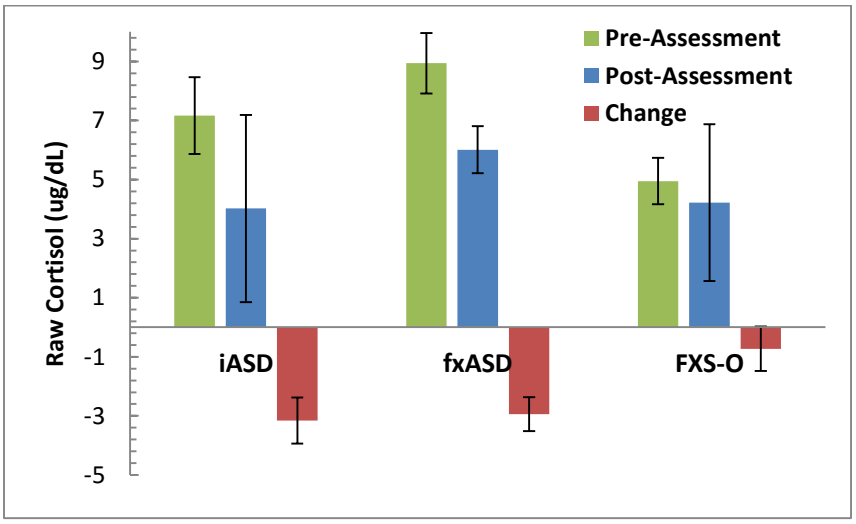

Figure 3.2. Day 2 Pre-Assessment, Post-Assessment and Change Cortisol Stress Response Patterns in iASD and FXS Males

Table 3.3. *Repeated Measure ANOVAs of Pre-Assessment Cortisol Stress Response Patterns in iASD and FXS Males with and without ASD*

	Pre-Assessment Cortisol				
Source	SS	df	MS	*F*	*p*
Day	0.02	1	0.02	0.46	0.50
Day x Diagnosis	0.50*	2	0.25	5.57*	0.01
Error	2.99	66	0.05		

		Day 1		Day 2	
Diagnostic Group	N	Mean	*SE*	Mean	*SE*
iASD	15	5.00[a]	.098	7.17[c]	.087
fxASD	33	8.08[b]	.066	8.94[c]	.059
FXS-O	21	6.61[b]	.083	4.95[d]	.074

Note. *p<.05. Pre-assessment cortisol is log transformed for normality.
Raw pre-assessment cortisol means are reported to demonstrate differences.
Means in the same column with different superscripts of a or b differ significantly at p <.05.
Means in the same column with different superscripts of c or d differ significantly at p <.10.

Table 3.4. *Repeated Measure ANOVAs of Change Cortisol Stress Response Patterns in iASD and FXS Males with and without ASD*

	Change Cortisol				
Source	SS	df	MS	*F*	*p*
Day	0.008	1	0.008	0.09	0.77
Day x Diagnosis	1.23*	2	0.62	7.23*	0.001
Error	5.62	66	0.09		

		Day 1		Day 2	
Diagnostic Group	N	Mean	*SE*	Mean	*SE*
iASD	15	.40[a]	.083	3.16[a]	.078
fxASD	33	2.31[a]	.056	2.94[b]	.178
FXS-O	21	3.38[b]	.071	.73[b]	.066

Note. *p<.05. Change cortisol is log transformed for normality.
Raw change cortisol means are reported to demonstrate differences.
Means in the same column with different superscripts of a or b differ significantly at p <.05.

Table 3.5. *Multivariate Linear Regression Results: Pre-Assessment Cortisol Levels on Day 1 Predicting Social Affect Deficits and Restricted and Repetitive Behaviors in iASD and FXS Males*

Multivariate Tests					
Effect	**Wilk's λ**	***df***	***F***	***p***	**η^2**
Nonverbal IQ	0.89	1	3.86*	0.03*	0.07
Group	0.66	2	7.04*	<0.01*	0.12
Pre-Assmt Cortisol Day 1	0.94	1	1.82	0.17	0.02
Group x Pre-Assmt Cortisol Day 1	0.96	4	0.24	0.92	0.02

