

Functional Brain Activation During Arithmetic Processing in Females With Fragile X Syndrome Is Related to *FMR1* Protein Expression

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Abstract: Arithmetic processing deficits in persons with fragile X Syndrome (fraX), the most common heritable cause of mental retardation, are well known. In this study, we characterize the neural underpinnings of these performance deficits using functional MRI. Given that a single gene defect (*FMR1*) is known to be responsible for this disorder, we also assess whether brain activation in arithmetic processing areas is related to amount of *FMR1* protein expression (FMRP). Subjects included 16 females with fraX, and 16 female age-matched controls. Subjects viewed arithmetic equations with two ($1 + 3 = 4$) or three ($2 + 3 - 1 = 5$) operands, and were asked to judge whether the results were correct or not. Subjects with fraX showed significant impairment in behavioral performance on the 3-operand but not the 2-operand arithmetic equations. Significant brain activation was observed bilaterally in the prefrontal and parietal cortices for unaffected subjects, and bilateral prefrontal and left angular gyrus for subjects with fraX, for both trial types. Subjects with fraX exhibited less overall activation than did unaffected subjects in both types of trials; and, unlike the unaffected group, did not show increased extent of activation in association with greater task difficulty. During the 3-operand trials, activation in bilateral prefrontal and motor/premotor, and left supramarginal and angular gyri were positively correlated with FMRP, suggesting that decreased *FMR1* protein expression underlies deficits in math performance in persons with fraX. More broadly, this investigation demonstrates a unique bridging of cognitive and molecular neuroscience and represents a useful approach for the study of brain development and function. *Hum. Brain Mapping* 16:206–218, 2002. © 2002 Wiley-Liss, Inc.

Key words: fMRI; arithmetic processing; fragile X syndrome; FMRP; molecular neuroscience; cognitive neuroscience; angular gyrus

INTRODUCTION

Fragile X syndrome (fraX) is the most common heritable cause of neurodevelopmental disability, with an estimated prevalence of ~1 in every 2,000–4,000 live births [Turner et al., 1996]. In 1991, a single mutated gene (*FMR1*) was identified as the cause of fraX. The typical mechanism by which the normal *FMR1* gene becomes mutated involves an increase in trinucleotide (CGG) repeats, resulting in an abnormally long deoxyribonucleic acid (DNA) segment. When the number of repeats increases beyond ~200, the chemical

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modification process called DNA methylation is more likely to occur. This, in turn, inactivates the *FMR1* gene, leading to decreased *FMR1* protein and deficits in cognitive processing. The cognitive profile of fraX includes deficits in visuospatial processing and working memory, visual-motor coordination, and poor arithmetic skills [Baumgardner et al., 1994; Freund and Reiss, 1991; Grigsby et al., 1990; Kwon et al., 2001; Mazzocco et al., 1993].

Females with fraX have been identified as having a pattern of relative weakness on arithmetic reasoning and digit span [Brainard et al., 1991; Kemper et al., 1986; Miezieski et al., 1986], and to be significantly worse on the four symptoms of Gerstmann syndrome, including acalculia, the inability to do simple arithmetic [Grigsby et al., 1990]. In this study, we characterize both the arithmetic performance of females with fraX and the neural underpinnings of their performance deficits using functional magnetic resonance imaging (fMRI). In addition, we assess whether activation in arithmetic processing areas is related to amount of *FMR1* protein expression (FMRP).

Much of what is known about brain localization of arithmetic processing comes from lesion studies. Although classically the parietal cortex is thought to underlie the acalculia component of Gerstmann syndrome, acalculia has also been reported in patients with lesions to both prefrontal [Fasotti et al., 1992; Luria, 1966] and parietal cortex [Benson and Weir, 1972; Henschen, 1920; McCarthy and Warrington, 1988; Takayama et al., 1994; Warrington, 1982; Whalen et al., 1997] as well as subcortical structures including the thalamus [Ojemann, 1974]. More recently, brain imaging studies have identified a number of brain regions involved in performance of arithmetic tasks [Burbaud et al., 1995, 2000; Dehaene et al., 1999; Gruber et al., 2001; Rueckert et al., 1996]. Across these studies, activation is reported in both prefrontal and parietal areas. In particular, Menon et al. [Menon et al. 2000a,c] identified the angular gyrus as being a region specifically activated in response to increasing arithmetic complexity.

In the present study, subjects viewed arithmetic equations in the form " $a + b = c$ " or " $a + c - b = d$ " and were asked to judge whether the results were correct or not. We hypothesized that subjects with fraX would show significant impairment in performance on the task, particularly in the more difficult, 3-operand equations. We also hypothesized that they would show significant deficits in activation of brain regions known to be involved in arithmetic processing—particularly, the inferior and middle frontal gyri, and the supramarginal and angular gyri of the parietal

cortex, and that activation in these areas would be related to FMRP.

