fMRI Study of Cognitive Interference Processing in Females with Fragile X Syndrome

**Leanne Tamm, Vinod Menon, Cindy K. Johnston,
David R. Hessl, and Allan L. Reiss**

Abstract

■ Females with fragile X syndrome, the most common form of inherited developmental and learning problems, are known to be impaired in executive function. The current study is the first to investigate the performance of females with fragile X on a cognitive interference task utilizing functional magnetic resonance imaging (fMRI). Fourteen females with fragile X and 14 age-matched healthy controls were imaged while they performed a counting Stroop interference task. Compared to controls, females with fragile X appeared to have longer reaction times during the interference condition of the task, and adopted a strategy trading speed for accuracy. Females with fragile X also had a significantly different pattern of activation than controls. Whereas controls showed significant activation in the inferior/ middle frontal gyrus and inferior/superior parietal lobe, females with fragile X showed more extensive activation in the anterior region of the prefrontal cortex, and failed to show expected activation in the inferior/superior parietal lobe. Further, between-group analyses revealed that females with fragile X had reduced activation in the left orbitofrontal gyrus, thought to be involved in modulating goal-directed behavior. Females with fragile X also demonstrated a markedly different pattern of deactivation from controls. These findings suggest that deficits in cognitive interference processing during the counting Stroop task observed in females with fragile X may arise from inability to appropriately recruit and modulate lateral prefrontal and parietal resources. ■

INTRODUCTION

Fragile X syndrome is the most common heritable cause of developmental disability in males and females (Donnenfield, 1998; Freund, Reiss, & Abrams, 1993). Prevalence estimates for fragile X are approximately 1/1000 for males and 1/2000 for females (Donnenfield, 1998; Morton et al., 1997). The syndrome arises from disruption in expression of the FMR1 gene. Females are heterozygous for the disorder and typically display less severe pathology than males with fragile X (Welch & Williams, 1999).

Deficits in cognitive ability and behavioral performance are frequently observed in fragile X syndrome. Cognitively, females with the disorder typically score in the mildly mentally retarded range or normal cognitive range with learning disabilities, most often in math (Riddle et al., 1998). Neuropsychological testing reveals that females with this condition have short-term memory deficits, higher verbal than performance IQs, a characteristic Wechsler IQ test profile (i.e., poor performance on arithmetic, digit span, and block design subtests), and frontal lobe-related deficits (Jakala et al., 1997; Borhgraef, Umans, Steyaert, Legius, & Fryns, 1996; Mazzocco, Hagerman, Cronister-Silverman, & Penning-

ton, 1992; Hagerman & Sobesky, 1989). Behaviorally, females with fragile X often demonstrate difficulties with attention, anxiety, and socialization (Freund et al., 1993). Several studies have reported a moderate to high frequency of difficulties with attention and comorbidity with attention deficit hyperactivity disorder in females with fragile X (Freund et al., 1993; Hagerman et al., 1992; Borhgraef, Fryns, & Van den Berghe, 1990; Hagerman & Sobesky, 1989). Specifically, females with fragile X are known to have difficulties focusing and organizing tasks, and demonstrate impulsivity or problems with inhibition of behavior.

Because of the importance of cognitive and behavioral deficits in the everyday functioning of persons with fragile X and the lack of information on their neural basis, the present study was designed to further investigate deficits in executive functioning and attentional processing in females with fragile X. The experimental paradigm used is a variant of the Stroop task (Stroop, 1935). The Stroop task is a simple, yet remarkably reliable, measure of cognitive interference where the processing of one stimulus interferes with the simultaneous processing of another (Smith & Nyman, 1974; Jensen, 1965; Santos & Montgomery, 1962; Stroop, 1935). Specifically, the interference component of the task involves a subject naming the color of the ink of an incongruent word- color

stimulus (e.g., BLUE printed in red ink). Since verbalizing responses can result in excessive head movements, the original Stroop task is not an ideal task for use with functional magnetic resonance imaging (fMRI). The counting Stroop, a Stroop-variant that does not require an overt verbal response, was recently developed for use in fMRI research (Bush et al., 1998).

Preliminary studies conducted by Bush et al. (1998, 1999) indicated activation of the anterior cingulate, middle frontal gyrus, inferior temporal gyrus, precentral gyrus, premotor cortex, and superior parietal lobe occurs during the interference condition of the counting Stroop task. Similarly, another recent report (unpublished) of the counting Stroop demonstrated activation in the frontopolar cortex, ventrolateral prefrontal cortex, intraparietal sulcus, and calcarine sulcus (Zysset, Muller, Lohmann, & von Cramon, 2000). These findings are generally consistent with the majority of studies reporting on Stroop-like tasks that have variously reported activation in the anterior cingulate cognitive division, lateral prefrontal cortex, inferior frontal gyrus, right orbitofrontal area, inferior parietal lobule, left premotor cortex, supplementary motor area, and left putamen (Bush et al., 1999; Taylor, Kornblum, Lauber, Minoshima, & Koeppe, 1997; Carter, Mintun, & Cohen, 1995; George et al., 1994; Bench et al., 1993; Corbetta, Miezen, Dobmeyer, Shulman, & Petersen, 1991; Pardo, Pardo, Janer, & Raichle, 1990).

The primary objective of the current study was to examine more closely the cognitive, attentional, and inhibition abilities and deficits of females with fragile X utilizing the counting Stroop interference task. A second objective was to assess whether females with fragile X and unaffected females (controls) differed in terms of brain activation during task performance as assessed with fMRI, and to further elucidate the brain areas involved in resolving interference effects. In this study it was hypothesized that females with fragile X would perform less well than controls in terms of both speed and accuracy on the counting Stroop task. Activation in the prefrontal cortex, parietal lobes, and anterior cingulate was predicted for controls, and reduced activation in these same areas was expected for females with fragile X.

RESULTS

Wechsler IQ Scales/Child Behavior Checklist (CBCL)

The Full Scale IQ scores of the females with fragile X ($M = 84.43$, $SD = 15.79$) were significantly lower than those of the controls ($M = 117.93$, $SD = 13.21$; $t(26) = 6.09$, $p < .001$). It was of particular interest to address the extent to which the groups differed once the influence of IQ was controlled; thus, analyses of the behavioral and brain activation data were conducted with IQ as a covariate.