	Social Affect Deficits		**Restricted/Repetitive Behaviors**		**Overall ASD Behaviors**	
	B (SE)	**t**	**B (SE)**	**t**	**B (SE)**	**t**
Intercept	41.99 (18.09)	2.32*	13.44 (9.00)	1.49	55.43 (20.41)	2.72**
FXS-O (reference group)	--	--	--	--	--	--
fxASD	3.42(2.48)	1.38	2.73(1.23)	2.21*	6.15(2.80)	2.20*
iASD	3.28(3.10)	1.06	1.46(1.54)	.95	4.74(3.50)	1.36
Nonverbal IQ	-.08(.04)	-2.07*	-.03(.02)	-1.42	-.11(.04)	-2.46*
Pre-Assessment Cortisol Day 1	-.91(2.18)	-.42	-1.19(1.09)	-1.10	-2.10(2.50)	-.86
fxASD x Pre-Assmt Cortisol Day 1	-.44(3.00)	-.15	.94(1.49)	.63	.49(3.38)	.15
iASD x Pre-Assmt Cortisol Day 1	-1.06(3.21)	-.33	-.49(1.60)	-.31	-1.54(3.62)	-.43

*Note. *p<.05, **p<.001*

Social Affect Deficits: R^2=.189, *F(6,62)*=3.656, *p*<.05. Restricted/Repetitive Behaviors: R^2=.205, *F(6,62)*=3.925, *p*<.05. Overall ASD Behaviors: R^2=.328, *F(6,62)*=6.529, *p*<.001.

Pre-assessment cortisol (ug/dL) is log transformed for normality.

Social Affect Deficits, Restricted/Repetitive Behaviors and Overall ASD Behaviors from the Autism Diagnostic Schedule-2. FXS-O, fragile X syndrome – only, established as the reference group within the multivariate regression model. fxASD, fragile X syndrome and autism spectrum diagnosis; iASD, idiopathic autism spectrum diagnosis.

Table 3.6. *Multivariate Linear Regression Results: Pre-Assessment Cortisol Levels on Day 2 Predicting ASD Behaviors in iASD and FXS Males*

Multivariate Tests					
Effect	**Wilk's λ**	***df***	***F***	***p***	**η^2**
Nonverbal IQ	.88	1	4.02	.02	.11
Group	.66	2	6.99	<.001	.14
Pre-Assmt Cortisol Day 2	.99	1	.34	.71	.01
Group x Pre-Assmt Cortisol Day 2	.96	4	.70	.59	.004

	Social Affect Deficits		**Restricted/Repetitive Behaviors**		**Overall ASD Behaviors**	
	B (SE)	**t**	**B (SE)**	**t**	**B (SE)**	**t**
Intercept	41.58(19.31)	2.15*	17.87(9.68)	1.85^t	59.45(21.83)	2.72**
FXS-O (reference group)	--	--	--	--	--	--
fxASD	5.33(2.92)	1.82^t	2.40(1.47)	1.64	7.73(3.31)	2.34*
iASD	8.05(3.40)	2.37*	4.17(1.71)	2.45*	12.23(3.85)	3.18**
Nonverbal IQ	-.08(.04)	-2.00*	-.04(.02)	-1.82^t	-.12(.05)	-2.58*
Pre-Assessment Cortisol Day 2	-2.09(2.31)	-.90	-1.24(1.16)	-1.07	-3.33(2.62)	-1.27
fxASD x Pre-Assmt Cortisol Day 2	1.97(3.28)	.60	.41(1.64)	.25	2.38(3.71)	.64
iASD x Pre-Assmt Cortisol Day 2	4.84(4.16)	1.16	2.57(2.09)	1.23	7.41(4.70)	1.57

Note. ^{t}*p<.10, *p<.05, **p<.001*

Social Affect Deficits: R^2=.192, *F(6,62)*=3.685, *p*<.05. Restricted/Repetitive Behaviors: R^2=.195, *F(6,62)*=3.741, *p*<.05. Overall ASD Behaviors: R^2=.326, *F(6,62)*=6.480, *p*<.001.

Pre-assessment cortisol (ug/dL) is log transformed for normality.

Social Affect Deficits, Restricted/Repetitive Behaviors and Overall ASD Behaviors from the Autism Diagnostic Schedule-2. FXS-O, fragile X syndrome – only, established as the reference group within the multivariate regression model. fxASD, fragile X syndrome and autism spectrum diagnosis; iASD, idiopathic autism spectrum diagnosis.