MATERIALS AND METHODS

Subjects, diagnosis, and molecular measures

Sixteen young female subjects with a diagnosis of fraX (range: 10.12–22.73 years; mean: 16.17 years) were recruited from throughout the US. Subjects were recruited through advertisements in national fraX newsletters, through prior clinical contacts, and through contact at national fraX conferences. The diagnosis of fraX was confirmed by DNA analysis. Standardized Southern blot and polymerase chain reaction (PCR) analyses were performed followed by *FMR1*-specific probe hybridization [Oberle et al., 1991]. The CGG repeat number was calculated from the Southern blot autoradiogram images. FMRP levels were ascertained by calculating the percentage of peripheral lymphocytes containing FMRP using immunostaining techniques [Willemsen et al., 1995]. All subjects with fraX had the full mutation. Sixteen female unaffected subjects also participated (range: 10.85–22.67 years; mean: 16.97 years). The human subjects committee at the Stanford University School of Medicine approved all protocols used in this study, and all subjects (or their guardians) gave written informed consent.

Neuropsychological assessment

IQ was determined using the Wechsler Intelligence Scales. The Wechsler Intelligence Scale for Children, Third Edition (1991) was administered to those under 17 years of age, and the Wechsler Adult Intelligence Scale-Third Edition (1997) was administered to those 17 years of age or older. The Digit Span, Arithmetic, and Coding subtests, present in both the WISC-III and the WAIS-III, were selected for analyses as behavioral measures of mathematical ability or working memory.

Math task experimental design

The experiment began with a 30-sec rest epoch followed by six alternating 30-sec epochs of "easy" (2-operand equation) experimental trials and control trials. These six easy experimental epochs were followed by a second 30-sec rest epoch. After the second rest there were six alternating 30-sec epochs of "difficult" (3-operand equation) experimental trials and control trials. After these six difficult experimental epochs was a third 30-sec rest epoch. During the rest condition, subjects passively viewed a blank screen. Easy

experimental epochs consisted of nine, 2-operand addition or subtraction problems (randomly intermixed) with either a correct or an incorrect resultant (e.g., $1 + 2 = 3$ or $5 - 2 = 4$). Difficult experimental epochs consisted of nine, 3-operand addition/subtraction problems with either a correct or an incorrect resultant (e.g., $6 - 3 + 5 = 8$ or $6 + 2 - 3 = 4$). All experimental and control stimuli were presented for 2,625 msec, with an ISI of 708 msec. Sixty-six percent of the results were correct and required a button press and the other 34% were incorrect. Equal numbers of button presses were required for experimental and control trials. Of the incorrect-resultant trials, half of the results were one more than the correct answer, and half were one less than the correct answer. During all experimental epochs the instructions "Push if Correct" were displayed for the entire length of the epoch. Subjects were instructed to respond with a key press only when the resultant of the math equation was correct. Control epochs for the blocks of easy experimental trials were comprised of nine stimuli each consisting of a string of five single digits. Control epochs for the blocks of difficult experimental trials were comprised of nine stimuli each consisting of a string of seven single digits. During the control epochs the instructions "Push for 0" was displayed the entire length of the epoch. Subjects were instructed to respond with a key press only when a zero appeared in the string of digits.

Behavioral data analysis

Percentage of correct responses, reaction time (RT) and false alarms were compared using an analysis of covariance (ANCOVA).

fMRI acquisition

Images were acquired on a 1.5 T GE Signa scanner with EchoSpeed gradients using a custom-built whole head coil that provides a 50% advantage in signal to noise ratio over that of the standard GE coil [Hayes and Mathias, 1996]. A custom-built head holder was used to prevent head movement. Eighteen axial slices (6 mm thick, 1 mm skip) parallel to the anterior and posterior commissure covering the whole brain were imaged with a temporal resolution of 2 sec using a T2* weighted gradient echo spiral pulse sequence (TR = 2,000 msec, TE = 40 msec, flip angle = 89° and 1 interleave) [Glover and Lai, 1998]. The field of view was 240 mm and the effective in-plane spatial resolution was 3.75 mm. To aid in localization of functional data, high resolution T1 weighted spoiled grass gra-

dient recalled (SPGR) 3D MRI sequence with the following parameters was used: TR = 24 msec; TE = 5 msec; flip angle = 40° ; 24 cm field of view; 124 slices in sagittal plane; 256×192 matrix; acquired resolution = $1.5 \times 0.9 \times 1.2$ mm. The images were reconstructed as a $124 \times 256 \times 256$ matrix with a $1.5 \times 0.9 \times 0.9$ mm spatial resolution. Structural and functional images were acquired in the same scan session.

The task was programmed using Psyscope [Cohen et al., 1993] on a Macintosh (Sunnyvale, CA) notebook computer. Initiation of scan and task was synchronized using a TTL pulse delivered to the scanner timing microprocessor board from a 'CMU Button Box' microprocessor (<http://poppy.psy.cmu.edu/psyscope>) connected to the Macintosh. Stimuli were presented visually at the center of a screen using a custom-built magnet compatible projection system (Resonance Technology, CA).

Image preprocessing

Images were reconstructed, by inverse Fourier transform, for each of the 225 time points into $64 \times 64 \times 18$ image matrices (voxel size: $3.75 \times 3.75 \times 7$ mm). fMRI data were pre-processed using SPM99 (<http://www.fil.ion.ucl.ac.uk/spm>). Images were corrected for movement using least square minimization without higher-order corrections for spin history, and normalized to stereotaxic Talairach coordinates [Talairach and Tournoux, 1988]. Images were then resampled every 2 mm 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 using the general linear model and the theory of Gaussian random fields as implemented in SPM99 [Friston et al., 1995]. This method takes advantage of multivariate regression analysis and corrects for temporal and spatial autocorrelations in the fMRI data. Activation foci were superposed on high-resolution T1-weighted images and their locations interpreted using known neuroanatomical landmarks [Duvernoy and Bourgouin, 1999; Mai et al., 1997].