Information was obtained from the CBCL (Achenbach, 1991) for 9 subjects in the control group and 11 subjects in the fragile X group. An independent samples t test was conducted to compare the two groups on the Attention Problems subscale and the Thought Problems subscale. The results (assuming unequal variances) revealed a significant difference between groups [$t(10.42) = 2.33$, $p < .05$] with controls having a lower CBCL Attention subscale score ($M = 50.56$, $SD = 1.3$) than the females with fragile X ($M = 57.8$, $SD = 10.22$). Similarly, the results (assuming unequal variances) for the Thought Problems subscale [$t(10.00) = 2.66$, $p < .05$] revealed that controls ($M = 50.0$, $SD = 0$) had significantly lower scores than females with fragile X ($M = 56.00$, $SD = 7.5$). However, it should be noted that neither group mean was rated in the clinically significant range (i.e., $T > 70$) for either attention problems or thought problems.

Behavioral Analyses

Dependent variables included reaction time for correct trials for the interference and neutral conditions as well as number correct. Performance variables were not analyzed for 3 of the 14 females with fragile X and 2 of the 14 controls due to response box malfunction. The means and standard deviations for each dependent behavioral variable are listed in Table 1.

Interference Effect?

To address whether the counting Stroop produced a significant interference effect in healthy controls, a paired samples t test on reaction time to correct trials for the neutral and interference conditions was conducted for this group only. The results indicated that there was a significant interference effect with longer reaction times in the interference than the neutral condition [$t(11) = 8.52$, $p < .001$].

Reaction Time for Correct Trials

The results of the 2 (group: controls, females with fragile X) × 2 (experimental condition: neutral, interference) repeated measures ANCOVA, covarying IQ, revealed a significant diagnosis by condition interaction [$F(1,20) = 8.73$, $p < .001$]. Follow-up univariate ANCOVAs, covarying IQ, to examine the diagnosis by condition interaction, however, were not significant. Examination of the data in graphical form suggested that this interaction arose from the fact that controls had shorter reaction times than females with fragile X in the interference condition, but the two groups did not differ from one another in the neutral condition.

Number Correct

The results of a 2 (group: controls, females with fragile X) × 2 (experimental condition: neutral, interference)

Table 1. Dependent Variables for Females with Fragile X and Healthy Control Females

Dependent Variable	Fragile X (Mean, SD)	Control (Mean, SD)
Reaction Time (msec) for Correct Trials—Interference	1067.27 (169.31)	887.05 (90.73)
Reaction Time (msec) for Correct Trials—Neutral	938.95 (184.60)	810.89 (80.73)
Number Correct—Interference	74.73 (14.96)	84.33 (8.03)
Number Correct—Neutral	70.09 (17.78)	82.25 (10.02)

repeated measures ANCOVA, covarying IQ, on the number correct dependent variable did not reveal any significant main effects or interactions.

Speed/Accuracy Tradeoff

A partial correlation, controlling for IQ, was conducted for each group between the speed and accuracy variables. There was a significant negative correlation between reaction times for correct trials and number correct for both the interference and neutral conditions for the females with fragile X ($r = -.67$, $p < .05$ and $r = -.68$, $p < .05$, respectively).

No significant correlations emerged for the control group, however.

Brain Activation

An initial examination of within-group activation was made for each group on the two comparisons of interest, interference minus neutral (activation) and neutral minus interference (deactivation). A between-group analysis was then conducted to examine differences in activation. Because of significant differences in IQ, the between-group brain activation data were analyzed utilizing IQ as a covariate of no interest. This approach allowed for an

Table 2. Brain Areas Showing Significant Activation and Deactivation

Activated Regions	Voxels	Z Max	Peak Location
Interference minus neutral			
Controls			
Right inferior/superior parietal lobe (BA 7); superior/middle occipital gyrus (BA 18/19)	1271	4.27	34, −66, 50
Left superior parietal lobe (BA 7)	919	4.07	−22, −74, 50
Left inferior and middle frontal gyri (BA 9, 46, 47); anterior insula; orbitofrontal gyrus	1615	3.81	−52, 10, −2
Females with fragile X			
Left inferior/middle frontal gyri (BA 45, 46)	1846	3.87	−54, 30, 18
Left supplementary motor area/superior frontal gyrus (BA 6)	1416	3.81	−4, −8, 68
Right middle/inferior frontal gyrus (BA 9/47)	1725	3.57	38, 26, 24
Neutral minus interference (deactivation)			
Controls			
Left middle/posterior cingulate gyrus (BA 24); cerebellum; lingual gyrus; precuneus/cuneus	6402	5.19	−4, −12, 40
Left ventromedial prefrontal cortex (BA 11)	1058	3.42	−10, 38, −18
Females with fragile X			
Left globus pallidus/putamen/insular cortex extending to hippocampus, parahippocampal gyrus	4266	4.00	−28, −22, 0
Right superior temporal gyrus (BA 22); posterior insula; putamen	1305	3.78	32, 8, −24

