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RESEARCH ARTICLE

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Cortisol profiles differentiated in adolescents and young adult males with fragile X syndrome versus autism spectrum disorder

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Background: Fragile X syndrome (FXS) and non-syndromic autism spectrum disorder (ASD) are distinct disorders with overlapping behavioral features. Both disorders are also highly associated with anxiety with abnormal physiological regulation implied mechanistically. Some reports suggest atypical hypothalamus-pituitary-adrenal (HPA) axis function, indexed via aberrant cortisol reactivity, in both FXS and non-syndromic ASD. However, no study has compared cortisol reactivity across these two disorders, or its relationship to ASD symptom severity.

Methods: Cortisol reactivity (prior to and following a day of assessments) was measured in 54 adolescent/young adult males with FXS contrasted to 15 males with non-syndromic ASD who had low cognitive abilities.

Results: Greater ASD symptom severity was related to increased cortisol reactivity and higher levels at the end of the day, but only in the non-syndromic ASD group. Elevated anxiety was associated with increased HPA activation in the group with FXS alone.

Conclusions: Taken together, findings suggest a unique neuroendocrine profile that distinguishes adolescent/young adult males with FXS from those with non-syndromic ASD. Severity of ASD symptoms appears to be related to cortisol reactivity in the non-syndromic ASD sample, but not in FXS; while anxiety symptoms are associated with HPA activation in the FXS sample, but not in ASD despite a high prevalence of ASD, anxiety and physiological dysregulation characteristic in both populations.

KEYWORDS
anxiety, ASD, FXS, HPA Axis

1 | INTRODUCTION

Fragile X syndrome (FXS) affects approximately 1 in 5,000 males and is the most common inherited form of intellectual disability (Tassone, 2014). The condition is caused by a trinucleotide expansion of Cytosine Guanine Guanine (CGG) polymorphism of the *Fragile X Mental Retardation-1* (*FMR1*) gene on the X chromosome (Pieretti et al., 1991). This expansion causes hypermethylation of the *FMR1* gene resulting in diminished or absent production of the Fragile X Mental Retardation Protein (FMRP), which is involved in the regulation of protein synthesis for typical brain development (Schneider, Hagerman, & Hessl, 2009). Refinement of the FXS phenotype has been of increasing focus given the well-documented genetic characterization of the syndrome and the presence of behavioral features in FXS that overlap with other conditions including anxiety and autism spectrum disorder (ASD; Cordeiro, Ballinger, Hagerman, & Hessl, 2011; Cornish, Cole, Longhi, Karmiloff-Smith, & Scerif, 2013; Roberts et al., 2009).

Research has supported a physiological basis for many of these behavioral symptoms of the FXS phenotype including social anxiety and ASD symptomatology (Hatton et al., 2006; Roberts et al., 2009). However, the relationship between physiological activity and behavioral impairments in FXS is complex and associated with multiple factors including chronological age, and severity of features (e.g., Klusek, Roberts, & Losh, 2015). Dynamic systems theory recognizes the interplay of physiological systems and behavioral responsivity across development and provides a useful framework for questions regarding biobehavioral relationships, such as those hypothesized for FXS (Fidler, Lunkenheimer, & Hahn, 2011). Applying this theoretical model, the behavioral symptoms associated with ASD and anxiety in FXS could be seen as rooted, in part, in atypical physiological arousal regulation that leads to social avoidance and anxiety associated with social interaction that becomes more pronounced over time given repeated social difficulties. Thus, FXS provides a model for understanding the nature and mechanistic underpinnings of ASD and anxiety that could be shared among other disorders with similar profiles (Abrahams & Geschwind, 2010; Belmonte & Bourgeron, 2006; Devlin & Scherer, 2012).

1.1 | Cortisol as a measure of stress vulnerability

The hypothalamic-pituitary-adrenal (HPA) axis contributes to the maintenance of adaptive physiological stress response states in individuals. Regulation of the HPA axis involves the interplay between dynamic feedback systems of the hypothalamus, pituitary, and adrenal glands that are mediated through secretion of adrenal glucocorticoid hormones. Salivary cortisol, an index of arousal modulation, is a hormonal response involved in normative coping processes to environmental stressors with chronic elevation or dysregulation in cortisol stress responses resulting in health, cognitive, and social problems in otherwise intellectually typical individuals (de Kloet, Joëls, & Holsboer, 2005; McEwen, 2004; Sapolsky, 2000). Thus, cortisol provides a physiological biomarker to study stress vulnerability in FXS.

An emerging body of evidence suggests that HPA activity contributes to social and cognitive impairment in males with FXS, with some studies focused on circadian cycles (Hessl et al., 2002; Hessl, Glaser, Dyer-Friedman, & Reiss, 2006; Wisbeck et al., 2000), whereas other studies report relationships with baseline or reactivity indices of HPA activity (Roberts et al., 2009; Scherr, Hahn, Hooper, Hatton, & Roberts, 2016). Initial studies documented the presence of an atypical circadian cycle characterized by normal levels of awakening and morning expression followed by reduced diurnal decline later in the day (Hessl et al., 2002; Wisbeck et al., 2000). This work indicated that males with FXS displayed elevated cortisol expression on both standard research assessment days as well as on "non-stressful" typical days (Wisbeck et al., 2000) and with elevated cortisol associated with problem behaviors (Hessl et al., 2002). A number of subsequent studies have reported elevated baseline/pre-challenge cortisol levels in comparison to typically developing controls or unaffected family members (Hessl et al., 2002; Roberts et al., 2009), and a recent study found that elevated baseline cortisol was associated with working memory deficits in school-age boys with FXS (Scherr et al., 2016).

