Article

Functional Neuroanatomy of Visuospatial Working Memory in Fragile X Syndrome: Relation to Behavioral and Molecular Measures

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Objective: Fragile X syndrome is a neurogenetic disorder that is the most common known heritable cause of neurodevelopmental disability. This study examined the neural substrates of working memory in female subjects with fragile X syndrome. Possible correlations among behavioral measures, brain activation, and the FMR1 gene product (FMRP expression), as well as between IQ and behavioral measures, were investigated.

Method: Functional magnetic resonance imaging was used to examine visuospatial working memory in 10 female subjects with fragile X syndrome and 15 typically developing female subjects (ages 10–23 years). Subjects performed standard 1-back and 2-back visuospatial working memory tasks. Brain activation was examined in four regions of the cortex known to play a critical role in visuospatial working memory. Correlations between behavioral, neuroimaging, and molecular measures were examined.

Results: Relative to the comparison group, subjects with fragile X syndrome performed significantly worse on the 2-back task but not on the 1-back task. In a region-of-interest analysis focused on the inferior frontal gyrus, middle frontal gyrus, superior parietal lobule, and supramarginal gyrus, comparison subjects showed significantly increased brain activation between the 1-back and 2-back tasks, but subjects with fragile X syndrome showed no change in activation between the two tasks. Significant correlations were found in comparison subjects between activation in the frontal and parietal regions and the rate of correct responses on the 2-back task, but not on the 1-back task. In subjects with fragile X syndrome, significant correlations were found during the 2-back task between FMRP expression and activation in the right inferior and bilateral middle frontal gyri and the bilateral supramarginal gyri.

Conclusions: Subjects with fragile X syndrome are unable to modulate activation in the prefrontal and parietal cortex in response to an increasing working memory load, and these deficits are related to a lower level of FMRP expression in fragile X syndrome subjects than in normal comparison subjects. The observed correlations between biological markers and brain activation provide new evidence for links between gene expression and cognition.

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Fragile X syndrome is the most common inherited cause of neurodevelopmental disability, occurring at a frequency of approximately one in every 2,000 to 4,000 live births (1). The syndrome arises from disruption in expression of the FMR1 gene, which is most commonly caused by expansion of a CGG repeat stretch in the gene, with resultant methylation and silencing of expression. The absence of the FMR1 gene product (FMRP) in neurons is associated with abnormal morphology of dendritic spines and a reduction in the length of synapses in the cortex (2, 3). The neuropsychological profile of fragile X syndrome is notable for mental retardation, as well as difficulties in visual memory and perception, mental manipulation of visuospatial relationships among objects, visual-motor coordination, processing of sequential information, and executive function (4–6). In this study, we used a working memory task to investigate deficits in higher-order cognition in subjects with fragile X syndrome. Impairment in visuospatial working memory may be an important contributing factor to the behavioral profile of people with fragile X syndrome. For example, impairment in the processing and retention of information in social situations, an area of cognition known to be heavily dependent on nonverbal skills, may be implicated in difficulties known to occur in subjects with fragile X syndrome, such as poor social relatedness, avoidance, and anxiety (7–9).

Working memory is the ability to hold and manipulate information online in the brain (10–12). The component processes involved in working memory—encoding, rehearsal, storage, and executive processes on the contents of stored memory—represent key cognitive operations of the human brain. Neurophysiological studies have sug-

TABLE 1. Demographic and Neuropsychological Characteristics of Female Subjects With Fragile X Syndrome and Healthy Comparison Subjects in a Study of Visuospatial Working Memory

	Subjects With Fragile X Syndrome (N=10)			Comparison Subjects (N=15)			Analysis		
Characteristic	Mean	SD	Range	Mean	SD	Range	t	df	p
Age (years)	17.23	4.49	10.2–22.7	15.05	4.58	7.66–21.6	1.17	23	0.25
IQ									
Full-scale	84	12	65–108	117	13	93–137	6.46	23	<0.001
Verbal	86	13	66–111	115	12	94–134	5.67	23	<0.001
Performance	85	13	62–104	117	13	93–132	6.09	23	<0.001
Judgment of Line Orientation score	11.1	9.6	0.0–24.0	25.3	4.0	18.0–30.0	4.23	22	<0.001
Woodcock-Johnson spatial reasoning test score	41.0	7.7	31.0–53.0	61.9	7.3	50.0–75.0	6.37	20	<0.001

gested that the prefrontal cortex plays a critical role in working memory (13–16), although other brain regions, notably the parietal cortex, also play an important role (17, 18). Positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) studies have investigated the neural substrates of visuospatial working memory and its components, and most studies have shown significant prefrontal cortex as well as parietal cortex involvement (19–23).

Although there are a few behavioral and functional neuroimaging studies involving subjects with fragile X syndrome (24–26), we are aware of no studies that have specifically examined working memory in this population, even by using behavioral measures. In this study, we investigated the neural substrates of working memory in female subjects with fragile X syndrome. Given that subjects with fragile X syndrome show significant deficits in visuospatial cognition as well as in executive function, we hypothesized that they would show significant impairment in performance on visuospatial 1-back and 2-back working memory tasks. We also hypothesized that they would show significant deficits in activation in brain regions known to be involved in working memory—particularly, the inferior and middle frontal gyri, the dorsolateral prefrontal cortex, and the parietal cortex. Whole-brain voxel-by-voxel analyses were utilized to investigate differences in areas outside the regions of interest. We then examined possible correlations among behavioral measures, brain activation, and FMRP expression, as well as between IQ and behavioral measures.

Method

Subjects, Diagnosis, and Molecular Measures

Ten female subjects with a diagnosis of fragile X syndrome (mean age=17.2 years, SD=4.5, range=10–23) were recruited from throughout the United States (Table 1). Subjects were recruited through advertisements in national newsletters for fragile X syndrome patients and their caregivers, through prior clinical contacts, and through contact at national conferences. After complete description of the study to the subjects and caregivers, written informed consent was obtained. Fifteen female comparison subjects also participated (mean age=15.1 years, SD=4.6, range=8–22). The diagnosis of fragile X syndrome (presence of an FMR1 full mutation) was confirmed by DNA analysis. Standard Southern blot and polymerase chain reaction analyses were performed, followed by FMR1-specific probe hybridization (27). The CGG repeat number was calculated from the Southern blot autoradiogram images. A PhosphorImager (Molecular Dynamics, Inc., Sunnyvale, Calif.) was used to quantitate the radioactive intensity of the normal methylated and unmethylated bands of each sample on the Southern blot. The FMR1 gene activation ratio, the intensity of the normal unmethylated band divided by the sum of the intensities of both the normal unmethylated and methylated bands, was calculated (28). The FMR1 gene activation ratio represents the proportion of the normal (nonmutant) FMR1 gene that is on the active X chromosome and reflects the proportion of cells that can express FMRP. FMRP expression was ascertained by calculating the percentage of peripheral lymphocytes containing FMRP by using immunostaining techniques (29).

