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Brain Research 877 (2000) 367–370

BRAIN RESEARCH

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Short communication

Functional brain activation during cognition is related to FMR1 gene expression

Vinod Menon[a,*], Hower Kwon[a], Stephan Eliez[a], Annette K. Taylor[b], Allan L. Reiss[a]

[a]*Departments of Psychiatry and Behavioral Sciences, 401 Quarry Road, Stanford University School of Medicine, Stanford, CA 94305-5719, USA*
[b]*Kimball Genetics, Inc., Denver, CO 80206, USA*

Accepted 13 June 2000

Abstract

Fragile X syndrome, the most common known cause of inherited mental retardation, is caused by alterations of the FMR1 gene encoding the FMRP protein. We investigated the relation between FMRP protein levels and functional brain activation during a working memory task. Our study provides the first evidence for a relation between FMR1 gene expression and neural activity during higher-order cognition. More broadly, our findings provide the first demonstration of how gene-brain-behavior investigations can help to bridge the gap between molecular and systems neuroscience. © 2000 Elsevier Science B.V. All rights reserved.

Theme: Disorders of the nervous system

Topic: Genetic models

Keywords: Fragile X syndrome; Working memory; Functional magnetic resonance imaging; Molecular correlation; FMRP.

The analysis of genetic mutations in mice has been enormously successful in enhancing our understanding of the molecular basis of memory and learning; however, the use of molecular genetic approaches to understanding human cognition has been limited to date [6]. Fragile X syndrome (fraX), one of the most common causes of inherited mental retardation [3], results from the silencing of the FMR1 gene. The cognitive phenotype of fraX includes deficits in executive function, visuo-spatial memory and attention [7], and decreased IQ [15]. FraX, therefore, provides a useful model to investigate specific genetic influences on human cognitive functioning. In the present report, we describe the results of the first functional magnetic resonance imaging (fMRI) study to investigate the relationship between FMR1 gene expression and brain activation during cognition.

Cognitive functioning was investigated using a working memory (WM) task known to involve critical components of higher-order cognition, including encoding, rehearsal, storage and executive functions [1]. Disruption in FMR1 gene expression results in decreased FMR1 protein (FMRP) levels in the brain and is associated with abnormal morphology of dendritic spines in the cortex [11,16]. Specifically, we examined the relation between FMRP (measured as the percent of lymphocytes positive for FMRP) [20] and brain activation in the dorsolateral prefrontal cortex and parietal cortex, regions known to be involved in WM. Ten female subjects who were heterozygous for the fraX full mutation as shown by standard DNA analysis [12] (ages 10–22 years; mean 17.2 years) were imaged while they performed a visuo-spatial WM task.

Ten female subjects with a diagnosis of fragile X syndrome (range 10–22 years; mean 17.2 years) were recruited from throughout the US. 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 [17]. The CGG repeat number was calculated from the Southern blot autoradiogram images. The data were then also used to calculate the activation ratio. FMRP levels were ascertained by calculating the percentage of peripheral lymphocytes containing FMRP using immunostaining techniques [20].

*Corresponding author. Tel.: +1-650-498-6737; fax: +1-520-244-6474.

E-mail address: menon@stanford.edu (V. Menon).

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PII: S0006-8993(00)02617-2

In alternating 30-s epochs subjects performed either a working memory (WM) or control task. In the WM task, subjects viewed a symbol ('O') presented once every 2 s at one of nine distinct spatial locations on a screen. They responded with a key press if the current location of the symbol was identical to its location two steps back. In the control task, subjects responded if the symbol appeared in the center of the screen. There were six epochs each of the WM and control tasks. Each control and experimental epoch consisted of 16 stimuli presented for 500 ms each, with a 1500 ms inter-stimulus interval.

