ORIGINAL ARTICLE

Here's Looking at You, Kid

Neural Systems Underlying Face and Gaze Processing in Fragile X Syndrome

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Background: Children with fragile X syndrome (fraX) are at risk for manifesting abnormalities in social function that overlap with features of autism and social anxiety disorder. In this study, we analyzed brain activation in response to face and gaze stimuli to better understand neural functioning associated with social perception in fraX.

Methods: Eleven female subjects with fraX, aged 10 to 22 years, were compared with age-matched female control subjects. Photographs of forward-facing and angled faces, each having direct and averted gaze (4 types of stimuli), were presented in an event-related design during functional magnetic resonance imaging. Subjects were instructed to determine the direction of gaze for each photograph. Activation in brain regions known to respond to face and gaze stimuli, the fusiform gyrus (FG) and superior temporal sulcus (STS), were compared between groups to isolate neural abnormalities in the perception of directed social stimuli.

Results: The fraX subjects had decreased accuracy in determining the direction of gaze compared with controls. Region of interest analysis of the FG revealed a significant interaction between diagnostic group and face orientation. Specifically, control subjects had greater FG activation to forward than to angled faces, whereas fraX subjects had no difference in FG activation to forward and angled faces. Controls showed greater left STS activation to all stimuli compared with fraX subjects.

Conclusions: Our results suggest that gaze aversion in fraX subjects is related to decreased specialization of the FG in the perception of face orientation. Decreased STS activation in fraX suggests aberrant processing of gaze. These data suggest that gaze aversion in fraX may be related to dysfunction of neural systems underlying both face and gaze processing.

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FRAGILE X SYNDROME (FRAX) IS an inherited neurodevelopmental disorder caused by disrupted expression of the fragile X mental retardation (*FMR1*) gene. In this relatively common syndrome, *FMR1* gene expression is reduced, and the resulting lack of FMR1 protein (FMRP) protein leads to altered synaptic function and abnormal dendritic spine morphology.[1] Fragile X syndrome is an X-linked condition; therefore, female subjects have 1 affected and 1 unaffected X chromosome, resulting in FMRP levels that are reduced by approximately 50%. In addition, the level of FMRP can vary among affected male and female individuals, thus contributing to variation in the level of impairment. As a neuropsychiatric phenotype, fraX is associated with increased risk for impairment in several domains, including sustained attention, working memory, visuospatial analysis, visuomotor coordination, executive function, and social function.[2]

Social difficulties, often associated with a subset of autistic behaviors, are one of the earliest and most maladaptive symptoms of fraX. These difficulties may worsen during childhood, thus having a potential long-term effect on the child's adaptive behavior and mental health. One hallmark of fraX is the propensity to avoid eye contact and turn away during a social greeting, even while offering a handshake or socially acceptable remark.[3] Male individuals also show abnormalities in social play with peers and during verbal and nonverbal social communication.[4] Young girls with fraX are usually less affected than boys but also show social deficits, including withdrawal and avoidant behavior.[5] Even female subjects with fraX who have IQs in the normal range exhibit social dysfunction, which may be related to reports of increased frequencies of social anxiety and mood disorders[6] in these subjects. The social avoidance seen in individuals with fraX has been attributed to hyperarousal during social situations.[7,8] Together, these studies suggest that brain systems that process socially relevant stimuli may function differently in fraX than in typically developing subjects.

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In typically developing subjects, the neurofunctional correlates of social cognition have been investigated by observing responses to faces and the direction of eye gaze. Neuroimaging studies have found greater activation of the fusiform gyrus (FG) in response to faces compared with letter strings, scrambled faces, houses, or human hands.[9-11] Also, patients with lesions in this area have difficulty recognizing faces,[12] and intraoperative recordings from the FG show increased responses to faces.[13,14] However, the specificity of FG activation remains in question. Recently, Gauthier and colleagues[15,16] suggested that visual expertise in general, rather than the analysis of faces in particular, recruits the capabilities of the FG. An understanding of the variables that modulate FG activation in response to faces is beginning to emerge. Rossion and colleagues[17] showed that the left FG was particularly responsive to parts of faces rather than whole faces, whereas the opposite was true for the right FG. Forward and angled faces have been shown to evoke a greater FG response than profiles of faces.[14] Attention to faces increases FG activation,[18] and reduced accuracy at matching faces may decrease FG activation.[19]

The perception of gaze direction is less well studied, but neuroimaging data suggest the involvement of the superior temporal sulci (STS)[20,21] and the middle and inferior temporal gyri.[22] Some studies suggested that averted gaze activates the STS more than does direct gaze,[20] whereas other studies found no differences in STS activation to averted vs direct gaze.[22,23] A recent parametric analysis showed that increasing proportions of direct gaze are associated with increasing blood flow in the STS.[24]

We hypothesized that if the FG and the STS are typically activated in response to face and gaze stimuli, then studying activation of these regions in individuals with fraX may help us begin to understand the nature of social difficulties in this condition. Several studies have shown that individuals with fraX, unlike individuals with autism, recognize and recall faces normally.[2,25] Therefore, we predicted that the perception of faces, which is attributed to the FG, may function normally in fraX. However, because of the symptom of gaze aversion, the STS may show altered functioning, and brain regions associated with anxiety may show increased activation in fraX. We addressed our hypothesis by presenting 4 types of stimuli: Forward and angled faces with direct and averted gaze. Direct gaze and forward faces signify socially relevant stimuli. Averted gaze and angled faces are appropriate control stimuli because they possess similar visual characteristics. Combined with the inclusion of a homogeneous patient group with an identifiable etiology, this design allowed us to provide a foundational study of social information processing in fraX. To our knowledge, this study is the first to use event-related functional magnetic resonance imaging (fMRI) to examine functional brain abnormalities underlying the perception of face and gaze stimuli in fraX.

METHODS

SUBJECTS

Fifteen female subjects with a diagnosis of fraX (fraX subjects) were recruited through advertisements in national and regional fraX newsletters and referrals from physicians. Fifteen typically developing female control subjects were recruited through advertisement within the local community. We included only female subjects because they have milder symptoms than male fraX subjects and therefore are more likely to perform the task accurately and tolerate the scan. Including only female subjects also removes intersubject variance attributable to sex.