Neuropsychological Assessment

IQ was determined by using the Wechsler intelligence scales. The WISC-III (30) was administered to those under age 17 years, and the WAIS-III (31) was administered to those over age 17 years. Spatial reasoning and recognition were assessed by using the Woodcock-Johnson spatial reasoning test (32) and the Judgment of Line Orientation test (33).

Experimental Design

The visuospatial 1-back and 2-back working memory tasks consisted of rest, experimental (E) and control (C) epochs in the following order: rest-E-C-E-C-E-C-rest-E-C-E-C-E-C-rest. Thus, there were three rest epochs, six experimental epochs, and six control epochs in each task. Each rest epoch was 30 seconds long, during which subjects passively viewed a blank screen. Experimental epochs began with a 4-second display of the instructions, "Push for 1 Back," in the 1-back task, and "Push for 2 Back," in the 2-back task. Control epochs began with a 4-second display of the instructions, "Push for Center." Each control and experimental epoch consisted of 16 stimuli presented for 500 msec each, with a 1500-msec interstimulus interval. The stimulus was the letter "O" presented in one of nine distinct visuospatial locations in a 3×3 matrix. In the 1-back task, the subject was instructed to respond if the stimulus was in the same location as in the previous trial. In the 2-back task, the subject was instructed to respond if the stimulus was in the same location as in two trials back. In the control task, the subject was instructed to respond if the stimulus was in the center position.

Behavioral Data Analysis

The rate of correct responses refers to the percentage of stimulus trials in which the subject responded correctly, either with an appropriate button push or an appropriate inhibition. The miss rate is the percentage of trials in which the subject failed to respond appropriately. The false alarm rate is the percentage of stimuli to which the subject responded with a button push when no response was needed. Subjects' reaction times also were measured.

fMRI Acquisition

Images were acquired on a 1.5-T GE Signa scanner with Echospeed gradients (General Electric Medical Systems, Milwaukee) by using a custom-built whole-head coil that provides a 50% advantage in signal-to-noise ratio over that provided by the standard GE coil (34). Eighteen axial slices (6 mm thick, 1 mm skip) parallel to the anterior and posterior commissures covering the whole brain were imaged with a temporal resolution of 2 seconds by using a T_{2}*-weighted, gradient echo, spiral pulse sequence (TR=2000 msec, TE=40 msec, flip angle=89° and 1 interleave) (35). The field of view was 240 mm, and the effective in-plane spatial resolution was 4.35 mm. To aid in localization of functional data, a high-resolution T_1-weighted, spoiled gradient recalled echo acquisition in the steady state, three-dimensional MRI sequence with the following parameters was used: TR=35 msec, TE=6 msec, flip angle=45°, 24 cm field of view, 124 slices in coronal plane, 256 × 192 matrix, acquired resolution=1.5 × 0.9 × 1.2 mm. On several scans of subjects with fragile X syndrome, a faster protocol was utilized to decrease time of acquisition of the spoiled gradient recalled echo acquisition in the steady state image, with TR=11 msec, TE=2 msec, and flip angle=15°. The images were reconstructed as a 124 × 256 × 256 matrix with a 1.5 × 0.9 × 0.9 mm spatial resolution.

The task was programmed by using PsyScope (36) on a Macintosh (Sunnyvale, Calif.) computer. The initiation of the scan and the task was synchronized by using a TTL (transistor-transistor logic) pulse delivered to the scanner timing microprocessor board from a "CMU Button Box" microprocessor (http://psyscope.psy.cmu.edu) connected to the Macintosh computer. The stimuli were presented visually at the center of a screen by using a custom-built, magnet-compatible projection system (Resonance Technology, Los Angeles).

Image Preprocessing

The images were reconstructed, by using inverse Fourier transform, for each of the 261 time points into 64 × 64 × 18 image matrices (voxel size: 3.75 × 3.75 × 7 mm). The fMRI data were preprocessed by using statistical parametric mapping (SPM 99) software (Wellcome Department of Cognitive Neurology, Institute of Neurology, University College, London). The images were corrected for movement by using least-square minimization without higher-order corrections for spin history and were normalized to stereotaxic Talairach coordinates (37). The images were then resampled every 2 mm by using sinc interpolation and smoothed with a 4-mm Gaussian kernel to decrease spatial noise.

Statistical Analysis

Statistical analysis was performed on individual and group data by using the general linear model and the theory of Gaussian random fields as implemented in SPM 99. This method takes advantage of multivariate regression analysis and corrects for temporal and spatial autocorrelations in the fMRI data (38).

In the first step, a within-subject procedure was used to model all the effects of interest, covariates, and nuisance variables for each subject. The individual subject models were identical across subjects (i.e., a balanced design was used). Confounding effects of fluctuations in global mean were removed by proportional scaling where, for each time point, each voxel was scaled by the global mean at that time point. Low-frequency noise was removed with a high-pass filter (0.5 cycles/min) applied 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. We then defined the effects of interest for each subject with the relevant contrasts of the parameter estimates. For each of these contrasts, a corresponding contrast image was also generated. Voxel-wise t statistics were normalized to z scores to provide a statistical measure of activation that is independent of size of the study group. Finally, to determine the presence of significant clusters of activation, the joint expected probability distribution of the height and extent of the z scores (39), with height ($z>2.33$, $p<0.01$, with corrected p values) and extent ($p<0.05$) thresholds, was used to correct for spatial correlations in the data.

Whole brain analysis. The aim of this analysis was to determine which brain regions showed significant activation for each main effect and interaction of interest. The group analysis was performed by using a random-effects model with a two-stage hierarchical procedure. This model estimates the error variance for each condition of interest across subjects, rather than across scans (40), and therefore provides a stronger generalization to the population. This analysis consisted of two steps. In the first step, contrast images for each subject and each effect of interest were generated, as described above. In the second step, these contrast images were compared by using a general linear model analysis to determine appropriate t statistics. Thus, for example, with one contrast image per subject, reflecting activation during the 2-back experimental condition contrasted with the control condition, a one-way t test was used to determine group activation for that contrast. Finally, the t statistics were normalized to z scores, and significant clusters of activation were determined by using the joint expected probability distribution of height and extent of z scores, with height ($z>2.33$, $p<0.01$) and extent ($p<0.05$) thresholds.

Region-of-interest analysis. The aim of this analysis was to investigate the profile of regional task-related differences in activation. The group analysis was conducted by using z scores derived from the individual subject analysis, as described above. The percentage of voxels, in each region of interest, with $z>2.33$ ($p<0.01$) was determined for each relevant contrast and entered into an analysis of variance (ANOVA). A four-way repeated measures ANOVA with the factors region of interest (inferior frontal gyrus, middle frontal gyrus, superior parietal lobule, supramarginal gyrus), hemisphere (left, right), task (1-back, 2-back), and condition (experimental, control) was done. A standard analysis framework (41) was used to interpret interactions, simple effects, and main effects. An alpha level for significance of $p<0.05$ (two-tailed) was used.

