Supplemental Document

Aberrant Face and Gaze Habituation in Fragile X Syndrome

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SA1: Appendix
Eye movement analysis and results

Eye gaze avoidance is a known characteristic of FXS; therefore, it is possible that participants with FXS may not have fully fixated on the facial stimuli used in this study. While pupil eye trackers can directly measure the locations of pupil fixation these devices may not be reliable when used in a scanner because the head motions of young subjects may jar and displace the eye tracker. Instead, we used a novel method to detect large eye movements or saccades using properties of the fMRI timeseries in the eye region. While not as definitive as eye tracker data, these eye movement measurements provided additional evidence of compliance with the task and attention to the stimuli.

Previous research has shown that eye movements affect the variance of the BOLD timeseries in the eye region, and that this variance can be correlated with eye motions detected using an eye tracker(1). However, the variance of the time series does not reflect the timing of the eye movements, and does not discriminate between the different task conditions of an experiment. To obtain this information, we use the fact that rapid motions may cause spikes in an fMRI time series, a phenomenon known as spin history artifacts(2). For the present study, we hypothesized that rapid eye movements would cause spin history artifacts in the eye regions, but not in other regions of the brain.

In the current study, these artifacts can be detected only if the eye movement occurs while an image slice that includes the eye is being collected. Since five 4-mm slices will cover a typical eye (19 mm diameter), and there are 30 slices in the image, this detection method can find only a small fraction (about 1/6) of the total eye movements. Furthermore, the amplitude of the spike is highly variable because it depends on the relative timing of the eye movement to the slice timing sequence, the specific slice location, and the amplitude of the eye movement. Thus, we chose to count only the presence of a spike instead of measuring the amplitude of the spike.

Eye movement methodology: We delineated ROIs that covered each eye region. A control ROI of equal size was delineated in the parietal lobe. The threshold for spike detection was chosen to provide maximal sensitivity to time series spikes in the eye region, while allowing a minimal number of false positives in the control region. It is expected that the control region should have no false positives because the potential artifacts in the control region caused by rapid head motions were removed using

ArtRepair software (http://cibsr.stanford.edu/tools/methods/artrepair-software.html) during preprocessing. Pilot tests using a range of thresholds from 1% to 5% signal change showed that a 2% threshold detected the most spikes in the eye region while yielding virtually no spikes in the control region. Thus, a 2% threshold was used for the spike-counting analyses.

We calculated the spike rate for each condition (direct gaze, averted gaze and fixation) by counting the total number of spikes in the fMRI signal of the eye region during each condition and dividing that count by the total duration of the condition measured in frames, where TR = 2 seconds. Spike rates were then compared between conditions and between groups.

Eye movement results: There were no spikes detected at the 2% spike detection threshold in the control region for any condition. For the eye ROIs, both groups had significantly higher spike rates during fixation than during either task condition ($p<0.001$). All group comparisons were covaried by age due to the relatively large age range and skewed distribution of age in the comparison group. There was a significant main effect of run ($p=0.047$) but no main effect of group or gaze direction. There was a significant group by gaze direction interaction ($p<0.01$). The FXS group had significantly more eye movements relative to the comparison group for the direct gaze condition ($p< 0.01$), but there were no significant group differences in the averted or fixation conditions ($p>0.10$). There were no significant group by run interactions and no within group differences between eye movements in run 1 vs run 2 for any condition (all p's>0.10).

Previous research on eye motion in healthy adults using an eye tracker has shown that saccades were rare in a fearful face experiment(3). Conversely, our results with pediatric participants suggest a notably higher saccade rate for both pediatric populations, e.g. 0.2 spikes/TR suggests an estimated rate of 0.6 saccades/second assuming a detection probability of 1/6 and TR=2 seconds. Thus, the spike rates in the current experiment suggest these children have substantially higher eye saccade rates than have been previously reported for healthy adults.