All subjects reported that they were right-handed. All fraX subjects had the *FMR1* full mutation, as confirmed by DNA (Southern blot) analysis. Written informed consent was obtained from all participants, and the human subjects review committee at Stanford University School of Medicine, Stanford, Calif, approved all protocols.

All of the control subjects were medication free. Of the final 11 fraX subjects included in the study, 8 were medication free. The remaining 3 fraX subjects were taking the following medications: (1) guanfacine hydrochloride (Tenex) and venlafaxine hydrochloride (Effexor); (2) paroxetine (Paxil) and methylphenidate hydrochloride (Ritalin Hydrochloride); and (3) paroxetine, methylphenidate, and levothyroxine sodium (Synthroid). Subjects stopped taking methylphenidate and guanfacine for 24 hours before the scan, and continued taking all other medications. The fraX group consisted of 9 white, 1 Hispanic, and 1 Pacific Islander subject. The final control group consisted of 9 white, 1 Hispanic, and 1 Asian American subject.

The final subject groups did not differ significantly in age (fraX group, mean age, 16.4 years [SD, 4.09 years; range, 10-22 years]; control group, mean age, 15.5 years [SD, 3.41 years; range, 10-22 years]; $F_{1,20}<1$). The IQs were measured using the Wechsler Intelligence Scale for Children III for subjects younger than 17 years and the Wechsler Adult Intelligence Scale III for subjects 17 years and older. One fraX subject was removed for having an IQ significantly below the group mean (>2.5 SDs). To reduce the IQ disparity between groups, 3 control subjects were removed for having an IQ significantly greater than the group mean (>120, or 1.33 SDs). The final Full-Scale IQ scores for the fraX group were in the average range of intelligence (80-111), with a mean score of 93.7 (SD, 10.4). Full-Scale IQ scores for the control group ranged from 85 to 120, with a mean score of 107.0 (SD, 11.2). A between-groups *t* test verified that the control group had significantly higher Full-Scale IQ scores than the fraX group ($F_{1,20}=8.24$ [$P<.01$]).

EXPERIMENTAL TASKS

Stimuli

Color photographs of faces of 120 college-aged models with neutral facial expressions were taken against a common, solid-color background at a distance of about 2 m. Thirty photographs from each of the following 4 categories were used: (1) face forward with direct gaze, (2) face forward with averted gaze, (3) face angled with direct gaze, and (4) face angled with averted gaze. Angled faces and averted gaze were turned approximately 45° away from the camera. The photographs included 66 men and 54 women. Of these, 93 were white and 27 were African American, Hispanic, Asian American, or Indian. Sex and race were distributed similarly across stimulus categories. Examples of the stimuli are shown in **Figure 1**.

Task Designs

A research investigator (K.M.) practiced a training version of the task with each subject until confident that she understood the instructions and could respond accurately. Subjects performed 2 tasks. The event-related task used a jittered stimulus presentation, with a mean intertrial interval of 1572 milliseconds (SD, 1805 milliseconds)[26] and a range of 0.25 to 4.25 seconds. Stimuli were presented using PsyScope software,[27] which also triggered the initiation of the fMRI scan by sending a tran-

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Figure 1. Examples of photographs used as stimuli. Labels describe stimulus type. FD indicates forward face with direct gaze; FA, forward face with averted gaze; AD, angled face with direct gaze; and AA, angled face with averted gaze.

sistor-transistor logic pulse to the scanning processor. Stimuli were projected onto a screen attached to the head coil. Subjects looked directly upward at a mirror to view the stimuli. Each stimulus was presented for 1750 milliseconds, followed by a 250-millisecond duration fixation cross. Subjects were instructed to use the right index finger to press a button if the person in the photograph was looking at them, and to use the right second digit to press another adjacent button if the person was looking away from them. Correct and incorrect responses and reaction times were recorded if they occurred between 150 and 2000 milliseconds after the stimulus. Each subject performed 2 runs of the event-related task, with each run lasting 4 minutes 32 seconds. The runs were separated by 2 to 3 minutes to prevent subject fatigue. In each run, 15 stimuli of each condition were presented, so 2 runs contained 30 stimuli per condition.

The same stimuli described above were then presented in a block design to derive functional regions of interest (ROIs) defining the FG and STS. In particular, a block design was used to measure responses to all types of stimuli combined. This method of defining ROIs by the location of significant activation in response to related stimuli has been used in previous studies[28] and is a methodologically attractive way to create subject-specific ROIs based on functional anatomy. The task consisted of 8 alternating epochs, each lasting 30 seconds and presenting 15 stimuli for 1750 milliseconds each, with a 250-millisecond interstimulus interval. Half of the epochs contained a mix of all 4 types of face stimuli, whereas the alternating epochs contained scrambled pictures. Subjects were asked to indicate the direction of gaze for the faces and to alternate pressing the first and second button in response to the scrambled pictures.

MRI SCANNING

Images were acquired on a 1.5-T GE scanner (General Electric Company, Milwaukee, Wis) using a custom-built whole-head coil that provides a 50% advantage in signal-to-noise ratio over that of the standard GE head coil.[29] A custom-built head holder was used to prevent head movement. Eighteen axial slices (6-mm thickness; 1-mm gap) parallel to the anterior and posterior commissures and covering the whole brain were imaged using a T2-weighted gradient echo spiral pulse sequence (repetition time [TR], 2000 milliseconds; echo time [TE], 40 milliseconds; flip angle, 89° and 1 interleave[30]; field of view [FOV], 240 mm; in-plane resolution, 3.75 mm). To help localize activation, a high-resolution T1-weighted, spoiled GRASS (gradient recalled acquisition in a steady state) image (SPGR) 3-dimensional MRI sequence (TR, 24 milliseconds; echo time, 5 milliseconds; flip angle, 40°; FOV, 240 mm; 124 sagittal slices; 256×192 matrix; resolution, $1.5 \times 0.9 \times 1.2$ mm) was also collected.