Regions of Interest

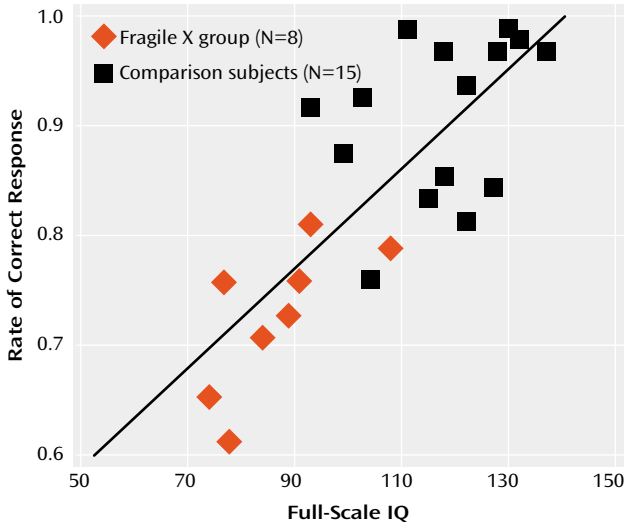
Regions of interest were demarcated separately in the left and right hemispheres from the average T_1-weighted Talairach normalized images.

Inferior frontal gyrus. By using a surface rendering of the average brain, the anterior border was identified by the junction of the lateral orbital sulcus and inferior frontal sulcus. The posterior border was defined by the precentral sulcus. The superior border was defined by the inferior frontal sulcus. The inferior border was defined by the lateral orbital sulcus until it could no longer be visualized. For the most posterior part of the inferior frontal gyrus, the inferior border was the horizontal ramus of the lateral fissure. Medially, the border was defined by a straight line joining the deepest point of the inferior frontal sulci and the deepest point of the lateral orbital sulci. These definitions closely followed surface sulci demarcated by Duvernoy (42) and Ono et al. (43).

Middle frontal gyrus. The superior frontal sulcus defined the medial boundary for this region. The frontomarginal sulcus and the inferior frontal sulcus defined the lateral boundary. At more posterior slices where the inferior frontal sulcus disappears, the superior precentral sulcus was used as the lateral boundary. The posterior boundary for this region was the precentral gyrus.

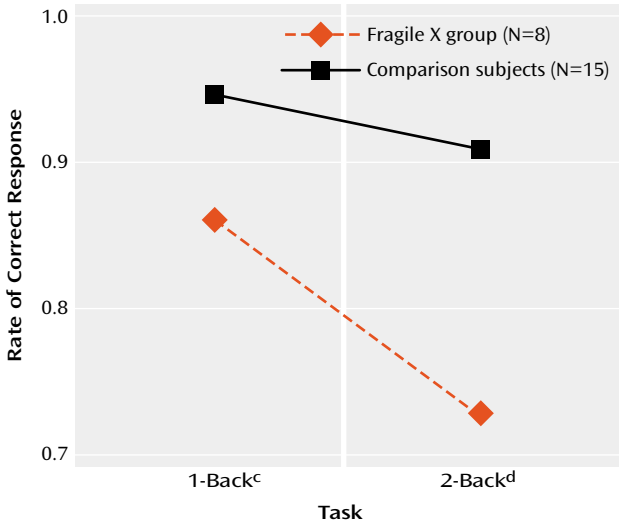
Parietal cortex. Information from triplanar views was used to delineate the parietal lobe and parcel the lobe into the superior parietal lobule, supramarginal gyrus, and angular gyrus. The pari-

FIGURE 1. Correlation Between Full-Scale IQ and Accuracy in the Experimental Condition of a 2-Back Visuospatial Working Memory Task[a] of Female Subjects With Fragile X Syndrome and Healthy Comparison Subjects



[a] Subjects were presented with a series of 3 × 3 matrices with nine distinct visuospatial locations. Subjects were instructed to respond if the stimulus was in the same location as two trials back.

FIGURE 2. Accuracy in Experimental Condition of 1-Back and 2-Back Visuospatial Working Memory Tasks[a] of Female Subjects With Fragile X Syndrome and Healthy Comparison Subjects[b]



[a] Subjects were presented with a series of 3 × 3 matrices with nine distinct visuospatial locations. Subjects were instructed to respond if the stimulus was in the same location as in the previous trial for the 1-back task or in two trials back for the 2-back task.
[b] Significant interaction of diagnosis and task, covaried by full-scale IQ (F=9.8, df=1, 21, p<0.005).
[c] Significant difference between groups (F=6.9, df=1, 21, p<0.02).
[d] Significant difference between groups (F=32.7, df=1, 21, p<0.0001).

etal lobe was delineated by first marking the postcentral sulcus as its anterior border. The inferior border was the sylvian fissure until it was no longer identifiable in the coronal section progressing in the anterior to posterior direction. At this point, the inferior and medial borders were defined by the parallel sulcus and the interhemispheric fissure, respectively. Next, in the midsagittal view, a line parallel to the parieto-occipital fissure was identified and linearly interpolated to the parieto-occipital notch on the lateral surface. This defined the posterior border of the parietal lobe. The parietal lobe was then parceled by using neuroanatomical landmarks identified in the axial plane. The intraparietal sulcus was used to demarcate the superior parietal lobule from the supramarginal gyrus and angular gyrus. The intraparietal sulcus and the ascending posterior segment of the parallel sulcus were then used to separate the supramarginal gyrus and angular gyrus.

Data Analysis

IQ data and results on the Woodcock-Johnson spatial reasoning test and the Judgment of Line Orientation test were compared between groups by using t tests and an alpha level of 0.05. Correct and incorrect response rates and reaction times on the visuospatial working memory tasks were compared by using a two-by-two factorial analysis of covariance (ANCOVA) with the factors diagnosis, task (1-back versus 2-back), and condition (experimental versus control); an alpha level of 0.05; and full-scale IQ as a nuisance covariate. Pearson product-moment correlation was used to analyze the relationship between performance on the working memory tasks and functional brain activation. Spearman correlations were also performed when data were suspected of being nonnormalized.

Results

Neuropsychological Measures

The mean full-scale IQ was 84 for the fragile X syndrome group and 117 for the comparison group, a significant difference (Table 1). Group differences on verbal IQ and performance IQ were also significant (Table 1). Significant group differences of similar magnitude were also observed on the Woodcock-Johnson spatial reasoning test and the Judgment of Line Orientation test (Table 1).

