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Reduced Hippocampal Activation During Recall is Associated with Elevated *FMR1* mRNA and Psychiatric Symptoms in Men with the Fragile X Premutation

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Abstract

Recent studies reveal that young carriers of the fragile X premutation are at increased risk for psychiatric conditions, memory problems and executive deficits. Post mortem and structural MRI studies suggest the hippocampus is preferentially affected by the premutation. The current study utilized magnetic resonance imaging (MRI) to explore the relationship between hippocampal structure and function as well as molecular/genetic and psychiatric measures in men with the fragile X premutation. Although the groups did not differ in hippocampal volume, the premutation group showed reduced left hippocampal activation and increased right parietal activation during a recall task relative to controls. These results suggest that brain function underlying memory recall is affected by premutation status. Left hippocampal activation was negatively correlated with both *FMR1* mRNA level and psychiatric symptomology in the premutation group. These associations support the theory that increased levels of *FMR1* mRNA affect brain function and contribute to psychiatric symptoms.

Keywords

Fragile X premutation; FMR1 mRNA; Hippocampus; fMRI; Recall; Memory

Introduction

Carriers of premutation expansions (55 to 200 CGG repeats) of the fragile X mental retardation 1 (*FMR1*) gene are at increased risk for social, emotional, and cognitive problems and of developing a late-onset neurodegenerative disorder, fragile X-associated tremor/ataxia syndrome (FXTAS; Dorn et al. 1994; Franke et al. 1998; Tassone et al. 2000a, b, c; Johnston et al. 2001; Hagerman and Hagerman 2002; Borghgraef et al. 2004; Moore et al. 2004a, b; Cornish et al. 2005; Hessler et al. 2005; Farzin et al. 2006). FXTAS involvement in premutation carriers, when it occurs, typically manifests itself in carriers over the age of 50, though in rare cases it has been reported earlier. Recently, data from our laboratory has suggested that brain function is also affected by premutation status in relatively young premutation carriers without FXTAS who demonstrate no overt neurological symptoms. Compared with controls on an fMRI task, men with the premutation showed diminished brain activation in the amygdala and several brain areas that mediate social cognition while viewing fearful faces (Hessler et al. 2007). The reduced amygdala activation in this group was also significantly associated with self-report of psychiatric symptoms on the Symptom Checklist-90-Revised (SCL-90-R). Additionally, these men displayed a lack of startle potentiation while viewing fearful faces and showed reduced skin conductance response when greeting an unfamiliar experimenter in comparison with the control group.

Several studies have suggested that brain structure itself is also affected by premutation status and report the hippocampus to be significantly affected. In a structural brain MRI study, Jäkälä and colleagues (Jäkälä et al. 1997) showed that, compared to controls, males and females with the premutation had significantly reduced hippocampal volumes and associated memory deficits. In a more recent study of 20 male premutation carriers and 20 age and IQ matched controls, Moore et al. (2004a, b) demonstrated significantly reduced grey matter density in several brain regions in the premutation group, including the amygdala-hippocampal complex. Within this group, increased age, increased CGG repeat size and decreases in the percentage of blood lymphocytes expressing fragile X mental retardation protein (FMRP) were associated with decreased grey matter density in the amygdala-hippocampal complex. Although these studies did not control for the possibility of FXTAS involvement, they show significant differences between groups even at younger ages, when the presence of FXTAS is unlikely.

Repeat lengths in the premutation range result in elevated *FMRI* mRNA levels (Tassone et al. 2000a, b, c), and mild reductions in FMRP production in some carriers with CGG repeat expansions in the upper premutation range (Tassone et al. 2000a, b, c; Kenneson et al. 2001). Results from several studies suggest that the hippocampus may be especially vulnerable to these molecular effects of the premutation. During normal fetal development, the hippocampus is one of the areas in which *FMRI* transcription is the highest (Abitbol et al. 1993) and also demonstrates one of the highest expression rates of *FMRI* mRNA in the human brain in adults (Tassone et al. 2004). In a study of the knock-in mouse model of the premutation (Entezam et al. 2007), FMRP expression was significantly reduced in several brain regions, including the hippocampus. In those brain areas sampled in post mortem studies of brain tissue from older premutation males with FXTAS, the hippocampus shows the largest percentage of cells with intranuclear inclusions, again suggesting that this brain region may be particularly affected in FXTAS (Greco et al. 2002, 2006). Our current working hypothesis for psychiatric and cognitive involvement among carriers of premutation alleles posits that clinical features arise through a combination of RNA toxicity and mild reductions of FMRP. If carrying the premutation allele has neural consequences, and if the hippocampus is particularly vulnerable, it is in this area that we might expect to see the clearest CNS manifestations through various imaging approaches.

Based on the previous molecular, genetic, and clinical findings illustrating the effects of the premutation on the hippocampus, we conducted a magnetic resonance brain imaging study to determine whether men with the *FMRI* premutation show functional changes in this brain region. Additionally, we sought to explore whether altered hippocampal function in this group is related to *FMRI* genetic measures, memory task performance, or psychiatric symptoms. The current study was restricted to males to avoid the confounding effect of X-chromosomal activation ratio in females.

Methods

Participants

Participants included 11 men with a confirmed premutation *FMRI* allele and a comparison group of 11 men without the premutation. All subjects whose data is reported here also participated in a study of amygdala function whose results are reported in the Hessel et al. (2007) paper referenced above. The two groups did not differ in age, Full Scale IQ (117.9, 113.4; $t=0.54$, $p=0.60$), level of education and overall psychiatric symptomology as measured by the SCL-90-R Global Severity Index (see Table 1). All participants except one control were right-handed. Four individuals were Latino (two in control group, two in premutation group), one East Indian (control group), and the remaining participants were Caucasian (self-report). Males with the premutation were recruited through screening of fragile X pedigrees of probands with fragile X syndrome. Controls were non-carrier males in families affected by fragile X. No participants were ascertained due to clinical symptoms or referred to a clinic after participation in this study. Neurological examinations on all participants were normal, including absence of tremor and ataxia.

Psychological assessment

Intelligence—Cognitive ability was based on Full Scale IQ using the Wechsler Adult Intelligence Scale, Third Edition (WAIS-III; (Wechsler 1997).