DATA ANALYSIS

A 3-way analysis of variance (ANOVA) was used to examine task accuracy (percentage correct) and response time. The factors included face orientation (forward and angled), gaze orientation (direct and averted), and group (control and fraX).

Functional images were reconstructed by means of the inverse Fourier transform for each of the 186 time points into $64 \times 64 \times 18$ image matrices (voxel size, $3.75 \times 3.75 \times 7$ mm). Functional MRI data were analyzed using SPM99 software (Statistical Parametric Mapping 99; Wellcome Department of Cognitive Neurology, Institute of Neurology, University College, London, England). Images were corrected for movement using least squares minimization without higher-order corrections for spin history, and normalized to stereotaxic Montreal Neurologic Institute coordinates.[31] Images were then resampled every 2 mm using sinc interpolation and smoothed with a 4-mm gaussian kernel to decrease spatial noise.

The general linear model and the theory of gaussian random fields implemented in SPM99 were used to complete statistical analyses of fMRI data.[30] For each subject, activation was calculated at each voxel and corrected for temporal autocorrelation. Confounding effects of fluctuations in the global mean were removed by proportional scaling. Low-frequency noise was removed by applying a high-pass filter (0.5 cycle/min) to the fMRI time series at each voxel. A temporal smoothing function (gaussian kernel corresponding to dispersion of 8 seconds) was applied to the fMRI time series to enhance the temporal signal-to-noise ratio.

For each subject, a t score image was generated for each contrast of interest, including (1) forward compared with angled faces, collapsed over gaze orientation, and (2) direct compared with averted gaze, collapsed over face orientation. Individual contrast images were combined into a group image using a random-effects model, which provides a stronger generalization to the population.[32] All comparisons between the fraX and control groups were controlled for differences in IQ by including IQ as a covariate. A mask was used to remove group differences arising from deactivation. Voxelwise t statistics were normalized to z scores to provide a statistical measure independent of sample size. Significant clusters of activation were determined using the joint expected probability of height ($z>1.96$ [$P<.05$]) and extent of z scores ($P<.05$),[33] yielding a clusterwise significance level of $P=.05$, corrected for multiple comparisons. The MNI coordinates were converted to Talairach coordinates using procedures described by Brett.[34] Activation foci were superimposed on high-resolution T1-weighted images and localized with reference to the stereotaxic atlas of Talairach and Tournoux.[31] Because the contrasts examined in this study were chosen a priori, activations from other contrasts are not reported.

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Table 1. Gaze Orientation Task Performance*

	fraX Group	Control Group
Accuracy, % correct		
AA	80.0 ± 18.8	95.2 ± 6.6
AD	75.7 ± 23.9	90.3 ± 8.2
FA	81.9 ± 19.2	96.4 ± 6.1
FD	96.7 ± 3.9	97.6 ± 5.2
Response time, ms		
AA	896.3 ± 160.6	886.80 ± 156.4
AD	1094.7 ± 106.1	1006.5 ± 138.1
FA	961.8 ± 207.0	938.20 ± 126.8
FD	932.5 ± 141.2	875.70 ± 111.5

Abbreviations: AA, angled face with averted gaze; AD, angled face with direct gaze; FA, forward face with averted gaze; FD, forward face with direct gaze; fraX, fragile X syndrome.

*Behavioral data from the gaze orientation task. Subjects pressed button 1 if the model in the picture was looking at them, and button 2 if the model was looking away. Accuracy and response time for each type of stimulus are listed. Data are expressed as mean ± SD.

ROI ANALYSIS

An ROI analysis was used to explore activation of the FG and STS within groups and in response to each type of stimulus. The boundaries of anatomical regions were drawn on each subject's spatially normalized structural MRI. These regions were further constrained by a mask constructed from each subject's activation during the block design task, which combined all face and gaze stimuli (minus scrambled stimuli), thresholded at $P<.05$. When conjoined with the anatomical outline, each subject's FG and STS regions were defined structurally and functionally. The ROI activation was calculated as the percentage of significant voxels ($z>1.67$) for each of the 4 stimulus conditions. A repeated measures ANOVA was conducted for each ROI with the factors of face orientation (forward and angled), gaze orientation (direct and averted), hemisphere (right and left), and group (fraX and control).

The anatomical boundaries of all regions were drawn on coronal slices using BrainImage software.[35] The FG begins at the coronal slice containing the largest cross section of the anterior commisure and proceeds back to the posterior transverse collateral sulcus. The medial border is the collateral sulcus, and the lateral border is the occipitotemporal sulcus. The superior temporal region includes the STS and the superior and middle temporal gyri. It begins at the onset of the anterior temporal pole and terminates posteriorly at the disappearance of the inferior temporal sulcus.

RESULTS

Three fraX subjects were removed from the sample for scoring below 50% on the gaze discrimination task, leaving only those subjects whose task accuracy indicated understanding and compliance with task demands. One control subject was removed for failure to respond to the task. Thus, the following results include 11 subjects in the fraX group and 11 subjects in the control group.

TASK ACCURACY

Group means and SDs for task accuracy are summarized in **Table 1**. Main effects were found for group, face orientation, and the interaction between face and gaze orientation. Control subjects had significantly greater accuracy compared with the fraX group ($F_{1,19}=13.39$ [$P=.002$]). For all subjects, accuracy was greater for forward than for angled faces ($F_{1,19}=10.88$ [$P=.004$]). The significant interaction between face and gaze orientation ($F_{1,19}=10.60$ [$P=.004$]) showed that for forward faces, accuracy was greater for direct gaze than for averted gaze, but for angled faces, accuracy was not different for direct compared with averted gaze conditions. This effect was driven primarily by the fact that all subjects had greater accuracy responding to forward faces with direct gaze than to any other stimulus.

Full-Scale IQ scores were significantly correlated with task accuracy for both groups combined ($r=0.56$ [$P=.003$, 1-tailed]). However, this appeared to reflect a categorical (ie, group) effect only, since IQ and task accuracy were not significantly correlated within each of the groups (control group, $r=0.36$ [$P=.14$]; fraX group, $r=0.35$ [$P=.15$]). Therefore, all subsequent between-group analyses were covaried for IQ, which also controlled for between-group differences in task accuracy.