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 [10]. 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 commissures covering the whole brain were imaged with a temporal resolution of 2 s using a T2* weighted gradient echo spiral pulse sequence (TR=2000 ms, TE=40 ms, flip angle=89° and 1 interleave) [9]. The field of view was 240 mm and the effective inplane spatial resolution was 4.35 mm. To aid in localization of functional data, high resolution T1 weighted spoiled grass gradient recalled (SPGR) 3D MRI sequence with the following parameters was used: TR=35 ms; TE=6 ms; 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.

Images were first corrected for movement using least square minimization without higher-order corrections for spin history, and normalized to stereotaxic Talairach coordinates [18]. Images were then resampled every 2 mm using sinc interpolation and smoothed with a 4 mm Gaussian kernel to decrease spatial noise. 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. This method takes advantage of multivariate regression analysis and corrects for temporal and spatial autocorrelations in the fMRI data [8]. A within-subject procedure was used to model all the effects of interest, covariates and nuisance variables for each subject. 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. For each subject, a general linear model was used to contrast brain activation during the WM and control tasks. Voxel-wise *t*-statistics were normalized to *Z* scores to provide a statistical measure of activation that is independent of sample size.

We demarcated two regions of interest (ROIs) in the prefrontal cortex — inferior and middle frontal gyrus (IFG, MFG), and two ROIs in the parietal cortex — superior parietal lobe (SPL) and supramarginal gyrus (SMG), based on known neuroanatomical surface and cross-sectional landmarks Duvernoy [4] and Ono et al. [13]. The fraction of voxels activated ($Z>2.33$; $P<0.01$) in each ROI was used as the measure of brain activation. The relation between fraction of voxels activated in each ROI and FMRP and Activation Ratio was investigated using Pearson correlations.

Consistent with previous imaging studies [2], the analysis revealed significant activation ($P<0.01$; corrected for multiple spatial comparisons) in a distributed network consisting of the left and right MFG, right IFG, right SMG, and right SPL, as well as the left and right superior frontal gyrus and pre-supplementary motor area (Fig. 1a). Significant correlations between FMRP and brain activation in the right IFG ($r=0.69$; $P=0.027$), left MFG ($r=0.81$; $P=0.004$), right MFG ($r=0.71$; $P=0.022$), left SMG ($r=0.70$; $P=0.024$), and right SMG ($r=0.70$; $P=0.024$) (Fig. 1b), but not in the left IFG ($r=0.62$; $P=0.055$), left SPL ($r=0.39$; $P=0.263$) or right SPL ($r=0.43$; $P=0.221$) were found. A virtually identical profile of correlations was observed between FMR1 activation ratio (fraction of cells with the FMR1 gene active) [15] and brain activation.

Our study demonstrates for the first time a relation between brain function during higher-order cognition and expression of a protein that affects brain function. These data provide essential information that can now be used to elucidate pathways underlying genetic and molecular mechanisms involved in the disruption of brain activation in fraX syndrome. FMRP has been hypothesized to regulate synaptic activity and plasticity by its role in the transport of mRNA to dendrites in response to neural stimulation [5,19]. Abnormally low levels of FMRP in fraX are associated with unusually long, thin dendritic spines in the neocortex, theoretically further contributing to diminished synaptic transmission [14]. Thus, the effects of low FMRP — both the long-term dysmorphology of dendrites as well as the disruption of dynamic synaptic responses — likely result in reduced transmission of neuronal signals, thereby restricting the neuronal network that can be recruited in response to cognitive task demands.

Further gene-brain-behavior investigations of FMRP will contribute significantly to our understanding of the neurobiological mechanisms of normal cognitive development, as well as how a single gene defect can so profoundly disrupt cognitive function. Our results suggest that fMRI may provide a sensitive measure to examine the role of FMR1 and other genes in cognition, emotion, and social interaction, thereby bridging the gap between systems and molecular neuroscience [6].

Figure 1a.

