Title

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Journal

Journal of Autism and Developmental Disorders, 44(10)

ISSN

0162-3257

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Publication Date

2014-10-01

DOI

10.1007/s10803-014-2125-7

Peer reviewed

Published in final edited form as:
J Autism Dev Disord. 2014 October ; 44(10): 2536–2546. doi:10.1007/s10803-014-2125-7.

Emotion Potentiated Startle in Fragile X Syndrome

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Abstract

Social avoidance and anxiety are prevalent in fragile X syndrome (FXS) and are potentially mediated by the amygdala, a brain region critical for social behavior. Unfortunately, fMRI investigation of the amygdala in FXS is limited by the difficulties experienced by intellectually impaired and anxious participants. We investigated the relationship between social avoidance and emotion-potentiated startle, a probe of amygdala activation, in children and adolescents with FXS, developmental disability without FXS (DD), and typical development. Individuals with FXS or DD demonstrated significantly reduced potentiation to fearful faces than a typically developing control group ($p<.05$). However, among individuals with FXS, social avoidance correlated positively with fearful-face potentiation ($p<.05$). This suggests that general intellectual disability blunts amygdalar response, but differential amygdala responsiveness to social stimuli contributes to phenotypic variability among individuals with FXS.

Keywords

Fragile X Syndrome; Social Anxiety; Amygdala; Startle; Autism

Fragile X syndrome (FXS) is the most common inherited cause of intellectual disability, and the most common known single gene cause of autism (Chudley and Hagerman 1987; Hagerman 1987; Brown et al. 1986). It is caused by a trinucleotide expansion in the 5’

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The authors declare that they have no conflict of interest.

untranslated region of the *FMR1* gene on the X chromosome which, when it exceeds 200 trinucleotide repeats, leads to gene silencing and subsequent absence of the *FMR1* gene product, FMRP (Verkerk et al. 1991; Rousseau et al. 1991). Transcripts in the brain that are normally regulated by FMRP become enhanced in number in its absence, leading to synaptic abnormalities (Garber et al. 2008; Feng et al. 1997).

The behavioral phenotype of FXS is characterized by hyperactivity, attention problems, repetitive or stereotyped behaviors, and a striking prevalence of anxiety and autism. Using parent-report diagnostic interviews and based on DSM-IV criteria, we evaluated 97 boys and girls with FXS and found that 82.5% of the sample met criteria for at least one anxiety disorder regardless of sex, age, autism, IQ or proband status (Cordeiro et al. 2010). Social phobia and selective mutism, considered a severe form of social anxiety, were especially prominent (Cordeiro et al. 2010). This social anxiety can manifest in social deficits, including excessive shyness, high incidences of avoidant personality disorders and clinically significant issues of social withdrawal (Freund et al. 1993; Lachiewicz 1992; Kau et al. 2004; Roberts et al. 2007).

In the brain, the amygdala plays an important role mediating social behavior and sensitizing the organism to potential threats in the environment, and responds robustly to arousing social-emotional stimuli such as fearful faces (T.W. Buchanan et al. 2009; Davis 1992; P.J. Whalen et al. 2009; Adolphs 2010; P. J. Whalen et al. 2001; Baird et al. 1999). Amygdalar neuropathology is a significant contributor to anxiety disorders and figures especially prominently in pathophysiology of social phobia (Shin et al. 2009; Amaral 2002; Birbaumer et al. 1998).

Because of its role in social behavior and anxiety, the amygdala has become a structure of great interest in FXS research. An early demonstration of amygdalar involvement in FXS involved a case study of twin girls with the FXS full mutation who were discordant for intellectual disability (ID) (Mazzocco et al. 1995). Though both girls exhibited low social competence scores and had similar genetic involvement, one twin had an IQ score in the typical range and a significantly smaller amygdala than the other, whose IQ score fell in the ID range (Mazzocco et al. 1995; Reiss et al. 1995). Early evidence from the mouse model of FXS shows a specific impairment of amygdala dependent behaviors (Paradee et al. 1999). More recent work in this model has found both pre and post synaptic deficits within the amygdala, including blunted long term potentiation (A. Suvrathan et al. 2010; Aparna Suvrathan and Chattarji 2011; Olmos-Serrano et al. 2010; Zhao 2005). However, studies of amygdalar activation in response to social stimuli in the FXS population have yielded conflicting results. Using functional brain resonance imaging (fMRI) techniques, Watson et al. showed that the amygdala became abnormally sensitized in male adolescents with FXS upon repeated exposure to direct gaze faces (Watson et al. 2008). In an fMRI study of facial processing in females with FXS, however, Hagan et al. did not report any differences in amygdala activation, though significantly reduced anterior cingulate activation was documented (Hagan et al. 2008). We documented reduced amygdalar activation in response to social stimuli among individuals who carry the *FMR1* premutation (50 to 200 CGG repeats), females with FXS and males with mosaic expression of the FXS allele as compared to controls with normal *FMR1* alleles (Hessl et al. 2007; Hessl et al. 2011; Kim et al. 2012).

This reduction in amygdalar activation to fearful faces appears to be a dose responsive effect of FMRP expression, therefore it may be particularly prominent among individuals with the FXS full mutation, in whom FMRP expression is the most dramatically reduced (Hessl et al. 2011; Kim et al. 2012). This finding may be particularly relevant to the FXS phenotype, as clinical measures of psychiatric symptoms, autistic symptoms and anxiety have all been associated with an "FMRP mediated blunted amygdala response" (Hessl et al. 2007; Hessl et al. 2011; Kim et al. 2012). To date these studies have largely focused on carriers of the premutation and on individuals with a variety of genetic protective mechanisms (e.g. female heterozygotes and males with mosaic expression of the FXS allele) whereas data on males with the full mutation, who are often the most severely affected, is limited. The demanding nature of fMRI protocols has generally precluded the study of these individuals, as issues of hyperactivity, high anxiety and intellectual disability may prevent the level of compliance and understanding necessary for successful completion of fMRI protocols.

Emotion potentiated startle offers a less invasive, biobehavioral probe of amygdala activation. Startle potentiation was originally studied in animals, where the startle reflex, evoked by an auditory stimulus, was demonstrably enhanced by fearful emotional states (Koch and Schnitzler 1997; Davis et al. 1993). The startle response in humans may be measured via electromyography (EMG) of the eye-blink and is not only potentiated by threatening circumstances but is also modulated by more subtle internal emotional states and external emotional stimuli (Cuthbert et al. 1996; Vrana et al. 1988). Startle reflex circuitry receives a direct projection from the central nucleus of the amygdala and it is this projection that is believed to modulate the startle response in the context of varying internal emotional states (Koch and Schnitzler 1997). Early animal studies demonstrated both that direct electrical stimulation of the amygdala potentiated the startle response and that amygdalar lesions abolished fear potentiation of the reflex (Rosen and Davis 1988; Hitchcock and Davis 1986). In humans, early evidence of the amygdala's role in startle modulation came from studies of rare patients with temporal lobe and amygdalar lesions, who demonstrated an absence of startle potentiation and emotion modulation (T. W. Buchanan et al. 2004; Funayama et al. 2001; Angrilli et al. 1996). Pissiota et al. used modern positron emission tomography imaging techniques to provide further evidence of a role for the amygdala in modulating the startle response: these authors evaluated startle responses during presentation of images of a feared object in patients with specific phobia and demonstrated simultaneous amygdalar activation and startle potentiation, supporting the idea of startle modulation as a readout of amygdalar activation (Pissiota et al. 2003). Emotion potentiated startle thus provides a physiologic probe of amygdalar activation whose less demanding nature may make it more appropriate for use in intellectually disabled populations than fMRI.