Table 3.7. *Multivariate Linear Regression Results: Change Cortisol Levels on Day 1 Predicting ASD Behaviors in iASD and FXS Males*

Multivariate Tests					
Effect	**Wilk's λ**	***df***	***F***	***p***	**η^2**
Nonverbal IQ	.89	1	3.7	.03	.11
Group	.66	2	7.01	<.001	.17
Change Cortisol Day 1	.97	1	.88	.42	.03
Group x Change Cortisol Day 1	.90	2	1.57	.19	.002

	Social Affect Deficits		**Restricted/Repetitive Behaviors**		**Overall ASD Behaviors**	
	B (SE)	**t**	**B (SE)**	**t**	**B (SE)**	**t**
Intercept	41.53(17.43)	2.38*	12.87(8.57)	1.50	54.40(19.86)	2.74**
FXS-O (reference group)	--	--	--	--	--	--
fxASD	4.39(1.28)	3.44**	1.49(.63)	2.37*	5.88(1.45)	4.04**
iASD	4.86(1.53)	3.18**	1.58(.75)	2.11*	6.44(1.74)	3.70**
Nonverbal IQ	-.08(.04)	-2.09*	-.02(.02)	-1.25	-.10(.04)	-2.37*
Change Cortisol Day 1	-2.64(2.72)	.97	1.58(1.34)	1.18	-1.05(3.10)	-.34
fxASD x Change Cortisol Day 1	4.16(3.68)	1.13	-2.78(1.81)	-1.81	1.38(4.19)	.33
iASD x Change Cortisol Day 1	4.78(3.63)	1.32	.64(1.78)	.36	5.42(4.14)	1.31

Note. $^{t}p<.10$, $*p<.05$, $**p<.001$
Social Affect Deficits: R^2=.200, $F(6,62)$=3.832, p<.05. Restricted/Repetitive Behaviors: R^2=.235, $F(6,62)$=4.475, p<.001. Overall ASD Behaviors: R^2=.323, $F(6,62)$=6.412, p<.001.
Pre-assessment cortisol (ug/dL) is log transformed for normality.
Social Affect Deficits, Restricted/Repetitive Behaviors and Overall ASD Behaviors from the Autism Diagnostic Schedule-2. FXS-O, fragile X syndrome – only, established as the reference group within the multivariate regression model. fxASD, fragile X syndrome and autism spectrum diagnosis; iASD, idiopathic autism spectrum diagnosis.

Table 3.8. *Multivariate Linear Regression Results: Change Cortisol Levels on Day 2 Predicting ASD Behaviors in iASD and FXS Males*

Multivariate Tests					
Effect	**Wilk's λ**	***df***	***F***	***p***	**η^2**
Nonverbal IQ	.89	1	3.86	.03	.10
Group	.67	2	6.81	<.001	.17
Change Cortisol Day 2	.97	1	.92	.40	.03
Group x Change Cortisol Day 2	.89	2	1.90	.11	.034

	Social Affect Deficits		**Restricted/Repetitive Behaviors**		**Overall ASD Behaviors**	
	B (SE)	**t**	**B (SE)**	**t**	**B (SE)**	**t**
Intercept	39.30(16.73)	2.35*	18.05(8.76)	2.06*	57.28(19.64)	2.92**
FXS-O (reference group)	--	--	--	--	--	--
fxASD	3.75(1.06)	3.55**	1.91(.55)	3.44**	5.65(1.24)	4.56**
iASD	1.01(1.88)	.55	2.78(.99)	2.82**	3.80(2.22)	1.73^t
Nonverbal IQ	-.07(.04)	-2.01*	-.04(.02)	-1.85^t	-.11(.04)	-2.53*
Change Cortisol Day 2	-.53(2.23)	-.24	1.26(1.17)	1.08	.73(2.62)	.28
fxASD x Change Cortisol Day 2	1.78(3.20)	.56	-1.12(1.68)	-.67	.67(3.76)	.18
iASD x Change Cortisol Day 2	-10.67(4.89)	-2.17*	.99(2.57)	.37	-9.68(5.76)	-1.68^t

Note. $^t p<.10$, *$p<.05$, **$p<.001$

Social Affect Deficits: $R^2=.257$, $F(6,62)=4.913$ $p<.001$. Restricted/Repetitive Behaviors: $R^2=.192$, $F(6,62)=3.685$ $p<.05$. Overall ASD Behaviors: $R^2=.332$, $F(6,62)=6.622$, $p<.001$.