Research on cortisol reactivity in males with FXS has largely focused on social responsiveness to discrete events or more generalized social and cognitive experiences rather than traditional stress reactivity paradigms. In a series of studies investigating cortisol reactivity to a social challenge in the home for males 6 to 17 years of age, evidence suggested that increased cortisol reactivity to such a challenge was related to parent ratings of social impairments and overall problem behaviors (Hessl et al., 2002; Hessl, Glaser, Dyer-Friedman, & Reiss, 2006). Increased cortisol reactivity to a challenge was also related to increased eye contact, which paralleled patterns seen in unaffected siblings. However, in a different study with same-aged males with FXS, increased cortisol reactivity was associated with reduced eye contact (Hall, DeBernardis, & Reiss, 2006). In preschool-aged boys with FXS, elevated baseline and blunted cortisol reactivity characterized those with FXS and a high level of ASD features, a pattern that was not found in boys with FXS who had a low degree of ASD features (Roberts et al., 2009). In contrast, elevated cortisol prior to an assessment of ASD was associated with less severe ASD features in 5- to 20-year-old males (Hall et al., 2008). Thus, although there is evidence of an association between salivary cortisol with social impairments and ASD symptoms in FXS, there are inconsistencies, which may reflect differences across studies as regards the ages of participants, the measures used, or the particular comparison group employed.

Like FXS, non-syndromic ASD, or ASD not associated with a specific genetic disorder (e.g., FXS, Rett syndrome, tuberous sclerosis), is characterized by impairments in social communication and a high prevalence of stress and anxiety (Corbett, Schupp, Levine, & Mendoza, 2009). As reported in a systematic review of cortisol studies in persons with non-syndromic ASD (Taylor & Corbett, 2014), cortisol reactivity appears sluggish in response to acute stress paradigms, such as the Trier Social Stress Test for Children, with high functioning individuals with non-syndromic ASD illustrating the absence of expected physiological responsivity or less reactivity relative to same-age peers (Corbett, Schupp, & Lanni, 2012; Jansen et al., 2000; Lanni, Schupp, Simon, & Corbett, 2012; Levine & Sheinkopf, 2012). Opposite patterns of dysregulation have been demonstrated based on environmental and social contexts in children with non-syndromic ASD in comparison to typically developing peers; with the former displaying hypo-responsivity to nonsocial, threatening and benign, social interaction challenges and heightened physiological responses to a blood draw, and non-evaluative, playground interactions with unfamiliar peers (Corbett et al., 2012; Corbett, Mendoza, Abdullah, Wegelin, & Levine, 2006; Corbett, Schupp, Simon, Ryan, & Mendoza, 2010; Lanni et al., 2012; Spratt et al., 2012). In addition, increased rates of stereotyped and sensory behaviors have been associated with higher levels of cortisol reactivity in adolescents with non-syndromic ASD (Bitsika et al., 2015; Lydon et al., 2015). Diurnal cycle abnormalities are found in low functioning, but not high functioning, children with ASD, suggesting differences in physiological underpinnings within ASD

(Corbett et al., 2009; Hoshino et al., 1987; Marinović-Ćurin et al., 2008; Richdale & Prior, 1992). Overall, these studies suggest that cortisol dysregulation is not consistent in individuals with non-syndromic ASD, and an increased focus on individual differences that may disentangle the complex associations of HPA activity and phenotypic heterogeneity in non-syndromic ASD has been called for (see Taylor & Corbett, 2014, for a review).

In summary, evidence supports cortisol dysfunction in both FXS and non-syndromic ASD with findings converging in some domains; yet, distinctions also appear evident both within and across disorders. Males with FXS appear to present with disrupted circadian rhythms characterized by a blunting of the afternoon decline observed in non-clinical populations. In contrast, evidence suggests that a circadian cortisol rhythm disruption may be restricted to persons with ASD who are low functioning and not present in those who are high functioning. Cortisol hyper-reactivity in response to social and cognitive challenges characterizes males with FXS, whereas an overall sluggish cortisol reactivity characterizes those with non-syndromic ASD in similar contexts. Despite clear evidence of a relationship of HPA axis function to problem behaviors in both FXS and non-syndromic ASD, no research has directly compared individuals with FXS to those ASD. Furthermore, no research has examined the relationship between cortisol reactivity and diagnostic measures of ASD or anxiety symptom severity in adolescents/young adults with FXS. Such data would contribute to our understanding of the mechanisms associated with problem behavior across both disorders, which can refine both assessment and intervention efforts for these populations (Thurman, McDuffie, Hagerman, & Abbeduto, 2014).

1.2 | Study rationale and hypotheses

The primary goal of this study was to characterize cortisol reactivity in adolescents/young adults with FXS and how it is associated with ASD and anxiety symptomatology. We accomplish this goal by contrasting cortisol profiles in a group of males with FXS to a group of males with non-syndromic ASD and examining the relationship of ASD symptom severity to cortisol reactivity to a task-based challenge within and across the groups. Finally, we investigate the relationship of anxiety behaviors (e.g., general anxiety and social avoidance) as a competing hypothesis to determine if cortisol is associated with ASD symptom severity, anxiety symptom severity, or both within and across groups. We hypothesized differing cortisol profiles between groups, such that there would be increased cortisol reactivity to challenge in the males with FXS and a sluggish response in males with non-syndromic ASD; however, we anticipated that ASD symptom severity would be associated with cortisol reactivity in both groups. Additionally, we predicted that a positive relationship between pre-assessment cortisol and anxious symptoms would be found in both groups, reflecting physiological responses of hyperarousal associated with elevated anxiety. The challenge was created by a demanding assessment protocol comprised of several standardized and experimental measures of language, cognition, and related domains, with cortisol assessed through saliva samples collected pre- and immediately post-assessment.