To our knowledge to date there have been only two studies of startle potentiation in the fragile X spectrum population. Our group first demonstrated reduced startle potentiation to fearful stimuli in a sample of adult men with the premutation (Hessl et al. 2007). More recently, we have conducted a study of autonomic dysregulation in a sample of males with autism spectrum disorders (ASD) and males with FXS both with and without ASD which included measures of startle potentiation (Cohen et al. 2013). Interestingly, this study demonstrated hyper reactivity to positive social images only among individuals with comorbid FXS and ASD. The current study examines startle potentiation in a much larger

group of children and adolescent males and females with the FXS full mutation in comparison to individuals with typical development and individuals with idiopathic intellectual disabilities and social impairments comparable to the group with FXS.

We had two primary aims:

1. To determine whether individuals with FXS demonstrate abnormal potentiation to human emotional faces relative to developmentally disabled and typically developing controls
2. To determine whether degree of emotion potentiation is associated with behavioral measures of social avoidance

METHODS

Participants

Participants enrolled in the study included 110 children and adolescents with FXS (43 female; 12.1 years +/− 5.0 years), 82 children and adolescents with intellectual disability and social impairments without FXS (DD; 40 female; 12.6 years +/− 4.9 years), and 79 typically developing children and adolescents (TD; 39 female; 10.0 years +/− 3.7 years). Of those enrolled, 35 participants with FXS (5 female), 23 with DD (13 female) and 6 with TD (0 female) did not complete the protocol successfully or experienced errors in data collection. Eight participants with FXS (2 female), 5 with DD (3 female) and 6 with TD (3 female) did not demonstrate a reliable startle to auditory stimuli for calculation of potentiation (table 1).

The final group used for analysis consisted of 67 participants with FXS (36 female) aged 5.5–25.9 (M:13.6, SD:5.1) 33 of whom had autism spectrum disorder (ASD), 54 with DD (24 female) aged 4.9–23.6 (M:13.7, SD:4.9) 17 of whom had ASD and 67 with TD (36 female) aged 4.9–18.2 (M:13.7 SD:4.9) (table 2). This sample was 72.3% Caucasian, 3.2% African American, 5.3% Asian, 2.7% American Indian/Alaskan Native, 6.4% more than one race, 5.3% other and 2.7% unknown. There were no significant differences in racial distribution between groups.

All participants were seen at the research clinic of the UC Davis MIND Institute. Participants were recruited as part of a larger physiology study. Participants with TD or DD were recruited from the MIND Institute recruitment core or advertisements in newsletters and clinics. Recruitment of the group with DD was designed such that rates of ASD in this group would be comparable to rates of ASD in the group with FXS, thus comparison of these groups may help to specify the effect of the *FMR1* gene mutation. Potential participants with TD with prior psychiatric diagnosis or treatment, learning disability, CNS involvement or siblings with ASD were excluded. Any individual with known hearing loss was also excluded. FXS status of participants diagnosed with FXS was confirmed by *FMR1* DNA testing using both PCR and Southern Blot analysis as previously described (Tassone et al. 2004; Saluto et al. 2005). Participants with DD who exhibited symptoms of FXS and had not been previously tested were also screened for FXS. Participants with FXS were recruited from a pool of patients referred to the MIND Institute for clinical visits and their families. Parental consent was obtained prior to the research assessment.

Physiological Assessment

All physiological assessments were conducted in a dedicated research laboratory which consisted of an experimental room and an observation/equipment control room. Prior to the experimental session, each participant was familiarized with the laboratory and electrodes were applied. In addition, many participants with FXS or DD were given electrode application materials (adhesive collars, etc.) and reviewed a DVD with their parent of a model participant completing the protocol prior to the session. Participants with FXS or DD and significant anxiety or limited language were shown a picture schedule of activities to improve understanding and compliance.

The protocol was administered using the James Long presentation and psychophysiology recording system (James Long Company, Caroga Lake, NY). Participants were shown happy, fearful, and neutral faces, and matched scrambled images of the faces obtained from the NimStim Face Stimulus library (Tottenham et al. 2009). Thirty-two images (8 of each type) were presented in a random order for 6 seconds each with a varying interstimulus interval of 4, 6 or 8s. A black screen with a white fixation cross at its center was presented during the interstimulus interval. Acoustic stimuli were presented binaurally through Telephonics highimpedance headphones. Startle stimuli were 50ms 105db white noise pulses, with 0ms rise and fall times delivered randomly at 1.5, 2.5, 3.5 or 4.5s after stimulus onset. During presentation of some images, no probe was administered. See figure 1 for sample visual stimuli and a schematic of the protocol.

Orbicularis oculi electromyogram (EMG) was recorded bipolarly from the right eye, with Electro-Cap International, Inc. (Eaton, OH) E21-6S 6mm tin cup electrodes 1.0cm apart, edge to edge, as close to the margin of the lower lid as possible, and the lateral electrode 0.6cm medial to the exterior canthus. A ground electrode was placed behind the right ear, on the mastoid. EMGs were amplified at a fixed gain (1,000, with an A/D input range of ±2.5V) and with band pass of 10–250Hz. Electrode impedances were generally maintained below 5kΩ and were measured before and after the procedure. All data were digitized at 1kHz.

Data Scrutiny and Analysis

The orbicularis oculi EMG data was scrutinized individually on each trial. Participants with no visual EMG response to more than 50% of the startle stimuli were deemed non-responders and were excluded from analysis as reported above. Raw EMG was digitally bandpass filtered at 80–240Hz. The data were analyzed in 75% overlapping 8ms windows, yielding a time resolution of 2ms. Baseline EMG activity was sampled 50ms before stimulus onset to 20ms after stimulus onset and aggregated across all trials. This aggregated baseline was used to detect confounding natural blinks exceeding baseline. Trials with baseline periods in which the threshold was exceeded (greater than 2 SD above aggregate baseline mean EMG) were rejected from analysis. The EMG peak magnitude between 20ms and 200ms post startle probe onset was analyzed for each trial.

An audiovisual recording of the participant's head and upper body was stored on DVD. Off-line coding to quantify gaze behavior and to mark events that typically produce artifacts was

done from the DVD recording using the Video Coding System (James Long Company, Caroga Lake, NY). Specifically, each participant's gaze fixation "on" vs "off" the screen was coded continuously. Trials were accepted for further analysis only if the participant was behaviorally compliant during administration of the auditory probe and had looked at the image prior to the onset of the auditory probe. Total gaze time was quantified.

Neuropsychiatric Evaluation

Each participant was administered a standardized IQ test by trained UC Davis MIND Institute personnel: 67.5% were given the Wechsler Abbreviated Scale of Intelligence, 9.8% the Wechsler Intelligence Scale for Children 4th Ed., 4.1% the Wechsler Adult Intelligence Scale 3rd Ed., 1.5% the Wechsler Preschool and Primary Scale of Intelligence 3rd Ed. (The Psychological Corporation, San Antonio, TX) and 13.4% the Stanford Binet 5th Ed. (Riverside Publishing, Rolling Meadows, IL). Valid IQ scores were not available for 16 out of 281 participants. These individuals did not differ significantly from the remainder of the sample in age, or on any physiological or behavioral measure.

