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Biobehavioral Indicators of Social Fear in Young Children with Fragile X Syndrome

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Abstract

Anxiety is among the most impairing conditions associated with Fragile X syndrome (FXS) and is putatively linked to atypical physiological arousal. However, few studies have examined this association in young children with FXS. The authors examined whether patterns of arousal and behavior during an experimental stranger approach paradigm differ between a cross-sectional sample of 21 young children with FXS and 19 controls (12–58 months old). Groups did not differ in mean levels of behavioral fear. Unlike the control group, however, the FXS group demonstrated increased facial fear at older ages, as well as age-dependent changes in associations between heart activity and distress vocalizations. These findings may inform theoretical models of anxiety etiology in FXS and early detection efforts.

Keywords

Fragile X; respiratory sinus arrhythmia; heart activity; anxiety; autism; attention

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Fragile X syndrome (FXS) is a single-gene disorder prevalent in approximately 1 in 4,000 males (Crawford, Acuna, & Sherman, 2001). The disorder is caused by a mutation of amplified CGG trinucleotide repeats on the *FMR1* gene at Xq 27.3, which results in reduced production of Fragile X mental retardation protein and subsequent atypical brain development (Bassell & Warren, 2008). Males with FXS, characterized by > 200 CGG repeats, typically demonstrate more impaired cognitive and behavioral profiles than females due to random inactivation of the X chromosome in females. Fragile X syndrome is the leading heritable cause of intellectual disability and is associated with a variety of co-occurring conditions, including developmental delay, attention problems, hyperactivity, autism, and anxiety (Bailey, Raspa, Olmsted, & Holiday, 2008). Anxiety disorders are among the most commonly diagnosed and treated conditions associated with FXS, with

70%–83% of males meeting anxiety disorder diagnostic criteria (Bailey et al., 2008; Cordeiro, Ballinger, Hagerman, & Hessl, 2011) and between 40% and 70% receiving psychopharmacological treatments for anxiety symptoms (Bailey et al., 2008, Bailey et al., 2012).

Despite the high prevalence of individuals with FXS and comorbid anxiety, syndrome-specific treatments for anxiety and other problem behaviors are lacking (Hall, 2009; Reiss & Hall, 2007). Several studies have begun laying the foundation for this work by characterizing early features of the FXS phenotype relevant to anxiety, such as atypical social approach (e.g., Hall, Lightbody, Huffman, Lazzeroni, & Reiss, 2009; Hessl, Glasser, Dyer-Friedman, & Reiss, 2006; Roberts, Mankowski, et al., 2009; Roberts, Weisenfeld, Hatton, Heath, & Kaufmann, 2007) and physiological arousal (e.g., Hall et al., 2009; Roberts, Boccia, Bailey, Hatton, & Skinner, 2001, Roberts, Tonnsen, Robinson, & Shinkareva, 2012). These studies suggest the expression of anxiety and other challenging behaviors in FXS may be partially attributed to atypical arousal modulation (Cornish et al., 2004; Hessl, Rivera, & Reiss, 2004). However, previous work has primarily examined these associations in adolescent or adult samples and has focused on isolated problem behavior outcomes rather than co-occurring conditions. Furthermore, few studies have examined physiological arousal during experimental presses for social anxiety, particularly in young children who benefit most from early detection and intervention.

Characterizing the emergence and specificity of anxiety risk factors in young children with FXS is essential to informing a theoretical model that could guide early detection and intervention efforts in this population. The present study aims to expand this critical area of research by examining behavioral and physiological indicators of social fear during an experimental social anxiety paradigm in young males with FXS and typically developing (TD) controls.

Anxiety in FXS

Anxiety and withdrawal are among the most frequent and impairing conditions associated with FXS, with approximately 70%–83% of affected individuals experiencing anxiety disorders (Bailey et al., 2008; Cordeiro et al., 2011; Kaufmann et al., 2004). The prevalence of anxiety disorders in FXS exceeds rates in other intellectual disabilities and in the general population (Cordeiro et al., 2011). Gender, autism status, and developmental delay are not associated with specific patterns of anxiety diagnoses in FXS, although individuals with autism exhibit slightly elevated rates of selective mutism, social phobia, and specific phobia (Cordeiro et al., 2011). Parents report that 70% of males and 56% of females with FXS have received treatments for anxiety disorders (Bailey et al., 2008), underscoring the clinical severity and pervasiveness of symptoms.