2 | METHODS

2.1 | Participants

Participants included 54 adolescents/young adult males with FXS and 15 males with non-syndromic ASD, who were drawn from a larger longitudinal, two-site study focused on language development during the transition into adulthood (PI: Abbeduto). Participants were recruited between the ages of 15 and 22 years. The mean age of the participants was 18 years (range: 15–22 years), with no significant group differences in age or race (see Table 1). In line with the eligibility criteria for the broader study, all participants were verbally communicative (using at least three-word combinations on occasion), spoke English as their primary language, and lived with the biological mother. The small non-syndromic ASD sample reflects the primary emphasis on FXS (see Table 1 for participant demographics and Table 2 for participant descriptives). Although the goal was to match the ASD sample to the FXS sample on non-verbal IQ, this was challenging given the range of IQs found in ASD; thus there was greater variability in the non-syndromic ASD sample with two participants having an IQ above 80. Nonverbal IQ was accounted for in the analyses, as detailed below.

Males with FXS had the full mutation of the *FMR1* gene (>200 CGG repeats) confirmed through molecular DNA testing. All participants with non-syndromic ASD had previously completed genetic testing to rule out FXS and, in many cases, other common syndromic causes of ASD (e.g., tuberous sclerosis), which was confirmed through review of medical records. Recruitment and assessment were split across the research sites at the MIND Institute at the University of California, Davis and the University of South Carolina. Participants with FXS were recruited nationally through parent listservs, social media, postings by the National Fragile X Foundation, and with the help of the Research Participant Registry Core of the Carolina Institute for Developmental Disabilities at the University of North Carolina at Chapel Hill and the research registry of the Intellectual and Developmental Disabilities Research Center of the MIND Institute. Participants with non-syndromic ASD were largely recruited locally to each research site, through a local university research registry, advertisements, social media, parent support groups, and the South Carolina Department of Disabilities and Special Needs, although a few participants lived outside of the immediate geographic region of the testing sites.

2.2 | Procedures

All data were drawn from each participant's first evaluation within a longitudinal study at the University of South Carolina or at the University of California-Davis as the focus in this study is on cortisol reactivity associated with the challenge of completing a research assessment in a novel environment with novel social partners (e.g., examiners). About 2 weeks prior to the scheduled

TABLE 1 Participant demographic information

	ASD $n = 15$	FXS $n = 54$	p value
Chronological age (years)			
M (SD)	18.0 (2.4)	18.3 (2.3)	.679
Range	15.1–21.9	15.0–23.1	
Nonverbal IQ composite[a]			
M (SD)	59.1 (23.1)	39.3 (5.4)	<.010
Range	36.0–111.0	36.0–56.0	
Nonverbal IQ growth scores[a]			
M (SD)	481.8 (13.0)	462.9 (13.2)	<.001
Range	458.0–507.0	420.0–490.0	
Race %			.931
Caucasian	73.3	85.2	
African–American	13.3	5.6	
American–Indian	0	1.9	
Asian	0	0	
Multiracial	0	0	
Other	13.3	7.4	
Medication %			
Anti-anxiety	20.0	29.6	
Anti-convulsant	0	1.9	
Anti-depressant	20.0	40.7	
Anti-psychotic	6.7	3.7	
Benzodiazepine	13.3	16.7	
Hypnotic	0	1.9	
SSRI/SNRI	6.7	20.4	
Stimulant	20.0	25.9	
α Adrenergic receptor agonist	0	14.8	
At Least One Medication %	46.7	64.8	.235
Number of Medications	1.37	.80	.124

[a]Measured with the Leiter International Performance Scale-Revised Brief IQ.

assessment families were mailed a packet of questionnaires to complete and bring with them to the assessment, which included a demographic questionnaire as well as information about the participant's current medication use. Participants were advised to continue medication use as usual during participation in the research study. Families were compensated for travel expenses and received a small honorarium for participation. Informed consent was obtained from the participant's mother (or from the participant if he was deemed to have the capacity to provide informed consent). The institutional review boards at the University of South Carolina and the University of California, Davis approved all study protocols.

TABLE 2 Participant descriptive information

	ASD $n = 15$	FXS $n = 54$	p value
Overall ASD severity[a]			
M (SD)	6.6 (1.5)	5.7 (2.2)	.072
Range	4.0–10.0	1.0–10.0	
ADAMS subscales			
General anxiety M (SD)	7.4 (4.7)	5.7 (3.1)	.249
Social avoidance M (SD)	8.4 (5.1)	7.0 (4.5)	.473
Cortisol stress response[b]			
Pre-assessment M (SD)	.18 (.15)	.27 (.22)	.076
Post-assessment M (SD)	.17 (.14)	.17 (.16)	.890
Reactivity M (SD)	−.01 (.19)	−.10 (.22)	.158

[a]Indexed by the ADOS-2 overall severity score.
[b]Reported in ug/dl and reactivity determined by subtracting pre-assessment from post-assessment values.

Testing sessions included two consecutive days of evaluation with data from Day 1 used in this study given our interest in initial reactions to novel social partners and the assessment. The assessment on Day 1 lasted approximately 5 hr and was standardized with language and cognitive tasks blocked following a fixed order (mean time of day: pre-assessment 9:20 AM onset, post-assessment 1:58 PM completion with 1 hr lunchbreak). The design of the study, where all participant assessments began and ended at approximately the same time of day, controlled for the impact of diurnal patterns on cortisol secretion, which did not differ across the two participant groups; pre- ($p = .917$) and post-assessment ($p = .752$). Pre-assessment time of day was marginally associated with morning cortisol levels ($p = .061$); however, post-assessment time of day was not associated with corresponding cortisol values ($p = .764$).

2.3 | Measures

2.3.1 | ASD symptomatology

Both the Autism Diagnostic Observation Schedule-Second Edition (ADOS-2; Lord et al., 2012) and Autism Diagnostic Interview-Revised (ADI-R; Rutter et al., 2003) were administered to all participants with adherence to Risi et al. (2006) caseness criteria for ASD diagnosis. In the case of non-syndromic ASD participants, the measures were used to confirm diagnosis. The ADOS-2 was also used to provide a metric of ASD symptom severity for participants in both diagnostic groups.