Participants with FXS or DD were evaluated for the presence of features of autism using the Autism Diagnostic Observation Schedule (ADOS) by a trained experimenter or licensed psychologist who had reached reliability for research studies (Gotham et al. 2007; Lord et al. 1999; Lord et al. 1989).

Parent Report Questionnaires

The Aberrant Behavior Checklist-Community Edition, a parent report questionnaire of behavior developed for use in populations with ID, was collected (ABC) (Aman et al. 1985). Our group recently completed a large psychometric study of the ABC in FXS, yielding a somewhat altered factor structure specific to FXS including 6 validated subscales: irritability, hyperactivity, socially unresponsive/lethargic behavior, social avoidance, stereotypy and inappropriate speech (Sansone et al. 2012). The Behavioral Assessment for Children, second edition (BASC-2) was also collected (Reynolds and Kamphaus 2004). This is a parent report questionnaire that evaluates a variety of behavioral subscales, including withdrawal. Raw scores and T-scores for various age groups and both typically developing and clinical populations are provided.

RESULTS

Factors associated with protocol completion

Factors associated with successful protocol completion among participants with FXS were evaluated (see table 3). Independent samples t-tests were used to investigate the contribution of varying IQ and age to the rate of successful data collection among participants with FXS. Successful data collection was associated with increased age ($t(94)=5.194$, $p<.0005$) and increased IQ ($t(78)=3.391$, $p=.001$) among individuals with FXS (equal variances not assumed) (see table 3).

Chi-squared tests using the Yate's correction for continuity were used to evaluate whether a diagnosis of ASD influenced rates of successful data collection among participants with

FXS. Participants with FXS comorbid with ASD were significantly less likely to successfully complete the protocol than those with FXS only, $\chi^2(1, N=108)=6.91$, $p=.009$.

Independent samples t-tests were used to investigate the contribution of varying levels of irritability and hyperactivity, as measured by the ABC, to the rate of successful data collection among participants with FXS. Successful data collection was associated with both decreased irritability scores ($t(101)=-2.56$, $p=.012$) and decreased hyperactivity scores ($t(97)= -3.28$, $p=.001$) among individuals with FXS (equal variances assumed) (see table 3).

Group differences in age and intellectual functioning

The subset of participants with valid physiological data was inspected for differences in age and IQ (table 2).

One way analysis of variance (ANOVA) revealed significant differences between groups on age among males ($F(2, 89)=11.592$, $p<.0005$). Post hoc tests using Tukey HSD revealed that males with TD were significantly younger than both males with DD ($p<.0005$) and males with FXS ($p<.0005$). Males with DD and males with FXS did not differ significantly on age ($p=.132$). Females in all groups were similar in age ($p=.236$).

An independent samples t-tests with equal variances not assumed revealed that females with FXS had significantly higher IQ's than females with DD ($t(56)=4.795$, $p<.0005$). An independent samples t-test with equal variances assumed revealed that males with DD did not differ significantly from males with FXS on the basis of IQ scores ($p=.446$).

A chi-squared test using the Yate's correction for continuity was used to compare rates of ASD among participants with DD and participants with FXS. The group with DD was well matched to the group with FXS in terms of social impairments: no significant differences were found ($p=.064$).

Visual attention to stimuli

One way ANOVA was used to compare gaze behavior between experimental groups. A significant difference was found between groups ($F(2, 181)=15.671$, $p<.0005$). Post hoc analyses using the Tukey HSD revealed that participants with TD spent significantly more time looking at the stimuli than both participants with DD ($p<.0005$) and participants with FXS ($p<.0005$), but the gaze behavior of participants with DD and participants with FXS did not differ significantly ($p=.979$).

To explore the impact that differential gaze behavior may have on the physiological data, we used Pearson correlations to investigate the association between time spent looking at the images and median startle response amplitude to each valence within each experimental group. No significant correlations were found.

Potentiated startle

To confirm the effectiveness of the protocol at eliciting emotion potentiated startle responses, a repeated measures ANOVA was conducted among the typically developing group to compare responses to each valence. Raw blink amplitudes for each trial were first

Z-scored within participant to eliminate interparticipant differences due to individual variability in baseline response strength. The means of these scores within each valence were used as dependent variables. Sex was included as a factor. There was a significant effect of valence: Wilks' Lambda=0.862, F=5.026, p=0.009, partial eta squared=.138. Fearful faces elicited the strongest response, followed by happy and finally neutral faces. Pair wise comparisons revealed that responses to fearful faces were significantly different than neutral (p=.008) but responses to happy faces did not differ significantly from those to fearful (p=.991) or neutral faces (p=.114). The interaction effect between sex and valence was non-significant (p=.606).

To address the first of our aims and determine whether individuals with FXS demonstrate enhanced potentiation in relation to DD and TD controls, potentiation was first quantified for each of the emotional faces using the following formula:

$$Potentiation\ to\ Emotional\ Face = \frac{Median\ Emotional\ Blink\ Amplitude - Median\ Neutral\ Blink\ Amplitude}{Median\ Neutral\ Blink\ Amplitude} \times 100$$

A two-way between groups ANOVA was conducted to explore the impact of group on the potentiation scores. Both gender and group were included as factors. IQ, age and looking time were excluded from the model due to lack of correlation with the physiology (see table 4). There was a significant difference between groups on fearful face potentiation (FFP): $F(2,163)$=4.392, p=.014, partial eta squared=.051 (see figure 2). Post hoc tests using the Tukey HSD revealed that participants with TD had significantly stronger FFP than both the group with FXS (p=.045) and the group with DD (p=.016). The group with FXS showed stronger potentiation than the group with DD, though this difference did not reach significance (p=.876). There was no significant main effect of gender (p=.142) or interaction effects between group and gender (p=.737). There was no significant difference between groups on happy face potentiation (HFP): p=.618 (see figure 2). There was no significant main effect of gender (p=.725). There was a significant interaction effect between group and gender (p=.040): among males, participants with TD exhibited the strongest HFP, followed by participants with FXS and finally participants with DD. Among females, participants with DD exhibited the strongest HFP, followed by participants with TD and finally participants with FXS. However, this effect did not survive adjustment of the significance level to .01 to account for violation of the assumption of homogeneity of variances.

To assess the impact of autism status on potentiated startle, a three-way between groups ANOVA was conducted on data from the groups with DD and FXS only. Group, gender and autism status were included as factors. IQ, age and looking time were excluded from the model due to lack of correlation with the physiology. There was no main effect of autism status on FFP (p=.391), but there was a significant group by autism status interaction: F(1,92)=4.909, p=.029, partial eta squared=.051. No other significant effects on FFP were found. Follow-up subgroup analyses using independent samples t-tests revealed that FFP was not significantly different between individuals with FXS and ASD and individuals with FXS without ASD (p=.311). However, individuals with DD and ASD had significantly

lower FFP than individuals with DD without ASD: $t(44)=2.350$, $p=.023$. There was no main effect of autism status on HFP ($p=.767$) and no interaction effects were found.