Anxiety symptoms are generally attributed to limbic system dysfunction, particularly in hippocampal and amygdala regions that function to modulate emotional and physiological stress response (see Martin, Ressler, Binder, & Nemeroff, 2010, for review). In his classic theoretical model, Barlow (1988, 2002) describes anxiety as a cyclical process involving situational cues, negative affect, hypervigilance, and cognitive biases. These interactions are

intensified by shifts in attention and increases in arousal, which the individual may attempt to reduce using coping strategies such as avoidance or worry. Both biological (e.g., physiology, genetics) and psychological (e.g., learning patterns) factors create synergistic vulnerabilities that predispose an individual to increased anxiety risk (Barlow, 2002). Similarly, the tripartite model of depression and anxiety proposes that anxiety stems from hyperarousal and elevated negative affect (Clark & Watson, 1991). Although the typical age of onset for phobias and separation anxiety disorders in the general population is between 7 and 14 years (Kessler et al., 2007), several studies have associated behaviorally inhibited temperament in infancy and early childhood with later anxiety outcomes (e.g., Chronis-Tuscano et al., 2009; Hirshfeld-Becker et al., 2007).

These theoretical models of anxiety are consistent with several phenotypic features of FXS, including limbic system atypicalities (see Hessl et al., 2004, for review), features of withdrawal (e.g., Freund, Peebles, Aylward, & Reiss, 1995), and hyperarousal (e.g., Hall et al., 2009; Roberts et al., 2001; Roberts et al., 2012); as well as with recent evidence that early patterns of negative affect and approach predict later anxiety symptoms in young children with FXS (Tonnsen, Malone, Hatton, & Roberts, 2013). However, it is unclear how these phenotypic features emerge and interact to produce anxiety vulnerability in FXS and other neurodevelopmental disorders, particularly in young children.

Previous research has characterized the behavioral phenotype of anxiety in FXS using both experimental social tasks and naturalistic interactions. During experimental presses for social anxiety, children with FXS have demonstrated gaze aversion, discomfort, task avoidance, and poorer vocal quality (Hessl et al., 2006; mean age 10.89 ± 2.82 years), and greater eye-gaze avoidance (Hall et al., 2009; mean age 14.57 ± 2.77 years) compared with unaffected siblings. Similar patterns have been observed through examiner ratings of naturalistic interactions at the beginning and end of research assessments. Compared with TD controls, individuals with FXS have shown atypical overall social approach behaviors, as measured by facial expression, eye contact, and physical movement (Roberts, Mankowski, et al., 2009; FXS mean age = 3.99 ± 2.25 yeqrs). Consistent with findings in older children (Hall et al., 2009), young children with FXS have shown improvements in approach behaviors over the course of assessments, suggesting a “warm up” effect after initial periods of social anxiety. Notably, this warm up effect is less robust in children with FXS and high autistic features, indicating that variability in social approach behaviors may differentiate comorbid autism in FXS (Roberts, Mankowski, et al., 2009). Poorer social approach in FXS has also been associated with lower adaptive functioning, parent ratings of social avoidance and indifference, school versus home environments (Roberts et al., 2007); lower intellectual ability, younger age, less favorable home environments (Hessl et al., 2006); and social versus nonsocial tasks (Hall, DeBernardis, & Reiss, 2006).

The expression of problem behaviors in FXS, including anxiety and withdrawal, has been associated with atypicalities in physiological arousal (e.g., Hall et al., 2009; Roberts et al., 2001, Roberts et al., 2012). Physiological functioning is often examined using heart activity, reflected by both interbeat interval (IBI), an index of both parasympathetic and sympathetic heart activity, and respiratory sinus arrhythmia (RSA), a reflection of respiration on vagal activity of the heart. Reduction of RSA during social challenges reflects attention and

vigilance to environmental risk, permitting an individual to quickly exhibit defense behaviors if situations become threatening (Porges, 1995). Compared with typical controls, individuals with FXS have consistently demonstrated shorter IBI (faster heart rate), lower RSA, and poorer modulation of heart activity across infancy (Roberts et al., 2012), early childhood (e.g., Roberts et al., 2001), and later childhood and adolescence (Hall et al., 2009; Heilman, Harden, Zageris, Berry-Kravis, & Porges, 2011). Compared with typical patterns of decreased RSA during social challenges, children and adolescents with FXS have shown increased RSA, indicating poor preparedness to adjust to new task demands (Heilman et al., 2011). Similarly, young children with FXS have shown less modulation (greater change) in IBI and RSA across alternating passive and active tasks, indicating difficulty modulating arousal with increased task demands (Roberts et al., 2001). However, the specific associations among physiology, behavior, and anxiety are unclear. For example, Hall et al. (2009) reported atypical patterns of both heart activity and eye gaze avoidance in children and adolescents with FXS during a 25-min conversational task; however, participants' heart activity did not relate to behavioral avoidance. These studies suggest children with FXS may be more vulnerable to problematic outcomes due to decreased capacity to physiologically adjust to changing task demands, although further research is needed to clarify these potential associations and implications for intervention.