The low detection rates in the control parietal region suggest these eye motion results are valid. As further confirmation of our methodology, we also analyzed eye gaze data from a separate experiment in which healthy children viewed social movies and needed to report whether or not a red dot was located in the center of the movie images. Using the same methods and spike threshold, healthy children showed an average spike rate of 0.043 spikes/TR. Since this spike rate is well below the current experiment, it is likely that the high spike rates reported for the FSX and DD populations reflect eye movement differences between clinical pediatric subjects and healthy adults.

ST1: Raw data for spike rates per TR

Group - run	Facing	Averted	Combined	Fixation
FXS – run 1	0.217	0.205	0.211	0.514
FXS – run 2	0.238	0.208	0.223	0.525
Comparison – run 1	0.166	0.178	0.172	0.474
Comparison – run 2	0.195	0.214	0.205	0.467
FXS – control region average	0.003	0.003	0.003	0.005
Comparison – control region average	0.001	0.001	0.001	0.003

Data are spike rates per TR (repetition time) for eye regions of interest. Control region data is from a region delineated in the parietal lobe.

ST2: Regions with group difference in habituation to eye gaze (direct + averted gaze combined) Only for participants with >50% correct

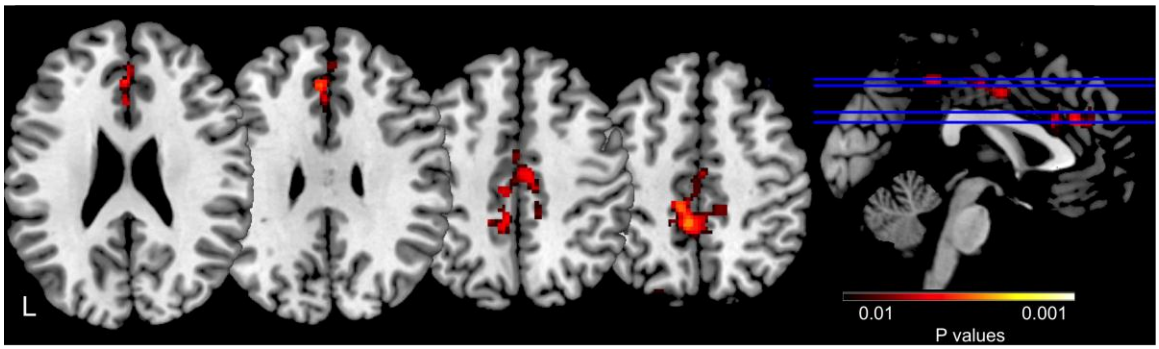
Brain Region	BA	# Voxels	Peak T	X	Y	Z	Follow-up within-group T tests[§]	
							FXS	Comparison
cingulate/left precuneus	7/31	513	5.11	-6	-42	48	Sensitization	Habituation
anterior cingulate	32/9	182	5.41	-4	34	28	Sensitization	Habituation
left lateral occipital	7	6	4.35	-22	-78	48	NS	NS

Peak coordinates in MNI space, for significant clusters $p < 0.01$ FWE corrected and > 20 voxels; BA = Brodmann area. Including only those participants whose accuracy was >50%, N = 46[§]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. Sensitization indicates there was a significant increase in activation from run 1 to run 2. NS indicates no significant change in activation from run 1 to run 2.

SF1: Examples of facial stimuli used in gaze habituation task.

Two examples of the facial stimuli used in the gaze habituation task. On the left is an example of a direct gaze stimulus, and on the right is an example of an averted gaze stimulus.

SF2: Group difference in habituation to direct and averted gaze stimuli combined, only including participants with < 50% accuracy on the gaze task.



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

SR1: References

1. Beauchamp MS: Detection of eye movements from fMRI data. Magnetic resonance in medicine 2003; 49:376–380

2. Friston KJ, Williams S, Howard R, Frackowiak RSJ, Turner R: Movement-Related effects in fMRI time-series. Magnetic resonance in medicine 1996; 35:346–355

3. Vuilleumier P, Armony JL, Driver J, Dolan RJ: Effects of Attention and Emotion on Face Processing in the Human Brain. Neuron 2001; 30:829–841