Social avoidance behavior ratings and association with potentiated startle

Social avoidance behavior among participants with FXS or DD was measured using the social avoidance subscale of the ABC (SA)(Sansone et al. 2012). An independent samples t-test revealed that SA scores were similar among participants with FXS ($M=1.75$, $SD=2.26$) and participants with DD ($M=2.09$, $SD=2.77$; $p=.482$). Social avoidance behavior among participants with TD was measured using T-scores on the withdrawal subscale of the BASC-2 (SW) (Reynolds and Kamphaus 2004). To address our second aim, we used Pearson correlation to assess the degree of association between emotion potentiated startle and SA or SW scores among participants with FXS, DD or TD (Sansone et al. 2012; Reynolds and Kamphaus 2004). Age, IQ and looking time were excluded due to lack of correlation with the physiology. There was a significant, though modest, two-tailed, positive correlation between FFP and SA among the group with FXS: $r(51)=.374$, $p=.006$. No other significant correlations were found.

DISCUSSION

This study showed that children and adolescents with FXS have an abnormally reduced potentiation to fearful face stimuli, a biobehavioral probe of the amygdala. The potential clinical relevance of this finding is demonstrated by a positive correlation between emotion potentiated startle and measures of social avoidance among individuals with FXS.

This blunting of amygdalar activation to fearful faces is in keeping with the results of previous studies of individuals affected by *FMR1* mutations (Kim et al. 2012; Hessl et al. 2011; Hessl et al. 2007). For example, blunted potentiated startle to fearful faces (as well as reduced amygdala response to fearful faces using fMRI) was reported in a sample of males with the *FMR1* premutation as compared to a sample of typically developing controls (Hessl et al. 2007).

Here we show that blunted amygdalar response among individuals with FXS is specific to fearful faces. Startle potentiation by happy faces among individuals with FXS was not significantly different from either comparison group. These results are in contrast with a recent study by our group of startle potentiation among adolescent males with FXS, in which we found no significant results in relation to negative stimuli and increased potentiation to positive stimuli among males with comorbid FXS and ASD (Cohen et al. 2013). Advantages of the current study include a much larger sample size and decreased possibility of type II error, inclusion of a developmentally delayed comparison group, and collection of data on gaze behavior and attention to stimuli, which facilitated the employment of strict trial by trial inclusion criteria based on gaze compliance. Furthermore, the current protocol employed carefully constructed control stimuli (neutral faces) in calculation of the potentiated startle measures of interest. The current findings are likely to be more specific to the amygdala: past studies of individuals with temporal lobe lesions, including lesions of the amygdala, and animal models with lesions of the amygdala, have found specific perturbations of modulation of the startle response by negative stimuli, with startle

modulation by positive stimuli remaining largely intact (Koch et al. 1996; Funayama et al. 2001). Therefore the current result of specific perturbation of startle modulation by negative and not positive stimuli suggests abnormalities specific to the amygdala. This is in keeping with work in the mouse model of FXS, which has demonstrated blunted long term potentiation in the amygdala and impaired fear potentiated startle, the homologous behavioral assay in rodents (Paradee et al. 1999; A. Suvrathan et al. 2010; Zhao 2005; Olmos-Serrano et al. 2010).

Neuroanatomical studies of the amygdala in individuals with FXS have demonstrated it to be reduced in size (Gothelf et al. 2008; Hazlett et al. 2009). It is possible, therefore, that the blunting of amygdalar responses seen here may be attributable to differences in amygdala size, which could not be measured by our techniques. However, past studies of startle potentiation among individuals with partial lesions of the temporal lobe and amygdala have shown that the degree of potentiation expressed does not appear to be related to the size of the amygdala (T. W. Buchanan et al. 2004). It is therefore unlikely that the blunted response demonstrated here can be accounted for purely by neuroanatomical differences.

Potentiation to fearful faces among individuals with FXS was significantly different from TD controls, but was not significantly different from a group of DD controls matched on both intellectual and social impairment. The specific deficit in FFP found here, then, may alternatively be characteristic of developmental delay or social impairment and not specific to the *FMR1* mutation itself. However, among individuals with FXS only, a positive association between potentiation and social avoidance behaviors was found, suggesting that differential activity of the amygdala within the group may contribute uniquely to phenotypic variation in individuals with FXS. This positive association between clinical phenotypic features and startle potentiation to fearful faces, even in the presence of an overall reduction in amygdalar activation has been previously demonstrated by our laboratory in a sample of men with the *FMR1* premutation (Hessl et al. 2007). Furthermore, we demonstrated a significant group by ASD interaction effect on FFP in the absence of a main effect of ASD on potentiated startle. This finding adds to a growing body of evidence suggesting that the neuroanatomical and neuropathological underpinnings of ASD differ between individuals with FXS and individuals with idiopathic autism (Hazlett et al. 2009; Yuhas et al. 2011; Hoeft et al. 2011)

The finding of reduced amygdalar activation among individuals with FXS is consistent, but counterintuitive, as anxious individuals without FXS have classically been associated with hyperactive amygdalae (Shin et al. 2009). Individuals with social phobia, for example, demonstrate increased amygdala reactivity in response to social stimuli and this hyperactivity is correlated with increased severity in anxiety symptoms (Phan et al. 2006; Thomas et al. 2001). FXS, which brings with it a host of anxiety-related clinical problems, might be expected to mimic these findings. However, here we have demonstrated the opposite pattern of amygdalar hypo-activity. Work by Wolfensberger and colleagues (2008) may provide insight into this seemingly unexpected result. These authors investigated amygdalar activation to negative faces among monozygotic twins who were either concordant or discordant for anxiety (Wolfensberger et al. 2008). Among twins who were discordant for anxiety, in whom the anxiety risk is presumably incurred by environmental

factors, anxious twins demonstrated the expected hyperactive amygdalar responses (Wolfensberger et al. 2008). However, among twins who were concordant for anxiety, in whom risk for anxiety is presumably incurred by genetic factors, anxious individuals actually showed amygdalar hypoactivity (Wolfensberger et al. 2008). In light of these results, it may not be as surprising that individuals with FXS, a genetic risk for anxiety, would show hypoactivation of the amygdala and would not necessarily mimic a typically developing population of individuals with anxiety. Furthermore, Frenkel and colleagues (2011) have demonstrated blunted EEG responses to fearful faces in individuals with anxiety, contradicting the classical view of anxiety as an internal state of hypervigilance and hyperarousal and instead depicting it as a state of blunted responsiveness (Frenkel and Bar-Haim 2011). Thus the reduced amygdalar activation seen among individuals with FXS may represent a significant blunting of social responsiveness that impairs processing of emotional faces and renders social interactions more ambiguous, contributing to social anxiety in this population.