Although anxiety is prevalent in FXS and has been putatively linked to atypical arousal modulation, the emergence and specificity of this association is unclear. Most psychophysiological studies in FXS have focused on hypothalamic-pituitary-adrenal (HPA) axis functioning, such as cortisol, with fewer studies indexing heart activity during experimental paradigms. Measuring heart activity permits the benefit of real-time synchrony with behavior, unlike cortisol, which provides a more diffuse measure of arousal. Although individuals with FXS have demonstrated atypical heart activity during social (e.g., Hall et al., 2009; Heilman et al., 2011) and nonsocial (e.g., Roberts et al., 2001) challenges, only two studies have identified associations between atypical patterns of heart activity and problem behaviors (Roberts et al., 2001; Roberts et al., 2012), both during nonsocial tasks. Additional work is needed to characterize the emergence of and specificity of atypical physiological arousal as a potential diathesis for anxiety, particularly in infants and young children, who often benefit most from early identification and intervention. The present study aims to expand this area of literature by addressing whether patterns of behavior and physiological arousal differ between infants and toddlers with FXS and typical controls during a stressful social paradigm. We hypothesized that compared with controls, infants and toddlers with FXS would display greater facial fear, distress vocalizations, and attempts to escape during the stranger task. We expected these atypical patterns of behavior would be reflected by shorter IBI, lower RSA, and less modulation of heart activity during a stressful social task.

Method

Participants

Data were drawn from two interrelated, longitudinal studies on the early development of children with FXS. Participants with FXS were recruited nationally through ongoing

research studies and FXS parent support groups, and FXS diagnoses were verified with genetic reports. Our control group was recruited locally via flyers and word of mouth. Control participants were born full term, had no parent-reported developmental or genetic concerns, and received scores in the average range on the Mullen Scales of Early Learning (Mullen, 1995). Human subjects procedures were approved by the Institutional Review Board, and all parents provided written consent.

The present study includes 40 male participants from the longitudinal studies with complete physiological data for at least one assessment, yielding 21 males with FXS (ages 12–58 months, mean age = 32.38 months) and 19 typical controls (ages 12–57 months, mean age = 33.26 months). Nineteen participants from the FXS group were also included in Roberts et al.'s (2012) previous study on heart activity and autistic behaviors in FXS; however, the age of assessments varied across studies. Although several participants with FXS had multiple data sessions available, we selected each participants' earliest data point, consistent with our focus on biobehavioral regulation in early childhood. Control participants were selected to match the FXS group on gender and chronological age. Table 1 includes a summary of participant characteristics.

Measures

Social anxiety—We used the stranger approach episode of the Laboratory Temperament Assessment Battery (Lab-TAB; Gange, Van Hulle, Aksan, Essex, & Goldsmith, 2011; Goldsmith & Rothbart, 1996) to elicit behavioral and physiological markers of social shyness and anxiety. The stranger approach episode has been previously used with similar age groups to elicit behavioral variables (e.g., Aktar, Majdanzic, de Vente, & Bogels, 2012; Diaz & Bell, 2011; Kochanska, 1998). The stranger approach episode occurred at the same point in each child's assessment, directly following a nonstressful, engaging task. At the start of the stranger approach episode, the child was in a neutral emotional state and seated comfortably in his mother's lap. A female examiner entered the room disguised in a standardized uniform of baseball cap, dark sunglasses, oversized sweatshirt, and long skirt. The "stranger" then approached the child using prescribed verbalization and timing per the manualized procedures. This paradigm yielded four phases: an initial phase of unstructured time prior to stranger entry (30 s "initial"); an approach phase in which the stranger knocked, announced herself, stated she would be coming closer to the child, and approached the child (30 s "approach"); a prolonged period in which the stranger knelt silently in front of the child with a neutral expression (120 s "kneel"); and a final phase in which the stranger stood and silently exited the room (30 s "recovery"). The total paradigm lasted 3 min and 30 s.

Data were coded offline by trained research assistants who began coding after reaching training reliability standards of at least 80% agreement. Reliability coding was completed on 20% of the data, with a Cohen's kappa coefficient of 0.80 with a master coder across all codes. First, we calculated the proportion of time each participant exhibited any level each fear variable (*response prevalence*). We also calculated the intensity of each fear response (*response intensity*) using the following procedures: For each variable, data were coded by number of seconds spent at each level of intensity per manualized facial and body coding

guidelines (e.g., *no fear* = 0, *low* = 1, *moderate* = 2, *high* = 3). Using procedures by Gagne et al. (2011), we calculated an average intensity score by weighting the number of seconds spent at each level of intensity across the observation, then divided the total observation time (e.g., 10 s at none = 10 × 0, 3 s at low = 3 × 1, 4 s at moderate = 4 × 2, total = 3 + 8, average = 11/17). Each variable was calculated across the four phases of the stranger approach episode. The original videos are no longer available, thus codes could not be calculated within each physiological phase and instead span the entire stranger approach episode. Negatively skewed variables were transformed using the square root function. We then standardized each variable to *z* scores, as each variable used different intensity scoring scales and criteria. Two behavioral sessions (5%), both of participants with FXS, were excluded from behavioral analyses due to equipment problems during data collection.

