

Reduced basal forebrain and hippocampal activation during memory encoding in girls with fragile X syndrome

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Fragile X syndrome (FraX), the most common heritable cause of developmental disability, is associated with IQ, memory, and visuospatial processing deficits. The fragile X gene (*FMR1*) is prominently transcribed in two regions critical to memory encoding and attention: the hippocampus and the basal forebrain. To probe functional MRI activation abnormalities associated with the disorder, girls with FraX and age-matched, normally-developing girls were scanned during a test of visual memory encoding. While there

were considerable similarities in activation patterns between the two groups, the girls with FraX showed significantly less activation in the hippocampus and the basal forebrain. This is the first study, to our knowledge, demonstrating functional deficits in FraX subjects in brain regions known to have the highest *FMR1* transcription. *NeuroReport* 15:1579–1583 © 2004 Lippincott Williams & Wilkins.

Key words: Acetylcholine; Basal forebrain; Episodic memory; fMRI; Fragile X syndrome; Hippocampus; Nucleus basalis

INTRODUCTION

Fragile X syndrome is the most prevalent genetically inherited cause of mental retardation, affecting approximately 1:2000–1:4000 live births [1]. The disorder is caused by an expanded CGG repeat within a specific gene on the long arm of the X chromosome [2]. Abnormal methylation of this repeat and adjacent areas impedes transcription of the fragile X mental retardation gene (*FMR1*), resulting in reduced production of the fragile X mental retardation protein (FMRP) [3]. This protein is expressed in neurons, with particularly high levels of gene transcription occurring in the nucleus basalis and hippocampus [4]. Given the regional predilection of *FMR1* gene transcription and the prominent role of the hippocampus in episodic memory [5] it is not surprising that females with FraX, while generally less impaired than males, show impairments in episodic memory [6]. While minor structural abnormalities have been detected in the hippocampus [7], functional deficits in brain regions most prone to FMRP loss have not been reported. In an effort to probe functional hippocampal deficits, we undertook a functional MRI (fMRI) study of visuospatial encoding in females with FraX.

SUBJECTS AND METHODS

Subjects: After obtaining written, informed consent from subjects or their parents 23 females with DNA-confirmed

FraX participated in this study. Of the 23 subjects with FraX, we limited our analysis to the 12 who showed successful encoding during the task (see below). FraX subjects ranged in age from 7 to 22 years (mean (\pm s.d.) 15.2 ± 4.7). Informed consent was also obtained for a group of 16 healthy, age-matched females (ages 9–22 years, mean 14.9 ± 3.8) who served as control subjects. All subjects were determined to be right-hand dominant and screened for neurological, psychiatric, and developmental disorders. Approval for the study was given by the Institutional Review Board of Stanford University.

Fragile X diagnosis: The diagnosis of fragile X was confirmed by standard DNA (Southern blot) analysis. All subjects had one allele with the fragile X full mutation.

Neuropsychological assessment: IQ estimates based on the Wechsler Adult Intelligence Scale, Third Edition (WAIS-III) [8] for subjects over 16 and the Wechsler Intelligence Scale for Children-III (WISC-III) [9] for subjects age <16 years were obtained for each participant. Verbal IQ, performance IQ, and full scale IQ (FSIQ) scores were then derived for each subject.

Experimental design: During the encoding task subjects viewed color photographs of outdoor scenes presented in one of two conditions: (1) novel and (2) repeat. The

photographs depicted natural landscapes and cityscapes; examples can be seen in a separate publication [10]. In the novel, or experimental condition, subjects viewed a new visual scene every 3 s. In the repeat, or control condition, the same two scenes were alternated every 3 s. The experiment began with a 30 s passive fixation epoch, followed by twelve 24 s epochs of viewing images in each of the two task conditions, and ended with a 30 s passive fixation epoch (total time 5 min 48 s). One subject in the FraX group had only a 20 s fixation at the end of the scan. Subjects were instructed to study and remember the pictures. In a subsequent scan ~10 min later, they performed a yes/no recognition task with 64 visual scenes; two-thirds of the scenes were chosen from the stimuli previously seen in the encoding task, the remaining scenes were similar in composition, but had not been previously seen. We did not analyze the imaging data from the second scan but used the behavioral data from the recognition task to confirm that subjects were performing the encoding task properly. Details of the task have been described elsewhere [10].