Pre-assessment cortisol (ug/dL) is log transformed for normality.

Social Affect Deficits, Restricted/Repetitive Behaviors and Overall ASD Behaviors from the Autism Diagnostic Schedule-2. FXS-O, fragile X syndrome – only, established as the reference group within the multivariate regression model. fxASD, fragile X syndrome and autism spectrum diagnosis; iASD, idiopathic autism spectrum diagnosis.

CHAPTER 4

DISCUSSION

The current study included low functioning young adult males with FXS and idiopathic ASD in order to examine differences in cortisol stress response patterns to a series of language, cognitive, social, and experimental assessments and the mediating effect of this pathophysiological mechanism on core traits of ASD, such as social affect deficits, restrictive interests/repetitive behaviors and overall autistic behaviors. Data were collected on two consecutive days in an effort to gain insight of stress regulation in response to a novel testing environment and assessments and its predictive value on these core behavioral features related to ASD. Detection of pathophysiological biomarkers can facilitate intervention services through diagnosis and treatment of behavioral features related to ASD.

4.1 SALIVARY CORTISOL PRE-ASSESSMENT AND CHANGE STRESS RESPONSE PATTERNS IN iASD AND FXS

This study confirms previously-reported findings of higher initial stress responses in FXS than idiopathic ASD to novel assessments with greater dysregulation in ASD regardless of genetic status; yet, expands this literature to illustrate a complex interplay between physiological processes, environment and behaviors in these populations. In contrast to prior research, this study focused on pre-assessment and change cortisol stress response across two days of testing. Two new findings emerge when examining

physiological stress response patterns in FXS with and without ASD in comparison to idiopathic ASD across two testing days. First, a significant interaction was found between diagnostic group and testing day on pre-assessment and change cortisol stress response on both days of evaluations. On the first day, males with FXS demonstrated significantly elevated pre-assessment cortisol stress response compared to males with ASD regardless of genetic status. Males with idiopathic ASD regulated significantly less throughout the day than males with only FXS. Secondly, day 2 is marked with habituation to testing settings in males with only FXS and a lack of habituation to settings with greater stress response regulation in idiopathic ASD. Additionally, complementary analyses highlight the maladaptive role of increased autism symptomatology on cortisol patterns, as demonstrated by an increase in stress response from pre-to post-assessment on day 1 of evaluations. Although this finding appears to only be present in males with idiopathic ASD as no such relationship when examining specifically in FXS.

These findings support research that illustrates atypical autonomic activity and reactivity in FXS, particularly to novel assessments and settings on Day 1. However, habituation to stress was exhibited in FXS only males, demonstrating adaptive physiological regulation which has not been reported in previous findings. Additionally, variable cortisol stress response patterns have been demonstrated in idiopathic ASD with typical cortisol awakening response; yet, significant variability in diurnal rhythms in children with high functioning ASD(Corbett et al., 2007; Zinke et al., 2010). Variability in HPA responsiveness has been exhibited in response to environmental settings with findings of HPA hypo-responsiveness during social evaluative stressors, such as the Trier Social Stress Test for Children, and hyper-responsive to unpleasant, benign social

situations such as a blood draw procedure or MRI (Taylor & Corbett, 2014). Our results support autonomic dysregulation in ASD, regardless of genetic status, with opposing physiological reactions to testing sessions compared to FXS only males.

These results suggest that young adult males with FXS only and those with ASD demonstrate distinctly opposing physiological stress response patterns. FXS is marked by heightened pre-assessment cortisol patterns, unlike males with idiopathic ASD. However, variable cortisol responses were found in ASD, regardless of genetic status, with heightened levels on day 2 rather than day 1 of evaluation. While participants with idiopathic ASD demonstrated the greatest regulation on day 2, these findings are counterintuitive to typical physiological expectation as habituation effects to the environment would be expected.

4.2 CORTISOL STRESS RESPONSE PREDICTING ASD RELATED BEHAVIORS IN YOUNG ADULT MALES WITH iASD and FXS

In regards to the second aim, cortisol stress response levels did not predict social affect deficits, restricted interests/repetitive behaviors or overall autistic behaviors in young adult males with FXS regardless of ASD status; however, greater physiological regulation on day 2 was predictive of lower social affect deficits in idiopathic ASD. Collapsing diagnostic groups highlighted the deleterious effects of physiological stress response from pre- to post-assessment on restricted and repetitive behaviors with increasing levels associated with greater restricted and repetitive behaviors.