In the first step, a within-subjects 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 sec) 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 sample size. Finally, the t -statistics were normalized to Z -scores, and significant clusters of activation were determined using the joint expected probability distribution of height and extent of Z -scores [Poline et al., 1997], with height ($Z > 2.33$; $P < 0.01$) and extent thresholds ($P < 0.05$).

Group analysis was performed using a random-effects model that incorporated a two-stage hierarchical procedure. This model estimates the error variance for each condition of interest across subjects, rather than across scans [Holmes and Friston, 1998] and therefore provides a stronger generalization to the population from which data are acquired. This analysis proceeded in 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 analyzed using a general linear model to determine voxel-wise t -statistics. One contrast image was generated per subject, per effect of interest (e.g., 2-operand condition, minus the control condition). A one-way t -test was then used to determine group activation for each effect. The following contrast images were calculated for each subject: 1) *2-operand – control*; 2) *3-operand – control*; and 3) *3-operand – 2-operand*. For both within- and between-group comparisons, we used a cluster-wise (corrected) significance level of $P = 0.01$.

RESULTS

Wechsler iq scales

The full-scale IQ scores of the fraX sample ranged from 52–108 with a mean score of 83.63. The mean of this group is just over one standard deviation (SD) below the scaled mean of the Wechsler tests. Only two subjects had an IQ below 70. In comparison, the unaffected group full-scale IQ scores ranged from 99–142 with a mean score of 122.69, which is more than one SD above the scaled mean. The difference between these group means was statistically significant ($t = 8.20$, $P < 0.001$). The group differences on Verbal

and Performance IQ Scores were consistent and similar to the full-scale IQ scores. The fraX group was roughly one SD below the scaled mean of the test on VIQ and PIQ (mean = 85.31 and 85.00, respectively) and the unaffected group was more than one standard deviation above the scaled mean on VIQ and PIQ (mean = 122.63 and 117.94, respectively). Group differences were significant for both VIQ ($t = 7.90$, $P < 0.001$) and PIQ ($t = 6.74$, $P < 0.001$).

Wechsler subtests

Three Wechsler subtests were selected as behavioral measures of mathematical ability or working memory: Arithmetic, Digit Span, and Coding. Two of these subtests (i.e., Arithmetic and Digit Span) use auditory stimuli whereas the other uses visually perceived stimuli. Independent Samples t -tests were conducted to measure group differences. On Arithmetic, the fraX group had a mean scaled score of 5.44 (SD 2.61) and the unaffected group had a mean scaled score of 13.25 (SD 3.30), $t = 7.436$, $P < 0.001$. On Digit Span, the fraX group had a mean scaled score of 6.50 (SD 2.80) and the unaffected group had a mean scaled score of 10.75 (SD 3.21), $t = 3.985$, $P < 0.001$. On Coding, the fraX group had a mean scaled score of 8.31 (SD 3.82) and the unaffected group had a mean scaled score of 13.31 (SD 2.94), $t = 4.147$, $P < 0.001$. Thus, regardless of the type of stimuli, the group differences were significant on all three subtests.

Behavioral performance

Performance data from the tasks were not analyzed for four of the 16 subjects with fraX, and for one of the 16 unaffected subjects. For the unaffected subject and two of the subjects with fraX, performance data were not recorded due to a response box malfunction. For the other two subjects with fraX, performance data were not used because their responses on the experimental trials of the second half of the experiment (when the task difficulty increased due to the introduction of 3-operand equations) were too few to reliably calculate performance. Because unaffected and fraX groups differed significantly in IQ, FSIQ was used as a covariate in all behavioral performance analyses described below. There was a statistically significant correlation between FSIQ and percent correct on the 3-operand equations for combined group samples (Spearman $R = 0.76$, $P < 0.001$). When examined separately, however, a marginally significant correlation was seen between FSIQ and percent correct on 3-operand equations in the fraX group ($R = 0.57$, P