Psychiatric symptoms—We assessed psychiatric symptoms using the SCL-90-R (Derogatis 1994), a standardized self-report inventory of current psychiatric symptoms. Although not standard for thorough diagnostic assessment, the SCL-90-R has been extensively used in research paradigms to assess current psychiatric symptoms. Ninety items, each rated

on a five-point scale of distress, are clustered into the following symptom dimensions: Somatization, Obsessive–Compulsive, Interpersonal Sensitivity, Depression, Anxiety, Hostility, Phobic Anxiety, Paranoid Ideation, and Psychoticism. The Global Severity Index (GSI) is an indicator of overall level of psychiatric disturbance within the past week.

Molecular genetic measures

CGG repeat size—Genomic DNA was isolated from peripheral blood lymphocytes (5 ml of whole blood using standard methods (Puregene Kit; Gentra Inc.). For Southern blot analysis, 5–10 µg of isolated DNA was digested with EcoRI and NruI. Hybridization was performed using the *FMRI* genomic dig-labeled StB12.3 probe. Genomic DNA was also amplified by PCR using primers c and f (Fu et al. 1991). Hybridization was performed with a dig-end-labeled oligonucleotide probe (CGG)₁₀. Analysis and calculation of the repeat size for both Southern blot and PCR analysis were carried out using an Alpha Innotech FluorChem 8800 Image Detection System.

FMR1 mRNA—Total cellular RNA was purified from 3–5 ml of peripheral blood using standard methods (Purescript kits; Gentra Inc.; Trizol; BRL). All quantifications of *FMRI* mRNA were performed using a 7700 Sequence detector (PE Biosystems) as previously described (Tassone et al. 2000a, b, c).

Brain volume and function

Brain image acquisition—Images were acquired on a 1.5T GE Signa scanner with Echospeed gradients and a standard GE whole head coil. FMRI was performed using a single-shot gradient recalled echo–echo planar imaging sequence with TR 2,000 ms, TE 32 ms, Flip angle 90°, FOV 22 cm, 4 mm slice thickness, 1 mm slice gap, 64×64 matrix, 27 slices, 194 NEX, and 62.5 KHz bandwidth and coronal orientation. A T1 weighted spoiled grass gradient recalled (SPGR) 3D MRI sequence with 1.3 mm³ resolution, 256×256 matrix, Flip angle=15° and FOV 22 cm was acquired in the same scan session to aid in localization of functional data. The functional tasks were programmed using Presentation™ software and presented visually using a head-coil mounted mirror and projection to a screen at the participant's feet. Initiation of scan and task were synchronized using a TTL pulse delivered to the scanner

Image preprocessing—Images were reconstructed, by inverse Fourier transform, for each of the time points into 64×64×18 image matrices (voxel size: 3.75×3.75×7 mm) utilizing SPM 99 (Friston et al. 1995). Images were corrected for movement using least square minimization without higher-order corrections for spin history, and normalized to stereotaxic MNI (Montreal Neurological Institute) coordinates. Images were then resampled every 2 mm using sinc interpolation and smoothed with a 4 mm Gaussian kernel to decrease spatial noise.

Total brain volume—Non-brain elements were manually removed from structural images by operator-guided tracing using a custom-written computer program operating on a UNIX, Solaris platform (Quanta 6.1) These images were automatically segmented into cerebrospinal fluid and brain matter components according to previously published methods in order to obtain a measure of total brain volume (DeCarli et al. 1992, 1995, 1996).

Hippocampal volume—Quantification of hippocampal volume was performed on coronal 3D SPGR images that were reoriented perpendicular to the long axis of the hippocampus. The sampled hippocampal volume included the CA1–CA4 fields, dentate gyrus, and the subicular complex, and were quantified by operator-guided tracing as described previously (Wu et al. 2002). All hippocampal volumes were adjusted for total brain volume.

Intrarater reliability for these methods was good, with intraclass correlation coefficients of 0.96 for the left hippocampus and 0.97 for the right hippocampus. A single rater performed all of the analysis and was blind to participant's experimental condition and demographic information.

FMRI recall task—Associative memory recall tasks done during functional MRI scanning have repeatedly been shown to result in robust activation of the hippocampal formation (e.g., Killgore 2000; Sperling et al. 2001; Stark and Squire 2001; Yonelinas et al. 2001; Duzel et al. 2003; Sperling et al. 2003; Giovanello et al. 2004). These studies, and others, document both the role of the hippocampus in recall tasks and the ability of fMRI to measure the functioning of the hippocampus during such tasks. The use of fMRI also allows the study of other brain regions that are active during recall – regions that may be able to compensate for deficits in hippocampal function.

To evaluate the activity of the hippocampus during a recall task, we followed a design similar to that used by Yonelinas et al. (2001). Twenty-four hours before the MRI session, participants were presented with 244 line drawings from the Snodgrass set of pictures (Snodgrass and Vanderwart 1980). These pictures were either presented in green or in red and participants were instructed to remember the pictures by creating a short, one-sentence explanation of why the object was that particular color. They were given six seconds to memorize each picture before the presentation of the next picture. Immediately after the memorization phase, participants were given a recall test where all pictures presented during the memorization phase were presented again, this time as black line drawings on a white background. Participants responded with the color each picture had been when originally presented and feedback was given. The next day, during the MRI session, subjects were again presented with the black and white drawings, this time for only 2 s, and asked to indicate by a button press whether the picture had initially been presented in red or green. A control task was also presented, during which participants were asked to indicate which simple shape on the screen was larger (right or left). Experimental and control pictures were presented in alternating 24 s blocks, with each block consisting of 12 pictures. A 24 s block of simple fixation occurred at both the beginning and the end of the scanning run. (See Fig. 1.)

FMRI analysis—Statistical analysis was performed on both individual and group data using the modified General Linear Model and the theory of Gaussian random fields as implemented in SPM99 (Friston et al. 1995). For both within-group and between-group comparisons, significant voxels were defined as those that exceeded a threshold value x equivalent to a one-tailed t -test $p < 0.05$ (Bonferroni corrected for multiple comparisons at the cluster level). Once subjected to threshold analysis, the activation was superimposed on the normalized high-resolution SPGR and localized manually using atlases of the human brain (Talairach and Tournoux 1998; Duvernoy and Bourgouin 1999). Group analyses were overlaid on images created by averaging all individuals' normalized SPGR images.

All effects of interest were modeled using a standard within-subjects procedure for each participant by contrasting experimental and control blocks (e.g. blocks of object recall—blocks of shape comparison). Models for individuals were identical across participants. A random-effects model incorporating a two-stage hierarchical procedure was utilized in performing group analyses. This model estimates the error variance for each condition of interest across participants rather than across scans (Holmes and Friston 1998). The contrast images for each participant for each effect of interest were generated first, as described above. These contrast images were then analyzed using a general linear model to determine voxel-wise t statistics and generating one contrast image per participant, per effect of interest.