BA = Brodmann's area; for each significant cluster ($p < .05$), region of activation, number of voxels activated, maximum Z score, and location of peak (Talairach coordinates) are shown.
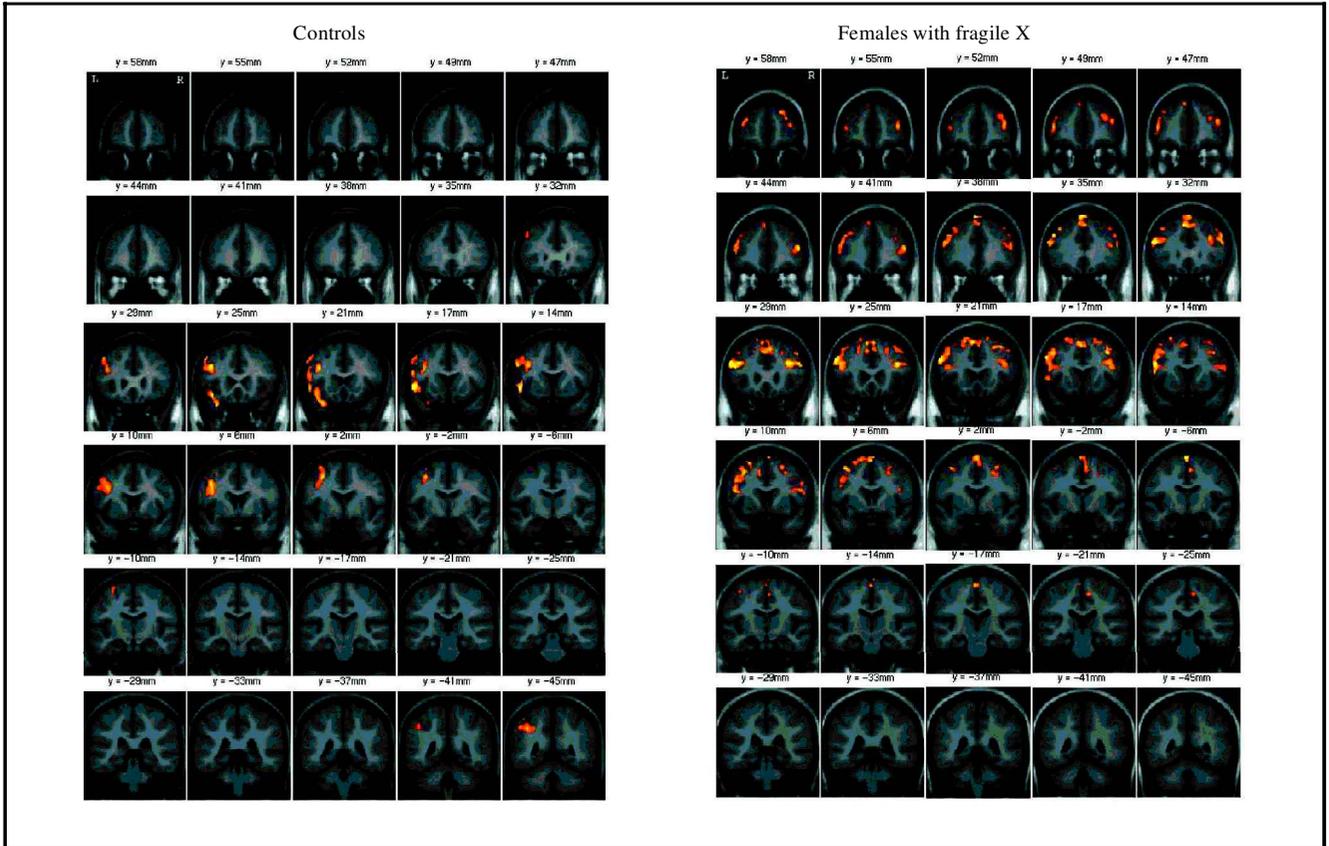
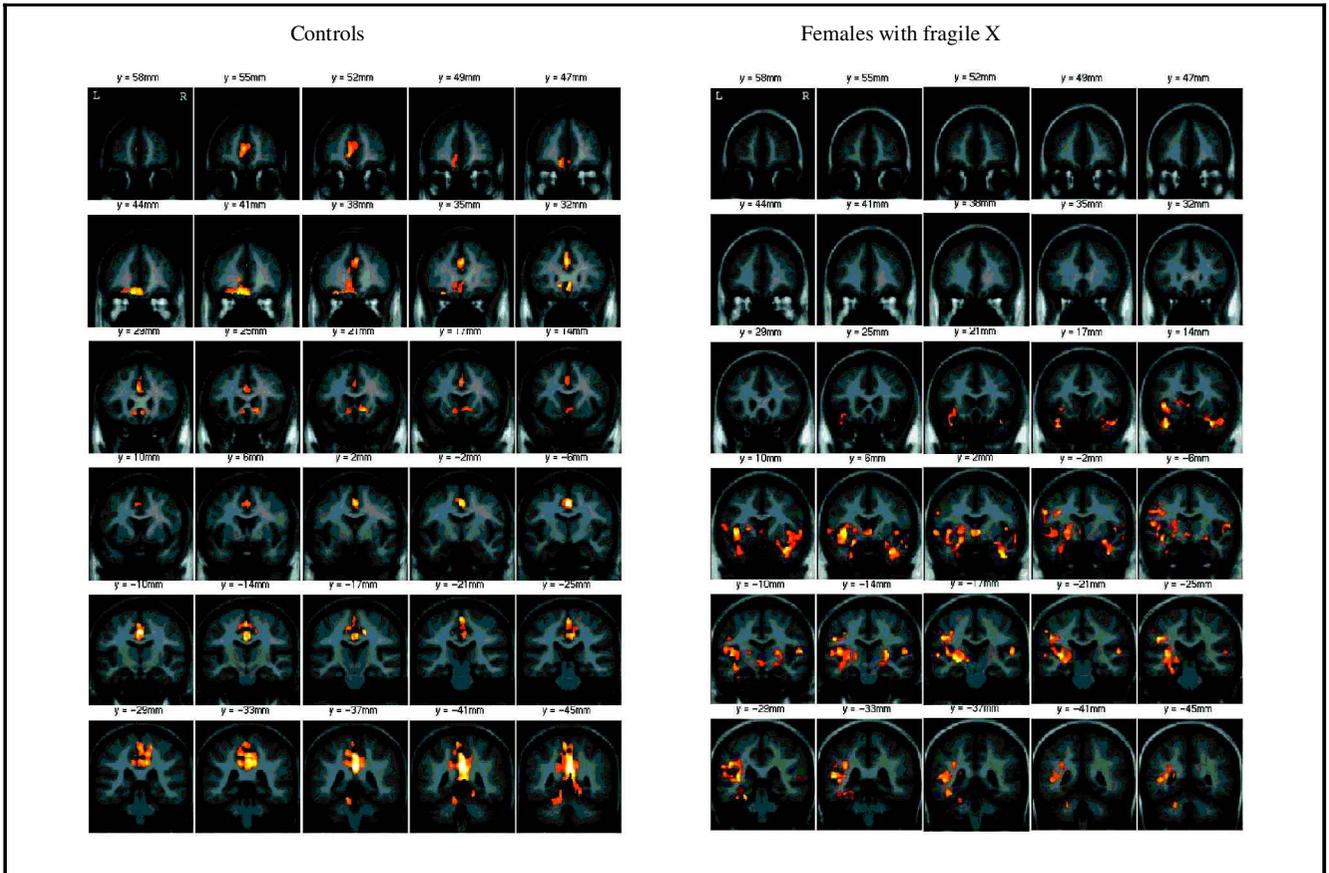**Figure 1.** Brain areas showing significant activation for the interference minus neutral condition.

Figure 2. Brain areas showing significant deactivation (neutral minus interference condition).

evaluation of the deficits specific to females with fragile X beyond those arising due to group differences in IQ.

Activation

Controls showed significant activation in the left inferior/middle frontal gyrus, right inferior parietal lobe, and left superior parietal lobe (Table 2, Figure 1). Females with fragile X showed significant activation bilaterally in the middle/inferior frontal gyrus and left supplementary motor area (Table 2, Figure 1).

Deactivation

Controls showed significant deactivation in the left middle/posterior cingulate gyrus and left ventromedial prefrontal cortex (Table 2, Figure 2). In contrast, females with fragile X showed significant deactivation in the left globus pallidus, left insular cortex, and right superior temporal gyrus (Table 2, Figure 2).