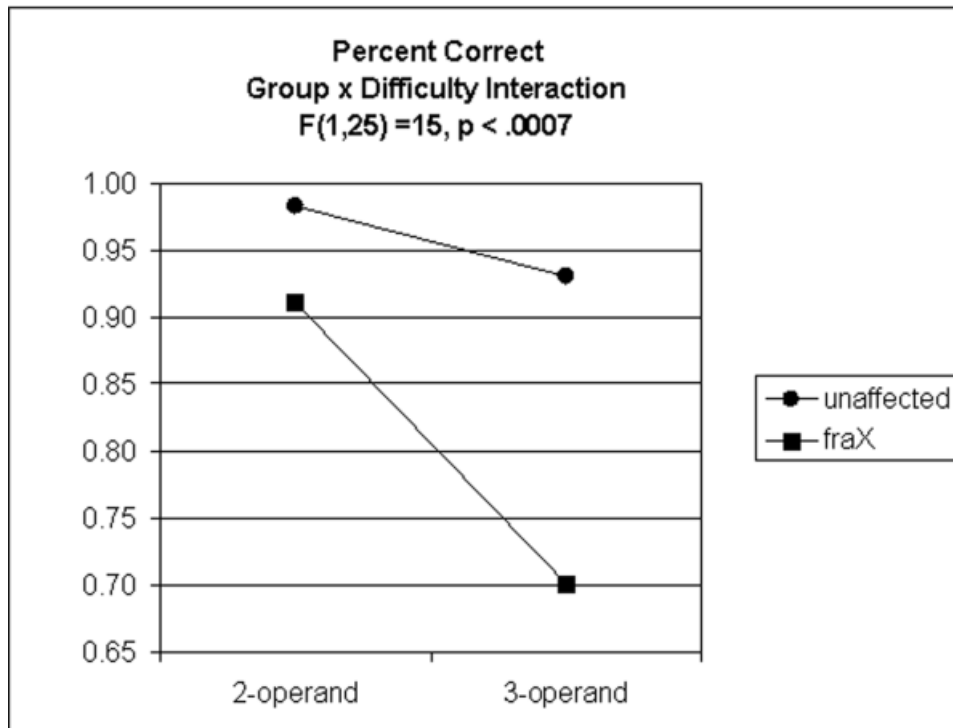


Figure 1.
Percent correct on 2- and 3-operand arithmetic equations for unaffected subjects and subjects with fraX.

= 0.054) but not in the unaffected group ($R = 0.32$, $P = 0.247$). No significant correlations were observed between IQ and performance on the 2-operand equations.

Mean accuracy, reaction times (RTs) and false alarms for the two experimental conditions were analyzed separately, using analysis of covariance (ANCOVA) with a between-subjects factor of Group (fraX, unaffected) and a within-subjects factor of difficulty (2-operand, 3-operand equations). Full scale IQ (FSIQ) was used as a covariate.

The analysis of accuracy revealed a significant main effect of Difficulty, $F(1,25) = 41.15$, $P < 0.0001$. In addition, there emerged a significant Group \times Difficulty interaction $F(1,25) = 14.99$, $P < 0.001$ (Fig. 1). When examined separately, subjects with fraX showed a significant decrease in performance from 2 to 3 operands, $F(1,11) = 50.06$, $P < 0.0001$; whereas unaffected subjects did not, $F(1,14) = 3.50$, $P = 0.082$.

The analysis of RT revealed a significant main effect of Difficulty, $F(1,25) = 86.36$, $P < 0.0001$. This main effect was due to the fact that RT was, on average, faster for the 2-operand ($M = 1,344.16$ msec) than for the 3-operand ($M = 2,091.03$ msec) equations. Neither the main effect of Group nor the Group \times Difficulty interaction was significant for the analysis of RT.

The analysis of false alarms revealed no significant main effects or interactions.

Brain activation, within-group comparisons

2-Operand activation

In the 2-operand trials compared to the control trials, unaffected subjects showed significant activation in the right middle frontal gyrus (MFG); bilateral insular/orbitofrontal gyrus; left inferior frontal gyrus (IFG); bilateral superior frontal gyrus (SFG), bilateral angular gyrus (ANG), left superior parietal lobe (SUPAR), left temporal/parieto-occipital junction and right middle occipital gyrus (Fig. 2a.).

On this same contrast, subjects with fraX showed significant activation bilaterally in the MFG, left premotor, bilateral insular/orbitofrontal gyrus, left IFG; bilateral SFG; left inferior temporal gyrus (ITG), left fusiform gyrus, left ANG, left middle occipital gyrus, and left cerebellum (Fig. 2b).

3-Operand activation

In the 3-operand trials compared to the control trials, unaffected subjects showed significant activation bilaterally in MFG and SFG, right insular/orbitofrontal gyrus, bilateral ANG, left superior, middle and inferior occipital gyri, and left lingual and fusiform gyri (Fig. 2c).