Within-group analyses of each contrast were performed to identify voxels/brain regions showing similar response modulation across participants in each group for a given contrast (e.g., recall-control). In addition, between-group analyses were performed to determine how the two groups differed in their average activation in response to each contrast of interest (i.e., which regions were more active in those with the premutation than in controls, and vice versa). Region of interest (ROI) analyses were carried out using Marsbar (Brett et al. 2002), a MATLAB toolbox written to be implemented within SPM. Contrasts were first defined as described above. Each contrast of interest was then analyzed only in voxels that fell either within the MNI (Montreal Neurological Institute Atlas) template of the area of interest (e.g. the hippocampus) provided within Marsbar or in a functionally or statistically defined region of interest (e.g. a region defined functionally by the group which is then assessed within individuals). A *t*-statistic termed “contrast value” was then calculated as the average of the contrast values of the voxels falling within the defined ROI. The contrast value in these analyses is comparable to the *Z* score reported in the whole-brain analyses tables.

Results

Total brain and hippocampal volume

Independent sample *t*-tests revealed no differences between groups in total brain volume or in right, left or total hippocampal volumes (see Table 2) even when hippocampal volumes were adjusted for total brain size (right: $t=-0.55, p=0.59$, left: $t=0.23, p=0.83$, total: $t=-0.18, p=0.86$). Neither CGG repeat size nor blood levels of *FMR1* mRNA was significantly correlated with total brain volume or adjusted hippocampal volumes (Pearson’s $r<0.40, p>0.30$).

Behavioral memory performance

For the immediate recall test on day1, a significant difference was found between the accuracy for the control group ($M, 82.7$; $SD, 8.02$;) and the premutation group ($M, 74.6$; $SD, 9.65$; range, 60.1–90.1; $t=2.13, p=0.045$). Behavioral data from the fMRI paradigm on day 2 (Fig. 2) showed no significant differences between groups on accuracy on the recall task (control: $M, 71.34$; $SD, 7.34$; premutation: $M, 67.97$; $SD, 9.09$; $t=0.937, p=0.36$), control task (control: $M, 93.94$; $SD, 5.49$; premutation: $M, 95.76$; $SD, 3.17$; $t=-0.919, p=0.37$), reaction time (in ms) during recall (control: $M, 1017$; $SD, 86.4$; premutation: $M, 1224$; $SD, 523.1$; $t=-1.30, p=0.21$) or reaction time during the control task (control: $M, 656$; $SD, 49.1$; premutation: $M, 668$; $SD, 103.6$; $t=-0.32, p=0.75$).

Within group fMRI analysis

During the associative memory probe, when compared with the control task, premutation carriers showed overall brain activation patterns that were quite similar to those evidenced by the control group (see Fig. 3 and Table 3). These areas included anterior cingulate cortex, bilateral dorsolateral prefrontal areas, left parietal cortex and left fusiform gyrus. Despite these overall similarities, the two groups showed different activation patterns in two areas: within the right parietal cortex and in the left hippocampal and parahippocampal regions. The premutation group showed significant activation in right parietal areas ($p<0.01$) while the control group did not. The control group showed significant activation in the left hippocampus and left parahippocampal region ($p<0.01$) while the premutation group did not (Fig. 4a). Neither group showed significant activation in the right hippocampus or right parahippocampal region.

Between-groups fMRI analysis

As would be predicted by the within-groups analysis, the control group showed greater activation than the premutation group in the left parahippocampal and hippocampal regions

(Fig. 4b), as well as areas in the right cuneus, right lingual gyrus and right caudate nucleus ($p<0.01$). The premutation group showed more activation than controls in right intraparietal sulcus, supramarginal gyrus and angular gyrus as well as the left caudate nucleus ($p<0.01$; see Table 4).

Correlation analyses

As we expected that abnormal hippocampal activation might be associated with *FMRI* measures and/or reflected in psychiatric symptoms, we also investigated hippocampal activity as a function of *FMRI* mRNA expression in blood, CGG repeat size and SCL-90-R scores. In conducting these analyses, we entered these variables as covariates of interest in analyzing activation elicited during the recall-control contrast. In those clusters that showed a significant relationship in this group analysis, we then performed a ROI analysis to look at the correlation between the mean contrast value for voxels in that cluster for each individual and their molecular measures or SCL-90-R score. We explored the relationship between brain activation and molecular measures only in the premutation group, as CGG repeat number and *FMRI* mRNA levels in the control group lacked sufficient variance to conduct meaningful correlation analyses. No significant correlation between CGG repeat number and hippocampal or parietal activation was revealed in the group data. A significant negative association between left hippocampal activation and increased blood levels of *FMRI* mRNA was evident in the premutation group (74 voxels, $\rho=-0.791$, $p=0.004$). This finding must be treated with some caution, however, as the group included a single participant whose blood mRNA level was quite high (5.1-fold above normal) and a statistical outlier. We primarily addressed this concern by using a nonparametric test to look at the correlation between mRNA and activation, but also looked at the correlation when his data were removed from the analysis. Without his data, the correlation was still strong ($\rho=-0.721$) but reduced in significance ($p=0.02$). A relationship between greater right-parietal activation (angular gyrus, IPS and supramarginal gyrus) and increased mRNA was also found within the premutation group (140 voxels, $\rho=0.955$, $p=0.004$). Unlike the correlation between mRNA and left hippocampal activation, the strength of this relationship was unaffected by the removal of the subject with the highest mRNA level from the data set. A significant negative correlation between SCL-90-R GSI score and left hippocampal activation was also evident in a whole-brain covariate of interest analysis (threshold $p<0.05$) in the premutation group (see Fig. 5). Despite a similar range of SCL-90-R GSI scores, this association was not evident in the control group. In the premutation group (331 voxels, $\rho=-0.645$, $p=0.032$), but not in the control group (331 voxels, $\rho=0.43$, $p=0.46$), severity of psychiatric symptoms as measured by the SCL-90-R GSI was negatively correlated with left hippocampal activation.