A limitation to this study is the lack of eye tracking data to document at exactly which area of the social stimuli participants were looking. Past studies have demonstrated that sympathetic activation among individuals with FXS is associated with time spent looking at the eye region in images of static faces, and that individuals with FXS demonstrate an abnormal sensitization of amygdalar reactivity upon repeated exposure to direct gaze faces (Farzin et al. 2009; Watson et al. 2008). Given the predominance of gaze avoidance in individuals with FXS, it has been suggested that individuals may avoid looking at faces to mediate this hyperarousal (Farzin et al. 2009). Also, cortisol, a stress hormone, is reduced when children with FXS demonstrate prominent gaze avoidance during social encounters (Hessl et al. 2006). Our results might therefore be explained by differing looking behavior between groups; for example individuals with FXS may find emotional faces to be aversive and respond by avoiding looking at arousing stimuli. Though we found time spent looking at the stimuli was not associated with increased startle amplitude in our sample, this measure did not evaluate time spent looking at specific regions of interest on the stimuli (ie the eye region) and therefore may be too low resolution to detect the relevant gaze behavior. This same limitation is shared by previous work demonstrating reduced startle potentiation among men with the *FMR1* premutation, therefore an alternative interpretation of these studies is that differing gaze behavior among individuals with *FMR1* mutations leads to attenuated amygdalar responses to social stimuli.

Although this study provides vital data on amygdalar function in males with FXS, a significantly impaired population which has been difficult to study with classical MRI techniques, the current study was nevertheless affected by several behavioral factors that limited compliance. Only 60% of individuals with FXS successfully completed the protocol and successful data collection was associated with increased IQ and age and reduced irritability, hyperactivity and social impairment, suggesting that the most severely affected individuals remain unmeasured.

Here we have presented evidence of reduced potentiated startle among individuals with FXS. Future studies may build upon these results by incorporating high resolution eye tracking data and determining whether this reduction is due to an inherent property of the

amygdala among individuals with FXS, or to differences in gaze behavior between individuals with FXS and typically developing individuals. Another exciting avenue for future research lies in investigation of the developmental time course of emotional face response among individuals with FXS and DD. Neural response to emotional faces is known to change throughout childhood and adolescence among typically developing children, and therefore it is possible that the blunted responses seen among individuals with FXS and DD in this study reflect an altered developmental time course of this response (Batty and Taylor 2006). Finally, this protocol has potential for providing essential data on an increased portion of the population of individuals with FXS if included in future treatment outcome studies, though it is most appropriate for use in older, higher functioning individuals.

Acknowledgments

The authors thank Lori Abucayan, Dr. Aaron Campbell, Susan Harris, Maisha Ollison, Dr. Andrea Schneider and Dr. Jennifer Yuhas-Duffy for their contribution to this research. Additionally, thanks to Dr. David Amaral for his suggestion to examine fear potentiated startle as a probe of amygdala involvement in the fragile X syndrome phenotype. We are especially thankful to the participants and their families for their contribution to the understanding of FXS. This work was supported by National Institute of Mental Health Grant MH7754.

WORKS CITED

Adolphs R. What does the amygdala contribute to social cognition? Annals of the New York Academy of Sciences. 2010; 1191(1):42–61. [PubMed: 20392275]

Aman MG, Singh NN, Stewart AW, Field CJ. The aberrant behavior checklist: a behavior rating scale for the assessment of treatment effects. Am J Ment Defic. 1985; 89(5):485–491. [PubMed: 3993694]

Amaral DG. The primate amygdala and the neurobiology of social behavior: implications for understanding social anxiety. Biol Psychiatry. 2002; 51(1):11–17. [PubMed: 11801227]

Angrilli A, Mauri A, Palomba D, Flor H, Birbaumer N, Sartori G, et al. Startle reflex and emotion modulation impairment after a right amygdala lesion. Brain. 1996; 119:1991–2000. [PubMed: 9010003]

Baird AA, Gruber SA, Fein DA, Maas LC, Steingard RJ, Renshaw PF, et al. Functional magnetic resonance imaging of facial affect recognition in children and adolescents. J Am Acad Child Adolesc Psychiatry. 1999; 38(2):195–199. [PubMed: 9951219]

Batty M, Taylor MJ. The development of emotional face processing during childhood. Developmental Science. 2006; 9(2):207–220. [PubMed: 16472321]

Birbaumer N, Grodd W, Diedrich O, Klose U, Erb M, Lotze M, et al. fMRI reveals amygdala activation to human faces in social phobics. Neuroreport. 1998; 9(6):1223–1226. [PubMed: 9601698]

Brown WT, Jenkins EC, Cohen IL, Fisch GS, Wolf-Schein EG, Gross A, et al. Fragile X and autism: a multicenter survey. American Journal of Medical Genetics. 1986; 23(1–2):341–352. [PubMed: 3513570]

Buchanan TW, Tranel D, Adolphs R. Anteromedial Temporal Lobe Damage Blocks Startle Modulation by Fear and Disgust. Behavioral Neuroscience. 2004; 118(2):429–437. [PubMed: 15113270]

Buchanan, TW.; Tranel, D.; Adolphs, R. The Human Amygdala in Social Function. In: Whalen, PJ.; Phelps, EA., editors. The Human Amygdala. New York: The Guilford Press; 2009. p. 289-318.

Chudley AE, Hagerman RJ. Fragile X Syndrome. The Journal of Pediatrics. 1987; 110(6):821–831. [PubMed: 3295158]

Cohen S, Masyn K, Mastergeorge A, Hessl D. Psychophysiological Responses to Emotional Stimuli in Children and Adolescents with Autism and Fragile X Syndrome. Journal of Clinical Child & Adolescent Psychology. 2013:1–14.

Cordeiro L, Ballinger E, Hagerman RJ, Hessl D. Clinical assessment of DSM-IV anxiety disorders in fragile X syndrome: prevalence and characterization. Journal of Neurodevelopmental Disorders. 2010:1–11. [PubMed: 22127836]

Cuthbert BN, Bradley MM, Lang PJ. Probing picture perception: Activation and emotion. Psychophysiology. 1996; 33:103–111. [PubMed: 8851238]

Davis M. The Role of the Amygdala in Fear and Anxiety. Annu Rev Neurosci. 1992; 15:353–375. [PubMed: 1575447]

Davis M, Falls WA, Campeau S, Kim M. Fear-potentiated startle: a neural and pharmacological analysis. Behavioural Brain Research. 1993; 58:175–198. [PubMed: 8136044]

Farzin F, Rivera SM, Hessl D. Brief Report: Visual Processing of Faces in Individuals with Fragile X Syndrome: An Eye Tracking Study. Journal of Autism and Developmental Disorders. 2009; 39(6): 946–952. [PubMed: 19399604]

Feng Y, Absher D, Eberhart DE, Brown V, Malter HE, Warren ST. FMRP Associates with Polyribosomes as an mRNP, and the I304N Mutation of Severe Fragile X Syndrome Abolishes This Association. Molecular cell. 1997; 1(1):109–118. [PubMed: 9659908]

Frenkel TI, Bar-Haim Y. Neural activation during the processing of ambiguous fearful facial expressions: An ERP study in anxious and nonanxious individuals. Biological Psychology. 2011; 88(2–3):188–195. [PubMed: 21846487]

Freund LS, Reiss AL, Abrams MT. Psychiatric Disorders Associated With Fragile X in the Young Female. Pediatrics. 1993; 91:321–329. [PubMed: 8380924]

Funayama ES, Grillon C, Davis M, Phelps EA. A Double Dissociation in the Affective Modulation of Startle in Humans: Effects of Unilateral Temporal Lobectomy. Journal of Cognitive Neuroscience. 2001; 13(6):721–729. [PubMed: 11564317]

Garber KB, Visootak J, Warren ST. Fragile X syndrome. European Journal of Human Genetics. 2008; 16:666–672. [PubMed: 18398441]

Gotham K, Risi S, Pickles A, Lord C. The Autism Diagnostic Observation Schedule: revised algorithms for improved diagnostic validity. J Autism Dev Disord. 2007; 37(4):613–627. [PubMed: 17180459]

Gothelf D, Furfaro JA, Hoeft F, Eckert MA, Hall SS, O'Hara R, et al. Neuroanatomy of fragile X syndrome is associated with aberrant behavior and the fragile X mental retardation protein (FMRP). Annals of Neurology. 2008; 63(1):40–51. [PubMed: 17932962]

Hagan CC, Hoeft F, Mackey A, Mobbs D, Reiss AL. Aberrant Neural Function During Emotion Attribution in Female Subjects With Fragile X Syndrome. Journal of the American Academy of Child & Adolescent Psychiatry. 2008; 47(12):1443–1454. [PubMed: 18981933]

Hagerman RJ. Fragile X Syndrome. Current Problems in Pediatrics. 1987:626–674.