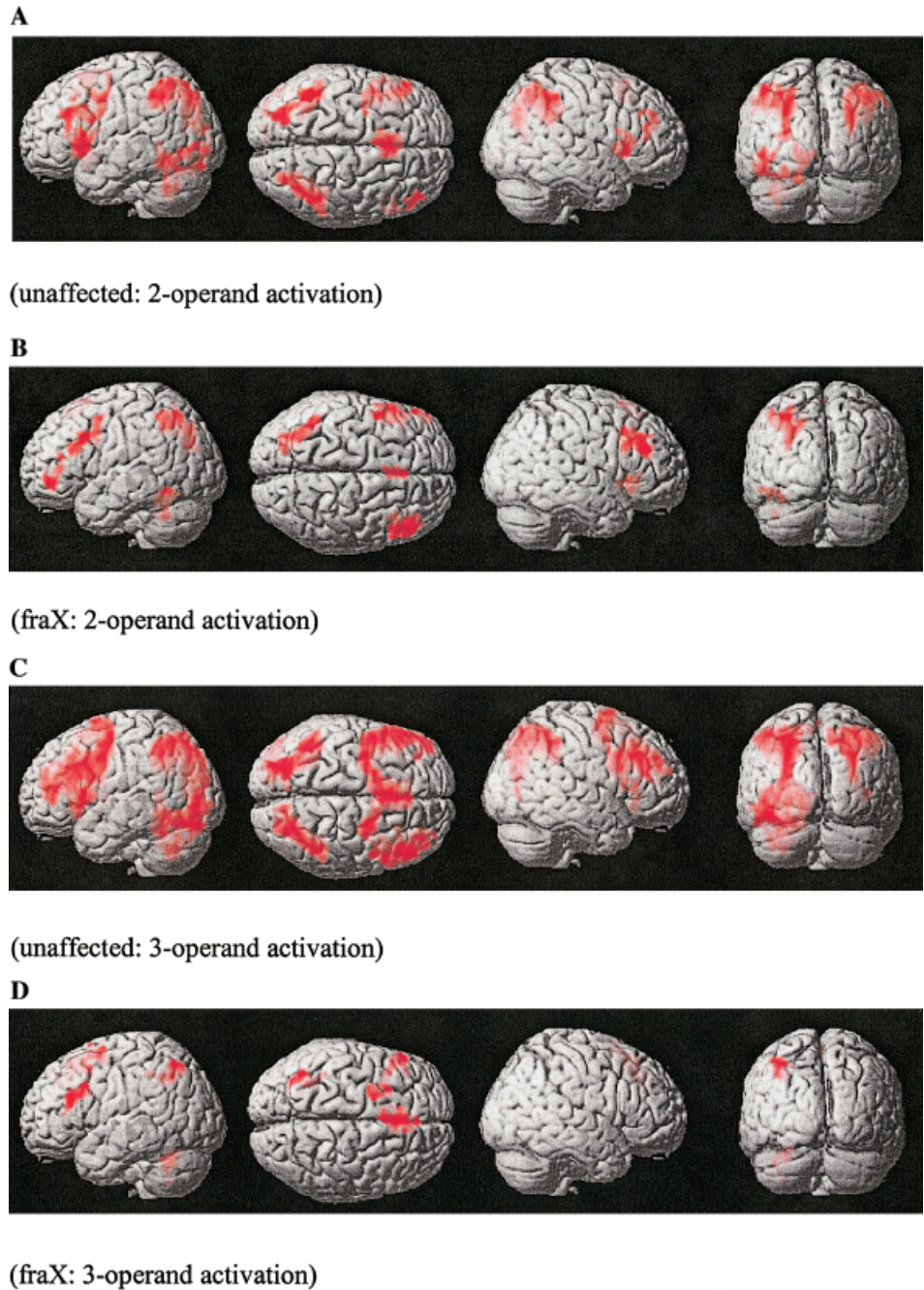


Figure 2.

Brain areas that show significantly greater activation during 2-operand arithmetic equations for (a) unaffected subjects and (b) subjects with fraX; and during 3-operand arithmetic equations for (c) unaffected subjects and (d) subjects with fraX. All activations reported were significant after height ($Z > 2.33$; $P < 0.01$) and extent ($P < 0.01$) thresholding.

On this same contrast, subjects with fraX showed significant activation in the left MFG, IFG, SFG, and ANG, as well as left intraparietal sulcus and middle cerebellum (Fig. 2d).

In both the 2- and 3-operand contrasts, the voxel extent (i.e., size of the clusters of activation) was much greater for the unaffected subjects than for the subjects with fraX (Table I).

All within-group fMRI analyses also were conducted omitting the five subjects (one unaffected and

four subjects with fraX) for whom behavioral data was not analyzable. These analyses yielded a very similar pattern of results as when the subjects were included. For the 2-operand trials, unaffected subjects ($n = 15$) showed significant activation in right MFG, bilateral orbitofrontal, left IFG and precentral gyrus, bilateral SFG and ANG, and left lingual and fusiform gyrus. On this same contrast, subjects with fraX ($n = 12$) showed significant activation bilaterally in the MFG, right middle temporal gyrus (MTG), and left ANG

TABLE I. Brain areas showing significantly greater activation during two types of experimental trials (2- and 3-operand arithmetic equations), compared to control trials, in unaffected subjects and subjects with fraX*

