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Aberrant face and gaze habituation in fragile X syndrome

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Abstract

Objective—We sought to investigate neural system habituation to face and eye gaze in fragile X syndrome (FXS), a disorder characterized by eye gaze aversion, among other social and cognitive deficits.

Method—Participants (age 15–25 years) included 30 individuals with FXS (14 females) and a comparison group of 25 individuals without FXS (12 females) matched for general cognitive ability and autism symptoms. We used fMRI to assess brain activation during a gaze habituation task. Participants viewed repeated presentations of 4 unique faces with either direct or averted eye gaze and judged the direction of eye gaze.

Results—Four participants (all males, 3 FXS) were excluded due to excessive head motion during fMRI scanning. Behavioral performance did not differ between groups. Less neural habituation (and significant sensitization) for the FXS group was found for all faces (direct and averted gaze) in cingulate gyrus, fusiform gyrus, and frontal cortex. Left fusiform habituation in female participants was directly correlated with higher, more typical, levels of fragile X mental retardation protein ($r(10)=0.67$, $p=0.02$), and inversely correlated with autism symptoms ($r_s(12)=-0.72$, $p<0.004$). There was no evidence for differential habituation to direct versus averted gaze within or between groups.

Conclusions—Impaired habituation and accentuated sensitization to face/eye gaze was distributed across multiple levels of neural processing. These results could help inform interventions such as desensitization therapy, which may help patients with FXS modulate anxiety and arousal associated with eye gaze thereby improving social functioning.

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Keywords

fMRI; habituation; social anxiety; eye gaze; fragile X syndrome; autism

Introduction

Fragile X syndrome (FXS), the most common inherited cause of intellectual disability, is the leading monogenic cause of autism spectrum disorder (ASD)(1). FXS results from a trinucleotide CGG repeat expansion (locus Xq27.3) leading to hypermethylation of the fragile X mental retardation 1 gene (FMR1) promoter region and reduced levels of *FMR1* protein (FMRP)(2). FMRP is involved in regulation of synaptic plasticity and dendritic pruning, both critical in neurodevelopment(3;4).

Reduced levels of FMRP are associated with cognitive impairment(5) as well as social deficits that overlap with characteristics of ASD and social anxiety disorder(6). Despite similarities, specific characteristics of some social deficits are unique to FXS. In the case of eye contact avoidance, individuals with FXS are sensitive to gaze initiation and find eye contact aversive whereas, in general, individuals with ASD are insensitive to social gaze(7). Eye gaze aversion in individuals with FXS is also associated with changes in skin conductance(8), cortisol reactivity(9) and pupillary reactivity(10), suggesting hyperarousal(11).

Eye gaze avoidance in FXS may also be linked to abnormalities in the neural circuitry underlying face/gaze processing as suggested by aberrant morphology in the fusiform gyrus, superior temporal gyrus and amygdala(12;13). Functional magnetic resonance imaging (fMRI) studies of gaze processing(14;15), indicate abnormal activation in brain regions supporting visual and social processing (fusiform and superior temporal gyri)(14), as well as regions underlying emotion processing (insula and amygdala)(15;16). Therefore, eye gaze avoidance may be linked to emotional responses to eye gaze, such as social anxiety symptoms known to be present behaviorally in FXS(17).

If social anxiety underlies eye gaze avoidance in FXS, then reducing anxiety with behavioral interventions such as exposure therapy may prove therapeutic. Such treatments have been shown to be effective in reducing symptoms of social anxiety disorder(18) and in behavioral shaping, a technique showing promise in FXS(19). Habituation to anxiety provoking stimuli is critical for successful desensitization and neural system plasticity is essential for habituation(20). We know little about neural system habituation in FXS, however deficits in synaptic plasticity have been established(4). These deficits may be associated with aberrant neural system habituation and understanding the extent of any habituation impairment will be important for developing behavioral treatments.