Group Differences

Controls showed significantly more activation than females with fragile X in the right orbitofrontal gyrus, left insular cortex, and orbitofrontal gyrus bordering on the frontal operculum, as well as in the left superior temporal gyrus (Table 3, Figure 3). In contrast, the females with fragile X did not demonstrate significantly greater activation than controls in any brain region.

Exploratory Analyses

In order to examine the relationship between brain activation in the orbitofrontal gyrus and behavioral performance on the counting Stroop task, partial correlations controlling for IQ were conducted for each group between the dependent variables for the interference condition and percent voxels activated in the left orbitofrontal gyrus (a region in which females with fragile X activated significantly less than controls). For the control group, these partial correlations were not statistically significant, and the correlations were relatively small in magnitude ($r < .3$) for both reaction time and number correct in the interference condition. In contrast, for the females with fragile X there was a significant negative correlation between percent voxels activated in the left orbitofrontal gyrus and reaction time in the interference condition ($r = -.84$, $p < .001$) and a nonsignificant, but moderate, correlation for the number correct variable ($r = .58$, $p < .15$).

In addition, partial correlations, controlling for IQ, were also conducted between percent voxels activated in the left orbitofrontal gyrus and behavioral measures collected outside the scanner hypothesized to be associated cognitive interference in females with fragile X. Specifically, the CBCL subscales Attention and Thought Problems were correlated with brain activation in the left orbitofrontal gyrus for the females with fragile X. The results of these analyses were not significant.

DISCUSSION

This study is the first to examine performance and brain activation in females with fragile X on the counting Stroop, a cognitive interference task designed to be compatible with fMRI. The behavioral results demonstrated the anticipated interference effect (i.e., longer reaction times in the interference condition) related to the two cognitive processes of counting and reading. Females with fragile X appeared to be more affected by the interference condition of the task (slower reaction times) than controls. Interestingly, there also was evidence to suggest that females with fragile X adopted a

Table 3. Brain Areas Where Controls Show Significantly Greater Activation Than Females with Fragile X After the Effects of IQ Were Covaried

Activated Regions	Number of Voxels	Z Max	Peak Location
IQ covaried			
Controls > females with fragile X			
Right orbitofrontal cortex; putamen; amygdala/hippocampus; anterior superior temporal gyrus	969	3.89	24, 12, −18
Left insular cortex/orbitofrontal cortex/frontal operculum; globus pallidus/putamen; lateral amygdala; superior/inferior temporal gyrus	2065	4.08	−28, −18, −2
Left superior temporal sulcus; superior temporal gyrus, parahippocampal gyrus/hippocampus, extending into cerebellum, and fusiform gyrus	996	4.17	−46, −40, 8
Females with fragile X > controls			
No significant differences			

For each significant cluster ($p < .05$), region of activation, number of voxels activated, maximum Z score, and location of peak (Talairach coordinates) are shown.

Figure 3. Brain areas in which controls show significantly more activation than females with fragile X for the interference minus neutral condition with IQ covaried. Specific deficit area in females with fragile X (orbitofrontal gyrus) circled in green.
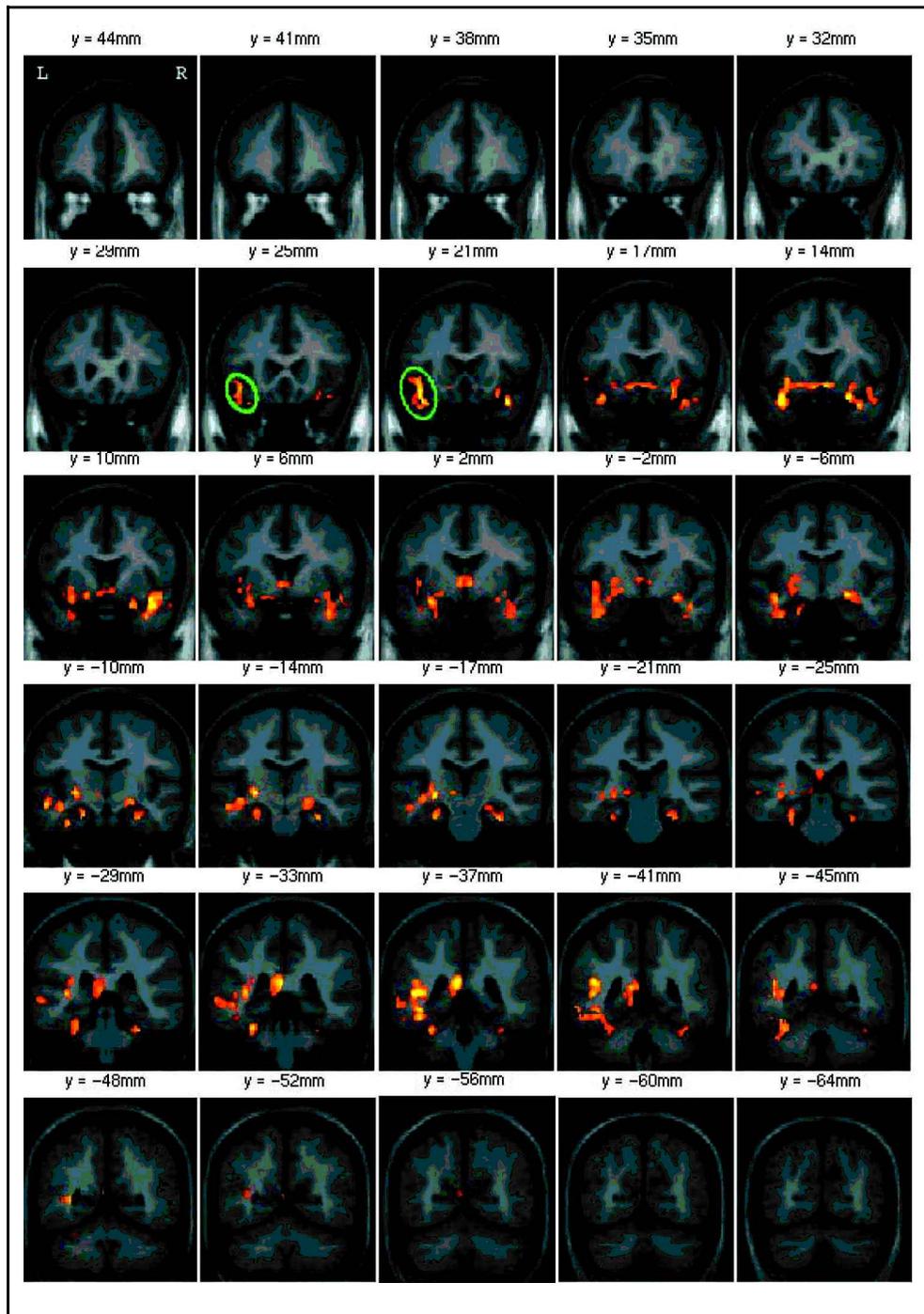
different strategy than controls to perform the task. Specifically, it appears that the females with fragile X adopted a strategy sacrificing speed for accuracy (i.e., a speed/accuracy tradeoff), while the controls did not.