Comparison	Area	Corrected <i>P</i> -value	Number of voxels in cluster	Z max	Peak coordinates
Unaffected: 2-operand – control					
	L ANG (BA 39)	0.000	2071	5.70	–44–44 44
	L SUPAR			4.40	–24–80 42
	R ANG	0.000	2054	5.34	30–74 28
	Middle occipital gyrus			4.64	42–56 52
	L orbitofrontal/insular gyrus	0.000	2568	5.20	–38 22 0
	IFG			4.85	–46 28 26
	R SFG	0.000	891	5.13	8 14 46
	L SFG			4.27	–8 14 52
	L temp/pariet/occip junction	0.000	1873	4.68	–46–56–14
	Fusiform gyrus			4.30	–4–90 –4
	R MFG	0.001	603	4.01	44 44 32
	R orbitofrontal gyrus			3.77	44 12 4
fraX: 2-operand – control					
	R SFG	0.024	228	4.58	2 14 64
	L SFG			3.21	2 30 56
	R MFG	0.000	686	4.47	50 36 26
	L ANG	0.000	874	4.42	–36–56 48
	Middle occipital gyrus			3.57	–32–74 28
	L MFG	0.000	559	4.18	–44 18 30
	Premotor cortex			3.52	–52 4 52
	L orbitofrontal gyrus	0.015	248	4.13	–50 44 –6
	L MFG			3.43	–54 36 16
	L IFG			2.68	–52 38 6
	L ITG	0.001	386	4.10	–50–58–16
	L Fusiform gyrus			3.79	–34–48–22
	L cerebellum			3.65	–42–54–34
	R insular cortex	0.003	310	3.32	30 12 –4
	R orbitofrontal gyrus			3.24	34 28 0
Unaffected: 3-operand – control					
	L ANG (BA 39)	0.000	7809	5.86	–24–74 50
	L inferior occipital			5.58	–42–70–18
	L lingual/fusiform			5.40	–44–60–14
	R ANG/SMG (BA 39/40)	0.000	2808	5.48	44–46 46
	L SFG	0.000	11639	5.47	–2 14 58
	L MFG (BA 45)			4.88	–42 0 40
	R SFG/MFG (BA ?/45)			4.42	26 –2 58
	R MFG (BA 45)	0.000	2406	4.54	48 8 22
	R insular/orbitofrontal	0.004	435	4.23	30 24 6
fraX: 3-operand – control					
	L ANG	0.004	341	4.09	–38–60 42
	L intraparietal sulcus			3.73	–32–54 46
	L SFG	0.013	287	3.90	–20 2 68
	L MFG (BA 45)			3.65	–30 10 48
	R SFG	0.000	828	3.77	–10 26 50
	L Cerebellum	0.051	222	3.69	–32–60–28
	L MFG (BA 45)	0.000	479	3.55	–40 12 28
	L IFG			3.44	–48 18 28

* For each significant cluster, region of activation, cluster-level significance level, number of voxels activated, maximum Z-score and location of peak are shown.

Figure 3.

Brain areas that show significantly greater activation for unaffected subjects than subjects with fraX during 3-operand, compared to 2-operand arithmetic equations.



and cerebellum. For the 3-operand trials, unaffected subjects showed significant activation bilaterally in MFG, SFG and ANG, left superior, middle and inferior occipital gyri, and left lingual and fusiform gyri. On this same contrast, subjects with fraX showed significant activation bilaterally in the MFG, and left SFG, MTG and ANG, as well as left intraparietal sulcus (ata not shown).

Brain activation, between-group comparisons

3-Operand minus 2-operand activation

In an effort to determine the interaction between group and task difficulty (i.e., differences in activation between the two groups as the task becomes more difficult), we compared activation on the 3-operand trials with activation on the 2-operand trials and subtracted fraX subjects' activation from unaffected subjects' activation (and vice-versa). In these comparisons, FSIQ was used as a nuisance covariate in an effort to minimize any differences in activation that may be attributable to the IQ difference between the groups.

As the task difficulty increased, unaffected subjects exhibited significantly more activation than fraX in the left SFG, bilateral motor cortex, bilateral SUPAR, right intraparietal sulcus, left ANG, left fusiform gyrus and left middle occipital gyrus (height threshold, $P < 0.01$, extent threshold, $P < 0.05$) (Fig. 3). Because we had a priori hypotheses (based on previous studies) about where activation would occur during these tasks, we were also able to use a voxel-wise (uncorrected) significance threshold to examine clusters of activation in those areas included in our directed hypotheses. Using this criteria and a significance level of $P < 0.01$, the right MFG and left cerebellum also emerged as regions where unaffected subjects showed greater activation than subjects with fraX as task difficulty increased. By contrast, there were no brain regions where subjects with fraX exhibited significantly more activation than unaffected subjects (Table II).

Relationship between brain activation and protein level in subjects with fraX

One goal of the present study was to assess whether activation in areas related to arithmetic processing is related to amount of *FMR1* protein expression in subjects with fraX. To investigate this proposed association, we used level of *FMR1* protein expression (as estimated by measuring the proportion of blood cells containing FMRP) as a covariate of interest to identify those brain areas in which activation increased as a function of FMRP. Age was used as a nuisance covariate because of evidence of differential cell selection in the blood across the lifespan (for those cells that have active normal X chromosomes, and thus produce FMRP [Rousseau et al., 1991]).

2-Operand activation

In the 2-operand trials, compared to the control trials, the brain areas in which activation increased as FMRP increased were: right orbitofrontal and MFG, bilateral cingulate cortex, left pre-supplementary motor area (pre-SMA), and left cerebellum. Brain activation correlated with FMRP was much more diffuse in this contrast than for the 3-operand analysis (Fig. 4a).

3-Operand activation

In the 3-operand trials, compared to the control trials, the brain areas in which activation increased as FMRP increased were: bilateral MFG, left IFG, right postcentral gyrus, right SFG, left motor/premotor cortex, and left caudate (Fig. 4b). We also used a voxel-wise (uncorrected) significance threshold to examine clusters of activation in areas included in our directed hypotheses. At this threshold, a significant cluster was also observed in the left SPMG/ANG (Table III).