RESPONSE TIME

Table 1 shows the group means and SDs for response time. No group differences were found in response time. Main effects were found for face orientation and the interaction between face and gaze orientation. For all subjects, reaction time was quicker for forward than for angled faces ($F_{1,19}=5.07$ [$P<.04$]). The interaction between face and gaze orientation ($F_{1,19}=35.03$ [$P<.001$]) showed that for forward faces, response time was similar for direct and averted gaze, but for angled faces, response time was longer for direct than for averted gaze ($F_{1,19}=21.2$ [$P<.001$]).

BRAIN ACTIVATION RELATED TO FORWARD COMPARED WITH ANGLED FACES

There were no significant between-group differences in the comparison of forward faces with angled faces (collapsed over gaze orientation). The ROI analysis was used to investigate activation of specific brain regions within each group and for each type of stimulus.

BRAIN ACTIVATION RELATED TO DIRECT COMPARED WITH AVERTED GAZE

Control > fraX

Three clusters of significantly greater activation were found in the control group. Activation maxima included the STS, lingual gyrus, and cerebellum. Other significantly activated regions are listed in **Table 2** and shown in **Figure 2**.

fraX > Control

Two clusters of significantly greater activation were found in the fraX group. Activation maxima included the right insula and cerebellum. Other significantly activated regions are listed in Table 2 and shown in Figure 2.

FUSIFORM GYRUS ROI

A significant interaction was detected between group and face orientation ($F_{1,20}=9.20$ [$P=.007$]). Although control subjects had significantly greater FG activation in re-

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Table 2. Brain Regions Showing Significantly Greater Activation to Direct Gaze Compared With Averted Gaze After Covarying for IQ*

Brain Regions (Brodmann Area)†	Direct Gaze > Avert Gaze: Corrected *P* Value of Cluster	No. of Voxels in Cluster	Peak *z* Score	Peak Location
Greater activation in control group than fraX group				
L cerebellum	.001	763	4.39	−20, −54, −26
R cerebellum			3.29	−18, −54, −30
L cerebellar vermis			2.42	−6, −58, −26
L lingual gyrus (18)			3.53	−20, −80, −16
L STS (20/22)	.001	1063	3.59	−46, −26, −8
L Heschl gyrus (41/42)			3.04	−42, −32, 8
L middle temporal sulcus (21)			3.34	−48, −18, −16
L insula			3.53	−36, −20, 2
L hippocampal gyrus			2.82	−32, −28, −20
L hippocampus			1.89	−26, −14, −22
L cuneus (17)	.029	493	3.49	−4, −74, 6
R cuneus (17)			2.55	16, −68, 0
L lingual gyrus (18)			3.09	−8, −84, −2
Greater activation in fraX group than control group				
L cerebellum	.032	485	4.22	−8, −66, −20
R/L midbrain			3.81	−2, −34, −10
R posterior thalamus			3.67	12, −22, 8
R IFG (44/45)	.001	755	3.49	48, 6, 8
R ventrolateral PFC (47)			2.96	32, 14, 6
R insula			2.95	38, 0, 10

Abbreviations: fraX, fragile X syndrome; IFG, inferior frontal gyrus; L, left; PFC, prefrontal cortex; R, right; STS, superior temporal sulcus.

*Statistical probability and cluster size are listed for each cluster. Talairach coordinates (peak location) are given for the peak voxel in each brain region that is included in that cluster.

†Numbers in parentheses indicate Brodmann areas.

sponse to forward compared with angled faces (post hoc t_{11}=6.42 [*P*=.02]), fraX subjects had no difference for forward compared with angled faces (*P*=.09) (**Figure 3**A). Activation of the FG to angled faces was not significantly different between the fraX and control groups (post hoc $F_{1,19}$=0.309 [*P*=.59]). A significant interaction between group and hemisphere ($F_{1,20}$=5.75 [*P*=.03]) showed that controls had significantly greater right than left FG activation to all stimuli, whereas fraX subjects had no hemispheric differences in FG response (Figure 3B). Activation of FG in the left hemisphere is not significantly different between groups ($F_{1,19}$=.55 [*P*=.47]).

SUPERIOR TEMPORAL SULCUS ROI

The analysis of the STS region showed significant main effects of group and hemisphere. Control subjects had greater STS activation than fraX subjects in response to all stimulus conditions combined ($F_{1,19}$=6.11 [*P*=.02]; **Figure 4**). In addition, all subjects had greater STS activation in the right hemisphere compared with the left ($F_{1,20}$=14.61 [*P*=.001]).

COMMENT

We examined abnormalities in neural responses to face and gaze stimuli to investigate the basis of alterations in social behavior, such as gaze aversion, in fraX individuals. Although all subjects had IQ scores within the average range of intelligence, the fraX group had lower IQ scores and decreased accuracy in determining gaze direction compared with controls. The ROI analysis of the FG showed a significant interaction between group and face orientation. Control subjects had greater FG activation to forward than to angled faces, whereas subjects with fraX had no difference in activation to forward compared with angled faces. Controls had significantly greater STS activation to all stimuli compared with fraX individuals. Therefore, our results suggest that gaze avoidance in fraX individuals may be related to reduced ability to perceive gaze and decreased specialization in the perception of face orientation.