The results of the neuroimaging study revealed that females with fragile X demonstrated a markedly different pattern of activation compared to controls. Although both groups recruited the prefrontal cortex (middle and inferior gyri) during the interference condition, for the females with fragile X this activation was bilateral, while for the controls this activation was primarily in the left hemisphere. Further, unlike the fragile X group, controls showed significant activation in the left superior parietal lobe and in the right inferior parietal lobe (angular gyrus). Between-group comparisons indicated that the females with fragile X did not show greater activation than controls in any brain area. In contrast, controls showed significantly greater task-related activation than females with fragile X in the left orbitofrontal gyrus, suggesting a specific deficit for females with fragile X in this brain region. The current findings and their implications for brain function in fragile X and

other neurodevelopmental disorders are discussed in detail below.

The present results are in agreement with a number of different studies that have suggested a role for specific regions of the lateral prefrontal cortex in resolving interference effects. Although the prefrontal cortex has been implicated in a wide range of cognitive functions (Duncan & Owen, 2000), several previous research studies have demonstrated that the dorsolateral prefrontal cortex, and specifically Brodmann's area (BA) 9/46, is involved in processing Stroop-related conflict and resolving interference effects (Menon, MacKenzie, Rivera, & Reiss, in preparation; Adleman, Menon, Blasey, White, & Reiss, under submission; Leung, Skudlarski, Gatenby, Peterson, & Gore, 2000, Macleod & MacDonald, 2000, Zysset et al., 2000; D'Esposito, Postle, Jonides, & Smith, 1999; D'Esposito, Postle, & Rypma, 2000; Jonides, Smith, Marshuetz, Koeppe, & Reuter-Lorenz, 1998; Taylor et al., 1997). Further, this region (BA 9/46) was activated in the interference condition of all three previous neuroimaging studies on the counting Stroop task (Zysset et al., 2000; Bush et al., 1998, 1999). Although both controls and females with fragile X activated the inferior and middle frontal gyri during the interference condition of the counting Stroop, females with fragile X showed different patterns of activation than controls. Specifically, prefrontal regions activated by females with fragile X were more anterior than those activated by controls (i.e., Talairach coordinates for females with fragile X = −54, 30, 18 and controls = −52, 10, −2). Further, females with fragile X demonstrated bilateral activation in the prefrontal cortex, while controls showed predominantly left hemisphere activation. Thus, compared to controls, females with fragile X recruited more prefrontal regions to perform the task, although behavioral data suggest that despite enhanced prefrontal activation, their performance was still poorer than that of controls.

A direct comparison between groups indicated that controls showed greater activation in the left insular cortex, left superior temporal gyrus, and bilaterally in the orbitofrontal gyrus, suggesting relative deficits in these areas in association with this genetic condition. However, some of these group differences appear to be arising both from increased activation in the control group and from deactivation in females with fragile X, while others appear to be arising solely from deactivation in females with fragile X. Deactivation in this study is operationalized as greater activation observed in response to the neutral condition than the interference condition. Specifically, between-group activation differences in the left orbitofrontal gyrus and insular regions appeared to result from both activation in controls (see Figure 1, interference minus neutral, for clarification) and deactivation in females with fragile X (see Figure 2, neutral minus interference, for clarification). In contrast,

controls show no activation in the right orbitofrontal gyrus and left superior temporal gyrus; these group differences appear to be due to deactivation in females with fragile X. Thus, the primary region in which females with fragile X activated less than controls (which does not appear to result from deactivation) was in the left orbitofrontal gyrus, suggesting a specific variation or deficit in females with fragile X in this region.

Studies suggest that the orbitofrontal cortex plays a specific role in controlling voluntary goal-directed behavior (Tremblay & Schultz, 2000; Schoenbaum, Chiba, & Gallagher, 1998). Further, the orbitofrontal gyrus is known to play a role in executive functions such as set shifting, decision making, working memory, and attentional control (Bechara, Damasio, & Damasio, 2000; Rolls, 1994). Given that females with fragile X typically demonstrate executive functioning deficits (Mazzocco, Pennington, & Hagerman, 1993), aberrant activation of the orbitofrontal gyrus may play a role in the behavioral symptomatology of individuals with this condition. This hypothesis was explored using correlational analyses between percent voxels activated in the orbitofrontal region and relevant behavioral measures collected both inside and outside the scanner. Findings from these analyses indicated that activation in the left orbitofrontal gyrus correlated with task performance for the females with fragile X and not the controls. Specifically, a significant negative correlation between activation and reaction time and a nonsignificant, moderate positive correlation between activation and number correct was observed for the females with fragile X (with the effects of IQ statistically controlled). These results suggest that the failure of the females with fragile X to activate this region may have contributed to their poorer performance of the task. There were no significant correlations between activation in this region and behavioral measures selected in an attempt to capture the functioning of females with fragile X. However, these behavioral measures are not specifically designed to assess the behavioral phenotype of females with fragile X. It is probable that dysregulation of the orbitofrontal cortex may in part underlie some of the behavioral symptoms commonly observed in fragile X syndrome, since attentional problems, mood lability, difficulty with socialization, and cognitive inflexibility or difficulty with set-shifting, are observed both in females with fragile X (Sobesky, Hull, & Hagerman, 1994; Freund et al., 1993; Hagerman et al., 1992; Edwards, Keppen, Ranells, & Gollin, 1988) and in persons with orbitofrontal lesions (Bechara et al., 2000; Rolls, 1994). Further investigation of links between orbitofrontal cortex activation and more precise measures of these symptoms in individuals with fragile X is warranted.