Hazlett HC, Poe MD, Lightbody AA, Gerig G, Macfall JR, Ross AK, et al. Teasing apart the heterogeneity of autism: Same behavior, different brains in toddlers with fragile X syndrome and autism. J Neurodev Disord. 2009; 1(1):81–90. [PubMed: 20700390]

Hessl D, Glaser B, Dyer-Friedman J, Reiss AL. Social behavior and cortisol reactivity in children with fragile X syndrome. Journal of Child Psychology and Psychiatry. 2006; 47(6):602–610. [PubMed: 16712637]

Hessl D, Rivera S, Koldewyn K, Cordeiro L, Adams J, Tassone F, et al. Amygdala dysfunction in men with the fragile X premutation. Brain. 2007; 130(2):404–416. [PubMed: 17166860]

Hessl D, Wang JM, Schneider A, Koldewyn K, Le L, Iwahashi C, et al. Decreased Fragile X Mental Retardation Protein Expression Underlies Amygdala Dysfunction in Carriers of the Fragile X Premutation. Biological Psychiatry. 2011; 70(9):859–865. [PubMed: 21783174]

Hitchcock J, Davis M. Lesions of the Amygdala, but Not of the Cerebellum or Red Nucleus, Block Conditioned Fear as Measured With the Potentiated Startle Paradigm. Behavioral Neuroscience. 1986; 100(1):11–22. [PubMed: 3954873]

Hoeft F, Walter E, Lightbody AA, Hazlett HC, Chang C, Piven J, et al. Neuroanatomical differences in toddler boys with fragile x syndrome and idiopathic autism. Arch Gen Psychiatry. 2011; 68(3): 295–305. [PubMed: 21041609]

Kau ASM, Tierney E, Bukelis I, Stump MH, Kates WR, Trescher WH, et al. Social behavior profile in young males with fragile X syndrome: Characteristics and specificity. [Article]. American Journal of Medical Genetics Part A. 2004; 126A(1):9–17. [PubMed: 15039968]

Kim SY, Burris J, Bassal F, Koldewyn K, Chattarji S, Tassone F, et al. Fear-Specific Amygdala Function in Children and Adolescents on the Fragile X Spectrum: A Dosage Response of the FMR1 Gene. Cerebral Cortex. 2012

Koch M, Schmid A, Schnitzler H-U. Pleasure-attenuation of startle is disrupted by lesions of the nucleus accumbens. Neuroreport. 1996; 7:1442–1446. [PubMed: 8856694]

Koch M, Schnitzler H-U. The acoustic startle response in rats-circuits mediating evocation, inhibition and potentiation. Behavioral Brain Research. 1997; 89:35–49.

Lachiewicz AM. Abnormal Behaviors of Young Girls With Fragile X Syndrome. American Journal of Medical Genetics. 1992; 43:72–77. [PubMed: 1605238]

Lord, C.; Rutter, M.; DiLavore, PC.; Risi, S. Autism Diagnostic Observation Schedule-WPS (ADOS-WPS). Los Angeles: Western Psychological Services; 1999.

Lord C, Rutter M, Goode S, Heemsbergen J, Jordan H, Mawhood L, et al. Autism diagnostic observation schedule: a standardized observation of communicative and social behavior. J Autism Dev Disord. 1989; 19(2):185–212. [PubMed: 2745388]

Mazzocco MMM, Freund LS, Baumgardner TL. Neuropsychological and Psychosoical Effects of the FMR-1 Full Mutation: Case Report of Monozygotic Twins Discordant for the Fragile X Syndrome. Neuropsychology. 1995; 9(4):470–480.

Olmos-Serrano JL, Paluszkiewicz SM, Martin BS, Kaufmann WE, Corbin JG, Huntsman MM. Defective GABAergic Neurotransmission and Pharmacological Rescue of Neuronal Hyperexcitability in the Amygdala in a Mouse Model of Fragile X Syndrome. Journal of Neuroscience. 2010; 30(29):9929–9938. [PubMed: 20660275]

Paradee W, Melikian HE, Rasmussen DL, Kenneson A, Conn PJ, Warren ST. Fragile X mouse: Strain effects of knockout phenotype and evidence suggesting deficient amygdala function. Neuroscience. 1999; 94(1):185–192. [PubMed: 10613508]

Phan KL, Fitzgerald DA, Nathan PJ, Tancer ME. Association between amygdala hyperactivity to harsh faces and severity of social anxiety in generalized social phobia. Biol Psychiatry. 2006; 59(5):424–429. doi:S0006-3223(05)01001-2 [pii]10.1016/j.biopsych.2005.08.012. [PubMed: 16256956]

Pissiota A, Frans O, Michelgard A, Appel L, Langstrom B, Flaten MA, et al. Amygdala and anterior cingulate cortex activation during affective startle modulation: a PET study of fear. European Journal of Neuroscience. 2003; 18:1325–1331. [PubMed: 12956731]

Reiss AL, Abrams MT, Greenlaw R, Freund LS, Denckla MB. Neurodevelopmental effects of the FMR-1 full mutation in humans. Nature Medicine. 1995; 1(2):159–167.

Reynolds, CR.; Kamphaus, RW. Behavior Assessment System for Children - Second Edition. I. American Guidance Service. , editor. MN: Circle Pines; 2004.

Roberts J, Weisenfeld L, Hatton D, Heath M, Kaufmann W. Social Approach and Autistic Behavior in Children with Fragile X Syndrome. Journal of Autism and Developmental Disorders. 2007; 37(9): 1748–1760. [PubMed: 17180715]

Rosen JB, Davis M. Enhancement of Acoustic Startle by Electrical Stimulation of the Amygdala. Behavioral Neuroscience. 1988; 102(2):195–202. [PubMed: 3365315]

Rousseau F, Heitz D, Biancalana V, Blumenfeld S, Kretz C, Boue J, et al. Direct Diagnosis by DNA Analysis of the Fragile X Syndrome of Mental Retardation. The New England Journal of Medicine. 1991; 325(24):1673–1681. [PubMed: 1944467]

Saluto A, Brussino A, Tassone F, Arduino C, Cagnoli C, Pappi P, et al. An enhanced polymerase chain reaction assay to detect pre- and full mutation alleles of the fragile X mental retardation 1 gene. J Mol Diagn. 2005; 7(5):605–612. [PubMed: 16258159]

Sansone SM, Widaman KF, Hall SS, Reiss AL, Lightbody A, Kaufmann WE, et al. Psychometric study of the Aberrant Behavior Checklist in Fragile X Syndrome and implications for targeted treatment. J Autism Dev Disord. 2012; 42(7):1377–1392. [PubMed: 21972117]

Shin, LM.; Rauch, SL.; Pitman, RK.; Whalen, PJ. The Human Amygdala in Anxiety Disorders. In: Whalen, PJ.; Phelps, EA., editors. The Human Amygdala. New York: The Guilford Press; 2009. p. 321-343.