The ADI-R is a standardized, semi-structured 93-item parent interview that provides a categorical diagnosis of ASD or no ASD based on the presence of operationally defined behaviors within the domains of communication, social interaction, presence of restricted and repetitive behaviors, and evidence of onset in early childhood. 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. The ADOS-2 yields an overall severity score, which can range from 1 to 10 and was used to capture ASD symptom severity in analyses.

Both the ADI-R and ADOS-2 were administered and scored live by graduate students or Ph.D.-level professionals, all of whom completed standard research reliability training (i.e., training with the instrument developers). Ten percent of the administrations for each diagnostic group were randomly selected and cross-site reliability across all examiners at both sites was assessed via videotaped administration. Consensus codes for each reliability administration were achieved through group discussion and mean percent agreement of each individual examiner relative to the consensus. Agreement of examiners with the consensus codes averaged 80% across all items for the ADOS-2 and 91% across all items on the ADI-R. Descriptive statistics for overall ASD severity scores are presented in Table 2. Seventy-four percent of the FXS sample met a classification for ASD (Risi et al., 2006).

2.3.2 | Anxiety

The Anxiety, Depression, and Mood Scale (ADAMS; Esbensen, Rojahn, Aman, & Ruedrich, 2003) was used to measure general anxiety and social avoidance. The ADAMS is a 28-item informant questionnaire that screens for psychiatric disorders in individuals with intellectual disabilities. Mothers rated their child's behaviors on a 4-point Likert scale ranging from 0 "not a problem" to 3 "severe problem." General Anxiety and Social Avoidance (i.e., Social Anxiety) were included in the present study, and these subscales have demonstrated good internal consistency with Cronbach's alpha of .83 for each. This questionnaire was mailed to mothers about two weeks in advance of the visit and was typically completed ahead of time. Any remaining items were completed during the assessment.

2.3.3 | Nonverbal intelligence

Nonverbal intellectual ability was measured with the Brief-IQ composite of the Leiter-R, which consists of Figure Ground, Form Completion, Sequential Order, and Repeated Patterns subscales. These composite subscales have shown consistent internal consistency reliability (α = .65 to .86). The Leiter-R, a nonverbal intelligence assessment, has proven particularly useful for measuring cognitive abilities in FXS as it reduces the impact of documented speech and language difficulties and eliminates verbal reasoning which may decrease the negative impact of anxiety on overall performance (Hooper, Hatton, Baranek, Roberts, & Bailey, 2000). Growth scale value scores were used in analyses, as they are less susceptible to flooring effects than standard scores and, unlike age-equivalent scores, are a true interval scale (Roid & Miller, 1997).

2.3.4 | Cortisol

Pre-assessment and post-assessment (assessment reactivity) samples of salivary cortisol were taken. All participant assessments began and ended at the same time of day to control for the effects of diurnal patterns on cortisol secretion. The pre-assessment sample was taken within 15 min of the participant's arrival at the testing site (mean time of day 9:20 AM) on the first day of testing and was intended to measure anticipation to the assessment and pre-social interaction cortisol levels. The post-assessment sample was taken at the end of all evaluations on that first day to depict cortisol levels after social interaction and experimental assessment challenges at the end of the day (mean time of day 1:58 PM). The assessment protocol followed a fixed order. A referential communication task lasting at least 20 min was typically the final task administered prior to the collection of post-assessment cortisol. This task requires the participant to verbally describe a series of known and ambiguous shapes to an examiner, capturing both language skills, and social collaborative behaviors (see Abbeduto et al., 2006 for detailed description of this task). Cortisol has a typical lag time of 20 min; thus, end of the day values for the present study likely represent physiological response both to the cumulative responses of the participant given the full day of assessment activities (i.e., some participants may become increasingly stressed over the course of the assessment while others may recover after each assessment) and to the activities that occurred prior to sampling. Cortisol collection was implemented at these approximate times to control for variation due to circadian rhythm patterns and to ensure roughly comparable levels of challenge during the day across participants.

Cortisol samples were collected using either a salivette that soaked in a participant's mouth for approximately 1 min (64% of sample) or using passive drool methods, depending on participant compliance. Comparability across the two methods was tested by collecting saliva via both methods on a subset of participants (n = 28). The values obtained across methods were highly correlated at r = .73. Participants were asked to avoid consuming citric acid and dairy products for at least 60 min prior to sampling in order to reduce contamination and provide valid identification of physiological measurements. All saliva samples were stored at −20°C until analysis. Cortisol levels were measured in micrograms/deciliters and determined by employing a competitive solid phase time-resolved fluorescence immunoassay with flouromeric end point detection (DELFIA) using radioimmunoassay. Salivary cortisol was processed using the Salimetrics Salivary Cortisol Enzyme Immunoassay kit (EIA) at the University of Trier in Germany. The intra-assay coefficient of variation was between 4.0–6.7%, and the corresponding inter-assay coefficients of variation were between 7.1–9.0%. Pre-assessment levels reflect the participant's cortisol levels upon arrival at the research site and prior to engaging with research staff and completing the study protocol; thus, they represent the participant's anticipation of the assessment. The post-assessment levels capture the participant's response to the cumulative experience of the assessment day coupled with their reaction to the challenge of a social collaborative language task. Reactivity scores were computed by subtracting pre-assessment levels from post-assessment; a higher reactivity score is indicative of more stress at post-assessment compared to pre-assessment.