The present findings underscore the importance of examining both activation and deactivation patterns when utilizing the subtraction method with neuroimaging data. The importance and role of deactivation in

interpreting neuroimaging findings is an issue that has begun to be addressed in the literature only recently. Although the source of deactivation remains poorly understood, one possibility is that the advent of the experimental task interrupts and inhibits ongoing conscious processes that are active during rest (Binder et al., 1999). Alternatively, deactivation may arise from inhibitory processes specific to the experimental condition or increased activation related to internal brain mechanisms during the neutral condition (Binder et al., 1999; Hutchinson et al., 1999; Shulman, Corbetta, et al., 1997, Shulman, Fiez, et al., 1997). Consistent with the current findings, generic deactivation has been reported, among other areas, in the precuneus/posterior cingulate (BA 31/7) and the temporal lobe (BA 20) during a variety of experimental tasks (Shulman, Fiez et al., 1997). Further, deactivation in the posterior cingulate/precuneus appears to be specific to language-related tasks (Shulman, Fiez, et al., 1997), a category into which the counting Stroop falls. In this study, females with fragile X showed markedly different patterns of deactivation than controls (see Figure 2). Specifically, controls showed deactivation in the posterior cingulate and ventromedial prefrontal cortex, while females with fragile X showed deactivation in the globus pallidus, insular, and superior temporal gyrus. It should also be noted that while the females with fragile X showed atypical patterns of deactivation compared to controls, the origin of these differences is not clear.

Although activation in the parietal lobe did not emerge as a statistically significant difference in the between-group comparison, the controls showed more activation in the inferior and superior parietal lobe than females with fragile X. The inferior parietal lobe activation observed in this study likely resulted from the arithmetic computation (counting) and possibly language processing (reading) required by this task. Previous research has suggested that the angular gyrus is involved in arithmetic processing (Menon, Rivera, White, Glover, & Reiss, 2000), as well as language processing (Crozier et al., 1999, Jessen et al., 1999). Imaging studies have also suggested a role for the superior parietal lobe in sustaining attention during relatively complex cognitive tasks (Rosen et al., 1999; Le, Pardo, & Hu, 1998; Bench et al., 1993; Pardo, Fox, & Raichle, 1991), as well as in implementing attentional demands of processing incongruent stimuli (Carter et al., 1995). Interestingly, parietal lobe activation (BA 7) was observed in the three previous studies reporting on the counting Stroop task (Zysset et al., 2000; Bush et al., 1998, 1999).

Given that a number of studies of the Stroop effect have reported anterior cingulate activation during cognitive interference, a post hoc examination of the control group data with $p < .05$ significance levels and no extent threshold was conducted. This examination still did not reveal activation in the anterior cingulate cogni-

tive division. Although it is unclear why activation was not detected in the anterior cingulate during the interference condition, this finding could potentially be explained by methodological issues. For example, a specified region of interest for the anterior cingulate was not defined and examined in this study, or the demands of the subtractive method and lack of power may have resulted in the anterior cingulate not crossing the statistical threshold. It may be, however, that the anterior cingulate plays a less significant or specific role in cognitive interference resolution than has previously been thought. This hypothesis is consistent with the work of Zysset et al. (2000) and Taylor et al. (1997), who have also suggested a less critical role for the anterior cingulate in resolving cognitive interference, particularly when the number of choices from which selections need to be made is limited. It has also been speculated that the anterior cingulate plays a more critical role in response formation and monitoring (Liddle, Kiehl, & Smith, 2001) or in the anticipation of and preparation for attentional activity (Sturm et al., 1999), rather than resolving cognitive interference.

Additional investigation with a larger sample size, groups matched on IQ, and males with fragile X is warranted to further explicate the current findings. Supplementary investigation utilizing an event-related design may also elucidate group activation differences. The event-related design is less susceptible than block designs to habituation and changes in behavioral strategies within and between blocks (Bush et al., 1998), and has the capacity to probe the time course of the signal change corresponding to interference (Leung et al., 2000).

Overall, the current findings suggest that, compared to healthy controls, females with fragile X show different patterns of activation, particularly in the prefrontal cortex, and a specific deficit in the left orbitofrontal gyrus, as well as strikingly different patterns of deactivation. Although IQ and deactivation may have driven some of the activation differences, there is still strong evidence to suggest that females with fragile X have anomalous brain activation during cognitive interference processing tasks and may fail to appropriately recruit and modulate lateral prefrontal cortex and parietal resources.

METHODS

Participants

Participants included 14 females with fragile X and 14 age-matched healthy control females, without the fragile X mutation. Participants ranged in age from 10-22 (mean age 15.43, $SD = 3.79$), and both groups were predominantly of white, non-Hispanic origin (86%). IQ estimates based on either the Wechsler Intelligence Scale for Children—Third Edition (WISC-III) or the

Wechsler Adult Intelligence Scale—Third Edition (WAIS-III) were obtained for each participant. Information was also obtained from the CBCL (Achenbach, 1991) for 9 subjects in the control group and 11 subjects in the fragile X group.

Task

The counting Stroop task was programmed using Psyscope (http://poppy.psy.cmu.edu/psyscope) on a Macintosh (Sunnyvale, CA) powerbook computer. Onset of scanning and task were synchronized using a TTL pulse delivered to the scanner timing microprocessor board from a 'CMU Button Box' microprocessor connected to the Macintosh with a serial cable. Stimuli were presented visually at the center of a screen using a custom-built magnet compatible projection system (Resonance Technology, CA).

The task consisted of 12 alternating experimental (interference) and control (neutral) conditions with a rest period at the beginning and end of the task. For both conditions, subjects were instructed to press the button that corresponded to the number of words on the screen. During the neutral task, the word ''fish'' was presented 1, 2, 3, or 4 times on the screen (15 trials). During the interference condition, subjects were presented the words ''one'' ''two'' ''three,'' and ''four,'' presented 1, 2, 3, or 4 times on the screen (15 trials). Stimuli were presented for 1350 msec at the rate of every 2 sec for a total of 180 trials (90 experimental, 90 control).

Image Acquisition and Analysis

MR images were acquired on a GE-Signa 1.5 T scanner (GE Imaging Systems, Milwaukee, WI) 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 & 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 commissures covering the whole brain were imaged with a temporal resolution of 2 sec using a T2*-weighted gradient echo spiral pulse sequence (TR = 2000 msec, TE = 40 msec, flip angle = 89° and 1 interleave). Although the whole brain was imaged, the presence of susceptibility artifacts that affect MRI data may have precluded observation of activation in the orbitofrontal and anterior temporal cortices, which are particularly sensitive to susceptibility artifact (Ojemann et al., 1997). In an attempt to mitigate the effects of susceptibility artifact and optimize the ability to detect activation, a strict threshold for movement (less than 3 mm translation and rotation), a coronal acquisition, and a relatively lower field strength magnet (1.5 T) were used. Detailed analyses of susceptibility artifact in spiral acquisitions will be provided elsewhere (Grecius, Krasnow, Reiss, & Menon, in prep-

aration). To aid in the localization of functional data, high resolution T1-weighted spoiled grass gradient recalled (SPGR) 3-D 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 the coronal plane; 256 × 192 matrix; acquired resolution = 1.5 × 0.9 × 1.2 mm. On a few subjects, a faster protocol was utilized to decrease time of acquisition of the SPGR image, with TR = 11 msec; TE = 2 msec; and flip angle = 15°. The images were reconstructed as a 124 × 256 × 256 with a 1.5 × 0.9 × 0.9 mm spatial resolution.