Physiological arousal—Heart activity was collected during the stranger response paradigm using the Mini-Logger 2000, a noninvasive telemetry system that fits discreetly under clothing and is tolerated by young children. Data were edited for artifacts and IBI and RSA derived using MxEdit software (Delta-Biometrics, 1989; Mini Mitter Co., Inc., 1994). Per MxEdit procedures, RSA was quantified using spectral analysis for the high-frequency band during each sequential 30-s epoch. We calculated IBI and RSA averages within the four stranger episode phases: initial, approach, kneel, and recovery. We also calculated mean IBI and RSA across all 30-s epochs to permit comparison with our behavioral variables. To simplify interpretation, IBI is hereafter described as “faster heart rate” (shorter IBI) and “slower heart rate” (longer IBI). Nine phase measurements (5%) were excluded due to technical problems during data collection (FXS: two initial, one approach, two recovery; TD: one initial, two approach, one recovery).

Procedures

The stranger approach episode was administered during a standardized assessment protocol; thus all participants were assessed at similar times of day and after completing similar assessment activities. Each assessment was conducted by at least two trained examiners who were either PhD-level clinicians or graduate students supervised by these clinicians. Behavioral responses were video recorded and coded offline by trained research assistants. We examined prevalence and intensity of three behavioral variables: facial fear, distress vocalizations, and escape behaviors.

Statistical Analysis

We used 2 (Group) × 3 (Fear Type) analysis of covariance (ANCOVA) to examine group differences in the proportion of time spent in each type of fear, controlling for mental and chronological age. Next, we used multivariate regression to test our hypothesis that children with FXS would exhibit greater intensity of facial fear, distress vocalizations, and escape behaviors than TD controls. We then conducted repeated measure ANCOVAs to test our hypotheses that children with FXS would demonstrate shorter IBI, lower RSA, and less modulation of IBI and RSA across phases. The normality of sampling distributions, linearity, homogeneity of variance, and homogeneity of regression assumptions were verified for all ANCOVA analyses. Finally, we examined the association between behavior

and physiology within each group using multivariate regression. All analyses were conducted using SAS 9.3.

Results

Group Differences in Behavior

We used ANCOVA to test whether the proportion of time spent in fear varied by fear type (escape behaviors, distress vocalizations, facial fear) or group (FXS, TD). Escape behaviors correlated with mental age ($r = .33$, $p = .05$); thus we included mental and chronological age as covariates. Results indicate a significant main effect of fear type, $F(2, 95) = 60.23$, $p < .001$, with Tukey–Kramer post hoc adjustments indicating greater escape behaviors than distress vocalizations or facial fear (ps < .05). No significant group differences or interactions emerged.

We next used multivariate regression to test whether groups differed in the intensity of our three behavioral indicators. We also examined whether age-related changes in these variables differed across groups by including Group × Age interactions. Distress vocalizations positively correlated with facial fear ($r = .33$, $p = .05$), supporting our examination of behavioral variables in a single multivariate model. The combined effects of the independent variables significantly predicted distress vocalizations, $F(4, 36) = 3.34$, $p = .02$, and facial fear, $F(4, 37) = 4.90$, $p = .003$, but not escape behaviors, $F(4, 37) = 1.01$, $p = .42$ (Table 2). The effect of group on facial fear varied across ages. Although the TD group showed decreased facial fear at older ages, the FXS group showed increased facial fear at older ages (Figure 2). Distress vocalizations did not significantly differ by group but were instead predicted by younger chronological and mental ages.

Group Differences in IBI and RSA

We examined group differences in physiology across phases using 2 (Group) × 4 (Phase: initial, approach, kneel, recovery) repeated measure ANCOVA, controlling mental and chronological age. Separate models were used to examine IBI and RSA. Both IBI and RSA significantly differed across phases, and the FXS group demonstrated lower RSA across the task (Table 3). Notably, the FXS group displayed faster heart rate without adjusting for mental age, $F(1, 37) = 8.03$, $p = .007$, suggesting that mental age largely accounts for this effect. Post hoc analyses using Tukey–Kramer adjustments indicated significant IBI differences between approach and each initial, kneel, and recovery phase, with similar trends emerging for RSA (ps ≤ .10, Table 4). Contrary to our hypotheses, no significant Phase × Group interactions emerged, suggesting that although mean RSA levels differed across groups, patterns across phase did not vary significantly. To ensure we did not mask potential Group × Phase interactions by collapsing physiological data across the four 30-s kneel epochs, we also conducted a 2 (Group) × 4 (Epoch) repeated measure ANCOVA. No Group × Phase interactions emerged for either IBI, $F(3, 104) = 0.67$, $p = .57$, or RSA, $F(3, 104) = 0.23$, $p = .87$. However, main effects of group (RSA: $p < .01$) and phase (RSA: $p = .02$, IBI: $p < .001$) were retained, with both groups showing a significant peak in RSA and IBI during the second 30-s epoch ($p < .05$). These similar patterns across groups support our decision to collapse data from the kneel phase within the broader repeated measure ANCOVA.