Behavioral Task Analysis

Because comparison and fragile X syndrome groups differed significantly in IQ, full-scale IQ was used as a covariate in all behavioral analyses described below. There was a statistically significant correlation between full-scale IQ and performance on the experimental condition of the 2-back task for the combined groups (r=0.78, df=21, p<0.001) (Figure 1). When data for each group were examined separately, no significant correlation was seen in either the fragile X syndrome group (r=0.70, df=7, p=0.051) or the comparison group (r=0.35, df=14, p=0.19), although the former correlation approached significance. Experimental behavioral data were successfully collected from all comparison subjects and from eight of the 10 subjects with fragile X syndrome.

The percentages of correct responses on the visuospatial working memory tasks in the two groups were compared by using ANCOVA (Table 2). Main effects of task (F=26.5, df=1, 21, p<0.0001) and condition (F=106.4, df=1, 21, p<0.0001) were observed, but a main effect of diagnosis was not (F=0.6, df=1, 20, p=0.44). There was a significant interaction of diagnosis, task, and condition (F=8.9, df=1, 21, p<0.008). A separate two-way ANCOVA examining the

TABLE 2. Performance on 1-Back and 2-Back Visuospatial Working Memory Tasks of Female Subjects With Fragile X Syndrome and Healthy Comparison Subjects

	Subjects With Fragile X Syndrome (N=8)								Comparison Subjects (N=15)							
	% Correct[b]		% Missed[c]		% False Alarms[d]		Reaction Time (msec)		% Correct[b]		% Missed[c]		% False Alarms[d]		Reaction Time (msec)	
Task and Condition[a]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1-back task																
Control condition	98	2	2	2	1	1	630	177	98	2	1	1	1	2	654	147
Experimental condition	86	11	6	5	8	8	714	195	95	4	4	3	2	3	684	150
2-back task																
Control condition	97	3	2	2	1	1	642	179	98	2	1	2	0	1	629	113
Experimental condition	73	7	15	3	13	8	727	190	91	7	6	5	3	5	725	215

[a] Subjects were presented with a series of 3 × 3 matrices with nine distinct visuospatial locations. In the experimental condition, subjects were instructed to respond if the stimulus was in the same location as in the previous trial for the 1-back task or in two trials back for the 2-back task. In the control condition, subjects were instructed to respond if the stimulus was in the center location.
[b] Percentage of stimulus trials in which the subject responded or inhibited response appropriately.
[c] Percentage of stimulus trials in which the subject failed to respond appropriately.
[d] Percentage of stimulus trials in which the subject responded when response should have been inhibited.

interaction of diagnosis and task was not significant for the control condition ($F=0.2$, df=1, 21, $p<0.70$) but was significant for the experimental condition (Figure 2). Planned comparisons of this two-way interaction showed that comparison subjects did not differ significantly in performance on the 2-back versus the 1-back task (91% correct responses on 2-back task versus 95% on the 1-back task) ($F=4.2$, df=1, 21, $p=0.052$), whereas subjects with fragile X syndrome performed significantly worse on the 2-back task than on the 1-back task (73% correct responses on 2-back task versus 86% on the 1-back task) ($F=28.9$, df=1, 21, $p<0.0001$). Moreover, subjects with fragile X syndrome performed worse than comparison group subjects on the experimental conditions of both tasks, with the difference statistically significant on both tasks (Figure 2).

Analysis of false alarms showed main effects of task ($F=24.5$, df=1, 21, $p<0.0001$) and condition ($F=26.2$, df=1, 21, $p<0.0001$) but not of diagnosis ($F<0.1$, df=1, 20, $p=0.92$). The two-way interaction of diagnosis and task was significant ($F=10.9$, df=1, 21, $p<0.004$). Subjects with fragile X syndrome had significantly higher false alarm rates ($F=26.1$, df=1, 21, $p<0.0001$) in the 2-back versus the 1-back task. The comparison subjects showed no difference ($F=1.9$, df=1, 21, $p=0.18$) in false alarms between the tasks. No significant three-way interaction (diagnosis-by-task-by-condition) was observed ($F=4.2$, df=1, 21, $p=0.053$). For reaction time, there were no significant interactions of diagnosis with any of the factors. There was no main effect of diagnosis ($F=0.4$, df=1, 20, $p=0.56$). A main effect of condition was observed ($F=18.6$, df=1, 21, $p<0.0003$).

Brain Activation: Whole Brain Analysis

Comparison subjects. In the 1-back experimental condition compared to the control condition, comparison subjects showed significant activation in the right middle frontal gyrus and superior frontal gyrus and in the left middle frontal gyrus and superior frontal gyrus (Table 3, Figure 3). In the 2-back experimental condition compared to the control condition, comparison subjects showed significant activation in the left pre-supplemental motor area, right middle frontal gyrus, right inferior frontal gyrus, right precuneus, right angular gyrus, right superior parietal lobule, right inferior parietal lobule, right supramarginal gyrus, left superior parietal lobule, left inferior parietal lobule, left middle frontal gyrus/precentral gyrus, and the right superior cerebellum bordering on the fusiform gyrus (Table 3, Figure 3). When the 1-back and 2-back tasks were directly compared, comparison group subjects showed significantly greater activation in the 2-back task in the pre-supplemental motor area, left inferior parietal lobule, left supramarginal gyrus, intraparietal sulcus, and right superior parietal lobule.

Subjects with fragile X syndrome. In the 1-back condition compared to the control condition, significant activation in subjects with fragile X syndrome was found in the right middle frontal gyrus, right superior frontal gyrus, right inferior frontal gyrus, right inferior parietal lobule, right insula, pre-supplemental motor area, left middle frontal gyrus, left superior parietal lobule, left precuneus, left inferior parietal lobule, and left angular gyrus (Table 3, Figure 3). In the 2-back condition compared to the control condition, subjects with fragile X syndrome activated the pre-supplemental motor area, right middle frontal gyrus, right inferior frontal gyrus, left superior frontal gyrus, left middle frontal gyrus, right inferior parietal lobule, right superior parietal lobule, and right supramarginal gyrus (Table 3, Figure 3). When the 1-back and 2-back tasks were directly compared, subjects with fragile X syndrome showed significantly greater activation in the 2-back task in the right superior frontal gyrus and left inferior frontal gyrus bordering on the left superior temporal gyrus.