Image Preprocessing

Images were reconstructed, by inverse Fourier transform, for each of the 225 time points into 64 × 64 × 18 image matrices (voxel size: 3.75 × 3.75 × 7 mm). fMRI data were preprocessed 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 & 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, Holmes, 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 superimposed on high-resolution T1-weighted images and their locations interpreted using known neuroanatomical landmarks (Duvernoy, Bourgouin, Cabanis, & Cattin, 1999; Mai, Assheuer, & Paxinos, 1997). MNI coordinates were transformed to Talairach coordinates using a nonlinear transformation (Brett, 2000).

A within-subjects procedure was first 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. Significant clusters of activation were determined using the joint expected probability distribution of height and extent of *Z* scores (Poline, Worsley, et al., 1997), with height ($Z > 2.33$; $p < .01$) and extent thresholds ($p < .05$).

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 & 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., interference condition minus the neutral condition). A two-sample *t* test was then used to determine group activation for each effect. The following contrast images were calculated for each subject: (i) activation: interference-neutral; (ii) deactivation: neutral−interference. For both within- and between-group comparisons, a cluster-wise (corrected) significance level of $p < .05$ was used.

Following these analyses, exploratory analyses were conducted to assess the relationship between brain activation and behavioral variables. Specifically, regions in which the females with fragile X activated significantly less than controls, namely, functional regions of interest (fROI), were identified. Partial correlations, controlling for IQ, between percentage of voxels activated (height threshold $Z > 2.33$) between the fROIs and relevant behavioral variables were then computed for each group.

REFERENCES

Achenbach, T. M. (1991). *Manual for the child behavior checklist/4-18 and 1991 profile.* Burlington: University of Vermont Department of Psychiatry Press.

Adleman, N. E., Menon, V., Blasey, C., White, C. D., & Reiss, A. L. (under submission). *A developmental fMRI study of the Stroop color word interference task.*

Bechara, A., Damasio, H., & Damasio, A. R. (2000). Emotion, decision making, and the orbitofrontal cortex. *Cerebral Cortex, 10,* 295- 307.

Bench, C. J., Frith, C. D., Grasby, P. M., Friston, K. J., Paulesu, E., Frackowiak, R. S. J., & Dolan, R. J. (1993). Investigations of the functional anatomy of attention using the Stroop test. *Neuropsychology, 31,* 907- 922.

Binder, J. R., Frost, J. A., Hammeke, T. A., Bellgowan, P. S. F., Rao, S. M., & Cox, R. W. (1999). Conceptual processing during the conscious resting state: A functional MRI study. *Journal of Cognitive Neuroscience, 11,* 80- 93.

Borhgraef, M., Fryns, J. P., & Van den Berghe, H. (1990). The female and the fra X syndrome: Data on the clinical and psychological findings in 7 fra X carriers. *Clinical Genetics, 37,* 341- 346.

Borhgraef, M., Umans, S., Steyaert, J., Legius, E., & Fryns, J. (1996). New findings in the behavioral profile of young fraX females. *American Journal of Medical Genetics, 64,* 346- 349.

Brett, M. (2000). http://www.mrc-cbu.cam.ac.uk/Imaging/mnis-pace.html.

Bush, G., Frazier, J. A., Rauch, S. L., Seidman, L. J., Whalen, P. J., Jenike, M. A., Rosen, B. R., Biederman, J. (1999). Anterior cingulate cortex dysfunction in attention deficit hyperactivity disorder revealed by fMRI and the counting stroop. *Biological Psychiatry, 45,* 1542- 1552.

Bush, G., Whalen, P. J., Rosen, B. R., Jenike, M. A., McInerney, S. C., & Rauch, S. L. (1998). The counting stroop: An interference task specialized for functional neuroimaging-Validation study with functional MRI. *Human Brain Mapping, 6,* 270- 282.

Carter, C. S., Mintun, M., & Cohen, J. D. (1995). Interference and facilitation effects during selective attention: An $H_2^{15}O$ PET study of Stroop task performance. *Neuroimage, 2,* 264- 272.

Corbetta, M., Miezen, F. M., Dobmeyer, S., Shulman, G. L., & Petersen, S. E. (1991). Selective and divided attention during visual discriminations of shape, color, and speed: Functional anatomy by positron emission topography. *Journal of Neuroscience, 11,* 2383- 2402.

Crozier, S., Sirigu, A., Lehericy, S., van de Moortele, P. F., Pillon, B., Grafman, J., Agid, Y., Dubois, B., & LeBihan, D. (1999). Distinct prefrontal activations in processing sequence at the sentence and script level: An fMRI study. *Neuropsychologia, 37,* 1469- 1476.

D'Esposito, M., Postle, B. R., Jonides, J., & Smith, E. E. (1999). The neural substrate and temporal dynamics of interference effects in working memory as revealed by event-related functional MRI. *Proceedings of the National Academy of Sciences, U.S.A., 96,* 7514- 7519.

D'Esposito, M., Postle, B. R., & Rypma, B. (2000). Prefrontal cortical contributions to working memory: Evidence from event-related fMRI studies. *Experimental Brain Research, 133,* 3- 11.

Donnenfield, A. E. (1998). Fragile X syndrome. *Indian Journal of Pediatrics, 65,* 513- 518.

Duncan, J., & Owen, A. M. (2000). Common regions of the human frontal lobe recruited by diverse cognitive demands. *Trends in Neuroscience, 23,* 475- 483.

Duvernoy, H. M., Bourgouin, P., Cabanis, E. A., & Cattin, F. (1999). *The human brain: Surface, three-dimensional sectional anatomy with MRI, and blood supply.* New York: Springer-Verlag.

Edwards, D. R., Keppen, L. D., Ranells, J. D., & Gollin, S. M. (1988). Autism in association with fragile X syndrome

in females: Implications for diagnosis and treatment in children. *Neurotoxicology, 93,* 359- 365.

Freund, L. S., Reiss, A. L., & Abrams, M. T. (1993). Psychiatric disorders associated with fragile X in the young female. *Pediatrics, 91,* 321- 329.