To test whether our null findings were driven by our modest sample size, we also conducted a one-way ANCOVA to test whether IBI and RSA during the approach phase varied by group, controlling for chronological age and initial physiology. The main effect of group was not significant for IBI ($t = -0.43$, $p = .67$) or RSA ($t = 0.65$, $p = .52$), corroborating the repeated measures model.

Physiology–behavior associations

Within each group, we used multivariate regression to test the combined effects of mental and chronological age, physiology, and the interaction of chronological age and physiology on intensity of our three dependent variables: escape behaviors, distress vocalizations, and facial fear. Physiological variables (mean IBI and mean VT) were tested in separate models, and mental age was included as a covariate in each model. Regions of significance were probed using methods by Johnson and Fay (1950; Hayes & Matthes, 2009). No main effects of interactions emerged for facial fear or escape behavior, despite overall significance of the facial fear IBI model, $F(4, 18) = 3.42$, $p = .04$. However, within the FXS group, distress vocalizations were predicted by the combined effects of the independent variables for both IBI, $F(4, 17) = 6.30$, $p = .005$, and RSA, $F(4, 17) = 4.74$, $p = .01$. Higher distress vocalizations were predicted by the interaction of chronological age and each mean IBI ($t = 3.08$, $p = .009$) and mean VT ($t = 2.46$, $p = .03$). Specifically, greater distress vocalizations in the FXS group were associated with faster heart rate in younger participants (< 29 months) but were associated with slower heart rate in older participants (> 51 months). Greater distress vocalizations were associated with higher RSA in participants older than 47 months. The effects of mental age were not significant in either model. These associations were not present in the TD group, which instead showed a marginal main effect of higher escape behaviors corresponding to faster heart rate, model: $F(4, 18) = 3.73$, $p = .03$; main effect: $t = -1.98$, $p = .07$. No other significant effects emerged.

Discussion

Understanding the etiology and emergence of anxiety and other problem behaviors in young children is challenged by the limited communication and developmental skills of pediatric samples, the paucity of well-validated measures appropriate at these ages, and an overreliance on parent rating scales. Central to these efforts is the need for a developmental framework that recognizes the unique behavioral and affective presentation of anxiety symptoms in very young children and how these features vary over time in a dynamic and transactional manner. Despite these challenges, recent work provides evidence that anxiety symptoms can be identified in toddlers and preschool-age children (Mian, Godoy, Briggs-Gowan, & Carter, 2012) and that temperamental vulnerabilities such as behavioral inhibition —characterized by physiological hyperarousal (Barlow, 2002), withdrawal from strangers, and fear in unfamiliar situations (Biederman et al., 2001)—are risk factors in the later development of anxiety disorders.

FXS is single-gene disorder characterized by a high degree of comorbidity, with intellectual disability and anxiety as two of the most prominent and debilitating conditions (Cordeiro et al., 2011). Despite the high occurrence of these conditions in FXS and other clinical

disorders (e.g., idiopathic autism), little work has been done to examine risk for comorbid conditions within FXS. To our knowledge, our study represents the initial work to investigate the early emergence and risk factors of anxiety in FXS that integrates both behavior and physiological factors into a biobehavioral framework. Our findings present both convergent and divergent patterns across the groups of FXS and TD controls and within the FXS group, underscoring the importance of multimethod assessment in the investigation of social fear as a potential early emerging indicator of anxiety in FXS. We first discuss the arousal findings, then we integrate the behavioral and arousal results to discuss these associations across groups within a developmental framework.

Our findings suggest generalized hyperarousal in the FXS group across both measures of heart activity, IBI and RSA, with evidence that IBI may be moderated by mental age, as has been previously reported (Roberts et al., 2001; Roberts et al., 2013). We also found task-specific arousal patterns that were largely parallel across both groups, with decreased arousal in response to the approach of the stranger. Although we hypothesized a change in arousal in response to the approach of the stranger, the direction of effect was opposite of our expectation. We anticipated an increase in arousal, reflecting fear and anxiety in response to the approach of a stranger. However, the reduction in arousal we instead observed suggests that although both groups recognized the stranger approach as a discrete event generating a physiological response to the changing task demand (Heilman et al., 2011), this discrete event was physiologically indexed as interest and attention toward a novel stimulus (Casey & Richards, 1988) rather than fear or anxiety that are often reflected by increased arousal. These data suggest that the arousal response of both groups of young children to the stranger approach may have been one of primarily interest and novelty detection.

Our examination of three behavioral components of social fear revealed varied patterns within and across groups. Contrary to expectations, children with FXS did not show elevated fear across all behavioral indicators. The FXS group displayed a variable profile across both behavioral and physiological measures, suggesting a unique profile that distinguished them from typical controls. Both FXS and control groups displayed escape behaviors most frequently, with similar levels of intensity across groups and ages. The prominence of escape behaviors in our sample is consistent with previous studies using a stranger episode with 9-month-old infants (Diaz & Bell, 2011). Despite the similar behavioral profiles of escape across FXS and TD groups, only the typical controls displayed an association of increased arousal with escape behavior. Escape behaviors did not relate to arousal in the FXS group.