Neural system habituation is routinely measured by quantifying changes in the magnitude of fMRI activation to repeated stimuli, as demonstrated in typically developing individuals within systems underlying emotion processing(21). Given the association between FMRP and synaptic plasticity, we hypothesized that individuals with FXS would display aberrant neural system habituation. We used an fMRI paradigm with repeated presentations of faces

to examine neural system habituation to eye gaze in FXS. We included faces with direct and averted eye gaze to test a secondary hypothesis that habituation is abnormal for direct but not averted eye gaze. We included individuals with FXS from both sexes because previous fMRI studies have examined either only females(14) or only males(15) although both reported aberrant neural systems underlying gaze processing. We sought to describe effects that were associated with FXS regardless of sex, and irrespective of IQ, autism symptoms and adaptive functioning; therefore, our comparison group was matched to the FXS group on these criteria. As such, differences in habituation could be attributed primarily to FXS and not to behavioral symptoms. We also sought to investigate the relationship between neural system habituation and individual differences in level of FMRP and autism symptoms.

Methods

Participants, aged 15-25 years, included 30 individuals with FXS and a comparison group of 25 individuals without FXS. FXS diagnosis was confirmed via Southern blot DNA analysis(Kimball Genetics, Denver, CO). One female and three males showed evidence of mosaicism(with both premutation and full mutation); the remaining individuals were diagnosed with full mutation. Blood was taken for each individual in the FXS group to estimate FMRP percentage; analysis was based on the percentage of peripheral lymphocytes containing FMRP, as assessed by immunostaining techniques(Kimball Genetics,(22)). Saliva samples were taken for each individual in the comparison group and PCR analyses were performed to exclude the possibility of FXS diagnosis(23). Participants in the comparison group were diagnosed with idiopathic developmental delay, intellectual disability, or learning disability and were free from any other known genetic condition, premature birth(<34weeks), low birth weight(<2000g), and any serious medical or neurological conditions affecting growth and development including seizure disorder, diabetes and heart disease. All participants were free from MRI contraindications and met screening criteria for ability to tolerate fMRI procedures (e.g. ability to hold still, minimal sensitivity to loud noises, cognitive level adequate to complete the behavioral component of the imaging task).

Participants with FXS were recruited across the United States and Canada and participants in the comparison group were recruited across Northern California. Both groups were recruited through advertisements, referrals and word of mouth. Nation-wide groups (FXS) and regional centers (comparison group) were also used. Participants and/or parents gave written informed consent and assent to participate in the study. Stanford University's Institutional Review Board approved all protocols.

The groups were matched on sex, intellectual functioning (IQ, Wechsler Abbreviated Scale for Intelligence (age $\geq$ 17years)(24) or Wechsler Intelligence Scale for Children (age<17years)(25)), autism symptoms (Autism Diagnostic Observation Schedule [ADOS] (26)) and adaptive behavior (Vineland Adaptive Behavior Scale(27)). Assessments were administered and scored by qualified personnel based on standard procedures listed in each testing manual. The ADOS was administered and scored by individuals trained by a research certified administrator and reliability was verified via consensus scoring.

Demographics and assessment scores for the participants who were included in the fMRI analysis (27 FXS, 24 comparison) are presented in Table 1 (see below for exclusion criteria). The groups did not differ significantly in sex, IQ, ADOS or Vineland scores (all p 's > 0.10).

Participants were scanned on a 3 Tesla General Electric Signa scanner (Milwaukee, WI) using one of two custom single-channel quadrature head coils (one head coil was decommissioned during the study). The number of participants scanned with each head coil did not differ between groups ($\chi^2=1.06$, $p>0.10$) and head coil type was not related to BOLD signal within group.