The results of the whole brain analysis of the FG could be interpreted as contradictory to the results of the ROI analysis of the same region. The ROI analysis showed that controls had significantly greater activation to forward than to angled faces, but the fraX group had no difference for forward vs angled faces. Therefore, for the whole brain analysis, we would expect control subjects to have greater FG activation than the fraX subjects for the group comparison of forward minus angled faces. However, the whole brain analysis showed no group differences. We believe that intersubject variability in the location of peak FG activation was responsible for this disparity. Group differences in FG activation in the whole brain analysis were in the same direction as the ROI-based results (control > fraX) but did not reach the significance threshold (*z*=3.69 [*P*=.001 uncorrected; *P*=.21 corrected]). This indicates how

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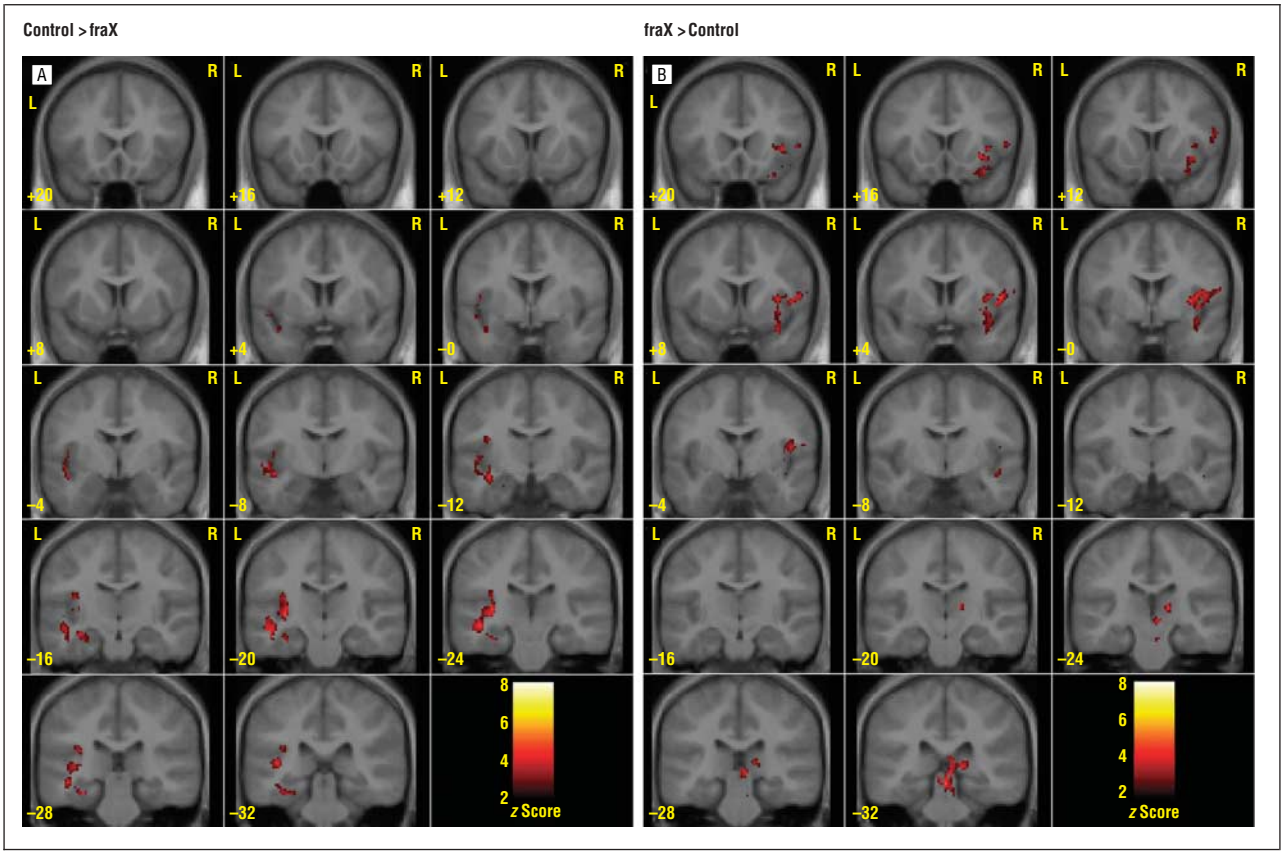

Figure 2. Location of activation that is significantly different between groups for the whole brain analysis of direct gaze compared with averted gaze, after covarying for group differences in IQ. The Talairach atlas y-coordinate is given in the lower left corner of each image. The right side of image indicates the right side of brain. All clusters are significant at $P<.05$. A, Greater activation in the control compared with the fragile X syndrome (fraX) group, including left superior temporal sulcus, middle temporal sulcus, and hippocampus. Additional significant clusters are listed in Table 2. B, Greater activation in the fraX compared with the control group in the right insula, posterior thalamus, and brainstem. Additional clusters are listed in Table 2.

the ROI analysis was helpful in controlling for individual variation in neuroanatomy, as intended.

Activation of FG is reliably found in response to faces, and is typically greater for forward than for angled faces,[9-11] perhaps because forward faces are perceived as more socially relevant. Lack of fusiform specialization may be associated with a relatively greater tendency of fraX individuals to look at faces when the faces are looking away.[36,37] Thus, they may develop a normal ability to process faces, but no preference for forward faces. On the other hand, fraX individuals avoid social gaze altogether, and therefore may not develop a normal ability to process gaze.

Another possibility is that the FG finding indicates difficulty processing angled faces, since subjects with fraX had significantly lower accuracy when responding to angled faces compared with forward faces. Decreased accuracy has been associated with increased activation of other visual cortical regions.[38] However, decreased accuracy also has been associated with decreased FG activation in previous studies involving control subjects.[16,19] Our data do not suggest that FG activation is related to accuracy in the current study. A post hoc analysis showed that there was no correlation between FG activation and accuracy for both groups combined or considered separately (combined, Pearson $r=-0.10$ for left FG and $r=0.09$ for right FG; fraX subjects, $r=0.15$ for left FG and $r=0.06$ for right FG; control subjects, $r=-0.05$ for left FG and $r=0.03$ for right FG).

Our results suggest that social abnormalities in fraX individuals are also related to a reduced ability to perceive social gaze, as evidenced by decreased STS activation to all categories of stimuli. Anatomical abnormalities in the STS have previously been reported in fraX.[39] Activation of this region has been associated with the perception of social gaze in control subjects.[20,21] Since fraX individuals typically avoid social gaze, they may not develop a normal ability to process gaze. Of course, we cannot determine whether alterations in the STS cause gaze aversion behavior, or whether gaze aversion behavior results in changes in these brain regions.