Likewise, there were no group differences in the behavioral display of distress vocalizations, which were associated with younger chronological and mental age across both groups. These data suggest that distress vocalizations represent a more “immature” response to social stress. The FXS group displayed an association of arousal to distress vocalizations that varied across development. Increased distress vocalizations were associated with elevated arousal at young ages (< 29 months) but with decreased arousal at older ages (> 51 months), with no association with arousal for the TD controls. Thus, the immature response of distress vocalizations within the FXS group may be rooted in arousal dysregulation that

shifts in the first years of life. This finding of developmental shifts in the association of behavior and arousal in infants and toddlers with FXS has been reported by our group previously in investigations of autistic behavior (Roberts, Tonnsen, et al., 2012), sensory reactivity (Baranek et al., 2008; Roberts, Tonnsen, et al., 2012), and visual attention (Roberts, Tonnsen et al., 2012). Dysregulated heart activity may indicate vulnerability for problematic outcomes given theoretical associations among anxiety, arousal, attention, and negative affect in typical populations (Barlow 1988, 2002; Clark & Watson, 1991) and infants with FXS (Roberts, Hatton, Long, Anello, & Colombo, 2012, Tonnsen et al., 2013).

Unlike escape behavior and distress vocalizations, facial fear differed across the groups. Whereas the typical controls displayed a trajectory of decreased facial fear expression at older ages, the FXS group displayed the opposite pattern of increased facial fear at older ages. In contrast, the physiological signature of facial fear was similar across groups, with neither group displaying an association of arousal with facial fear.

In summary, data from the present study reflect several biobehavioral patterns unique to our FXS group, including elevated generalized arousal, developmental shifts in associations between arousal and distress vocalizations, and increased expression of facial fear with age. Given the well-documented atypicalities in physiological arousal (e.g., Hall et al., 2009; Roberts et al., 2001; Roberts, Tonnsen, et al., 2012) and approach behaviors (e.g., Freund et al., 1995) exhibited by this population, as well as previous theories linking atypical arousal modulation to the expression of problem behaviors in FXS (e.g., Cornish et al., 2004; Hessl et al., 2004), it is possible these unique biobehavioral patterns signal a syndrome-specific dysregulation predictive of later problem behaviors. Future work may examine this possibility, attending in particular to the moderators that may sustain these putative associations. For example, higher anxiety symptoms in FXS have been associated with early patterns of elevated negative affect and longitudinal increases in temperamental approach, a measure of anticipation toward positive environmental stimuli (Tonnsen et al., 2013). Children with FXS may be vulnerable to decreased environment engagement due to compromised cognitive control and flexibility (e.g., Hooper et al., 2008) and therefore may be unable to effectively modulate behavior to reduce anxiety during social situations. This possibility is consistent with results from the present study and previous experimental evidence linking hypothalamic-pituitary-adrenal axis function and social anxiety symptoms in FXS (e.g., Hessl et al., 2006), as well as with preliminary findings that infants with FXS show difficulty disengaging attention from visual stimuli (Roberts, Hatton, et al., 2012). Notably, the associations among cognitive control of attention, arousal, and anxiety are also central to Barlow's (1988, 2002) model of anxious apprehension. However, these potential associations are speculative and have not yet been systematically investigated in FXS.

Limitations and Future Directions

Despite being among the first studies to examine behavioral and physiological responses to social stress in young children with FXS, our study is limited in several domains. First, we did not index real-time correlations between physiology and behavior, instead examining broad patterns derived across the duration of a paradigm designed to be socially stressful. Although our findings may inform links between behavior and physiology in FXS, future

work is needed to elucidate the specific mechanisms and processes underpinning these associations. Our sample was also restricted to a small cross-sectional group of males with FXS, which may be expanded through future work. Finally, approximately 10% of participants in both the FXS and TD groups did not tolerate the heart monitor and were not included in our sample. As such, our results may underrepresent children with heightened tactile sensitivity across both groups.

Future studies may examine the association between physiological arousal and psychopathology in individuals with the *FMR1* premutation, characterized by 50–200 CGG repeats. Given the identified vulnerabilities for social stress, depression, and anxiety in women with the *FMR1* premutation (Bailey et al., 2009; Roberts et al, 2009), informing physiological mechanisms of social challenges may indicate appropriate points of interventions and progress monitoring tools. Understanding the physiological signature of behavioral fear across the FXS spectrum of involvement may also inform attempts to characterize markers of treatment progress in preverbal or nonverbal children.