Whole brain T2*-weighted gradient echo spiral images were acquired with high-order shimming (echo time(TE)=30ms, repetition time(TR)=2000ms, flip angle=80°, FOV=22cm, acquisition matrix = 64×64, approximate voxel size = 4.0×3.4×3.4mm, 30 axial-oblique slices: 4.0mm thick, 1.0mm skip(28)). Participants viewed color photographs of four college-aged models (two male, two female; two Caucasian, one African American, one Asian) with neutral expressions (Supplemental Figure 1). Photos were selected from previous studies because they elicited differential activation patterns for individuals with FXS(14;15). Each face was presented 16 times, 8 times in each of two gaze orientations (directly toward or averted away from the participant) per run. Two identical consecutive runs lasted 430 seconds each. Habituation was defined as reduced activation in run 2 relative to run 1(29). Each face was presented for 4 seconds (2 multiples of the TR), separated by fixation periods during which participants were instructed to look at a fixation cross presented on a blank screen. Facial stimuli were presented in a fixed pseudo-random order with fixation durations ranging 2-10 seconds creating a jittered design with a variable inter-stimulus interval. Participants were instructed to press button 1 if the person in the picture was looking at them, or button 2 if looking away. Responses and reaction times were collected via button box. High-resolution T1-weighted anatomical images, collected during the same imaging session, facilitated normalization to standard space (TE=6ms, TR=35ms, flip angle=45°, FOV=24cm, slice thickness=1.5mm, 124 coronal slices; matrix=256×192; acquired resolution=0.94×1.25×1.5mm).

All participants practiced lying motionless in an MRI simulator and experienced the sounds and sights of an MRI scanner(30). Practice continued until the participant could hold motionless (movement<1mm measured by potentiometer) for 10 minutes. Participants also practiced the gaze habituation task with faces not used in the actual fMRI task.

Image preprocessing in SPM8(<http://www.fil.ion.ucl.ac.uk/spm/software/spm8/>) included realignment and correction for motion and global signal intensity artifacts using ArtRepair toolbox(<http://cibsr.stanford.edu/tools/methods/artrepair-software.html>). Volumes with movement>0.5mm per TR or global signal intensity fluctuations>1.5% were de-weighted and repaired using interpolation between the nearest non-outlier scans. Participants whose fMRI series required repair for>20% of volumes were removed from analyses. Images were co-registered to the participant's anatomical image, normalized to the MNI template, and spatially smoothed using a 4mm full-width at half maximum Gaussian kernel.

First level statistics included fixed effects modeling (SPM8) to identify activation to direct and averted gaze stimuli separately and overall (direct+averted). Habituation was measured as reduced activation in run 2 relative to run 1, for direct gaze, averted gaze, and overall. Group analyses in FSL (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSL>) included nonparametric permutation testing (Randomise, 10,000 permutations) and iterative cluster threshold finding (threshold free cluster estimation with family wise error correction for multiple comparisons, $p < 0.01(31)$), to minimize type II error. Large eye movements or saccades were estimated during fMRI to provide additional evidence of compliance with the task and attention to the stimuli (Supplemental).

Group comparisons for imaging and behavioral data were covaried by age due to the relatively wide range and skewed distribution of age in the comparison group, which tended to include younger individuals ($p = 0.051$). Accuracy and reaction time were compared using repeated measures ANOVA between groups: gaze direction and run were within subject factors and age was a covariate. Parallel analysis, including only individuals with $> 50\%$ accuracy, was performed because judging eye gaze is a known deficit among our study population. Pearson's or Spearman's Rho was used for correlation between habituation and individual differences in clinical measures. Activation or habituation values for specific regions of interest (ROIs) were extracted using MARSBAR (<http://marsbar.sourceforge.net/>) and fslmeants (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSL>).

Results

Four participants (all males, 3 FXS) were excluded from analysis due to excessive head motion ($> 20\%$ of fMRI volumes had scan-to-scan motion $> 0.5\text{mm/TR}$).

Task Performance

Accuracy and reaction time data were missing for one of the two runs for three participants (2 male FXS, 1 female comparison); in these cases remaining data were used for group comparisons. Accuracy and reaction time comparisons indicated no significant main effects of group, run or gaze direction, and no significant interactions. Results remained the same when participants with task accuracy $< 50\%$ were excluded.

fMRI Group differences

Voxel-wise group analyses of activation (both runs combined) revealed no significant main effect of gaze direction within group and no significant group by gaze interaction. Voxel-wise group analyses of habituation of activation revealed a significant main effect of group; the FXS group displayed less habituation for all stimuli (direct+averted) relative to the comparison group in widespread regions including cingulate gyrus, fusiform gyrus, frontal lobe and occipital lobe (Table 2, Figure 1). There was no significant group by gaze interaction for habituation and no within group differences in habituation to direct > averted gaze. The main effect of group on habituation of fMRI data remained significant when covaried for IQ; significant clusters were present in the same areas reported in Table 2. This result also remained significant when participants with task accuracy $< 50\%$ were excluded, although significant clusters were smaller (Supplemental Figure 2, Supplemental Table 2).