An alternative explanation for these findings is that fraX subjects looked away from the photographs. Although we cannot rule this out, because we did not measure eye movements, we did not find different activity in the frontal eye fields (Brodmann areas 6/8) of fraX subjects compared with controls. Also, fraX subjects did not have decreased accuracy in response to forward gaze stimuli. Finally, the participants in this study were chosen to be mildly affected patients with less severe gaze aversion behavior.

Social problems in fraX have been attributed to hyperarousal and anxiety.[7,8] Although our study did not directly assess the role of anxiety in social gaze perception, we did not see increased activation of the amygdala to direct gaze in fraX subjects. However, we saw increased activation of the right anterior insula, ventral pre-

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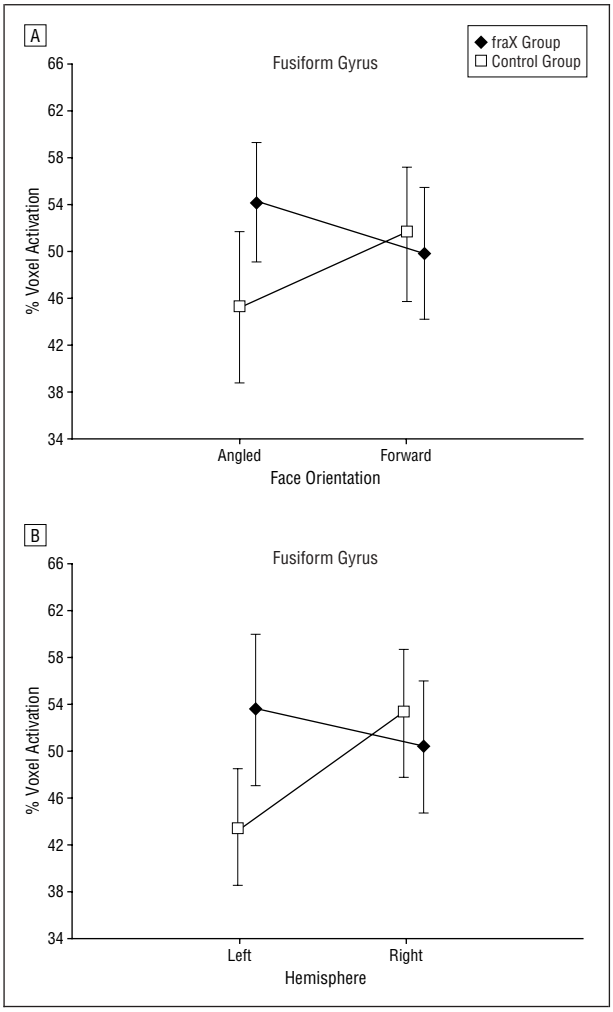

Figure 3. Fusiform gyrus (FG) region of interest. A, Significant interaction between group and face orientation ($F_{1,20}$=9.20 [P=.007]). Control subjects show significantly greater FG activation for forward faces than for angled faces, and subjects with fragile X syndrome (fraX) show no difference between forward and angled faces. Activation of FG to angled faces is not significantly different between groups. B, Significant interaction between group and hemisphere ($F_{1,20}$=5.75 [P=.03]). Control subjects have greater right than left hemisphere FG activation to all stimuli, whereas fraX subjects have no significant difference in activation of the FG between hemispheres. Data points indicate means; error bars, SEM.

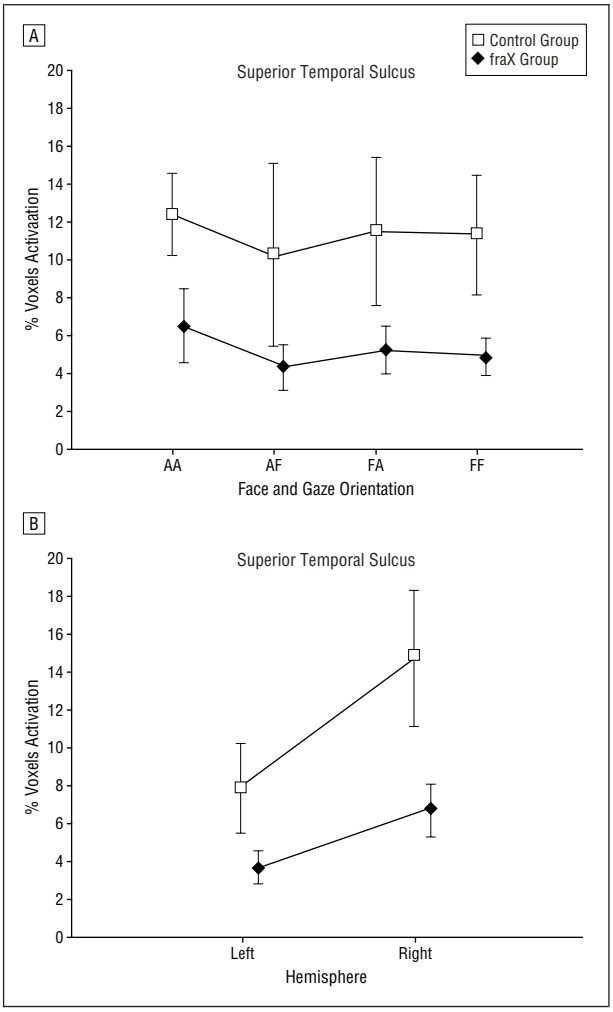

Figure 4. Superior temporal sulcus (STS) region of interest. A, The control group has significantly greater STS activation overall compared with the fragile X syndrome (fraX) group ($F_{1,19}$=6.11 [P=.02]). B, All subjects had greater right than left hemisphere STS activation ($F_{1,20}$=14.61 [P=.001]). Post hoc comparisons show that this effect is carried by control subjects, who had greater right than left hemisphere STS activation ($F_{1,20}$=13.93 [P=.001]). AA indicates angled face with averted gaze; AD, angled face with direct gaze; FA, forward face with averted gaze; FD, forward face with direct gaze; data points, means; and error bars, SEM.

frontal cortex, and midbrain. The ventrolateral prefrontal cortex has sensory and limbic inputs[40] and is activated during the experience of emotions, including sadness[41] and anxiety.[42] Similarly, the midbrain has been activated in neuroimaging studies of several emotions, including anxiety.[43] Insula activation is related to the experience of visceral and emotional symptoms, including chest constriction, fear, and uneasiness.[44,45] Activation of this constellation of regions suggests an increased emotional response to direct gaze in the fraX group. However, activation in these regions is not specific to anxiety. In addition, greater left posterior insula activation was seen in the control group. More research will be needed to determine whether social anxiety is related to altered gaze processing in fraX. It is possible that the photographs did not provoke anxiety in the fraX individuals because the scan did not involve an actual social situation, but only stimuli associated with social interactions. The knowledge that no real social interaction would take place during the fMRI task could have helped to lessen the social anxiety typically observed in these subjects.