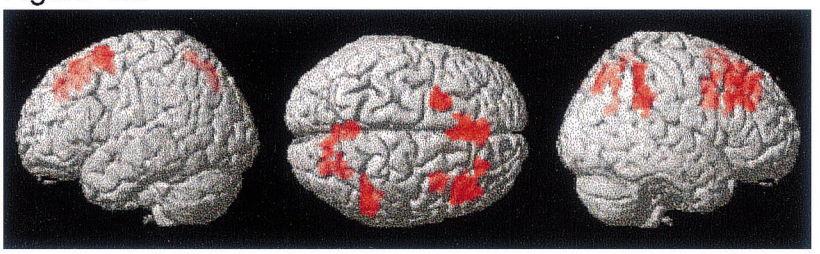

Figure 1b.

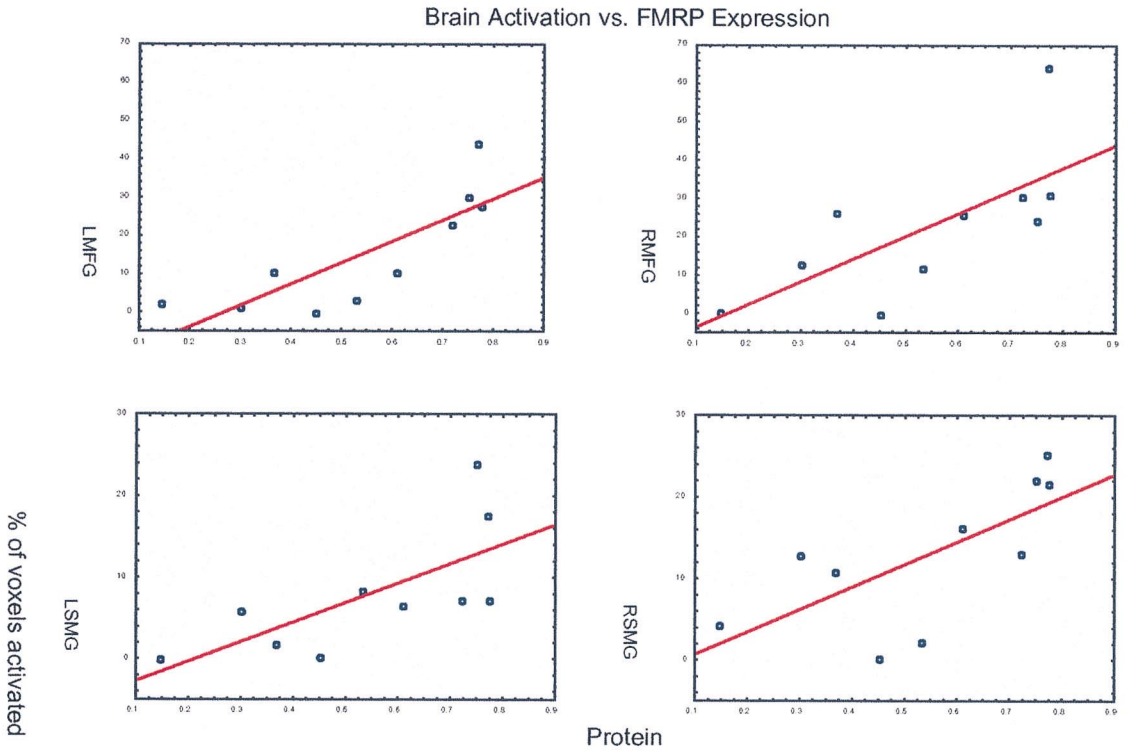

Fig. 1. (a) Brain areas that showed significantly greater activation during working memory, contrasted to the control condition, included the bilateral middle frontal gyri (LMFG and RMFG) and bilateral supramarginal gyri (LSMG and RSMG). (b) Brain activation in the MFG and SMG were significantly correlated with the Fragile-X mental retardation protein (FMRP) expression. FMRP may regulate the neuronal network that can be recruited in response to cognitive task demands.

Acknowledgements

Supported by NIH grants MH50047, MH01142 and HD31715, a grant from the M.I.N.D. Institute and the 'Lynda and Scott Canel Fund for Fragile X Research'.

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