fMRI acquisition: Images were acquired on a 1.5 T GE Signa scanner using a custom-built whole head coil that provided a 50% advantage in signal to noise ratio over that of the standard GE coil [11]. A custom-built head holder was used to prevent head movement. Eighteen axial slices (6 mm thick, 1 mm skip) parallel to the anterior commissure-posterior commissure line and covering the whole brain were acquired using a T2* weighted gradient echo spiral pulse sequence (TR=2000 ms, TE=40 ms, flip angle=89° and 1 interleave) [12]. The field of view was 24 cm and the effective inplane spatial resolution was 4.35 mm. To aid in localization of functional data, a high resolution T1-weighted spoiled grass gradient recalled (SPGR) 3D MRI sequence with the following parameters was used: TR=24 ms; TE=5 ms; flip angle=40°; 24 cm field of view; 124 slices in coronal plane; 256 × 192 matrix.

fMRI analysis: Images were reconstructed, by inverse Fourier transform, for each frame into 64 × 64 × 18 image matrices (voxel size 3.75 × 3.75 × 7 mm). Functional MRI data were pre-processed using Statistical Parametric Mapping (SPM99) software (Wellcome Department of Cognitive Neurology, London, UK). Images were corrected for movement, and normalized to Talairach space [13]. Subjects with >3 cm of translation or 3° of rotation on any axis on >10% of all images were excluded. One FraX subject and none of the control subjects were excluded on this basis. Otherwise, individual images with movement >3 mm translation in the x, y and z directions or rotation around the x, y and z axes were removed and replaced with the SPM-generated average of the two images surrounding it. Images were then resampled every 2 mm using sinc interpolation and smoothed with a 4 mm Gaussian kernel.

Statistical analysis was performed with SPM99. Voxel-wise t-statistics were normalized to Z scores to provide a statistical measure of activation that is independent of sample size. Group whole-brain activation analysis was performed using a random-effects model using a two-stage hierarchical procedure [14]. In the first step, an image was generated for each subject contrasting the experimental and control conditions. In the second step, these contrast

images were analyzed using a general linear model to determine voxelwise t-statistics. In order to determine the presence of significant clusters of activation, the joint expected probability distribution method [15] was used. Group statistical maps were generated using a one-sample t-test (height threshold $p < 0.01$, extent threshold $p < 0.05$). Group contrast maps were generated using a two-sample t-test (height and extent thresholds $p < 0.05$). There was a significant difference between groups in FSIQ and a trend towards a significant difference in performance on the recognition task (see below). Further, FSIQ and performance were not significantly correlated. As such, these two variables were treated as nuisance co-variates in the two-sample t-test.

Region of interest analysis: Hippocampal regions of interest (ROIs) were drawn on the group-averaged structural scans for each group (15 individual scans were averaged for the control structural image and 16 individual scans for the FraX structural image). The ROIs were drawn on the left and right hippocampus by a research staff member blinded to group status using a standard protocol [16]. These ROIs were used to determine the percentage of voxels within the left and right hippocampus that showed greater activity ($p < 0.05$) during experimental blocks versus control blocks. Percentage (rather than raw number) of voxels activated was chosen as the dependent variable to eliminate the potential confound of between-group functional differences arising on the basis of between-group differences in hippocampal volume.

Behavioral data analysis: We chose to limit our imaging analysis to those subjects who were performing the encoding task correctly (i.e. at least attempting to encode the images). Because there was no performance measure in the encoding task, we used encoding success, determined by analyzing subject's performance on the subsequent yes/no recognition task, as evidence that subjects were performing the encoding task correctly. Recognition accuracy was defined as the number of correct responses (both recognition of previously viewed scenes and rejection of foils) over the total number of scenes in the recognition task (64). All control subjects used in this study had ≥ 32 correct responses in the recognition task. Of the original 23 FraX subjects, one was excluded for excessive movement. Only nine of the 22 FraX remaining subjects had ≥ 32 correct responses. From 13 subjects who did not have ≥ 32 correct responses, we included three who showed evidence for successful encoding based on analysis of those questions to which they responded. Given this more lenient criterion, these subjects had to respond correctly to $\geq 67\%$ of the questions they did answer. These three subjects were correct in 24/24, 23/30, and 25/35 responses.

Overall, therefore, 12 FraX subjects from the original 23 were included; one subject was excluded based on the movement criteria and 10 were excluded based on the performance criteria. All of 16 control subjects met both the movement and behavior criteria for inclusion.

RESULTS

All results reported here are from the 12 FraX subjects and 16 controls included in the fMRI analysis.

IQ: FSIQ scores differed significantly between groups with a mean of 113 ± 15 in healthy controls and 90 ± 19 in FraX subjects ($p < 0.001$, two-sample *t*-test).