We also investigated correlations between activation in response to the memory-control contrast and accuracy scores from the in-scanner recall task. To assess the strength of these correlations, we performed an ROI analysis looking at the contrast value in response to the memory-control contrast for each individual in those voxels in parietal cortex that showed this association in the group data. Contrast values for individuals were then correlated with individual accuracy scores. Neither group showed a significant correlation between hippocampal activation and accuracy. The strongest association between activation and accuracy, in both groups, was in clusters within bilateral parietal cortex. The association was more extensive on the right in the premutation group (291 voxels, $\rho=0.890$, $p=0.001$) while the control group showed more voxels correlated with accuracy on the left than the premutation group (137 voxels, $\rho=0.840$, $p=0.001$). While the higher parietal recruitment in premutation carriers is suggestive of compensation, we were not able to document a negative correlation between hippocampal activation and right parietal activation in the premutation group ($\rho=-0.182$, $p=0.503$).

Discussion

The present study provides evidence that men with the fragile X premutation have a reduced ability to recruit the left hippocampus during recall. Relative to well-matched controls, men with the premutation were significantly worse on an immediate recall test but were not significantly worse on the in-scanner recall task 24 h later. Reduced hippocampal activation in the premutation group was accompanied by increased activation in both frontal and parietal areas, particularly right parietal areas, perhaps allowing them to compensate for decreased hippocampal involvement by compensatory recruitment in these areas. Both the decrease in left hippocampal activation and the increase in right parietal activation were correlated with increased *FMRI* mRNA levels in the premutation carriers. Additionally, the clinical relevance of these findings is suggested by the fact that hippocampal activity was negatively correlated with psychiatric symptomology in the men with the premutation. It is of particular interest that this association was absent in controls, who exhibited similar levels of psychiatric symptoms. This difference suggests that carrying the premutation allele causes brain changes that affect both hippocampal activity and psychiatric symptomology, while the etiology of psychiatric symptoms in controls varies and is unrelated to brain activity during recall.

That mRNA levels were significantly correlated with brain activation measures while correlations with CGG repeat number did not reach significance may be an important finding but must be treated with caution given our small sample size. Previous data from our group has shown that mRNA levels may be more important to psychiatric symptomology than CGG repeat number in premutation carriers (Hessl et al. 2005), perhaps due to intrinsic variation in mRNA level among individuals with similar CGG repeat expansions. Our data suggests that mRNA levels may also be a stronger factor in brain activation changes specific to premutation carriers. However, these distinctions are difficult to establish unambiguously, as CGG repeat number and *FMRI* mRNA levels are strongly correlated. The correlation between CGG repeat number and blood levels of *FMRI* mRNA in the current sample, for example, was quite strong ($\rho=0.764$, $p=0.006$). Additional factors (other than CGG repeat number) that modulate mRNA levels in premutation carriers have not been identified. Further examination of the factors contributing to increased *FMRI* mRNA levels and the relationship between mRNA levels, FMRP levels and brain function will be necessary to untangle how each factor contributes to the neural phenotype in premutation carriers.

We chose to investigate hippocampal function through a recall paradigm, expecting that the premutation group would be somewhat impaired on this task. Instead, the premutation group performed as well as the control group during the in-scanner recall task while unexpectedly showing a significant deficit during the immediate recall test. That the two groups performed comparably on the in-scanner task simplifies the interpretation of our functional results, giving us confidence that differences between the groups were not primarily a function of differences in their ability to perform the task. The immediate recall deficit demonstrated by the premutation group suggests that there is a significant difference between groups in their ability to encode information into memory and that there is some disparity in the rate of forgetting between the groups. From the data collected we cannot resolve whether hippocampal activation differences between groups are primarily due to differences in encoding ability (which is itself highly dependent on hippocampal function) or is truly reflective of hippocampal activity during recall. In order to address these questions, we are currently investigating hippocampal function during *encoding* of complex scenes utilizing an event-related design that will allow us to pull apart brain activation during correctly encoded scenes from those scenes that are later forgotten in a post-scan immediate recall test.

The reduced activity in the left hippocampus seen in our results could be reflective of premutation-specific developmental changes. There is some indication in the recent literature

that boys with the premutation are at increased risk for neurodevelopmental disorders, including autism spectrum disorders and Attention Deficit Hyperactivity Disorder (ADHD; Aziz et al. 2003; Goodlin-Jones et al. 2004; Farzin et al. 2006). Although it is not immediately clear how this connects to possible dysfunction of the hippocampus in premutation carriers, it is an indication that even young boys show some effect of their premutation status. Such developmental consequences could be caused either by elevated *FMRI* mRNA (Tassone et al. 2000a, b, c; Jacquemont et al. 2003; Oostra and Willemsen 2003; Allen et al. 2004; Hagerman and Hagerman 2004) or a mild reduction in FMRP as is known to occur in individuals with the premutation, especially with high CGG repeat numbers (Tassone et al. 2000a,b,c; Kenneson et al. 2001; Entezam et al. 2007). We would expect lower FMRP to result in abnormally reduced pruning during development (Irwin et al. 2000; Bagni and Greenough 2005; McKinney et al. 2005; Grossman et al. 2006) which would in theory result in larger and potentially more active hippocampi. It is possible that lowered FMRP and increased mRNA, which can co-occur in carriers with the premutation, are affecting hippocampal structure and function in different ways, complicating the picture and making molecular/fMRI associations difficult to clearly discern, especially in so small a sample. We were unable to measure FMRP levels in the current study but gathering such data in combination with measures of *FMRI* mRNA levels, CGG repeat lengths and functional data will be important in future investigations to pinpoint the molecular mechanism at work.

Alternatively, or in addition to a developmental effect, our findings could represent early pre-symptomatic brain changes that precede, but which are ultimately associated with, FXTAS. The intranuclear inclusions found in post mortem brain tissue of those who died with FXTAS could be accumulating throughout the lifespan of susceptible premutation carriers. However, it is not known whether inclusion formation, *per se*, adversely affects brain function. Indeed, although mice develop far greater inclusion loads in neurons than do humans with severe FXTAS, the mice develop only mild neurological features and relatively less neurodegeneration. (Willemsen et al. 2003).