This study is significant in distinguishing brain responses to social stimuli in fraX from those previously reported in autism. A subset of the social deficits seen in fraX is observed in autism, and hence the 2 developmental disorders have long been compared and contrasted.[4] For example, both fraX and autistic individuals experience difficulties with verbal and nonverbal social communication.[46] However, unlike autistic children, children with fraX show a propensity to engage in social behaviors with caregivers,[4] and they correctly identify facial and auditory emotion.[25,47] Cohen and colleagues[36,37] showed that, although both fraX and autistic children avoided social interactions, male fraX subjects avoided a stranger more than a parent, and autistic children avoided stranger and parent equally. Furthermore, analysis of dyadic social gaze patterns suggest that male fraX subjects are

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sensitive to eye gaze but avoid it because they find it aversive, whereas autistic subjects are insensitive to gaze and do not engage in social gaze, probably because of lack of interest or attention.[36,37,48] Our study furthers the distinction between fraX and autistic subjects by showing differences in neurofunctional responses to social stimuli. Previously, Schultz and colleagues[49] found reduced right FG activation to forward faces in autistic subjects. Similarly, Pierce and colleagues[50] found low or absent FG activation in response to face stimuli in autistic subjects, but not controls. In contrast, our study found activation of the FG in response to forward and angled faces in subjects with fraX, although with less differentiation of FG response to face orientation.

Finally, this study did not test whether activation differences in the FG and STS in fraX are specific to face and eye gaze stimuli or can be generalized to other visual stimuli. Also, further studies are needed to determine whether increased anxiety is related to STS dysfunction in fraX. Monitoring heart rate and eye gaze during scanning may help us to answer these questions in future investigations.