Salivary cortisol involves a complex interplay between the HPA axis and biochemical pathways involving the sympathetic and parasympathetic nervous systems. A number of psychotropic medications are believed to influence salivary cortisol either indirectly due to altering of subjective experience of stress (i.e., anti-depressants, anti-anxiety, antipsychotics, selective serotonin reuptake inhibitors (SSRI), serotonin-norepinephrine reuptake inhibitor (SNRI), benzodiazepines, and stimulants) or by influencing salivary composition through the sympathetic nervous system (SNS, i.e., alpha adrenergic receptor agonist, beta adrenergic receptor antagonist) or parasympathetic nervous system (PNS, i.e., anti-cholinergic and cholinergic) (Granger, Hibel, Fortunato, & Kapelewski, 2009). Sixty-one percent of participants were taking psychotropic medications that may influence their subjective experience of a challenge and, of these participants, 19% were also taking medications that may influence salivary composition through the SNS. Additionally, none were solely taking medications that influence SNS pathways, nor was anyone taking medications that impact cortisol through the PNS. Reporting of psychotropic medication use is provided in Table 1, and the influence of specific classes of psychotropic medication on cortisol levels are reported in detail below.

2.4 | Data analysis

Analyses were conducted in SAS 9.4 (SAS Institute, 2013). Cortisol reactivity values were log transformed due to positive skew. Missing data cases were listwise deleted (8% due to noncompliance, extreme artifacts or insufficient saliva to measure cortisol data). Potential confounding variables were examined using Pearson or Point-Biserial correlations within each diagnostic group between the cortisol values and chronological age, site, nonverbal IQ, and medication use (Table 3). Chronological age ($r = -.11$, $p = .364$) and study site ($r = .04$, $p = .751$) were not correlated with cortisol reactivity, so were not included in the statistical model. Nonverbal IQ was also not correlated with reactivity ($r = .11$, $p = .368$); however, we included it in the models to ensure that we examined its effect given the differing IQs across diagnostic groups. Overall medication use (characterized as participants taking psychotropic and/or SNS medications) was not correlated with reactivity cortisol levels ($r = .08$, $p = .503$). The number of general psychotropic medication taken (r's < .04, p's > .729) and the number taken influencing SNS pathways (r's < .07, p's > .535) were not significantly associated with pre-, post- or reactivity cortisol levels. Additional analyses of variance (ANOVA) confirmed that cortisol levels did not differ between those who were and were not taking medication (p's > .535).

A general linear model was conducted to test the main effects of group (FXS or non-syndromic ASD), ASD symptom severity, and their interaction as predictors of cortisol reactivity, controlling for nonverbal cognitive ability (i.e., the Leiter-R Brief IQ Growth Scale Value). Interaction contrasts were used to determine the effect of ASD symptom severity on cortisol reactivity for each group also controlling for nonverbal cognitive ability. We also conducted the models after removing the two participants with non-syndromic ASD who had IQ's above 80 as a further control for the effect of IQ on our results. Results failed to demonstrate an influence of two participants with ASD who had higher IQ on the pattern of findings so we retained them in the final dataset and report those analyses given the small sample size. To complement the two-group model, a general linear model was conducted to examine results when the FXS group was broken into those with and without ASD (FXS with ASD, FXS without ASD, and non-syndromic ASD). Eta squared (η^2) were computed from interaction contrasts for effect sizes; with values of η^2 at .01, .06, and .14 considered "small," "medium," and "large" (Cohen, 1969). Lastly, Pearson correlations tested the relationship between anxiety symptoms (i.e., social avoidance, generally anxious behaviors) to cortisol levels prior to and following a day of assessment within each diagnostic group (FXS or non-syndromic ASD). These findings illustrate the influence of anxiety, ASD symptoms, or a combination on cortisol levels within and across groups.

TABLE 3 Correlation matrix among study variables

Measure	1	2	3	4	5	6	7	8
1 Nonverbal cognitive ability[a]	1.00							
2 Chronological age	.11	1.00						
3 ASD severity[b]	−.18	−.11	1.00					
4 General anxiety[c]	.08	.08	.20	1.00				
5 Social avoidance[c]	−.03	−.01	.33**	.51**	1.00			
6 Pre-assmt cortisol[d]	−.13	.09	−.08	.15	−.06	1.00		
7 Post-assmt cortisol[d]	−.11	−.04	.10	.15	.21	.55**	1.00	
8 Reactivity cortisol[d]	.06	−.14	.18	−.05	.25*	−.70**	.21	1.00

[a]Measured with the Leiter International Performance Scale-Revised Brief IQ Growth Scores.
[b]Indexed by the ADOS-2 overall severity score.
[c]Indexed by the ADAMS General Anxiety and Social Avoidance subscales.
[d]Log transformation of cortisol values (ug/dL) and reactivity determined by subtracting pre-assessment from post-assessment values.
*$p < .05$; ** $p < .01$.

3 | RESULTS

3.1 | Group differences in cortisol reactivity and the impact of ASD symptomatology

The mean pre-assessment cortisol level was .27 for the FXS and .18 for the non-syndromic ASD group, and the mean post-assessment cortisol level was .17 for both groups. The mean cortisol reactivity to the assessment protocol was −.10 for the FXS group and −.01 for the non-syndromic ASD group (Table 2). The overall general linear model demonstrated that the combined influence of group (FXS or non-syndromic ASD), ASD symptom severity, and their interaction controlling for nonverbal cognitive ability accounted for significant variance in cortisol reactivity, F (4, 64) = 2.54, p = .048; R^2 = .14. The main effects of group (B = .66, p = .120) and ASD symptom severity (B = .01, p = .705) alone were not significant (see Table 4). However, the interaction representing the group X ASD symptom severity was significant (see Table 4). Post-hoc interaction contrasts (see Figure 1) indicated that the effect of ASD symptom severity was related to greater cortisol reactivity, but only in the ASD group (F [1, 63] = 5.48, p = .023), with a η^2 of 0.074 consistent with a medium effect (Cohen, 1969). There was not a significant effect of ASD symptom severity on cortisol reactivity for the FXS group (F [1, 63] = 0.14, p = .706), with a η^2 of 0.002.