Suvrathan A, Chattarji S. Fragile X syndrome and the amygdala. Current Opinion in Neurobiology. 2011; 21(3):509–515. [PubMed: 21555214]

Suvrathan A, Hoeffer CA, Wong H, Klann E, Chattarji S. Characterization and reversal of synaptic defects in the amygdala in a mouse model of fragile X syndrome. Proceedings of the National Academy of Sciences. 2010; 107(25):11591–11596.

Tassone F, Iwahashi C, Hagerman PJ. FMR1 RNA within the intranuclear inclusions of fragile X-associated tremor/ataxia syndrome (FXTAS). RNA Biol. 2004; 1(2):103–105. [PubMed: 17179750]

Thomas KM, Drevets WC, Dahl RE, Ryan ND, Birmaher B, Eccard CH, et al. Amygdala response to fearful faces in anxious and depressed children. Arch Gen Psychiatry. 2001; 58(11):1057–1063. [PubMed: 11695953]

Tottenham N, Tanaka JW, Leon AC, McCarry T, Nurse M, Hare TA, et al. The NimStim set of facial expressions: judgments from untrained research participants. Psychiatry Res. 2009; 168(3):242–249. doi:S0165-1781(08)00148-0 [pii]10.1016/j.psychres.2008.05.006. [PubMed: 19564050]

Verkerk AJMH, Pieretti M, Sutcliffe JS, Fu Y-H, Kuhl DPA, Pizzuti A, et al. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell. 1991; 65(5):905–914. [PubMed: 1710175]

Vrana SR, Spence EL, Lang PJ. The Startle Probe Response: A New Measure of Emotion. Journal of Abnormal Psychology. 1988; 97(4):487–491. [PubMed: 3204235]

Watson C, Hoeft F, Garrett AS, Hall SS, Reiss AL. Aberrant Brain Activation During Gaze Processing in Boys With Fragile X Syndrome. Archives of General Psychiatry. 2008; 65(11):1315–1323. [PubMed: 18981343]

Whalen, PJ.; Davis, FC.; Oler, JA.; Kim, H.; Kim, MJ.; Neta, M. Human Amygdala Responses to Facial Expressions of Emotion. In: Whalen, PJ.; Phelps, EA., editors. The Human Amygdala. New York: The Guilford Press; 2009. p. 265-288.

Whalen PJ, Shin LM, McInerney SC, Fischer H, Wright CI, Rauch SL. A functional MRI study of human amygdala responses to facial expressions of fear versus anger. Emotion. 2001; 1(1):70–83. [PubMed: 12894812]

Wolfensberger SPA, Veltman DJ, Hoogendijk WJG, Boomsma DI, de Geus EJC. Amygdala responses to emotional faces in twins discordant or concordant for the risk for anxiety and depression. NeuroImage. 2008; 41(2):544–552. [PubMed: 18396414]

Yuhas J, Cordeiro L, Tassone F, Ballinger E, Schneider A, Long JM, et al. Brief report: Sensorimotor gating in idiopathic autism and autism associated with fragile X syndrome. J Autism Dev Disord. 2011; 41(2):248–253. [PubMed: 20521090]

Zhao MG. Deficits in Trace Fear Memory and Long-Term Potentiation in a Mouse Model for Fragile X Syndrome. Journal of Neuroscience. 2005; 25(32):7385–7392. [PubMed: 16093389]

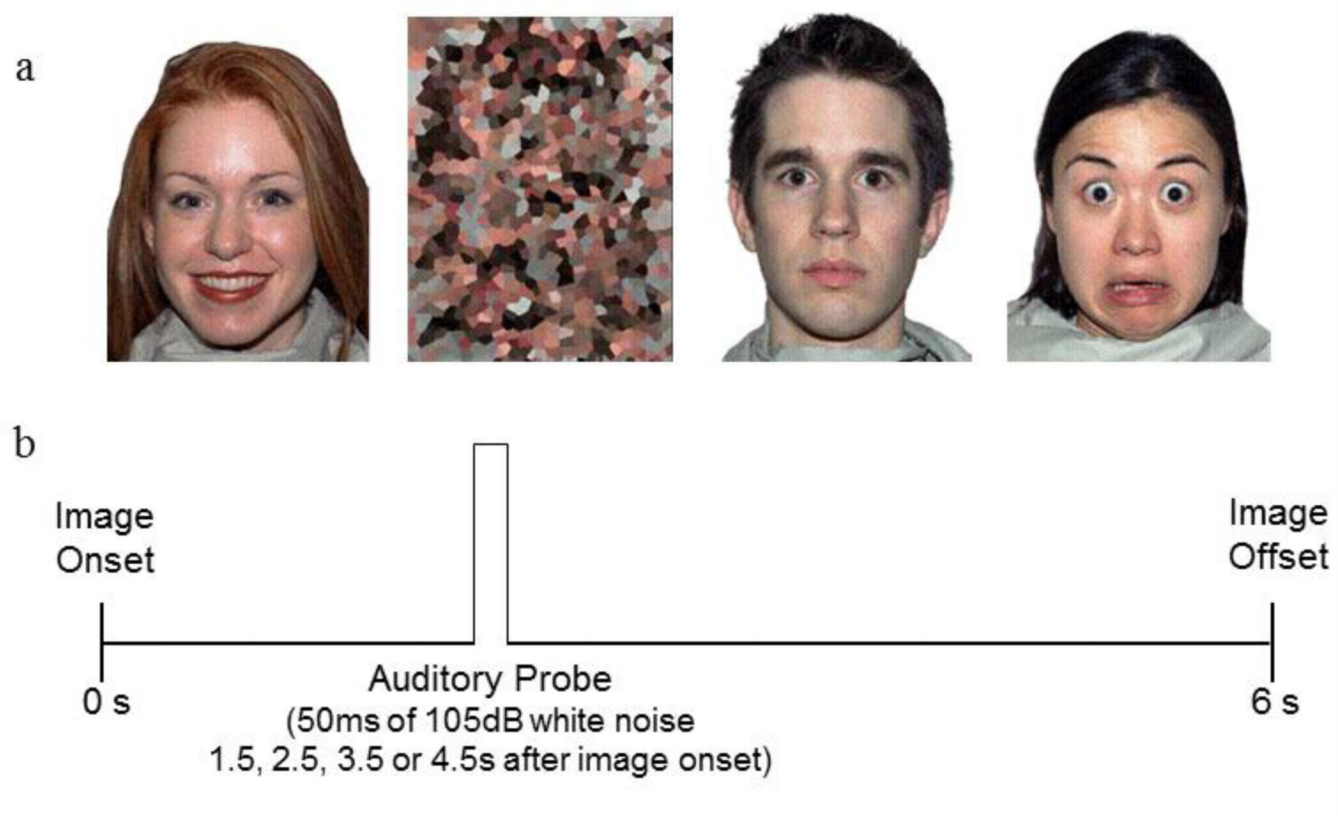

Figure 1.