Unlike previous research in FXS, no associations were shown between cortisol levels and social difficulties or overall ASD behaviors . Prior analyses examined the predictive nature of cortisol levels in school age children with FXS, while this study

consisted of adolescents and young adult males, possibly explaining the lack of association in the present study. Additionally, methodologies for measuring social, emotional and behavioral problems differ across studies with only Hall and colleagues (2008) use of the ADOS-G as an outcome measure. Hall and colleagues (2008) found a significant negative association between ADOS-G total score and pre-ADOS salivary cortisol samples in males with FXS with lower salivary cortisol levels prior to the assessment correlated with greater autistic behaviors. This sample differs from the current study with inclusion of children to adult males from 5 to 20 years of age, collection of salivary cortisol across four time points in one day, and exclusion of the effects of intellectual functioning on this relationship. These differences likely account for the lack of similar findings between pre-assessment cortisol and overall autistic behaviors in males with FXS found in the present study. Secondly, literature in ASD has focused on associations between cortisol levels and repetitive, compulsive and self-injurious behaviors (Bitsika et al., 2015; Gabriels et al., 2013; Lydon et al., 2015). Direct associations have been found between stress and stereotypy in children with ASD; however, directionality of these relationships has differed depending on population. Furthermore, research supports a lack of association between stress and repetitive behaviors in adolescence, which is upheld by the present study (Bitsika et al., 2015).

The present study expands this work illustrating the adaptive nature of physiological regulation in ASD as demonstrated by reduced rates of social affect deficits. Greater reduction in cortisol stress response was indicative of better social communication and affect skills, but only in young adult males with idiopathic ASD. Findings suggest that physiological regulation of stress in males with FXS is not

associated with behaviors related to ASD as individuals progress into young adulthood; however, physiological response may influence the severity of stereotypies or restricted/repetitive behaviors as evidenced by a positive association between physiological response and restricted behaviors after collapsing diagnostic groups.

4.3 LIMITATIONS

There were four main limitations in the study. First, cross-sectional approaches were used to examine patterns of cortisol stress response and the predictive nature of this pathophysiological mechanism on ASD symptomatology. Cross-sectional approaches provide a snap shot of the relationships; thus, replication of this study will be needed to cement these findings. The second and third limitations were the inclusion of only males and individuals with low cognitive abilities (IQ less than 85). These results do not generalize to females with FXS or idiopathic ASD or high functioning individuals. Lastly, the current study utilized the ADOS-2 as a measure of autism symptomatology. While the ADOS-2 is considered the 'gold standard' tool for directly measuring behaviors related to ASD, it provides only one piece of a larger picture. Behaviors captured by the ADOS-2 may actually be better explained by another comorbid condition. This issue becomes problematic, particularly in FXS due to the increased risk of numerous co-occurring conditions such as intellectual disability, attention problems and anxiety which may influence behaviors captured during the ADOS-2 administration.

4.4 FUTURE DIRECTIONS AND CONCLUSIONS

Concerning future directions, it would be beneficial to examine core traits of ASD across direct observation and parent reported measures. Inclusion of additional observation and parent reported tools provides additional sources of information on ASD

symptomatology and their association with physiological stress responses. Also, more longitudinal research should be conducted to examine the persistence of stress response patterns across the developmental trajectory into adulthood for males with FXS and idiopathic ASD. Following participants throughout early adulthood would be informative to the presentation of pathophysiological stress responses and ASD symptomatology and how they may change during maturation.

The current study presents the first examination of cortisol stress response patterns, one form of a pathophysiological biomarker, as indicators of concurrent ASD symptomatology in young adult males with FXS with and without ASD compared to males with idiopathic ASD. The results of the this study provide insight into possible varying physiological stress responses in this population and the association of this mechanism with social affect deficits found only in idiopathic ASD. Future studies should continue to investigate the interplay of physiological and environmental contributors to the ASD symptomatology in males with FXS and idiopathic ASD. The identification of physiological dysregulation underlying core traits of ASD symptomatology may contribute to strategies for diagnosis and treatment in this sample as well as broader, non-ASD populations.

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