As this study was designed and conducted as a preliminary examination of the effect of the fragile X premutation on hippocampal function, it had some limitations. The primary limitation was the small sample sizes of our groups. This limited our power to detect true volumetric differences in the hippocampus that might be present as well as limiting our power in investigating interaction effects between molecular, behavioral and brain imaging data. Our sample size also limited the variance in the molecular measures we collected and our ability to thoroughly investigate relationships between those measures and brain activation in response to our task. Because we expect those with greater levels of *FMRI* mRNA to be more affected, our study would have been strengthened by inclusion of more subjects with very high mRNA levels. We did have one individual with the premutation whose blood *FMRI* mRNA level was 5 times above normal. This same individual also exhibited the *least* hippocampal activation, the poorest memory performance, as well as the most severe psychiatric symptoms. Thus, rather than being an “outlier” in our sample, this individual may represent the upper end of a continuum of severity of dysfunction in this population. Indeed, while blood *FMRI* mRNA levels as high as tenfold above normal have been reported (Tassone et al. 2000a, b, c), we do not yet have brain MRI data available from individuals in this range.

The reduction of hippocampal activity observed in the premutation group was not accompanied by any measurable reduction of hippocampal volume. Although this study is primarily designed as an fMRI study of the hippocampus, our volumetric data strengthens our functional imaging results by showing that the hippocampal activation differences we measured are independent of gross morphological changes in the hippocampus in this particular sample. The sample sizes, however, preclude an adequately powered test of hippocampal volume differences between premutation carriers and typical controls in the wider population. The lack of hippocampal

volume effects associated with the premutation in the present study is in contrast both to Jäkälä et al. (1997), who found reduced volumes, and to Loesch et al. (2005) who found increased volumes in this region in male premutation carriers. The lack of consistent findings between these two studies may be due to specific cohort effects, especially if these cohorts included participants with and without FXTAS. The relative effects of decreased FMRP and/or elevated *FMR1* mRNA, which may have differential effects on brain development and structure across study participants, may also contribute to these inconsistencies.

It is also important to note that CGG repeat size and mRNA measures were ascertained from blood samples. As such, they may not accurately reflect levels in the brain. While a post mortem study carried out on a single premutation carrier and one control reported CGG-repeat length stability between tissue types, *FMR1* mRNA expression appears to vary across tissue type and brain region (Tassone et al. 2004). In general, however, the relative brain *FMR1* mRNA levels were found to be substantially higher than in peripheral blood leucocytes for both the premutation carrier and control, reflecting the important role this gene plays in brain tissue. *FMR1* mRNA levels were substantially higher in brain samples for the premutation compared to the control but not to as great an extent as in blood. From these data it appears likely that increased mRNA levels measured in the blood would be echoed by increased mRNA levels in the brain. Thus, while correlations with brain function measures must be treated with caution, mRNA levels measured in blood are our best current proxy for mRNA levels in the brain.

In conclusion, this study demonstrates reduced hippocampal activation during memory recall associated with parietal over-activation, psychiatric problems and abnormal elevation of *FMR1* mRNA in men with the fragile X premutation. These findings may be a result of RNA toxicity that in some may reflect developmental changes and in others may develop into a neurodegenerative disease, FXTAS, in later life.

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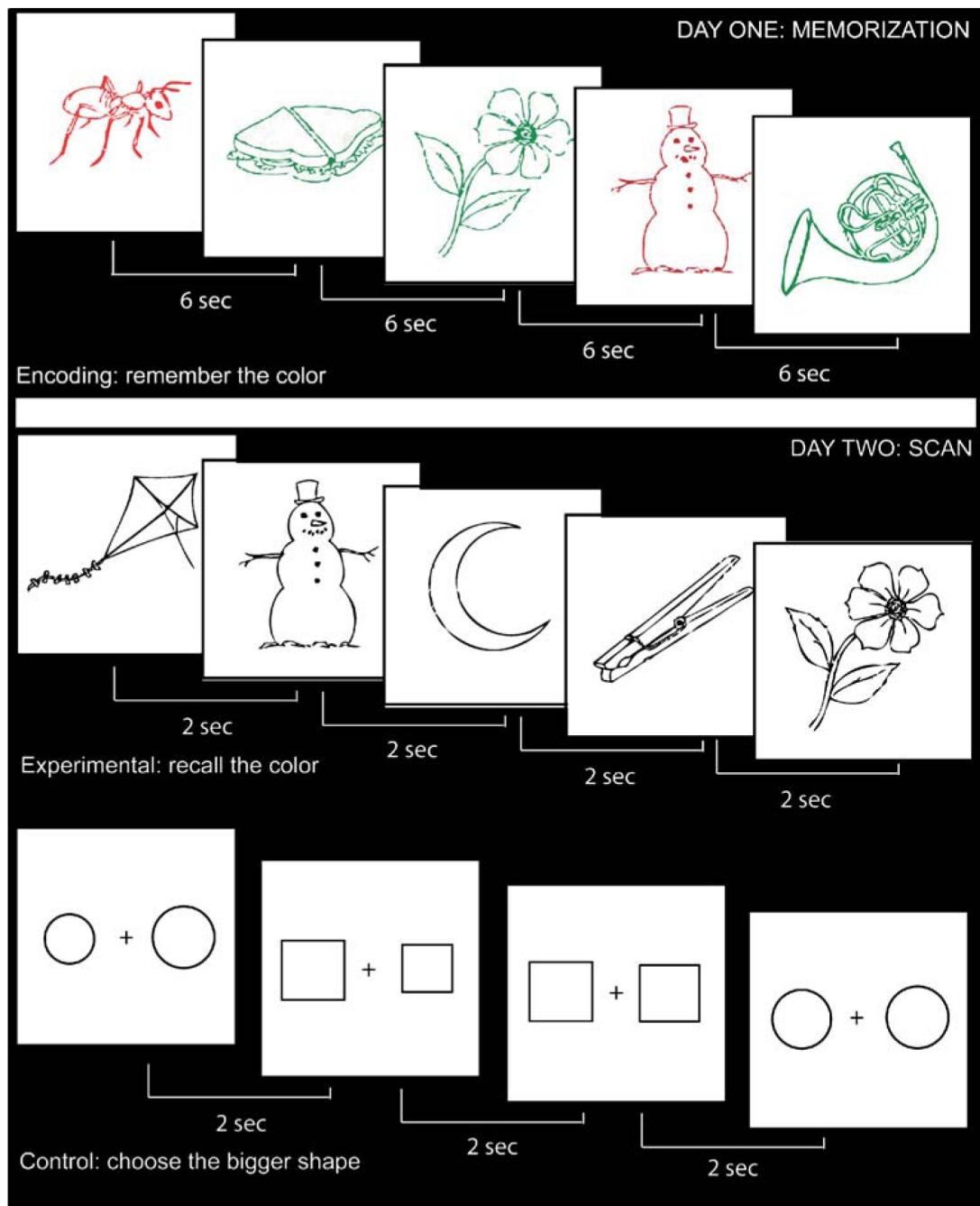