Brain Activation: Region-of-Interest Analysis

Regional differences in activation were investigated by using a four-way ANCOVA, with one between-group factor, diagnosis (comparison group versus fragile X syndrome group), and three within-group factors (region of

TABLE 3. Brain Regions With Significant Activation During 1-Back and 2-Back Visuospatial Working Memory Tasks, Relative to a Control Condition,[a] in Healthy Comparison Subjects and Female Subjects With Fragile X Syndrome

Subject Group, Task, and Activated Regions	Number of Voxels	z Score (maximum)	p (corrected for height and extent of z score)	Talairach Coordinates of Location of Peak Activation		
				x	y	z
Comparison group (N=15)						
1-back task						
Right middle frontal gyrus (Brodmann's areas 6 and 9)	1,507	4.49	<0.001	42	6	38
Right superior frontal gyrus (Brodmann's area 6)	774	4.41	<0.001	2	22	54
Left middle frontal gyrus, superior frontal gyrus (Brodmann's areas 6 and 45)	665	3.78	<0.001	–54	24	38
2-back task						
Pre-supplemental motor area, right middle frontal gyrus (Brodmann's area 6)	4,787	4.93	<0.001	–2	32	50
Right inferior frontal gyrus and middle frontal gyrus (Brodmann's area 47)	498	4.85	0.001	36	24	–6
Right precuneus, inferior parietal lobule, angular gyrus, superior parietal lobule, supramarginal gyrus (Brodmann's areas 7 and 19); left superior parietal lobule, inferior parietal lobule (Brodmann's area 40)	6,004	4.83	<0.001	2	–64	56
Left middle frontal gyrus, precentral gyrus (Brodmann's area 6)	2,679	4.75	<0.001	–26	2	60
Right fusiform/cerebellum (Brodmann's area 19)	272	3.73	<0.04	18	–58	–12
Fragile X syndrome group (N=10)						
1-back task						
Right inferior parietal lobule (Brodmann's area 40)	324	4.92	0.002	52	–42	46
Right superior frontal gyrus, middle frontal gyrus (Brodmann's areas 10 and 8)	1,444	4.70	<0.001	28	56	4
Left superior parietal lobule, precuneus (Brodmann's area 7)	224	4.22	<0.03	–6	–66	62
Right middle frontal gyrus, superior frontal gyrus (Brodmann's area 6)	265	3.95	0.007	28	8	56
Left superior parietal lobule, inferior parietal lobule, angular gyrus (Brodmann's areas 7 and 39)	241	3.85	<0.02	–42	–62	52
Right insula, middle frontal gyrus, inferior frontal gyrus (Brodmann's areas 45 and 47)	261	3.77	0.008	36	26	–2
Pre-supplemental motor area (Brodmann's area 8)	303	3.47	0.003	4	36	54
Left middle frontal gyrus – dorsolateral prefrontal cortex (Brodmann's areas 9 and 45)	295	3.40	0.003	–48	30	34
2-back task						
Pre-supplemental motor area (Brodmann's area 8)	751	5.30	<0.001	0	40	40
Left middle frontal gyrus, superior frontal gyrus (Brodmann's area 6)	226	4.12	<0.04	–24	8	60
Right inferior parietal lobule, superior parietal lobule (Brodmann's area 7)	438	4.05	<0.001	32	–62	44
Right supramarginal gyrus (Brodmann's area 40)	469	3.92	<0.001	54	–46	32
Right middle frontal gyrus (Brodmann's areas 8 and 9)	428	3.72	<0.001	30	36	42
Right middle frontal gyrus, inferior frontal gyrus (Brodmann's areas 8 and 44)	263	3.51	0.02	34	8	30

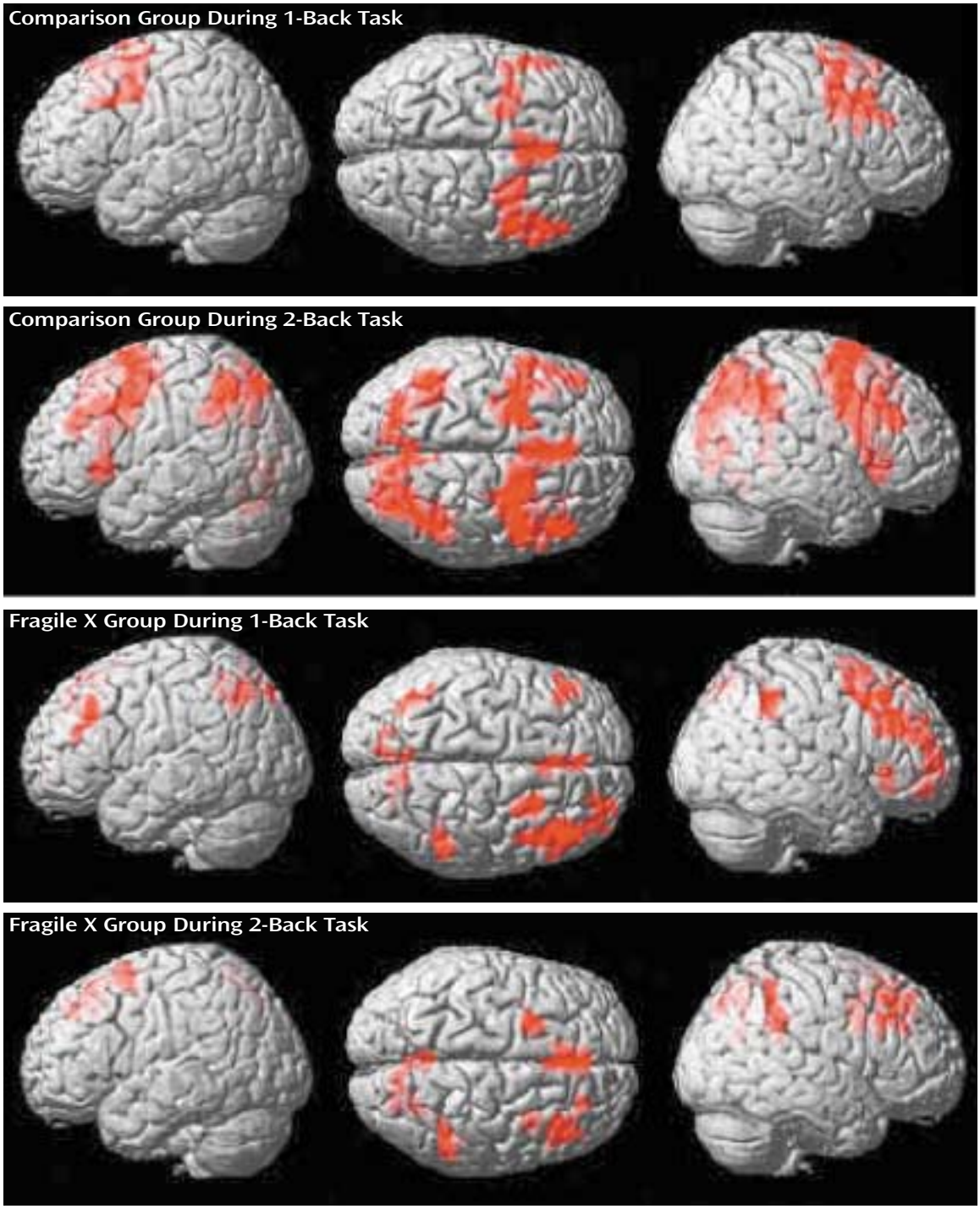
[a] Subjects were presented with a series of 3 × 3 matrices with nine distinct visuospatial locations. In the experimental condition, subjects were instructed to respond if the stimulus was in the same location as in the previous trial for the 1-back task or in two trials back for the 2-back task. In the control condition, subjects were instructed to respond if the stimulus was in the center location.

interest, task, and hemisphere), with full-scale IQ as a covariate.