DISCUSSION

Subjects with fraX were found to be significantly impaired in their performance on 3-operand arithmetic equations with respect to 2-operand equations,

TABLE II. Brain areas where control subjects show significantly greater activation than subjects with fraX during 3-operand, compared to 2-operand trials*

Comparison	Area	Corrected <i>P</i> -value	Number of voxels in cluster	Z max	Peak coordinates
Unaffected – fraX:					
3-operand – 2-operand	R motor cortex (BA 4)	0.000	1657	4.09	18 –28 72
	R SUPAR			3.94	28 –28 44
	R intraparietal sulcus			3.86	28 –42 36
	L middle occipital sulcus	0.000	432	3.96	–40 –52 2
	L ANG			4.06	–38 –62 2
	L fusiform gyrus			4.18	–34 –40 –6
	L SFG	0.006	325	4.18	–34 –40 –6
	L SFG	0.000	445	3.82	–20 –2 60
	L precentral gyrus			3.73	–42 –16 38
	L SUPAR	0.001	296	3.49	–22 –54 40
	L SUPAR	0.001	447	3.35	–10 –48 60
	L motor cortex			3.34	–36 –28 42
	R MFG ^a	0.013 ^b	101	3.82	30 16 44
	L cerebellum ^a	0.048 ^b	57	3.66	–6 –62 –38
FraX – Unaffected:					
3-operand – 2-operand	No suprathreshold voxels				

* For each significant cluster, region of activation, cluster-level significance level, number of voxels activated, maximum Z-score and location of peak are shown.

^a At the uncorrected level.

^b Uncorrected *P*-value.

and with respect to unaffected subjects. Although unaffected subjects' performance on 3-operand equations declined slightly from that of the 2-operand, this within-group difference was not statistically significant. Performance differences between subjects with fraX and unaffected subjects were observed after covarying for

IQ, suggesting that the poor arithmetic performance in the subjects with fraX was not due to a global IQ deficiency. This profile is in agreement with several studies reporting particular impairments in arithmetic processing in persons with fraX [Brainard et al., 1991; Grigsby et al., 1990; Kemper et al., 1986; Mizejeski et al., 1986].

A



fraX: correlation between FMRP and activation on 2-operand trials

B



fraX: correlation between FMRP and activation on 3-operand trials

Figure 4.

Brain areas that show, for subjects with fraX, a significant correlation between FMRP and brain activation for (a) 2-operand and (b) 3-operand arithmetic equations.

TABLE III. Brain areas where subjects with fraX show a positive correlation between brain activation and FMRP during 2-operand and 3-operand trials, compared to control trials*

Comparison: Correlation between FMRP and activation	Area	Corrected <i>P</i> -value	Number of voxels in cluster	Z max	Peak coordinates
2-operand – control	L Cingulate	0.000	22668	4.87	–16 –10 46
	R cingulate gyrus			4.78	22 4 40
	Pre-SMA			4.71	–20 –2 56
	R orbitofrontal gyrus	0.009	242	3.75	18 28 –18
	R MFG			3.29	14 24 –8
	L cerebellum	0.049	180	3.92	–18 –50 –36
3-operand – control	L motor cortex	0.000	819	4.44	–52 –10 42
	L MFG			4.16	–54 –2 34
	L MFG	0.002	357	4.44	–50 38 20
	L IFG			3.90	–50 40 –2
	L MFG			3.02	–42 38 –6
	L Caudate	0.001	424	3.68	–20 2 26
	Cingulate gyrus			3.52	0 2 28
	R postcentral gyrs	0.000	973	3.27	8 –32 68
	R SFG			3.26	20 –2 50
	R MFG	0.029	238	3.21	28 –6 40
	L SPMG/ANG ^a	0.001 ^b	168	3.26	–48 –46 42

* For each significant cluster, region of activation, cluster-level significance level, number of voxels activated, maximum Z-score and location of peak are shown.

^a At the uncorrected level.

^b Uncorrected *P*-value.

We further attempted to characterize the neural substrates of this decreased arithmetic performance in the fraX group. Within-group comparisons of brain activation on the task revealed significant activation bilaterally in the prefrontal and parietal cortices for unaffected subjects in both the 2-operand and the 3-operand trials. Subjects with fraX showed significant activation in bilateral prefrontal cortices and in the left angular gyrus for both the 2- and 3-operand trials. The activation sites for both groups fall squarely within areas known to be classically involved in arithmetic processing in non-impaired populations [Burbaud et al., 1995; Dehaene et al., 1999; Gruber et al., 2001; Menon et al., 2000a,c; Rickard et al., 2000]; however, activation patterns differed between the two groups in two important ways. First, the unaffected subjects exhibited more overall activation than subjects with fraX in both types of trials. Second, unlike the unaffected group, subjects with fraX did not show increased extent of activation in association with greater task difficulty. In fact, subjects with fraX showed a slightly *greater* left ANG activation in the 2-operand equations than the 3-operand equations (see Fig. 2).

To ensure that those subjects for whom behavioral data was not analyzable did not unduly influence these results (of particular concern being the two subjects with fraX whose button pressing on the experimental trials became infrequent with the introduction of the 3-operand trials) the fMRI analyses were conducted with all of those subjects removed. The same general pattern of activation emerged for both groups and both trial types.

To determine the “Group \times Difficulty” interaction, we subtracted activation associated with the 2-operand equations from activation associated with the 3-operand equations, and then compared this contrast across the two groups. Because there was a significant difference in IQ between the two groups, FSIQ was used as a nuisance covariate in an effort to minimize any differences in activation that may be attributable this IQ difference. In both left and right prefrontal, motor and parietal cortices, unaffected subjects increase activation in response to increased task difficulty more than do subjects with fraX. These areas have previously been found to be associated with arithmetic processing on this [Menon et al., 2000a,c]

and similar tasks [Burbaud et al., 1995; Dehaene et al., 1999; Rickard et al., 2000]. By contrast, there were no areas where subjects with fraX recruited more activation than unaffected subjects in association with increased task difficulty. Thus, although subjects with fraX perform significantly worse on 3-operand arithmetic calculations, they do not show a corresponding increase in activation in expected brain regions, as do unaffected subjects. These group differences appear to reflect a deficiency in recruitment of neural resources for arithmetic processing in subjects with fraX.