Fig. 1. Schematic of task design during the encoding task on day 1 and the in-scanner recall task on day 2

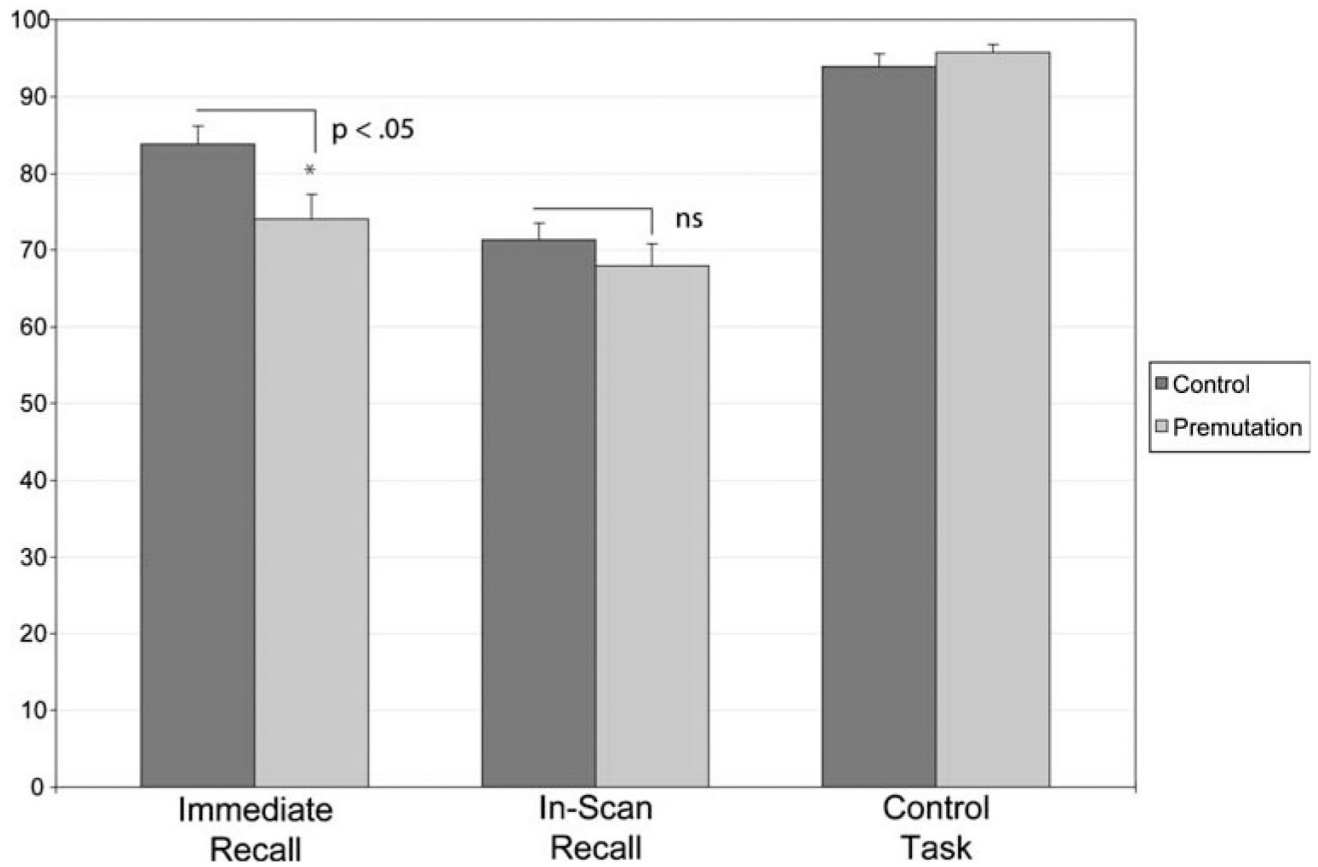


Fig. 2. Percent correct response on an immediate recall test (day 1) and in-scanner recall and control tasks (day 2)