Complementary three-group analyses (FXS with ASD, FXS without ASD, and non-syndromic ASD) were generally similar in direction and magnitude, but with results suggesting reduced power for both the overall model, F (3, 65) = 1.60, p = .199; R^2 = .07 and the interaction effects, F (6, 62) = 1.74, p = .127; R^2 = .14. Multiple regression analyses suggested no relationship between ASD symptom severity and cortisol reactivity in FXS with (p = .875) or without ASD (p = .903); consistent with the primary analyses; however, a trend was evident in the non-syndromic ASD group (B = .14, p = .076).

3.2 | Relationship between cortisol profiles and anxiety symptoms

Pearson correlations suggest that elevated symptoms of general anxiety were associated with increased pre-assessment cortisol in the FXS group (r = .302, p = .026) but not in the non-syndromic ASD group (r = .015, p = .956; see Table 5). Likewise, elevated social avoidance/social anxiety was associated with increased cortisol post-assessment in the FXS group (r = .283, p = .038) but not in the ASD group (r = .028, p = .919). No other significant relationships were found between social avoidance, general anxiety and cortisol levels in FXS or non-syndromic ASD (p's > .089). In summary, anxious and avoidant behaviors did not relate to physiological indices of stress in the ASD group; whereas, pre-assessment cortisol was associated with symptoms of general anxiety and post-assessment cortisol was related to symptoms of social avoidance/social anxiety in the FXS group; see Figures 2 and 3.

TABLE 4 Regression coefficients depicting ASD symptom severity on cortisol reactivity

Model	B	SE	t	p
Intercept	.13	1.47	.09	.928
Group[a]	.66	.42	1.58	.120
ASD severity	.01	.02	.38	.705
Group × ASD severity	.13	.06	2.11	.039*
Nonverbal cognitive ability[b]	-.001	.003	-.02	.811

[a]The non-syndromic ASD group was set as the reference category.
[b]Measured with the Leiter International Performance Scale-Revised Brief IQ Growth Scores.

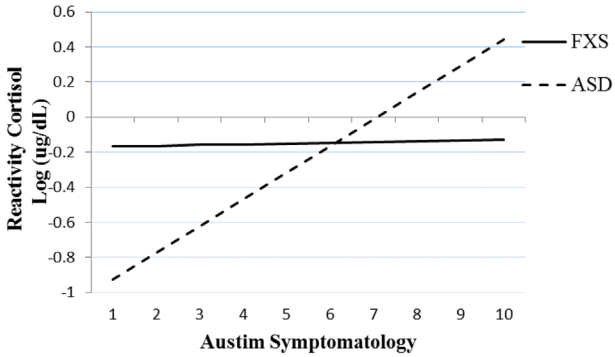

FIGURE 1 The interaction of ASD symptomatology and diagnostic group on modulation of cortisol after taking account of nonverbal intelligence. Higher cortisol level represents greater cortisol values at the end than beginning of the day. Adolescent males with non-syndromic ASD demonstrate greater reactivity with higher ASD symptoms. No such relationship is present in FXS

4 | DISCUSSION

The FXS phenotype includes anxiety, stress, and social impairments putatively linked to HPA axis dysfunction (Hatton et al., 2006; Hessl et al., 2002, 2006; Hessl, Rivera, & Reiss, 2004; Roberts et al., 2009). Significant phenotypic overlap exists between FXS and non-syndromic ASD with physiological dysregulation hypothesized to underlie these attributes. However, to date, no study has compared the relationship between ASD and anxiety symptomatology on HPA reactivity within these two disorders. Therefore, the overall aim of this study was to characterize cortisol reactivity to task-based challenge in adolescent and young adult males with FXS and its association with features of ASD and anxiety to that in non-syndromic ASD within the context of a standard research assessment.

Our results indicate that profiles of cortisol reactivity to challenge differentiate these two disorders despite their phenotypic overlap with differential contributions of ASD symptom severity versus anxiety symptom severity implied within and across groups. Evidence suggests both shared and distinct relationships across the groups with non-syndromic ASD and FXS. There was not a relationship of chronological age or nonverbal IQ to any cortisol index across both the non-syndromic ASD and FXS groups. A marginal circadian effect on cortisol was observed in morning samples, but not post-assessment levels, illustrating that afternoon cortisol was likely driven by our experiment.

TABLE 5 Pearson correlation coefficients between ADAMS subscale ratings and cortisol levels

	ADAMS subscale	
	Social avoidance (*r*)	General anxiety (*r*)
FXS (*n* = 54)		
Pre-assessment	.05	.30*
Post-assessment	.28*	.23
Reactivity	.12	−.15
ASD (*n* = 15)		
Pre-assessment	−.32	−.02
Post-assessment	−.03	−.03
Reactivity	.31	<.01

Cortisol values are log transformed in analyses, and reactivity determined by subtracting pre-assessment from post-assessment values. *$p < .05$.

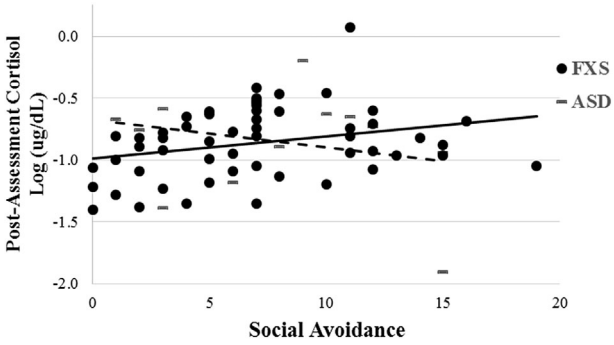

FIGURE 3 Correlation of social avoidance to post-assessment cortisol levels by group. Only a significant positive association in FXS with no relationship in ASD

In contrast, the groups with FXS and non-syndromic ASD differed across a number of aspects of cortisol reactivity in our study. First, the males with FXS displayed higher pre-assessment cortisol which differentiated the groups at a trend level. Second, a reduction in cortisol reactivity to challenge was observed in the FXS group, but not in the group with non-syndromic ASD. Third, ASD symptom severity was associated with the magnitude of cortisol reactivity in the non-syndromic ASD group whereas anxiety symptom severity was related to pre- and post-assessment cortisol levels in the group with FXS.