Group analysis of activation in run 1 only revealed no significant main effect of group or gaze direction and no significant group by gaze interaction.

ROI Analysis of Habituation

We conducted within-group post hoc analyses examining the direction and significance of change in activation from run 1 to run 2, in regions for which the FXS group displayed less habituation. Average activation within a 5mm sphere ROI centered on each local maxima from the group comparison of all stimuli was extracted for each participant, for each run. T-tests were used to compare values across runs. These results (Table 2, Figure 2) demonstrate a significant increase in activation from run 1 to run 2 (sensitization) for the FXS group and a significant decrease in activation from run 1 to run 2 (habituation) for the comparison group within most regions.

ROI Correlation with clinical measures

We examined correlations between clinical measures and habituation in two of the regions demonstrating significantly less habituation to eye gaze in FXS: the left fusiform and the anterior cingulate, because they have been previously implicated in aberrant social functioning in FXS(16). Females and males with FXS were analyzed separately because of the larger variability in FMRP levels and behavioral symptoms in females(5). We report correlations significant at $p < 0.025$, indicated by Bonferroni correction for testing two regions. Left fusiform habituation in females correlated significantly with FMRP levels ($r(10)=0.674$, $p=0.016$, FMRP data missing for 2 females) and with ADOS scores ($r_s(12)=-0.718$, $p<0.004$, Figure 3). The correlation between habituation and FMRP remained significant after controlling for IQ ($r(10)=0.686$, $p=0.020$). No other correlations reached significance.

Discussion

The present study investigated neural system habituation to eye gaze in individuals with FXS relative to a comparison group matched for sex, intellectual functioning, autism symptoms and adaptive behavior. Our primary result reveals less neural habituation for individuals with FXS to all facial stimuli, (direct+averted gaze), and the lack of group difference in activation to run 1 indicates that initial activation differences did not drive this result. There was no evidence for differential habituation to direct versus averted gaze within or between groups. Less habituation and neural system sensitization was found in the FXS group (Figure 2). This effect was evidenced in widespread cortical regions including lower and higher level visual processing (fusiform gyrus, bilateral occipital cortex), emotion processing (cingulate), as well as executive functioning regions (frontal). This suggests a deficit in habituation to face/eye gaze that is distributed across multiple levels of neural processing. The regions affected are consistent with reports of abnormal morphology and/or function in FXS(12;14;16). Significant correlations in females with FXS suggest habituation is related to individual differences in FMRP levels and autism symptoms.

The habituation observed in our comparison group is consistent with that seen in typically developing individuals – decreased activation to repeated stimuli(21;32), evidence of neural

plasticity reflecting more efficient neural processing(33). Conversely, the sensitization displayed by the FXS group indicates deficient modulation of neural responses to repeated gaze stimuli. Sensitization was seen in regions involved with emotion and social cognition (cingulate, fusiform and frontal cortex). One possible interpretation of this result is that neural system sensitization to gaze is related to the inability to modulate arousal(11). While the link between sensitization and physiological hyperarousal is not directly supported by our data, previous studies suggest support for this hypothesis. Neuroimaging in individuals with FXS demonstrates that both social anxiety and gaze durations are related to brain activation in regions supporting social cognition(34). Atypical eye gaze behavior has also been linked to aberrant physiology in individuals with FXS, including changes in skin conductance(8), cortisol reactivity(9) and pupillary reactivity(10). However, additional factors such as impaired attention and social cognition may also contribute to habituation deficits. Furthermore, our design does not allow us to address the specificity of habituation deficits because we did not include non-social stimuli. Future investigations of neural system habituation to social and non-social stimuli with simultaneous measurement of physiological arousal may help clarify the nature and specificity of habituation deficits.