Recognition task: There was a non-significant trend towards a difference in the raw number of correct responses between groups. Healthy control subjects averaged 42 ± 6 correct responses whereas FraX subjects averaged 36 ± 10 correct responses ($p = 0.058$, two-sample *t*-test).

Whole-brain activation: Whole-brain analyses revealed similar activation patterns across the groups with prominent recruitment of occipito-temporal regions in the ventral visual processing stream. Fusiform gyrus and hippocampal activation were also detected in each group (Fig. 1). A direct comparison between groups (co-varied for FSIQ and recognition performance) revealed increased activation of a basal forebrain cluster in the control group compared to the FraX group (Fig. 2a). Using a more stringent height threshold of $p < 0.01$, we performed a *post-hoc* analysis of a 29-voxel subcluster in the region of the left nucleus basalis. The mean activation t-score within this region differed significantly between the two groups ($p < 0.01$, two-sample *t*-test) as shown in Fig. 2b.

The FraX group showed increased activity compared to the control group in three clusters. Two of these were in the

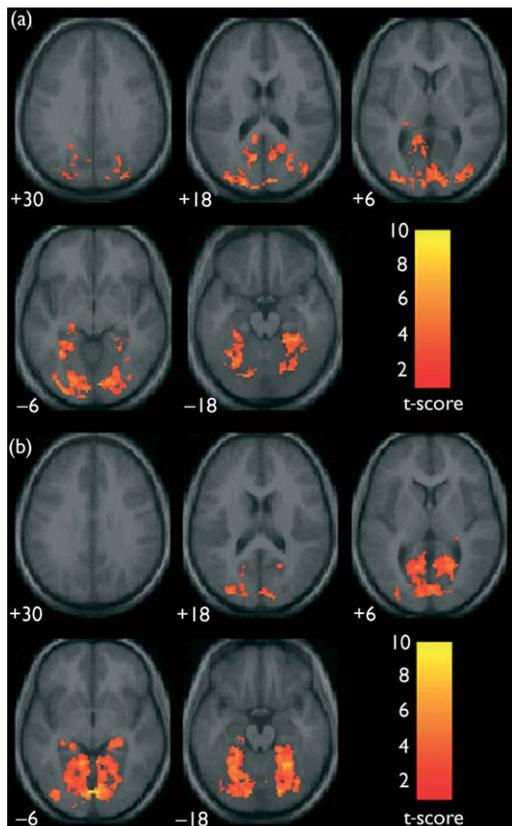


Fig. 1. Group activation during visual encoding. Axial images showing group activation during the visual encoding task for the FraX group (a) and control group (b). Functional images are overlaid on the respective group-averaged structural scans. The left side of the image corresponds to the left side of the brain.

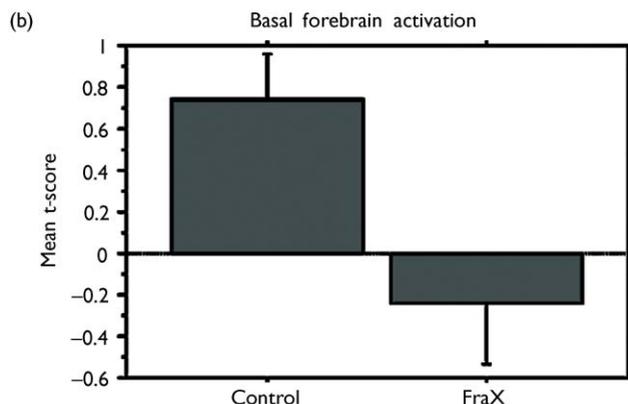
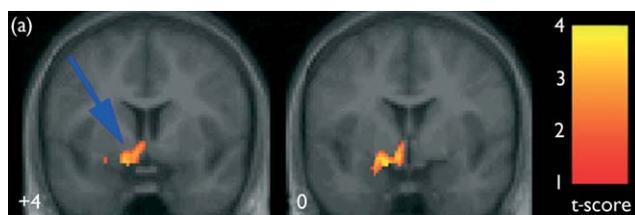


Fig. 2. Deficient basal forebrain activation in FraX. Coronal images showing regions activated more in the control group vs the FraX group after covarying for FSIQ and performance on the recognition task (a). The functional map was overlaid on the average structural map from the control group. The blue arrow indicates the approximate location of a 29 voxel subcluster in the region of the nucleus basalis whose average activation in the two groups is shown in (b). A two-sample *t*-test demonstrated a significant difference in the average activation for the nucleus basalis ROI after (and before) co-varying for IQ and performance ($df = 26$, $t = 2.79$, $p = 0.01$). After controlling for IQ and performance, the mean t-score was 0.74 (s.e. 0.22) for the control group and -0.25 (s.e. 0.29) for the FraX group. The black bars in (b) indicate s.e.

superior parietal region bilaterally and reflected greater activation in these regions in the FraX group (as also demonstrated from analysis and comparison of the +30 images in Fig. 1a,b). The third cluster was in the region of the right precentral gyrus. Unlike the two parietal clusters, this area was not activated in the FraX group as shown by the within-group contrast images (Fig. 1a). This region emerges as significant in the FraX vs control contrast as a result of greater deactivation (increased activity during control vs experimental epochs) in the control group. All significant within-group and between-group clusters are detailed in Table 1.