Elevated pre-assessment cortisol was associated with increased parent-reported symptoms of general anxiety disorder in only the FXS group despite the group with non-syndromic ASD having the same, or slightly higher, levels of anxiety. Likewise, there was a relationship between elevated social avoidance (i.e., social anxiety) and increased cortisol at the end of the assessment that is found only in the FXS group. This pattern of findings suggests that elevated anxiety in males with FXS is associated with activation of the HPA axis that is different from relationships observed in our sample of males with non-syndromic ASD. Specifically, our data suggest that males with FXS whose anxiety is triggered by a variety of stimuli (e.g., generalized anxiety disorder) found the anticipation of completing an assessment with unfamiliar people in a novel environment stressful as reflected by increased activation of the HPA axis. Likewise, our data indicate that males with FXS who have increased anxiety triggered by social interactions and performance-based expectations (e.g., social anxiety) find completing a prolonged research assessment culminating in a social collaborative language task, to be stressful, which resulted in elevated HPA activation. Males with non-syndromic ASD, however, do not display these same relationships despite having generally similar or elevated levels of parent-reported generalized and social anxiety. Males with non-syndromic ASD do not show a relationship of parent reported anxiety to cortisol expression. In fact, there is evidence of the opposite pattern with elevated symptoms of social anxiety associated reduced HPA activation in response to the onset of the assessment (e.g., pre-assessment cortisol) in the group with ASD.

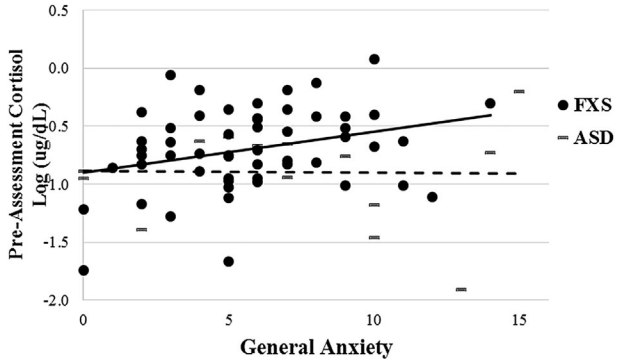

FIGURE 2 Correlation of general anxiety to pre-assessment cortisol levels by group. Only a significant positive association in FXS with no relationship in ASD

Within the non-syndromic ASD group, cortisol reactivity appears blunted at a group level, with a mean level of .01 change from pre- to post-assessment whereas the group with FXS displayed a mean level of .10 change. The restricted cortisol reactivity to challenge in the ASD group is striking considering the expected drop associated with the diurnal cycle which would be anticipated given that an average of 9 hr has elapsed between the morning pre- and early-afternoon post-assessment samples. The blunted cortisol reactivity in our study is consistent with reports of a dampened diurnal decline and blunted reactivity observed across the day in a subgroup of children and adolescents with ASD (Tomarken, Han, & Corbett, 2015; Tordjman et al., 2014). Interestingly, the magnitude of the blunted cortisol reactivity in ASD has been associated with increased ASD symptom severity in some studies (Tordjman et al., 2014) and with elevated anxiety in others (Hollocks, Howlin, Papadopoulos, Khnodoker, & Simonoff, 2014). In the present study, we found that increased ASD symptom severity was associated with increased reactivity but only in the non-syndromic ASD group.

Taken altogether these results suggest unique HPA axis profiles that imply different mechanistic factors reflecting opposing influences of ASD and anxiety symptoms across these two etiologically distinct neurodevelopmental disorders that nonetheless share a number of

behavioral features. Our findings are novel as no study has examined the relationship of cortisol reactivity to ASD features in adolescent and young adult aged males with FXS, and no published work has included a comparison group of males with non-syndromic ASD. This study expands previous literature to illustrate a complex interplay between physiological processes, environment and behaviors in males with FXS and males with non-syndromic ASD who are low functioning. In contrast to prior research in FXS, this study focused on reactivity of cortisol response in anticipation of and in response to completing a 6-hr research assessment requiring social interaction and performance on experimental, social collaborative language measures known to be challenging for males with FXS. Cortisol reactivity in the present study demonstrated a blunted response evidenced by a slight decrease (−.01) across the day in males with non-syndromic ASD when compared to FXS with a decrease of (−.10). These findings align with previous work indicating sluggish cortisol responses to challenging social interactions in males with non-syndromic ASD (Edmiston, Jones, & Corbett, 2016; Hollocks et al., 2014; Levine & Sheinkopf, 2012). Additionally, our findings support previous work in ASD illustrating a relationship between ASD symptom severity and reactivity, such that greater ASD behaviors were attributed to more of a physiological response following a social collaborative language task (Bitsika, Sharpley, Agnew, & Andronicos, 2015; Lydon et al., 2015).

Unlike previous work in FXS with Roberts et al. (2009), no relationship of ASD symptom severity to cortisol reactivity was present in this current study; these differences could be related to the differing ages across studies (preschool and early childhood versus late adolescence and young adulthood). Given that we examined the relationship of ASD symptom severity on cortisol reactivity through the primary two group analyses supplemented by a three-group analysis (FXS with ASD, FXS without ASD, ASD), our findings most likely represent a developmental effect. Conversely, the nature of the ASD measurement differs in this study and other work (e.g., ADOS-2 versus Childhood Autism Rating Scale). Alternatively, the severity of ASD symptoms may be higher in this adolescent/young adult sample than in previous work as suggested by findings that the degree of ASD symptom severity did not differ in the group of males with FXS contrasted to the non-syndromic ASD group. Although an age effect was not evident in the current study or in previous cross-sectional work (Hall et al., 2008), given our restricted age range, a developmental effect could still be present and should be examined in future longitudinal work. As it stands, however, the present results add to the growing body of literature suggesting that ASD symptoms in FXS and non-syndromic ASD may arise from largely different underlying psychological and physiological problems (Hardiman & Bratt, 2016; Klusek et al., 2015; Roberts, Boccia, Bailey, Hatton, & Skinner, 2001; Roberts et al., 2009; Thurman et al., 2014; Thurman, McDuffie, Kover, Hagerman, & Abbeduto, 2015).