Further study of habituation in FXS could significantly enhance our understanding of associated social avoidance behaviors and provide a useful quantitative measure for evaluating the efficacy of treatments for gaze aversion in FXS. Exposure therapy is one treatment technique with the potential to help normalize habituation to eye gaze and reduce gaze aversion. Exposure therapy involves repeated presentations of an aversive stimulus with the goal of decreasing fear or anxiety(35). Exposure therapy in the form of systematic desensitization has been shown to attenuate symptoms of auditory sensitivity in individuals with ASD (36)). Further, a study from our group indicated that behavioral shaping reduces gaze aversion in FXS(19). These findings suggest that repeated exposure combined with a well-designed behavioral program may facilitate direct gaze. Our results show increased neural system response to repeated presentations over the course of our relatively brief fMRI experiment, demonstrating plasticity but in the direction of sensitization rather than habituation. Sensitization may be due in part to the social anxiety present in FXS(17), suggesting that adjunctive pharmacological intervention may prove useful in reducing anxiety and physiological arousal thus enhancing the effectiveness of any behavioral (e.g., desensitization) treatment. In this regard, exposure therapy with graded desensitization to eye gaze may attenuate gaze aversion behavior and neural system habituation may be a useful outcome measure.

We attribute deficits in habituation primarily to FXS, and not to general cognitive ability, autism symptoms or adaptive behavior level because our comparison group was matched to the FXS group on IQ, ADOS and Vineland scores. Our finding of differential habituation between groups matched on autism symptoms indicates that the neurobiological mechanisms underlying gaze aversion in FXS are different from those underlying similar symptoms in other disorders. These findings underscore the importance of designing disease specific treatments for FXS.

Moreover, we revealed a correlation between degree of habituation and FMRP levels, suggesting a dose response relationship with the basic biomolecular component underlying

FXS. Although correlations with habituation were only significant among females with FXS, potentially due to the skewed distribution of FMRP in males, we note that the few males with FMRP above 20% appear to fall along the regression line generated for females (Figure 3A). FMRP level was correlated with brain morphology in a group of males and females(13) and with brain function in a study that examined only females(37). Future studies with larger sample sizes within each sex, including males and females with similar FMRP levels, will be important for determining the specificity of relationships between FMRP and neurobiological outcomes. The correlation between habituation deficits and autism symptoms in females with FXS supports the theory that habituation deficits underlie gaze aversion and impaired social functioning. Studies directly comparing habituation between individuals with FXS and individuals with similar social deficits, such as ASD and social anxiety, would also be beneficial for determining the specificity of these relationships. Furthermore, examining developmental trajectories of habituation, hyperarousal and autism symptoms will contribute to our understanding of causal pathways among these related deficits. These relationships between habituation and FMRP/autism symptoms were significant only within the fusiform gyrus, a face-processing region important for social functioning and known to have abnormal function(14;16) and morphology(12;13) in FXS.

Interestingly, we did not find significant group differences for habituation in the amygdala, despite its key role in emotion processing. We also did not find a group by gaze interaction, nor any within-group differences in habituation for direct versus averted gaze in this region. It is possible that our task demands resulted in sustained amygdala activation, thus habituation or sensitization between runs was not present. This finding is not consistent with a previous study by our group, which revealed significant within and between group differences in amygdala activation related to gaze direction(15). However, differences in experimental design and sampling frame may have contributed to the inconsistent results. Most notably, the present study examined habituation and thus presented a smaller number of unique faces (4 versus 120) for longer durations each (4sec versus 1.75sec) over a longer experimental period (~14minutes versus~9minutes). Video clips have been shown to elicit differential responses to gaze direction in individuals with autism(38) and may have elicited group differences in amygdala habituation and/or a significant effect of gaze direction in individuals with FXS.