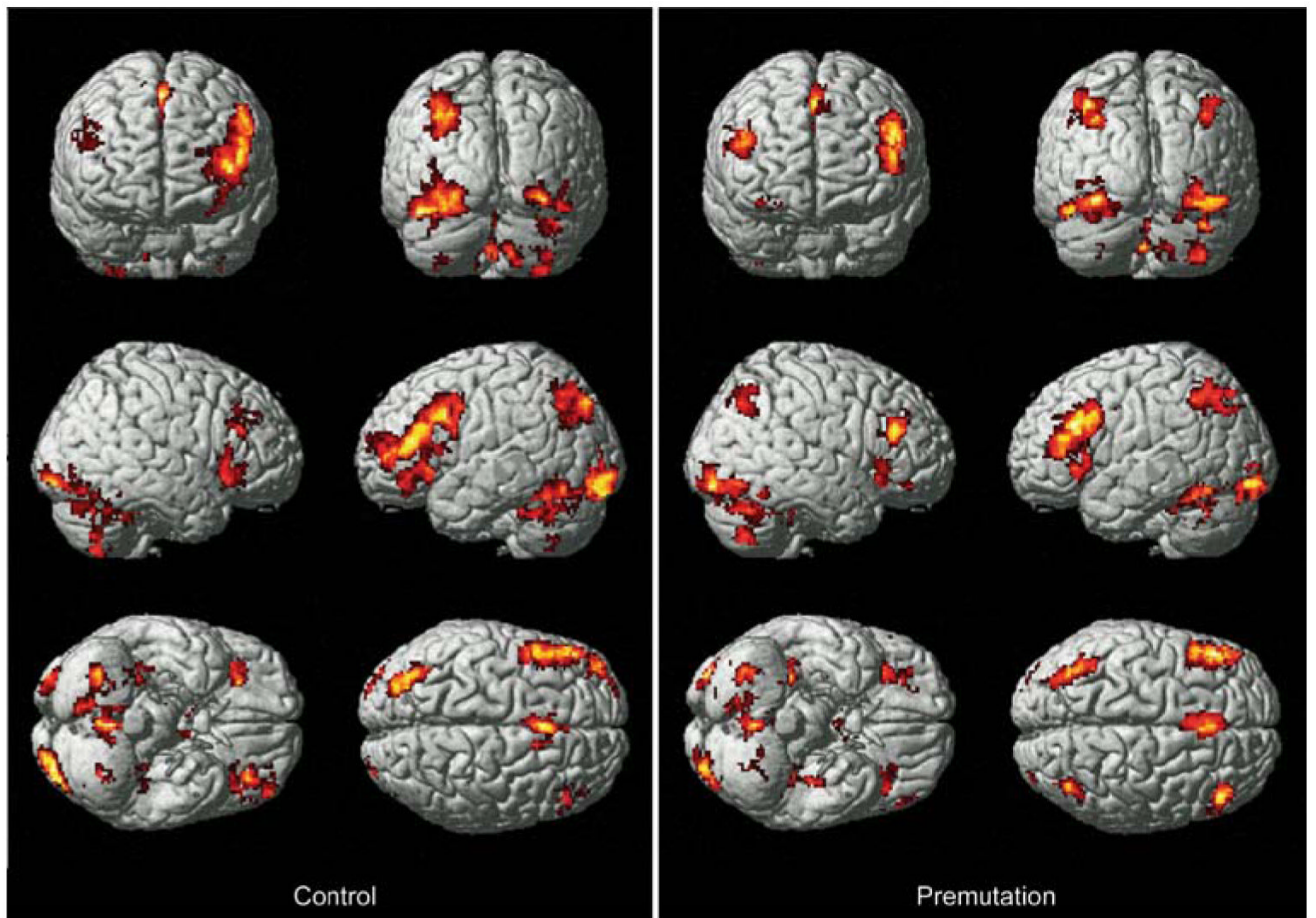


Fig. 3. Regions of activation rendered on the surface of a MNI-normalized template image for control (left) and premutation (right) groups during the associative memory recall task. Images are subject to threshold at $p < 0.01$ and corrected for multiple comparisons at the cluster level

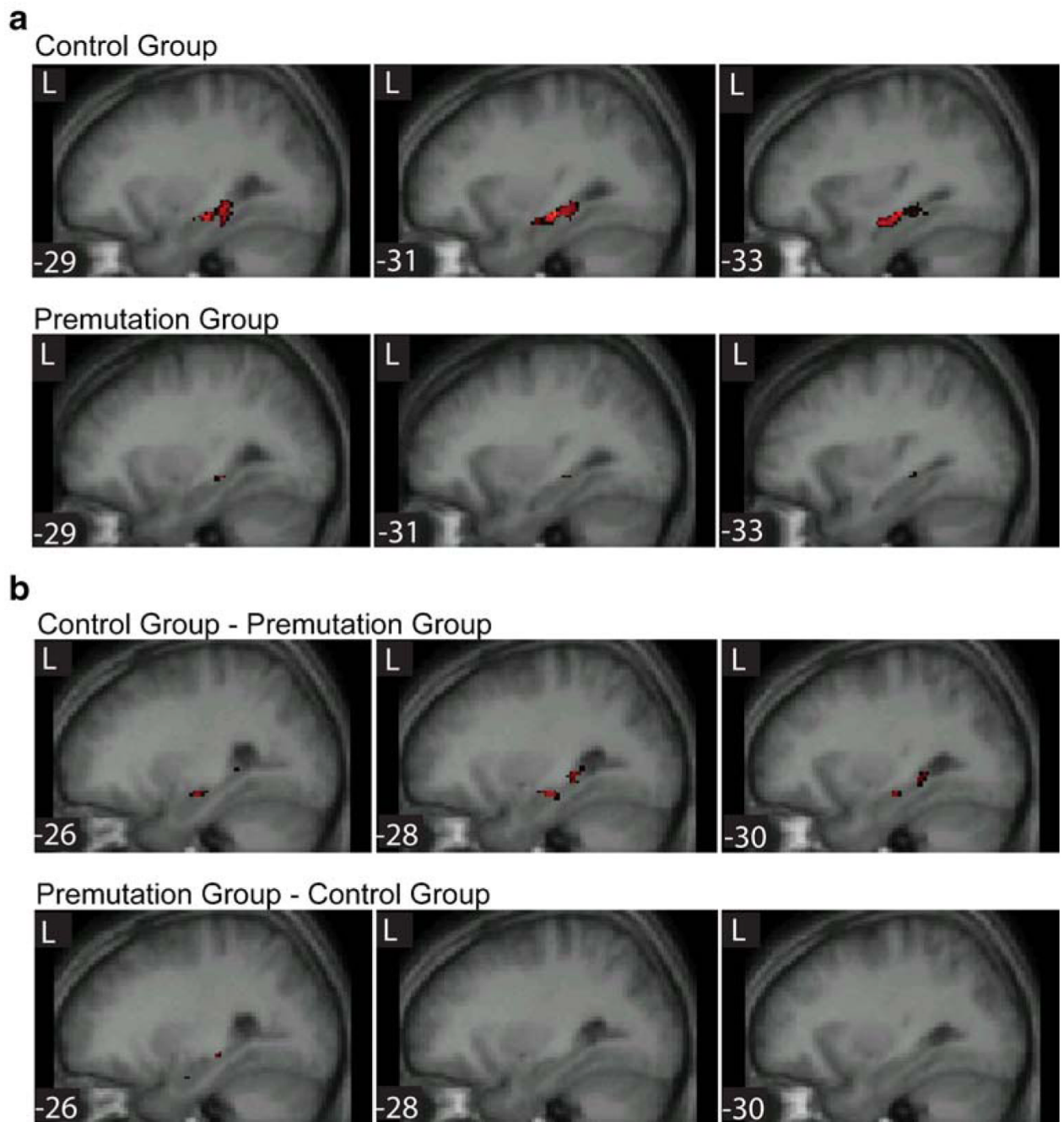


Fig. 4. Within-group (**a**) and between-group (**b**) activation in the left hippocampus during the recall task. Image threshold is set at $p < 0.05$ and cluster size > 10 voxels and images are masked to show only hippocampal activation