Region of interest: The ROI analysis demonstrated a significantly increased percentage of voxels activated in the left hippocampus of controls compared to FraX subjects ($df = 26$, $t = 2.38$, $p = 0.03$, two-sample *t*-test). Controls activated $54 \pm 22\%$ of voxels in the left hippocampus vs $36 \pm 17\%$ in subjects with FraX. There was no significant difference between activation in the right hippocampus ($df = 26$, $t = -0.19$, $p = 0.85$) where controls activated $43 \pm 19\%$ of voxels and FraX subjects activated $45 \pm 21\%$. Percentage of voxels activated in the left and right hippocampus did not correlate significantly with FSIQ or performance on the recognition task.

Table 1. Peak Talairach coordinates for within-group and between-group statistical maps.

Activated regions	Broadmann's Area (BA)	Cluster size (voxels)	Maximal z-score primary peak	Primary peak location
Control group				
Lingual/fusiform, parahippocampal, hippocampus	BA 18/19 BA 35 n/a	8660	5.62	-2, -78, -1
FraX group				
Lingual/fusiform, parahippocampal, hippocampus, superior parietal	BA 18/19 BA 35 n/a BA 7	6263	4.95	-22, -84, -6
Control-FraX				
Basal forebrain	n/a	607	3.50	-26, 11, -12
FraX-Control				
L. superior parietal	BA 7	2297	4.22	-24, -66, 35
R. superior parietal	BA 7	1699	3.47	24, -68, 37
R. precentral gyrus	BA 4/6	1053	3.40	24, -19, 53

DISCUSSION

We have demonstrated that FraX subjects performing a visual encoding task have deficient activation in two brain regions previously shown to have the highest levels of *FMRI* gene transcription: the hippocampus and the basal forebrain [4]. The basal forebrain activation abnormalities persisted after accounting for differences between the groups in FSIQ and encoding success (as determined by performance on a subsequent recognition task). The hippocampal activation did not correlate with FSIQ or encoding success. Thus, the deficient activation in the basal forebrain and hippocampus detected here does not reflect differences in FSIQ or task performance, but rather reflects differences in the neural substrate of these regions in FraX.

The hippocampus has long been implicated in memory function and is now fairly reliably activated in fMRI studies of episodic memory [17]. We have previously demonstrated mild structural differences in the hippocampus in FraX [7]. The current findings go further in suggesting that decreased FMRP in the hippocampus appears to translate into functional hippocampal abnormalities in girls with FraX. Although both groups showed evidence of bilateral hippocampal activation in the ROI analysis, there was a modest, but significant, reduction in left hippocampal activation in the FraX group. The increased superior parietal activity detected in the FraX group may reflect a compensatory mechanism related to deficient hippocampal activation. This interpretation is in keeping with the fMRI studies in Alzheimer's disease where impaired hippocampal activation during episodic memory tasks is often accompanied by increased compensatory activation in other regions including the parietal [18] and prefrontal cortices [19].

The nucleus basalis of Meynert, included within the basal forebrain cluster in this study, is a cholinergic nucleus with widespread connections to the neocortex. It is critical to visuospatial attention in rodents [20] and primates [21] and is presumed to play a similar role in humans. Recent functional imaging studies in humans have implicated the basal forebrain in episodic memory tasks [22,23]. In the current study, healthy controls showed increased activity in the basal forebrain/nucleus basalis region during the experimental blocks compared to the control blocks whereas subjects with FraX did not (Fig. 2b). This finding, considered in light of histological evidence showing high transcription

levels of *FMRI* in healthy nucleus basalis, suggests the possibility of a functional cholinergic deficit in FraX.

CONCLUSION

We have demonstrated functional abnormalities in the hippocampus and basal forebrain of girls with fragile X syndrome. These data converge with the finding that these two brain regions typically express the highest amount of *FMRI* mRNA in fetal brain tissue [4]. The basal forebrain deficit will be particularly important to confirm because it raises the possibility of treating this prevalent disorder with currently available medicines such as the acetylcholinesterase inhibitors.

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