Summary

Characterizing early emerging risk factors that may predict anxiety and other conditions in FXS is essential to refining the infant phenotype in FXS. This work is may also inform early intervention and treatment protocols in efforts to prevent the occurrence, or minimize the impact, of secondary conditions such as anxiety or autism in FXS. The present study suggests differential biobehavioral responses to social stress in FXS compared with TD controls, laying the groundwork for future investigation into the implications of these associations. This work is particularly important given the lack of syndrome-specific interventions available for young children with FXS (Reiss & Hall, 2007) and elevated anxiety reported in other clinical syndromes such as autism (White, Oswald, Ollendick, & Scahill, 2009).

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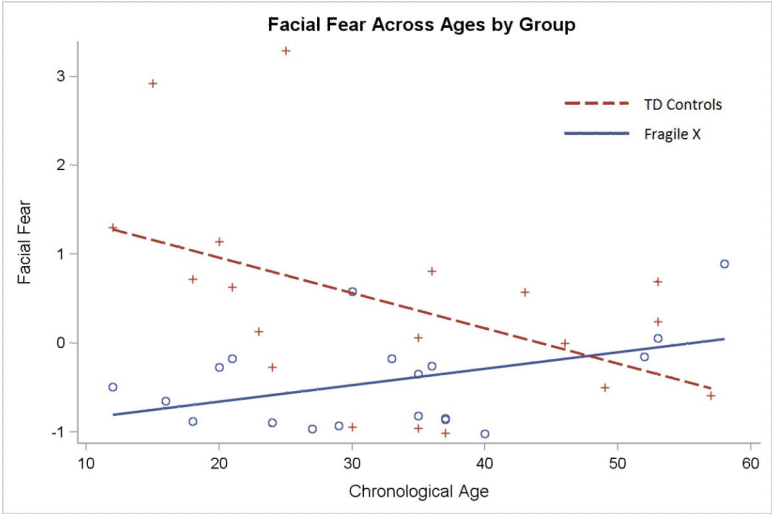