Statistically significant main effects were observed for region of interest (middle frontal gyrus>inferior frontal gyrus>superior parietal lobule>supramarginal gyrus; F=9.8, df=3, 69, p<0.0001), hemisphere (right>left; F=8.4, df=1, 23, p=0.008), and task (2-back>1-back; F=8.1, df=1, 23, p=0.009). There was no main effect of diagnosis (F=0.2, df=1, 22, p=0.65). However, a significant diagnosis-by-task interaction was observed (F=10.8, df=1, 23, p=0.003). The comparison subjects showed a significant increase in activation between the 1-back and 2-back tasks (F=23.6, df=1, 23, p<0.0001), but subjects with fragile X syndrome showed no difference between the two tasks (F=0.1, df=1, 23, p=0.78). When each task was examined separately, the comparison subjects and subjects with fragile X syndrome did not differ significantly on the 1-back (F=0.4, df=1, 22, p=0.51) or 2-back (F=0.1, df=1, 22, p=0.79) tasks. Therefore, the diagnosis-by-task interaction was driven almost entirely by the working memory load-specific increases in activation shown by the comparison subjects together with the absence of any such increase in subjects with fragile X syndrome. Furthermore, no regional differences in activation were observed as indicated by the lack of an interaction of region of interest, diagnosis, and task. Other higher-order interactions were nonsignificant.

The pattern for each region of interest was that of a significant increase in activation between the 1-back and 2-back tasks for comparison group subjects and a generally flat response for subjects with fragile X syndrome (Figure 4). All four regions of interest showed significant diagnosis-by-task interactions in activation. In the inferior frontal gyrus, comparison subjects showed significant increase in activation from the 1-back to the 2-back task (F=

FIGURE 3. Whole-Brain Activation During 1-Back and 2-Back Working Memory Tasks, Relative to a Control Condition,[a] in Female Subjects With Fragile X Syndrome (N=10) and Healthy Comparison Subjects (N=15)[b]



[a] Subjects were presented with a series of 3 × 3 matrices with nine distinct visuospatial locations in each task. In the experimental condition, subjects were instructed to respond if the stimulus was in the same location as in the previous trial for the 1-back task or in two trials back for the 2-back task. In the control condition, subjects were instructed to respond if the stimulus was in the center location.
[b] Areas of significant activation for subject groups determined by using a general linear model to yield images of group activation for each contrast.

6.1, df=1, 23, p=0.02), whereas subjects with fragile X syndrome showed a nonsignificant decrease (F=1.7, df=1, 23, p=0.20). Similarly, the comparison subjects showed significant increases in the middle frontal gyrus (F=10.7, df=1, 23, p=0.003), superior parietal lobule (F=44.3, df=1, 23, p<0.001), and supramarginal gyrus (F=34.6, df=1, 23, p<0.001), while subjects with fragile X syndrome did not show such increases (p>0.20).

Brain Activation and Performance

As a corollary to the analysis that showed an association between performance on the visuospatial working memory tasks and full-scale IQ (Figure 1), the relationship between brain activation and full-scale IQ was examined. When the two subject groups were combined (N=25), Pearson product-moment correlation analysis showed a significant correlation between full-scale IQ and activation during the 2-back task in the left inferior frontal gyrus (r=0.42, df=23, p<0.04), left superior parietal lobule (r=0.41, df=23, p<0.05), left supramarginal gyrus (r=0.50, df=23, p=0.01), and right supramarginal gyrus (r=0.42, df=23, p<0.04). No significant correlations between full-scale IQ and activation were observed in the 1-back task. However, when the groups were examined separately, neither group showed significant correlations between full-scale IQ and brain activation during the 2-back task, suggesting that the average group differences in full-scale IQ and activation were driving the correlation observed when the groups were combined. Nonparametric Spearman analysis also showed no significant correlation between full-scale IQ and brain activation, either within or across groups.

In the comparison subjects, response accuracy on the 2-back task was correlated with activation in the left inferior frontal gyrus, right inferior frontal gyrus, left middle frontal gyrus, right middle frontal gyrus, left superior parietal lobule, right superior parietal lobule, left supramarginal gyrus, and right supramarginal gyrus (Table 4). No such correlations were found in the subjects with fragile X syndrome. In the subjects with fragile X syndrome, reaction time on the 2-back task was significantly inversely correlated with activation in both frontal lobe regions of interest bilaterally (left inferior frontal gyrus, right inferior frontal gyrus, left middle frontal gyrus, and right middle frontal gyrus) (Table 4). The comparison subjects showed no such correlations on the 2-back task.

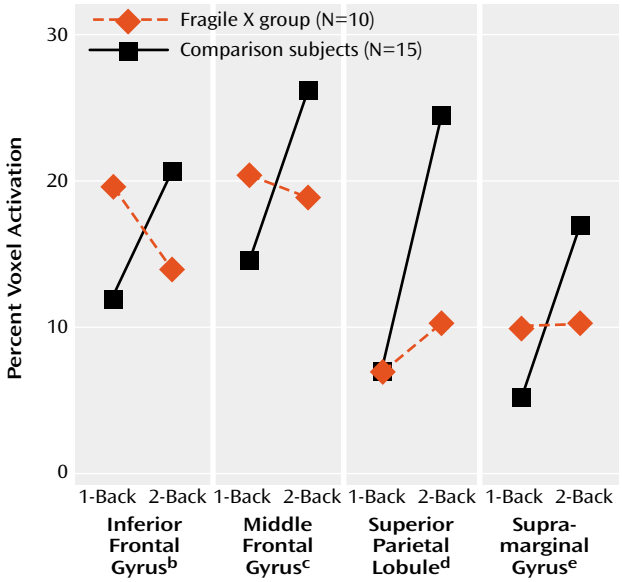
In the 1-back task, significant correlations were seen for reaction time but not for accuracy. Reaction time correlated positively in the comparison group (N=15) with activation in the right inferior frontal gyrus (r=0.56, df=13, p<0.03) and negatively in the fragile X syndrome group (N=8) with activation in the right inferior frontal gyrus (r=–0.80, df=6, p<0.02) and right superior parietal lobule (r=–0.78, df=6, p<0.03).