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REFERENCES

1. Irwin SA, Patel B, Idupulapati M, Harris JB, Crisostomo RA, Larsen BP, Kooy F, Willems PJ, Cras P, Kozlowski PB, Swain RA, Weiler IJ, Greenough WT. Abnormal dendritic spine characteristics in the temporal and visual cortices of patients with fragile-X syndrome. *Am J Med Genet.* 2001;98:161-167.
2. Cornish KM, Munir F, Cross G. Differential impact of the FMR-1 full mutation on memory and attention functioning. *J Cogn Neurosci.* 2001;13:144-150.
3. Wolff PH, Gardner J, Paccla J, Lappen J. The greeting behavior of fragile X males. *Am J Ment Retard.* 1989;93:406-411.
4. Reiss AL, Freund L. Behavioral phenotype of fragile X syndrome: *DSM-III-R* autistic behavior in male children. *Am J Med Genet.* 1992;43:35-46.
5. Lachiewicz AM. Abnormal behaviors of young girls with fragile X syndrome. *Am J Med Genet.* 1992;43:72-77.
6. Freund LS, Reiss AL, Abrams MT. Psychiatric disorders associated with fragile X in the young female. *Pediatrics.* 1993;91:321-329.
7. Belser RC, Sudhalter V. Conversational characteristics of children with fragile X syndrome: repetitive speech. *Am J Ment Retard.* 2001;106:28-38.
8. Hessl DR GB, Dyer-Friedman J, Blasey C, Hastie T, Gunnar M, Reiss AL. Cortisol and behavior in fragile X syndrome. *Psychoneuroendocrinology.* 2002;27:855-872.
9. Puce A, Allison T, Gore JC, McCarthy G. Face-sensitive regions in human extrastriate cortex studied by functional MRI. *J Neurophysiol.* 1995;74:1192-1199.
10. Kanwisher N, McDermott J, Chun MM. The fusiform face area. *J Neurosci.* 1997; 17:4302-4311.
11. Clark VP, Keil K, Maisog JM, Courtney S, Ungerleider LG, Haxby JV. Functional magnetic resonance imaging of human visual cortex during face matching: a comparison with positron emission tomography. *Neuroimage.* 1996;4:1-15.
12. Damasio AR, Damasio H, Van Hoesen GW. Prosopagnosia: anatomic basis and behavioral mechanisms. *Neurology.* 1982;32:331-341.
13. Allison T, Ginter H, McCarthy G, Nobre AC, Puce A, Luby M, Spencer DD. Face recognition in human extrastriate cortex. *J Neurophysiol.* 1994;71:821-825.
14. McCarthy G, Puce A, Belger A, Allison T. Electrophysiological studies of human face perception, II. *Cereb Cortex.* 1999;9:431-444.
15. Gauthier I, Tarr MJ, Moylan J, Skudlarski P, Gore JC, Anderson AW. The fusiform "face area" is part of a network that processes faces at the individual level. *J Cogn Neurosci.* 2000;12:495-504.
16. Gauthier I, Tarr MJ, Anderson AW, Skudlarski P, Gore JC. Activation of the middle fusiform "face area" increases with expertise in recognizing novel objects. *Nat Neurosci.* 1999;2:568-573.
17. Rossion B, Dricot L, Devolder A, Bodart JM, Crommelinck M, De Gelder B, Zoontjes R. Hemispheric asymmetries for whole-based and part-based face processing in the human fusiform gyrus. *J Cogn Neurosci.* 2000;12:793-802.
18. Wojciulik E, Kanwisher N, Driver J. Covert visual attention modulates face-specific activity in the human fusiform gyrus. *J Neurophysiol.* 1998;79:1574-1578.
19. Grady CL. Age-related changes in cortical blood flow activation during perception and memory. *Ann N Y Acad Sci.* 1996;777:14-21.
20. Hoffman EA, Haxby JV. Distinct representations of eye gaze and identity in the distributed human neural system for face perception. *Nat Neurosci.* 2000;3:80-84.
21. Campbell R, Heywood CA, Cowey A, Regard M, Landis T. Sensitivity to eye gaze in prosopagnosic patients and monkeys with superior temporal sulcus ablation. *Neuropsychologia.* 1990;28:1123-1142.
22. Wicker B, Michel F, Henaff MA, Decety J. Brain regions involved in the perception of gaze: a PET study. *Neuroimage.* 1998;8:221-227.
23. George N, Driver J, Dolan RJ. Seen gaze-direction modulates fusiform activity and its coupling with other brain areas during face processing. *Neuroimage.* 2001; 13:1102-1112.
24. Calder AJ, Lawrence AD, Keane J, Scott SK, Owen AM, Christoffels I, Young AW. Reading the mind from eye gaze. *Neuropsychologia.* 2002;40:1129-1138.
25. Turk J, Cornish K. Face recognition and emotion perception in boys with fragile-X syndrome. *J Intellect Disabil Res.* 1998;42:490-499.
26. Friston KJ, Zarahn E, Josephs O, Henson RN, Dale AM. Stochastic designs in event-related fMRI. *Neuroimage.* 1999;10:607-619.
27. Cohen JD, MacWhinney B, Flatt M, Provost J. PsyScope. *Behav Res Methods Instrum Comput.* 1993;25:257-271.
28. Ranganath C, D'Esposito M. Medial temporal lobe activity associated with active maintenance of novel information. *Neuron.* 2001;31:865-873.
29. Hayes C, Mathias C. Improved brain coil for fMRI and high resolution imaging [abstract]. In: *Proceedings of the Fourth Annual Meeting of the International Society for Magnetic Resonance in Medicine.* Berkeley, Calif: ISMRM; 1996:1414.
30. Glover GH, Lai S. Self-navigated spiral fMRI. *Magn Reson Med.* 1998;39:361-368.
31. Talairach J, Tournoux P. *Co-planar Stereotaxic Atlas of the Human Brain.* New York, NY: Thieme-Stratton Inc; 1988.
32. Holmes AP, Friston KJ. Generalizability, random effects, and population inference [abstract]. *Neuroimage.* 1998;7:S754.
33. Poline JB, Worsley KJ, Evans AC, Friston KJ. Combining spatial extent and peak intensity to test for activations in functional imaging. *Neuroimage.* 1997;5:83-96.
34. Brett M. The MNI brain and the Talairach atlas. Available at: http://www.mrc-cbu.cam.ac.uk/Imaging/mnispace.html. Accessed July 2001.
35. Reiss AL. *BrainImage.* 5.1.5 ed; Stanford, Calif: AL Reiss; 2002.
36. Cohen IL, Vietze PM, Sudhalter V, Jenkins EC, Brown WT. Parent-child dyadic gaze patterns in fragile X males and in non–fragile X males with autistic disorder. *J Child Psychol Psychiatry.* 1989;30:845-856.
37. Cohen IL, Vietze PM, Sudhalter V, Jenkins EC, Brown WT. Effects of age and communication level on eye contact in fragile X males and non–fragile X autistic males. *Am J Med Genet.* 1991;38:498-502.
38. Garrett AS, Flowers DL, Absher JR, Fahey FH, Gage HD, Keyes JW, Porrino LJ, Wood FB. Cortical activity related to accuracy of letter recognition. *Neuroimage.* 2000;11:111-123.
39. Reiss AL, Lee J, Freund L. Neuroanatomy of fragile X syndrome: the temporal lobe. *Neurology.* 1994;44:1317-1324.
40. Price JL. Prefrontal cortical networks related to visceral function and mood. *Ann N Y Acad Sci.* 1999;877:383-396.
41. Levesque J, Eugene F, Joanette Y, Paquette V, Mensour B, Beaudoin G, Leroux JM, Bourgouin P, Beauregard M. Neural circuitry underlying voluntary suppression of sadness. *Biol Psychiatry.* 2003;53:502-510.
42. Fredrikson M, Wik G, Annas P, Ericson K, Stone-Elander S. Functional neuroanatomy of visually elicited simple phobic fear: additional data and theoretical analysis. *Psychophysiology.* 1995;32:43-48.
43. Chua P, Krams M, Toni I, Passingham R, Dolan R. A functional anatomy of anticipatory anxiety. *Neuroimage.* 1999;9:563-571.
44. Bouilleret V, Dupont S, Spelle L, Baulac M, Samson Y, Semah F. Insular cortex involvement in mesiotemporal lobe epilepsy. *Ann Neurol.* 2002;51:202-208.
45. Augustine JR. Circuitry and functional aspects of the insular lobe in primates including humans. *Brain Res Brain Res Rev.* 1996;22:229-244.
46. Mazzocco MM, Kates WR, Baumgardner TL, Freund LS, Reiss AL. Autistic behaviors among girls with fragile X syndrome. *J Autism Dev Disord.* 1997;27:415-435.
47. Simon EW, Finucane BM. Facial emotion identification in males with fragile X syndrome. *Am J Med Genet.* 1996;67:77-80.
48. Bregman JD, Leckman JF, Ort SI. Fragile X syndrome: genetic predisposition to psychopathology. *J Autism Dev Disord.* 1988;18:343-354.
49. Schultz RT, Gauthier I, Klin A, Fulbright RK, Anderson AW, Volkmar F, Skudlarski P, Lacadie C, Cohen DJ, Gore JC. Abnormal ventral temporal cortical activity during face discrimination among individuals with autism and Asperger syndrome. *Arch Gen Psychiatry.* 2000;57:331-340.
50. Pierce K, Muller RA, Ambrose J, Allen G, Courchesne E. Face processing occurs outside the fusiform "face area" in autism: evidence from functional MRI. *Brain.* 2001;124:2059-20573.

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