It is difficult to say, with these data alone, whether this differential pattern of brain activation between fragile X and unaffected subjects is specific to arithmetic processing, or whether it belies a more general cognitive processing deficit in subjects with fragile X. Indeed, many of the brain areas reported here are not unique to arithmetic processing, but show up across a number of functional imaging tasks [see Gruber et al., 2001]. Studies that examined differences in brain activation between subjects with fraX and unaffected controls on a visuospatial working memory task [Kwon et al., 2001; Menon et al., 2000b], however, show differential activation patterns that are unlike those reported here. This suggests that the present findings are specific to the task at hand, and not a "signature" of generalized processing deficits in these subjects.

A growing body of research now suggests that FMRP plays a critical role in both the development and functioning of the human brain [Abrams et al., 1999; Kaufmann et al., 1999; Kwon et al., 2001; Menon et al., 2000b; Tassone et al., 1999]. In persons with fraX, abnormal morphology of dendritic spines and a reduction in the length of synapses in the cortex have been found to be associated with low levels of FMRP [Hinton et al., 1991; Rudelli et al., 1985]. This synaptic malfunction may result in a functional system not being able to recruit a sufficient number of neurons, leading to weaker overall activation for a given cognitive task. Recent studies [Kwon et al., 2001; Menon et al., 2000b] report significant correlations between FMRP and activation in dorsolateral prefrontal cortex and parietal cortex regions during a visuospatial working memory ("2-back") task in subjects with fraX. Here, we were interested in whether FMRP would be related to brain activation, particularly in those regions associated with arithmetic processing. Although we use an indicator of FMRP based on blood samples, research shows that leukocyte-based molecular measures of *FMR1* provide a good indicator of *FMR1* expression in the brain [Abrams et al., 1999; Tassone, 1999; Willemsen et al., 2000].

As hypothesized, with increasing levels of FMRP, subjects with fraX showed more activation during the 3-operand trials in bilateral prefrontal, bilateral motor/premotor, and left SUPMG/ANG, all areas that have been reported previously as being involved in arithmetic processing in typically-developing subjects [Burbaud et al., 1995; Dehaene et al., 1999; Menon et al., 2000a,c; Rickard et al., 2000]. Thus, these data directly suggest that decreased *FMR1* protein expression underlies deficits in arithmetic performance that are reported frequently in persons with fraX.

Activation also correlated with FMRP in the 2-operand trials, but the pattern was less focal than in the 3-operand trials. In this contrast, activation was diffusely distributed across bilateral frontal and parietal areas as well as cingulate gyrus. This marked difference in correlated activation patterns for the 3- vs. the 2-operand trials suggests that FMRP is related to efficient recruitment of neural resources to perform this arithmetic task, but only for those trials for which processing is less "automated." As noted above, subjects with fraX only made significantly more response errors than control subjects on the 3-operand trials, and not on the less-difficult 2-operand trials. It follows that more focal activation would be observed in brain regions related to arithmetic processing when subjects are forced to do actual arithmetic computations than when they are able to call upon instant solutions from memory, as is much more likely to be the case in the 2-operand trials. The fact that this increase of focal activation for the 3-operand over the 2-operand trials was not present in the fraX group before using FMRP as a covariate underscores the significance of *FMR1* gene expression in the cognitive functioning of this population.

It also is important to note that FMRP, in itself, was not correlated with IQ in our sample, either before ($r = 0.119$, $P = 0.66$) or after ($r = 0.000$, $P = 1.0$) the effects of age were removed. Thus, the pattern reported here, of increased brain activation with increasing FMRP, is not attributable to a simple relationship between increased activation in arithmetic-related brain regions and an increase in general intelligence.

FMRP was not correlated with performance (i.e., percent correct, reaction time or false alarms) on the trials. This lack of correlation could be due to the fact that there were relatively few subjects with fraX for whom performance data was available ($n = 12$). It is possible that with a larger sample of subjects with fraX, a correlation between FMRP and behavioral performance would emerge. Furthermore, it is possible that a parallel correlation between FMRP and task performance was not observed because the relation-

ship between brain activation and performance is not straightforward. There may be complex functional interactions between brain regions, which remain to be explored.

The present study presents the first attempt at characterizing the neural underpinnings of the well-described arithmetic deficits associated with fraX syndrome. The behavioral findings of significantly decreased calculation performance in the subjects studied here corroborate well with previous studies, and the imaging results help formulate a brain-based model for understanding the mechanisms behind the deficit. Furthermore, the finding of a correlation between FMRP and brain activation during arithmetic processing helps to further articulate the relationship between this single gene defect and higher cognitive functioning in persons with fraX. Future work in our laboratory will extend this research to males with fraX, and attempt to further tease apart the complex differences in network activation observed between this population and both typically and atypically developing children and young adults.

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