Brain Activation, FMRP Expression, and FMR1 Gene Activation Ratio

In the subjects with fragile X syndrome, brain activation during the 1-back task in the left middle frontal gyrus was significantly correlated with both the FMR1 gene activation ratio and FMRP expression (Table 5). In the 2-back task, the FMR1 gene activation ratio was significantly correlated with activity in the right inferior frontal gyrus, left middle frontal gyrus, and right middle frontal gyrus, while FMRP expression was significantly correlated with activity in the right inferior frontal gyrus, left middle frontal gyrus, right middle frontal gyrus, left supramarginal gyrus, and right supramarginal gyrus (Table 5).

No significant correlations were seen between the FMR1 gene activation ratio or FMRP expression and any of the behavioral measures on either task.

FIGURE 4. Brain Activation in Regions of Interest During 1-Back and 2-Back Working Memory Tasks, Relative to a Control Condition,[a] in Female Subjects With Fragile X Syndrome and Healthy Comparison Subjects



[a] Subjects were presented with a series of 3 × 3 matrices with nine distinct visuospatial locations in each task. In the experimental condition, subjects were instructed to respond if the stimulus was in the same location as in the previous trial for the 1-back task or in two trials back for the 2-back task. In the control condition, subjects were instructed to respond if the stimulus was in the center location.
[b] Significant interaction of diagnosis and task (F=6.7, df=1, 23, p=0.02).
[c] Significant interaction of diagnosis and task (F=5.4, df=1, 23, p=0.03).
[d] Significant interaction of diagnosis and task (F=11.8, df=1, 23, p=0.02).
[e] Significant interaction of diagnosis and task (F=12.8, df=1, 23, p=0.002).

Discussion

To our knowledge, this report presents the first study to examine the neural substrates of working memory in individuals with fragile X syndrome. Behaviorally, we found significant visuospatial working memory deficits in the subjects with fragile X syndrome, even after accounting for differences in IQ. Relative to the comparison subjects, the subjects with fragile X syndrome also showed significant differences in brain activation. Significant correlations were found between brain activation and both FMRP expression and performance in the 2-back task. Significant correlations also were seen on the 1-back task for both subject groups, but they were more limited in degree and extent.

TABLE 4. Correlation Between Two Measures of Performance on a 2-Back Visuospatial Working Memory Task[a] and Activation in Brain Regions of Interest in Healthy Comparison Subjects and Female Subjects With Fragile X Syndrome

	Comparison Group (N=15)				Fragile X Syndrome Group (N=8)			
	Correct Response Rate		Reaction Time		Correct Response Rate		Reaction Time	
Region of Interest	r	p	r	p	r	p	r	p
Left inferior frontal gyrus	0.72	0.002	–0.01	0.97	0.13	0.76	–0.85	0.008
Right inferior frontal gyrus	0.59	<0.03	0.12	0.67	–0.16	0.70	–0.89	0.003
Left middle frontal gyrus	0.59	<0.03	–0.09	0.74	0.19	0.66	–0.79	<0.02
Right middle frontal gyrus	0.55	<0.04	–0.05	0.86	0.11	0.80	–0.90	0.003
Left superior parietal lobule	0.66	0.008	–0.22	0.42	0.24	0.57	0.19	0.66
Right superior parietal lobule	0.61	<0.02	0.04	0.90	0.22	0.60	–0.26	0.53
Left supramarginal gyrus	0.54	0.04	–0.44	0.10	0.41	0.31	–0.19	0.65
Right supramarginal gyrus	0.61	<0.02	–0.04	0.90	0.51	0.20	–0.67	0.07

[a] Subjects were presented with a series of 3 × 3 matrices with nine distinct visuospatial locations. In the experimental condition, subjects were instructed to respond if the stimulus was in the same location as two trials back. In the control condition, subjects were instructed to respond if the stimulus was in the center location.

TABLE 5. Correlation of FMR1 Gene Activation Ratio and FMR1 Gene Product (FMRP) Expression With Brain Activation in Regions of Interest During 1-Back and 2-Back Visuospatial Working Memory Tasks[a] in Female Subjects With Fragile X Syndrome (N=10)

	1-Back Task				2-Back Task			
	FMR1 Gene Activation Ratio[b]		FMRP Expression[c]		FMR1 Gene Activation Ratio[b]		FMRP Expression[c]	
Region of Interest	r	p	r	p	r	p	r	p
Left inferior frontal gyrus	0.53	0.12	0.53	0.12	0.55	0.10	0.62	0.055
Right inferior frontal gyrus	0.59	0.07	0.48	0.17	0.70	<0.03	0.69	<0.03
Left middle frontal gyrus	0.67	<0.04	0.70	<0.03	0.77	0.009	0.81	0.004
Right middle frontal gyrus	0.57	0.09	0.56	0.09	0.66	<0.04	0.71	<0.03
Left superior parietal lobule	–0.18	0.62	–0.15	0.68	0.34	0.33	0.39	0.26
Right superior parietal lobule	–0.20	0.58	–0.19	0.59	0.39	0.27	0.43	0.22
Left supramarginal gyrus	0.15	0.68	0.19	0.59	0.61	0.06	0.70	<0.03
Right supramarginal gyrus	0.11	0.77	0.15	0.69	0.61	0.06	0.70	<0.03

[a] Subjects were presented with a series of 3 × 3 matrices with nine distinct visuospatial locations. In the experimental condition, subjects were instructed to respond if the stimulus was in the same location as in the previous trial for the 1-back task or in two trials back for the 2-back task. In the control condition, subjects were instructed to respond if the stimulus was in the center location.
[b] Represents the proportion of the normal (nonmutant) FMR1 gene on the active X chromosome, which reflects the proportion of cells that can express FMRP.
[c] Ascertained by calculating the percentage of lymphocytes containing FMRP.

Behavioral Results

Behavioral deficits in the fragile X syndrome group were observed after covarying for IQ, suggesting that even after accounting for overall cognitive level, subjects with fragile X syndrome may be especially deficient in working memory skills. Subjects with fragile X syndrome showed good performance on the control condition of the working memory tasks, suggesting intact attention and impulse control when working memory load is relatively low. Performance on the 2-back task was significantly worse than on the 1-back task for both subject groups. However, subjects with fragile X syndrome experienced a significantly greater decrease in accuracy (13% for the fragile X syndrome subjects versus 4% for the comparison subjects) with increased memory load. In addition to experiencing lower overall accuracy, subjects with fragile X syndrome had higher rates of errors of omission and commission. These findings may be related to clinical descriptions of greater difficulties with attention and impulsivity in this population (8, 44).

Our results agree with prior neuropsychological studies showing that visuospatial perception and short-term memory, attention, and manipulation of visuospatial relationships are impaired in the fragile X syndrome population (4, 6). Prior data also have suggested that subjects with fragile X syndrome are particularly weak in visual short-term memory and sequential processing relative to other cognitive domains (4). However, unlike these prior neuropsychological studies, which involved several cognitive processes in addition to working memory, the study reported here used a task that better operationalized working memory and provided direct evidence for specific deficits in working memory in subjects with fragile X syndrome. These deficits were observed even after differences in IQ were taken into account, implying that the ability to maintain and manipulate information in memory is specifically affected in fragile X syndrome.