Figure 1.
Facial fear across ages by groups, not adjusted for mental age

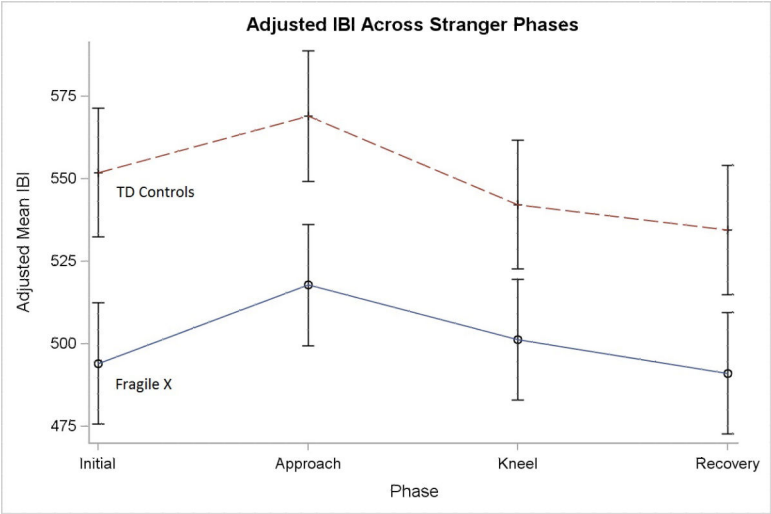

Figure 2.
IBI across stranger phases, adjusted for mental and chronological ages

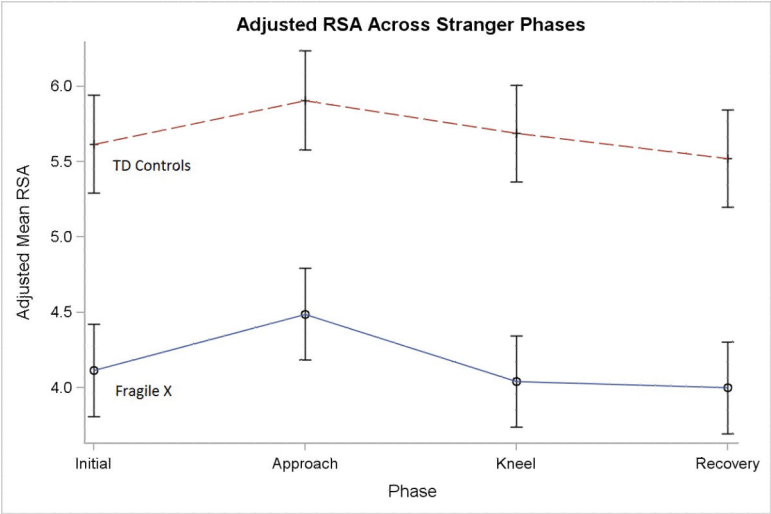

Figure 3.
RSA across stranger phases, adjusted for mental and chronological ages

Table 1

Descriptive Characteristics

	FXS (*n* = 21)				TD (*n* = 19)			
	Mean	***SD***	**Min**	**Max**	**Mean**	***SD***	**Min**	**Max**
Age at stranger	32.38	12.04	12	58	33.26	13.92	12	57
Mental age	26.67	10.41	8.75	45.25	35.80	17.30	14.00	66.00
CARS total score	29.69	5.94	16.5	40	--	--	--	--
Household income	$96,682	$86,422	$12,000	$400,000	$69,605	$19,939	$32,500	$100,000
Response intensity[a]								
Facial fear	0.08	0.06	0.03	0.23	0.20	0.21	0.03	0.79
Distress vocalizations	0.16	0.46	0	1.98	0.38	0.81	0	2.76
Escape behavior	0.86	0.71	0	2.34	1.06	0.49	0.07	1.78
Response prevalence[b]								
Facial fear	0.93	98.14	0	6.15	10.06	90.72	0	27.80
Distress vocalizations	6.38	86.27	0	58.32	12.49	76.98	0	73.96
Escape behavior	49.89	66.72	0	92.39	61.24	74.50	0	89.80
IBI								
Initial	496.45	69.42	413.15	660.22	557.86	57.44	432.00	679.27
Approach	518.00	77.41	412.70	697.86	580.59	54.85	475.00	700.50
Kneel	500.39	76.39	399.95	692.55	540.90	75.76	380.99	700.51
Recovery	483.42	56.99	394.83	613.95	533.79	75.86	371.25	675.04
RSA								
Initial	4.25	1.24	2.56	6.59	5.84	1.21	3.49	7.51
Approach	4.61	1.35	2.93	7.61	5.50	1.37	3.33	8.07
Kneel	4.21	1.29	2.64	7.61	5.46	1.56	1.89	7.42
Recovery	4.08	1.07	2.07	6.00	5.36	1.31	2.69	7.27

Note. Mental age was measured by averaging fine motor, expressive language, receptive language, and visual reception age equivalents on the Mullen Scales of Early Learning (MSEL; Mullen, 1995). CARS = Childhood Autism Rating Scale (Schopler, Reichler, & Renner, 1998). Min = minimum; Max = maximum. IBI = Interbeat interval; RSA = Respiratory sinus arrhythmia; Reactivity = Approach-initial.

[a] Response intensity was calculated by transforming and standardizing the weighted proportion of time spent at each level of intensity

[b] Response prevalence was calculated as the proportion of time participants displayed the behavior at any level of intensity

Table 2

Multivariate Regression Parameter Estimates for Response Intensity Model

Parameter	Facial fear				Distress vocalizations			
	Estimate	***SE***	***t***	***p***	**Estimate**	***SE***	***t***	***p***
Intercept	–0.96	0.56	–1.71	.10	1.38	0.60	2.31	.03
Group	**2.79**	**0.74**	**3.76**	**<.001**	–0.18	0.79	–0.23	.82
Chronological age	0.01	0.03	0.21	.83	**–0.10**	**0.03**	**–3.21**	**.003**
Group × Chronological Age	**–0.07**	**0.03**	**–2.37**	**.02**	–0.03	0.03	–1.08	.29
Mental age	0.02	0.04	0.51	.61	**0.09**	**0.04**	**2.42**	**.02**

Note. Significant values are listed in bold.

Table 3

Fixed Effects of Repeated Measure Analysis of Covariance for Heart Activity Analyses

Effect	*df*	Error	Interbeat interval		Respiratory sinus arrhythmia	
			F	***P***	***F***	***p***
Genetic	1	36	2.24	.14	**8.62**	**< .001**
Phase	3	104	**7.03**	**< .001**	**3.50**	**.02**
Genetic × Phase	3	104	0.67	.57	0.23	.87
Chronological age	1	36	**4.46**	**.04**	**12.50**	**.001**
Mental age	1	36	0.01	.90	1.02	.32

Note. Significant values are listed in bold.

Table 4

Differences of Least Square Means for Heart Activity Analyses

Effect	Phase 1	Phase 2	Interbeat interval					Respiratory sinus arrhythmia				
			Estimate	Standard error	*df*	*t*	Adjusted *p*	Estimate	Standard error	*df*	*t*	Adjusted *p*
Phase	Initial	Approach	–20.42	6.86	**104**	**–2.98**	**.02**	–0.33	0.14	104	–2.32	.10
	Initial	Kneel	1.23	6.63	104	0.19	1.00	0.00	0.14	104	0.01	1.00
	Initial	Recovery	10.22	6.80	104	1.50	.44	0.11	0.14	104	0.74	.88
	Approach	Kneel	21.64	6.72	**104**	**3.22**	**.01**	0.33	0.14	104	2.38	.09
	Approach	Recovery	30.64	6.89	**104**	**4.45**	**.00**	**0.44**	**0.14**	**104**	**3.05**	**.02**
	Kneel	Recovery	9.00	6.63	104	1.36	.53	0.10	0.14	104	0.75	.88
Genetic	Fragile X	Typical	–48.31	32.29	36	–1.50	.14	**–1.52**	**0.52**	**36**	**–2.94**	**.01**

Note. Significant values are listed in bold.