The current study suggests that cortisol reactivity to challenge in adolescent and young adult males with FXS is independent of ASD features, chronological age, and nonverbal IQ. We found a complex interplay between anxiety and ASD symptom severity on cortisol levels in FXS, as evidenced by generalized anxiety associated with higher pre-assessment levels whereas anxiety associated with social interaction and performance-based demands was associated with increased post-assessment levels. Although analyses examining these relationships in non-syndromic ASD were likely underpowered, they suggest opposing interactions between anxiety problem behaviors and cortisol responses than those found in FXS. These are important distinctions that need additional research given the high prevalence of ASD and anxiety in FXS and controversy that exists concerning the extent to which these symptoms in FXS represent the same set of underlying problems as in the non-syndromic case (Hall, Lightbody, Hirt, Rezvani, & Reiss, 2010; Thurman et al., 2014).

Inclusion of an etiologically distinct neurodevelopmental disorder as a comparison group for cortisol reactivity has not been done in either the FXS or non-syndromic ASD fields, and so this study contributes to both lines of research. Also, most of the published studies in non-syndromic ASD have focused on high functioning persons, so the fact that our study targets lower functioning adolescents and young adult males is important. Our results indicate key mechanistic differences within HPA axis that are distinct across these two groups who nonetheless share a number of phenotypic features.

4.1 | Limitations and future directions

Findings from this study are drawn from samples of adolescents and young adults most of whom are low functioning with nonverbal IQs in the moderately severe range. Thus, while this sample is generally homogenous with regard to age and ability level, findings cannot be extended to higher functioning individuals or those of different ages or sex. Secondly, the sample size for the non-syndromic ASD group was small, and this study was cross-sectional, so larger samples and longitudinal studies are needed to examine developmental and age effects. Yet, despite the sample size, we report a moderate effect size for the relationship between elevated ASD severity and increased reactivity in the non-syndromic ASD group suggesting that these relationships are robust. Thirdly, we focused on ASD and anxiety symptom severity as primary features of interest and future work that includes direct observations of anxiety from multiple sources would extend these findings. Additionally, this study design does not reflect results from a true stress protocol, such as Trier Social Stress Test, but rather a more naturalistic challenging research protocol. These findings may differ from studies with stress protocol designs that elicit more discreet responses to socially challenging situations. Moreover, future directions may include more frequent sampling throughout the day and to specific socially challenging study protocols in order to unravel the intersection between diurnal responses and arousal dysregulation attributed to environmental stressors in FXS and non-syndromic ASD. Lastly, a number of participants with FXS and non-syndromic ASD were taking psychotropic medications during participation of this study, which may have influenced their cortisol levels depending on their body size, metabolism, and development of drug tolerance (Blake Woodside, Winter, & Fisman, 1991; Granger et al., 2009; Tranfaglia, 2011). However, no associations were found between cortisol levels

and psychotropic medication use, nor were group differences present between participants on and off medications. Thus, psychotropic medication use was not an exclusionary criteria for the present study as the majority of individuals, 50–70%, with FXS and non-syndromic ASD take psychotropic medications to treat problem behaviors associated with these disorders (Esbensen, Bishop, Seltzer, Greenberg, & Taylor, 2010; Valdovinos, Parsa, & Alexander, 2009). Inclusion of participants on psychotropic medications allows for greater external validity and generalizability of these findings. Additionally, the present study was not a treatment study; thus asking participants to refrain from taking vital medications prior to participation, including a wash out period, brought up ethical and safety concerns. Future studies should continue to investigate the interplay of individual attributes and environmental contributors to physiological mechanisms in these populations and how these vary or remain constant across developmental trajectories and study designs.

5 | CONCLUSION

The current study presents the first examination of cortisol reactivity in adolescents and young adult males with FXS compared to males with non-syndromic ASD. We found HPA profiles distinguished these groups with more severe ASD features associated with a more reactive cortisol response only in the non-syndromic ASD group. There was no relationship of the severity of ASD features in the group of males with FXS with nonverbal IQ and age not related to cortisol modulation in either group. Anxiety, in contrast, was associated with cortisol levels in the FXS group only. These results provide insight into varying physiological stress responses across FXS and non-syndromic ASD, which share a number of overlapping features namely stress, anxiety, and ASD symptoms. Our findings suggest that HPA activity differentially contributes to the expression of ASD features across these etiologically distinct samples with cortisol reactivity associated with elevated severity of ASD features in non-syndromic ASD, but not FXS. Such findings suggest the need to "unpack" diagnostic categories or symptomatology that may reflect very different types of underlying mechanisms in different etiological groups. Thus, diagnostic efforts might be refined given recognition that stressful environments, such as novel, diagnostic sessions, might result in elevated ASD symptom severity expression in non-syndromic ASD populations (Lopata, Volker, Putnam, Thomeer, & Nida, 2008; Lydon et al., 2015). Physiological monitoring of distress to environmental changes may assist with understanding the individual's behavioral well-being. Also, treatment aimed at improving management of stress through mindfulness-based and other behavioral interventions may reduce the severity of ASD features in non-syndromic ASD (Hou, Ng, & Wan, 2015; Johnson, Jenks, Miles, Albert, & Cox, 2011; Miodrag, Lense, & Dykens, 2013; Scholey et al., 2009). Finally, while these results are associated with a moderate effect size, the variance explained (14%) is rather modest suggesting that other factors are clearly impacting these relationships and need to be investigated.

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CONFLICTS OF INTEREST

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