Neuroimaging Findings

In conjunction with these behavioral deficits, differences in patterns of brain activation between comparison subjects and subjects with fragile X syndrome were found. The activation patterns seen in the comparison group, namely involvement of bilateral prefrontal and parietal cortices, are in good agreement with previous findings that these regions play critical roles in working memory. For example, electrophysiological (14, 17) and lesion (45,

46) studies have consistently indicated that the prefrontal cortex is essential in successful working memory performance. Other fMRI studies have found bilateral dorsolateral prefrontal cortex involvement in visuospatial working memory processing (47, 48). Furthermore, several PET and fMRI studies have shown a correlation between activation increases in the dorsolateral prefrontal cortex and increased memory load (49, 50). A previous study using an n-back task similar to the task in our study reported robust activation in the middle frontal gyrus only during the 2-back task (48). Prior imaging studies have reported consistent activation of the parietal lobe during both verbal and visuospatial working memory tasks (51). Imaging studies also have suggested that the ventral aspects of the parietal cortex are involved in the short-term storage and retrieval of object-related material, while the dorsal aspects of the parietal areas are involved in the storage and retrieval of visuospatially encoded information (11). On the basis of prior findings of frontal and parietal lobe involvement in the storage, retrieval, and executive manipulations of the contents of working memory, we selected these regions as regions of interest for the current study.

The subjects with fragile X syndrome showed significant activation in bilateral frontal and parietal areas in both the 1-back and 2-back tasks. As such, they showed little change in the pattern of activation in a comparison between the tasks of differing working memory load. On the other hand, the comparison subjects had significantly greater activation in bilateral areas of the parietal cortex in the 2-back task compared to the 1-back task. To further clarify group differences, we proceeded to a region-of-interest analysis.

The statistical significance of a two-way ANCOVA of diagnosis and task across all regions of interest (Figure 4) confirmed our hypothesis that the subjects with fragile X syndrome had different patterns of brain activation than the comparison subjects. The level of activity seen in the subjects with fragile X syndrome in the experimental conditions may represent nonspecific, generalized activation that is not modulated in response to increasing demands. It is also possible that activation reaches a ceiling in subjects with fragile X syndrome during the 1-back task. In contrast, Callicott et al. (52) reported the presence in normally developing adults of a threshold in performance and recruitment of neural resources by a 3-back visuospatial working memory task.

Taken together, the behavioral and neuroimaging analyses reveal significant deficits in working memory systems in subjects with fragile X syndrome. These differences in brain activation may arise from synaptic and dendritic abnormalities known to be present in subjects with fragile X syndrome (2, 3). This, in turn, may lead to dysregulation of neural processes critical to working memory performance.

Relationship Between Brain Activation and Performance

In contrast to correlations between activation and accuracy on the 1-back or 2-back tasks in the comparison subjects, no correlation was found in subjects with fragile X syndrome. This finding supports the hypothesis of generalized nonspecific activation in subjects with fragile X syndrome. However, an inverse correlation was seen between reaction time on the 2-back task and brain activation for subjects with fragile X syndrome bilaterally in the inferior frontal gyrus and the middle frontal gyrus. This correlation was not observed in the comparison subjects. This finding suggests that despite a relative impairment of working memory function, subjects with fragile X syndrome show an association between activation in these frontal areas and the speed of working memory processing.

Effects of FMRP Expression on Neural Activation

The subjects with fragile X syndrome showed robust patterns of activation during the 1-back task in the bilateral prefrontal cortex and parietal cortices but showed a correlation between FMRP expression and brain activation during the 1-back task only in the left middle frontal gyrus. In contrast, significant correlations were seen in the 2-back task in the right inferior frontal gyrus, bilateral middle frontal gyrus, and bilateral supramarginal gyrus. These findings may be explained by a ceiling effect. The subjects with fragile X syndrome may have recruited all of their neural resources needed for working memory in the 1-back task, resulting in an absence of any correlation between brain activation and FMRP. In the 2-back task, the fragile X syndrome group experienced significant deterioration in performance along with the lack of coherent recruitment in areas subserving working memory. The degree to which subjects can recruit areas adequately appears to be related to FMRP expression. These results indicate that FMRP may play a dynamic role in modulating brain activation in response to working memory load.

Some studies have begun to elucidate the role of FMRP in neurons. These studies point to the function of FMRP as an RNA-binding protein, possibly involved in the transport and/or stabilization of RNA from the nucleus to the dendrites (53, 54). Levels of a rat homologue of FMRP have been shown to increase rapidly (on the order of minutes) in response to neuronal stimulation (54), indicating a possible role of FMRP in the regulation of protein synthesis in response to increased synaptic input. Furthermore, low levels of FMRP in subjects with fragile X syndrome have been associated with abnormal morphology of dendritic spines and a reduction in the length of synapses in the cortex (2, 3), although the precise mechanism by which this occurs is not known.

On the basis of these findings, we hypothesize that intracellular FMRP levels increase rapidly in normally developing individuals in response to a working memory task. In light of the role of FMRP in transporting and/or stabiliz-

ing mRNA, increased FMRP may act as a precursor to increased protein synthesis in dendritic processes. In the fragile X syndrome population, in which the level of FMRP is lower, the degree of dendritic protein synthesis may be limited by the ability of the neuron to synthesize FMRP for its role in modulating protein translation. The resulting limit in protein translation may lead to impairments in synaptic maturation or plasticity. These dynamic impairments in translation would occur in addition to the baseline abnormalities in neural activation likely to result from synaptic and dendritic dysmorphology.

This study is the first we are aware of to examine the relationship between brain activation and a molecular marker of genetic dysfunction in the context of working memory load. We plan future studies that will compare IQ-matched populations and will involve subjects with a more limited age range to avoid the confounding effects of development and age. Differences in neural architecture and functioning may exist between prepubertal and adolescent subjects, although a recent developmental study of spatial working memory reported similar areas of activation in frontal and parietal regions (23).

In conclusion, this study shows that subjects with fragile X syndrome exhibit significant visuospatial working memory deficits that extend beyond the effects of differences in IQ. These deficits are correlated with abnormalities in activation in brain areas that are known to be involved in working memory. In addition, FMRP expression is correlated with brain activation in subjects with fragile X syndrome, a finding that may reflect the involvement of this protein in the dynamic response to working memory load. These results bear possible clinical significance in light of behavioral problems exhibited by subjects with fragile X syndrome in the realm of social functioning. As the gene defect in this disorder can be readily quantified, we believe that fragile X syndrome may serve as a model for bridging the gap between the molecular biology of neurogenetic disorders and their neuronal and behavioral manifestations.

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