Our estimate of eye movement frequency, BOLD signal change in the eye region (Supplement), indicates that there was no significant group by run interaction and no within group differences in run 1 versus run 2 eye movements. Thus, a group difference in eye movements did not drive the difference in habituation. We conclude it is unlikely that group differences in interest or attention were responsible for the differential habituation we observed because such circumstances would be accompanied by changes in eye moments. We note more eye movements for the FXS group relative to the comparison group for direct gaze, suggesting atypical responses to direct gaze, perhaps in effort to avoid direct gaze. More eye movements during direct gaze may have contributed to the lack of difference between habituation to direct versus averted gaze in the FXS group and/or to our lack of a group difference within the direct gaze condition for run 1. Future studies employing an eye tracker could be used to determine where a participant is fixating, and to further delineate face/gaze processing in FXS.

Inclusion of individuals taking medications was a necessary limitation since medication use is high among the populations we studied. Importantly, FXS and comparison groups did not differ on the number of medications used (in general and by class, Table 1). Therefore, it is unlikely that our group differences were driven by medication usage.

In summary, individuals with FXS displayed a deficit in neural habituation and demonstrated sensitization to face/gaze that is distributed across multiple levels of processing. Sensitization in regions involved with emotion and social cognition may be related to an inability to modulate social anxiety given previous results demonstrating social anxiety and hyperarousal related to gaze processing. Correlation results suggest habituation is related to individual differences in FMRP levels and autism symptoms assessed outside the scanner. Although the relationships were only significant among females, they provide preliminary evidence supporting the relevance of neural system habituation as a biomarker for designing and assessing treatment trials such as exposure therapy for gaze aversion in FXS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Jennifer Lynn Bruno had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

JB carried out the statistical analysis and drafted the manuscript. ALR and ASG conceived of the study, participated in its design and coordination and helped to draft the manuscript. EMQ recruited participants, collected the data and helped to analyze the data and draft the manuscript. PKM contributed to the statistical analysis for eye movements. All authors read and approved the final manuscript.

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Figure 1.

Group difference in Habituation to direct and averted gaze stimuli combined.

Yellow/orange indicates regions for which individuals with fragile X syndrome demonstrate less habituation (T values, $p < 0.01$, FWE corrected) for both conditions combined. Left side of image = right side of brain

Figure 2.

Habituation of dorsal anterior cingulate activation by group and condition.

Habituation of dorsal anterior cingulate cortex is shown graphically as change in activation from run 1 to run2. Activation by group is for averted and direct gaze stimuli, for a for a 5mm sphere around the peak voxel in the dorsal anterior cingulate. Activation values are in arbitrary units and vertical bars represent standard error.

* Within group difference between run 1 and run 2 is significant ($p < 0.05$). For Direct gaze within the comparison group difference is marginally significant ($p = 0.09$).

Figure 3.

Relationship between fusiform habituation and individual differences in level of FMRP and social functioning.

A. Relationship between fusiform habituation and FMRP percentage in the FXS group. B. Relationship between fusiform habituation and ADOS scores in FXS and comparison groups. Fusiform habituation is defined as run 2 activation minus run 1 activation. Values greater than 0 indicate habituation (decreasing activation from run 1 to run 2) and values less than zero indicate sensitization (increasing activation from run 1 to run 2). The r , r_s and p values refer to FXS female only scores. FMRP = Fragile X mental retardation protein, higher percentage is associated with higher levels of cognitive and neurobiological functioning. ADOS = Autism Diagnostic Observation Schedule, higher scores indicate greater symptom severity.