Schematic representation of the protocol: a) Examples of the happy, scrambled, neutral and fearful images presented. b) Schematic representation of the startle stimulus.

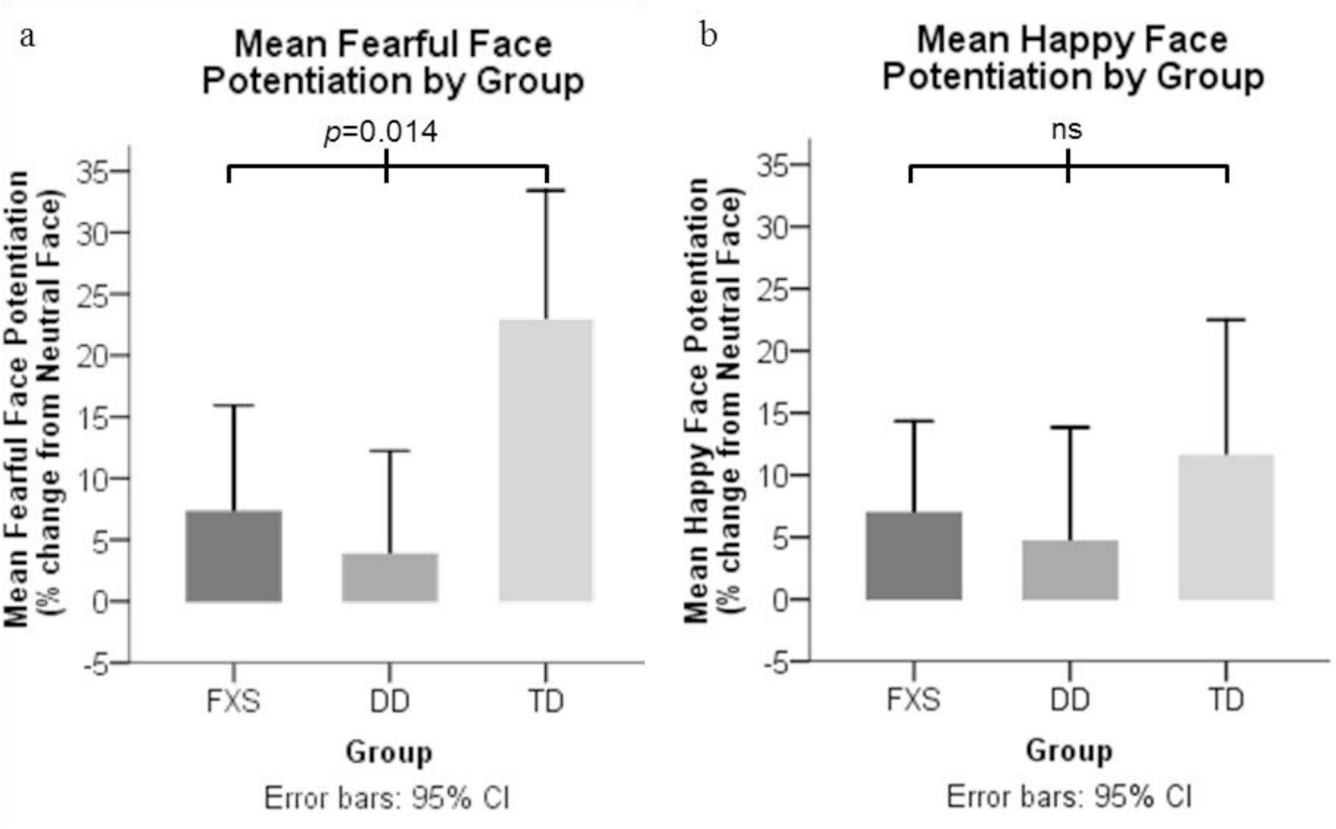

Figure 2.

Graphs of mean potentiation scores by group: a) Fearful potentiation by group. b) Happy potentiation by group.

Table 1

Categorization of data collection in each experimental group.

	FXS		DD		TD	
	N(female)	% Total N	N(female)	% Total N	N(female)	% Total N
Successful Physiological Data Collection	67(36)	60.9%	54(24)	65.9%	67(36)	84.8%
Non-Compliant or Erroneous Data Collection	35(5)	31.8%	23(13)	28.0%	6(0)	7.6%
Non-Responsive to Auditory Stimuli	8(2)	7.3%	5(3)	6.1%	6(3)	7.6%
Total	110(43)	100%	82(40)	100%	79(39)	100%

Table 2

Age, IQ, gaze behavior and frequency of ASD diagnoses in each experimental group.

		FXS		DD		TD		
		Mean (SD)	**Range**	**Mean (SD)**	**Range**	**Mean (SD)**	**Range**	
N*(% female)*		67*(53.7)*		54*(44.4)*		67*(53.7)*		
Age	Male	14.6(4.9)	6.4–24.0	15.1(4.8)	5.2–23.6	10.0(3.8)	5.0–18.2	****
	Female	12.7(5.1)	5.5–25.9	12.1(4.7)	4.9–22.0	10.6(3.8)	5.0–17.9	
IQ	Male	63.4(13.1)	45.0–87.0	61.0(10.9)	40.0–79.0	113.8(11.6)	88.0–129.0****	
	Female	80.8(17.8)	42.0–117.0	63.1(10.4)	40.0–83.0****	109.1(12.8)	84.0–137.0****	
Looking Time		83.6(20.5)	22.0–108.0	82.0(24.0)	19.6–108.0	97.5(14.4)	18.3–108.0****	
N w/ASD*(%)*		33*(49.3)*		17*(31.5)*				

Significant difference between FXS and relative control group at: ****$p \leq 0.0005$.

Table 3

Factors associated with successful protocol completion among participants with FXS.

	Protocol Completed		Protocol Not Completed		
	Mean (SD)	**Range**	**Mean (SD)**	**Range**	
N*(female)*	75*(38)*		35*(5)*		
Age	13.4(5.0)	5.5–25.9	9.2(3.4)	5.0–17.5	****
IQ	70.1(19.0)	30.0–117.0	59.2(12.9)	36.0–95.0	***
N with ASD *(%)*	40*(53.3)*		29*(82.9)*		**
Irritability Score	9.8(10.4)	0.0–41.0	15.2(8.9)	3.0–40.0	**
Hyperactivity Score	6.4(6.1)	0.0–25.0	11.0(6.9)	0.0–24.0	***

** $p \leq 0.01$;

*** $p \leq 0.001$;

**** $p \leq 0.0005$

Table 4

Relationship between age, IQ, looking time and emotional potentiated startle.

		Age		IQ		Looking Time	
		Pearson r	**Sig.**	**Pearson r**	**Sig.**	**Pearson r**	**Sig.**
FXS	FFP	−.055	.685	−.129	.357	−.095	.480
	HFP	.053	.696	−.092	.510	−.032	.816
DD	FFP	−.062	.678	−.022	.882	.162	.270
	HFP	.005	.975	.104	.490	−.079	.603
TD	FFP	.000	.997	−.124	.344	.070	.580
	HFP	.062	.634	−.026	.843	−.142	.272