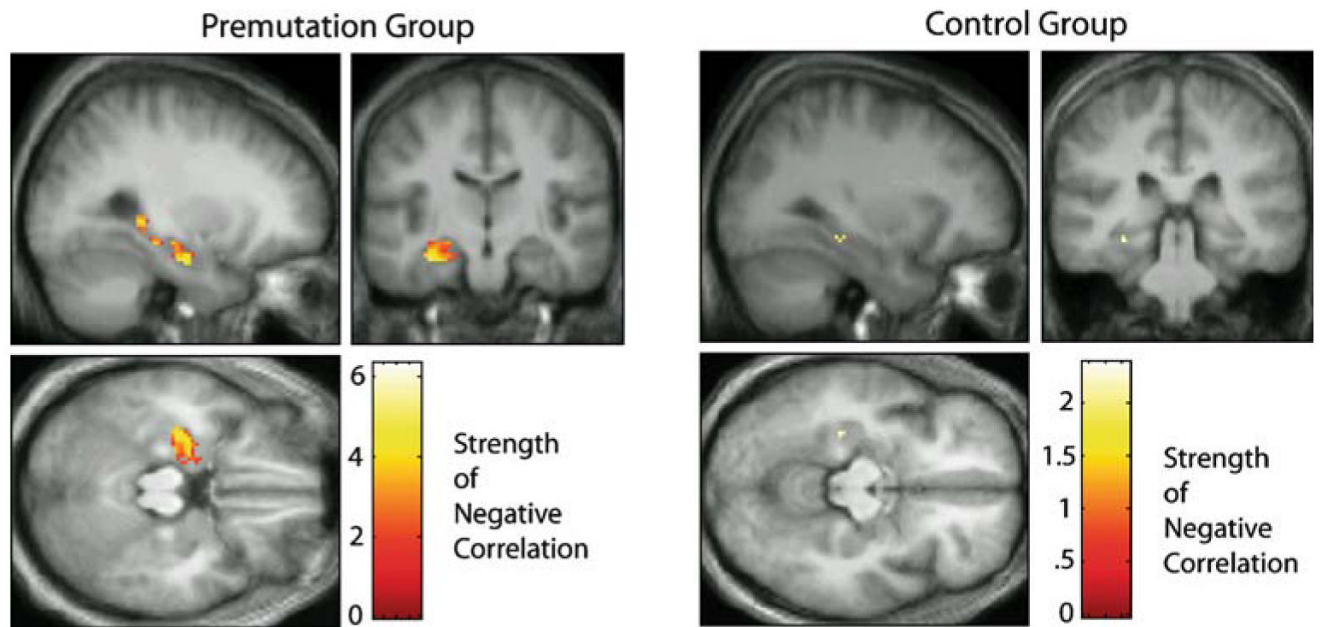


Fig. 5. Bright clusters represent voxels in the left hippocampus in which activation is *negatively* correlated with psychiatric symptoms as measured by the SCL-90-Revised. Images threshold is set at $p < 0.05$ and a cluster size of > 10 voxels and are masked to show this relationship only in the hippocampus

Table 1

Participant descriptive statistics and *FMRI* measures

Brain area	Control (<i>n</i> =11)			Premutation (<i>n</i> =11)			<i>t</i>	<i>p</i> -value
	Mean	SD	Range	Mean	SD	Range		
Age	40.1	7.7	26-55	42	7.4	28-56	-0.59	0.56
WAIS-III full scale IQ	113.4	18.5	84-148	117.9	19.7	83-152	-0.54	0.60
Education level (years)	15.0	2.5	12-18	15.5	3.4	10-20	-0.43	0.67
SCL-90-R GSI	59.8	12.0	41-81	54.5	10.9	42-81	1.10	0.29
<i>FMRI</i> CGG repeat size	27.4	5.5	17-32	94.3	31.18	59-150	-7.01	<0.0001
<i>FMRI</i> mRNA level	1.2	0.2	0.90-1.5	3.2	1.0	1.2-5.1	-6.19	<0.0001

Table 2
Total brain and hippocampal volumes for men with the *FMR1* premutation and matched controls

Brain area	Control (n=11)		Premutation (n=11)		p-value
	Mean	SD	Mean	SD	
Total brain (cc ³)	1,503.8	110.4	1,480.2	110.0	0.60
Hippocampus (cc ³)					
Left	1.95	0.24	1.89	0.23	0.39
Right	2.02	0.22	2.01	0.20	0.96
Total	3.99	0.43	3.90	0.42	0.64

Table 3
Stereotaxic locations and z-scores of activation peaks in the within-group maps

Memory-control contrast						
Group	Area	Number of voxels in cluster	Z max	Peak coordinates		
Control	R superior prefrontal gyrus, cingulate sulcus	584	4.96	2	16	54
	R lateral fissure	667	4.94	34	28	8
	L fusiform gyrus	1,981	4.88	-46	-56	-20
	L superior, middle and inferior frontal gyrus	3,026	4.88	-50	12	38
	L intraparietal sulcus, angular gyrus	964	4.76	-34	-62	38
	R inferior occipital gyrus	305	4.67	30	-94	-12
	R caudate	735	4.31	14	0	10
	R cerebellum (V)	1,724	4.27	32	-36	-28
	L hippocampus	183	4.22	-28	30	0
	L cingulated gyrus	148	3.86	10	20	28
	R cingulated gyrus	128	3.78	50	30	38
	R middle occipital gyrus	2,190	4.93	36	-88	-14
	L inferior frontal gyrus/sulcus, middle frontal gyrus	1,932	4.79	-48	30	30
	L superior frontal gyrus, cingulate sulcus	719	4.7	-2	26	50
	R inferior occipital gyrus	375	4.58	-32	-96	-12
L fusiform gyrus	1,163	4.5	-34	-36	-24	
Premutation	L angular gyrus, intraparietal sulcus	642	4.32	-32	-80	38
	R lateral fissure, posterior orbital gyrus	436	4.27	34	28	-8
	R caudate nucleus	227	4.26	14	10	10
	L caudate nucleus	441	4.04	-18	-2	24
	R angular gyrus, intraparietal sulcus	238	3.81	38	-68	40
	R middle frontal gyrus, inferior frontal sulcus	349	3.77	42	32	26

All clusters significant at $p < 0.01$, corrected for multiple comparisons.

Table 4
Stereotaxic locations and z-scores of activation peaks in the between-group maps

Memory-control contrast						
Comparison	Area	Number of voxels in cluster	Z Max	Peak coordinates		
Control > premutation	R cuneus, lingual gyrus, caudate	1,042	3.21	20	-82	10
	L parahippocampal gyrus, hippocampus, lateral fissure	593	3.06	-10	-52	8
Premutation > Control	R angular gyrus, intraparietal sulcus, superior parietal gyrus, supramarginal gyrus	848	4.38	38	-68	40
	L caudate nucleus	713	3.19	-8	10	12

All clusters significant at $p < 0.05$ corrected for multiple comparisons.