Table 1

Demographics, cognitive test scores, fMRI task performance and medications

	FXS group			Comparison group			Statistic and significance
Number of participants	27 (14 females)			24 (12 females)			$\chi^2 = 0.02, p=0.90$
	Mean	Range	SD	Mean	Range	SD	
Age (years)	20.93	16.05-25.33	2.75	19.00	15.14-25.77	3.15	$t = 2.00, p=0.051$
IQ	67.78	40-119	19.05	75.00	53-123	20.73	$F = 2.31, p=0.14$
ADOS	6.59	0-18	6.08	6.25	0-16	4.67	$F = 0.27, p=0.61$
Vineland	67.26	37-103	16.31	65.33	30-86	13.38	$F = 1.33, p=0.25$
fMRI task accuracy (% correct)	85.49	37.00-100	20.18	88.87	37.00-100	15.62	$F = 0.41, p=0.53$
fMRI task reaction time (msec)	1159	507-1951	336	1341	729-1924	341	$F = 2.36, p=0.13$
	N	%		N	%		
Medications (any)	10	35.71		9	37.50		$\chi^2 = 0.001, p=.97$
SSRI	6	21.43		4	16.67		$\chi^2 = 0.25, p=0.73^\ddagger$
Stimulants	3	10.71		6	25.00		$\chi^2 = 1.69, p=0.28^\ddagger$
Other	6	21.43		1	4.17		$\chi^2 = 3.50, p=0.10^\ddagger$

All values are mean (standard deviation) except for number of participants and medications, which are frequency counts. The statistic and significance values for *number of participants* refer to the male/female distribution between groups. IQ = Wechsler full-scale intelligence quotient, standard scores are reported. ADOS = Autism diagnostic observation schedule, social + communication composite, higher scores indicate more social/communication deficits. All participants were administered Module 3 or 4. Scores of 7 or greater are in the autism spectrum disorder range; 12 individuals in the FXS group and 9 individuals in the comparison group met or exceeded this threshold. Vineland = Vineland adaptive behavior scale, standard scores are reported, higher scores indicate greater adaptive functioning. Group comparisons of IQ, ADOS, Vineland, fMRI accuracy and fMRI reaction time include age covariate.

SSRI = selective serotonin reuptake inhibitor. *Other* includes neuroleptics, atypical antipsychotics and other drugs known to affect neurological functioning.

[‡]Indicates Fisher's correction for χ^2 was used due to counts fewer than 5.

Table 2

Significant clusters showing group differences in habituation to eye gaze (direct+averted gaze combined)

Brain Region	BA	# Voxels	Peak T	X	Y	Z	Follow-up within-group T tests [§]	
							FXS	Comparison
anterior cingulate	32	4182	5.46	-4	34	28	Sensitization	Habituation
right angular gyrus/ supramarginal gyrus	40/39	580	4.95	64	-46	28	NS	Habituation
left middle/lateral occipital gyrus	19	574	4.69	-34	-76	18	Sensitization	Habituation
left fusiform	37/36	538	4.46	-24	-46	-14	Sensitization	Habituation
left frontal	6	370	4.76	-18	6	58	Sensitization	Habituation
right occipito-temporal/ inferior temporal	37	355	4.6	48	-56	-8	NS	Habituation
right cerebellum	----	243	4.23	6	-58	-6	Sensitization	Habituation
left frontal	6	154	4.01	-2	4	70	NS	Habituation
left precentral/middle frontal gyrus	6	135	4.98	-52	4	40	Sensitization	Habituation
right lateral occipital/superior parietal	7	127	4.15	16	-72	48	Sensitization	NS
left middle frontal	8	59	3.89	-40	34	38	NS	NS
left inferior frontal	45/44	29	4.27	-46	32	14	Sensitization	Habituation
right precentral gyrus	6	28	5.06	48	-4	46	Sensitization	Habituation
left posterior cingulate	31	28	4.25	-22	-64	20	Sensitization	NS
right lateral occipital/ superior parietal	7	27	3.5	20	-70	58	Sensitization	Habituation

Peak coordinates in MNI space, for significant clusters $p < 0.01$ FWE corrected and > 20 voxels; BA = Brodmann area. Including all participants $N = 51$.

[§]Follow up T tests refer to within group comparison of change in activation from the run 1 to run 2. Habituation indicates there was a significant decrease in activation from run 1 to run 2 ($p < 0.05$). Sensitization indicates there was a significant increase in activation from run 1 to run 2 ($p < 0.05$). NS indicates no significant change in activation from run 1 to run 2.