Friston, K. J., Holmes, A. P., Worsley, K. J., Poline, J. B., Frith, C. D., & Frackowiak, R. S. J. (1995). Statistical parametric maps in functional imaging: A general linear approach. *Human Brain Mapping, 2,* 189- 210.

George, M. S. E. C., Ketter, T. A., Parekh, P. I., Rosinsky, N., Ring, H., Casey, B. J., Trimble, M. R., Horwitz, B., Herschovitch, P., & Post, R. M. (1994). Regional brain activity when selecting a response despite interference: An $H_2^{15}O$ PET study of the Stroop and an emotional Stroop. *Human Brain Mapping, 1,* 194- 209.

Grecius, M., Krasnow, B., Reiss, A. L., & Menon, V. (in preparation). *The effect of susceptibility artifact on BOLD signal detection in fMRI: How much signal loss is too much signal loss?*

Hagerman, R. J., Jackson, C., Amiri, K., Silverman, A. C., O'Connor, R., & Sobesky, W. (1992). Girls with fragile X syndrome: Physical and neurocognitive status and outcome. *Pediatrics, 89,* 395- 400.

Hagerman, R. J., & Sobesky, W. E. (1989). Psychopathology in fragile X syndrome. *American Journal of Orthopsychiatry, 59,* 142- 152.

Hayes, C., & Mathias, C. (1996). *Improved brain coil for fMRI and high resolution imaging.* Presented at the ISMRM 4th Annual Meeting Proceedings, New York.

Holmes, A. P., & Friston, K. J. (1998). Generalizability, random effects, and population inference. *Neuroimage, 7,* 754.

Hutchinson, M., Schiffer, W., Joseffer, S., Liu, A., Schlosser, R., Dikshit, S., Goldberg, E., & Brodie, J. D. (1999). Task-specific deactivation patterns in functional magnetic resonance imaging. *Magnetic Resonance Imaging, 17,* 1427- 1436.

Jakala, P., Hanninen, T., Ryyanen, M., Laakso, M., Partanen, K., Mannermaa, A., & Soininen, H. (1997). Fragile-X: Neuropsychological test performance, CGG triplet repeat lengths, and hippocampal volumes. *Journal of Clinical Investigation, 100,* 331- 338.

Jensen, A. R. (1965). Scoring the Stroop test. *Acta Psycologia, 24,* 398- 408.

Jessen, F., Erb, M., Klose, U., Lotz, M., Grodd, W., & Heun, R. (1999). Activation of human language processing brain regions after the presentation of random letter strings demonstrated with event-related functional magnetic resonance imaging. *Neuroscience Letters, 270,* 13- 16.

Jonides, J., Smith, E. E., Marshuetz, C., Koeppe, R. A., & Reuter-Lorenz, P. A. (1998). Inhibition in verbal working memory revealed by brain activation. *Proceedings of the National Academy of Sciences, U.S.A., 95,* 8410- 8413.

Le, T. H., Pardo, J. V., & Hu, X. (1998). 4 T-fMRI study of nonspatial shifting of selective attention: Cerebellar and parietal contributions. *Journal of Neurophysiology, 79,* 1535- 1548.

Leung, H. C., Skudlarski, P., Gatenby, J. C., Peterson, B. S., & Gore, J. C. (2000). An event-related functional MRI study of the stroop color word interference task. *Cerebral Cortex, 10,* 552- 560.

Liddle, P. F., Kiehl, K. A., & Smith, A. M. (2001). Event-related fMRI study of response inhibition. *Human Brain Mapping, 12,* 100- 109.

MacLeod, C. M., & MacDonald, P. A. (2000). Interdimensional interference in the Stroop effect: Uncovering the cognitive and neural anatomy of attention. *Trends in Cognitive Science, 4,* 383- 391.

Mai, J. K., Assheuer, J., & Paxinos, G. (1997). *Atlas of the human brain.* San Diego, CA: Academic Press.

Mazzocco, M. M., Hagerman, R. J., Cronister-Silverman, A., & Pennington, B. F. (1992). Specific frontal lobe deficits among women with the fragile X gene. *Journal of the American Academy of Child and Adolescent Psychiatry, 31,* 1141- 1148.

Mazzocco, M. M., Pennington, B. F., & Hagerman, R. J. (1993). The neurocognitive phenotype of female carriers of fragile X: additional evidence for specificity. *Journal of Developmental and Behavioral Pediatrics, 14,* 328- 355.

Menon, V., MacKenzie, K., Rivera, S., & Reiss, A. L. (in preparation). Prefrontal cortex involvement in processing incongruent arithmetic equations: Evidence from event-related fMRI.

Menon, V., Rivera, S. M., White, C. D., Glover, G. H., & Reiss, A. L. (2000). Dissociating prefrontal and parietal cortex activation during arithmetic processing. *Neuroimage, 12,* 357- 365.

Morton, J. E., Bundey, S., Webb, T. P., MacDonald, F., Rindle, P. M., & Bullock, S. (1997). Fragile X syndrome is less common than previously estimated. *Journal of Medical Genetics, 34,* 1- 5.

Ojemann, J. G., Akbudak, E., Snyder, A. Z., McKinstry, R. C., Raichle, M. E., & Conturo, T. E. (1997). Anatomic localization and quantitative analysis of gradient refocused echo-planar fMRI susceptibility artifacts. *Neuroimage, 6,* 156- 167.

Pardo, J. V., Fox, P. T., & Raichle, M. E. (1991). Localization of a human system for sustained attention by positron emission tomography. *Nature, 349,* 61- 64.

Pardo, J. V., Pardo, P. J., Janer, K. W., & Raichle, E. (1990). The anterior cingulate cortex mediates processing selection in the Stroop attentional conflict paradigm. *Proceedings of the National Academy of Sciences, U.S.A., 87,* 256- 259.

Poline, J. B., Worsley, K. J., Evans, A. C., & Friston, K. J. (1997). Combining spatial extent and peak intensity to test for activations in functional imaging. *Neuroimage, 5,* 83- 96.

Riddle, J. E., Cheema, A., Sobesky, W. E., Gardner, S. C., Taylor, A. K., Pennington, B. F., & Hagerman, R. J. (1998). Phenotypic involvement in females with the FMR1 gene mutation. *American Journal of Mental Retardation, 102,* 590- 601.

Rolls, E. T. (1994). The orbitofrontal cortex. In A. C. Roberts, T. W. Robbins, & L. Weiskrantz (Eds.), *The prefrontal cortex: Executive and cognitive functions* (pp. 67- 86). Oxford: Oxford University Press.

Rosen, A. C., Rao, S. M., Caffarra, P., Scaglioni, A., Bobholz, J. A., Woodley, S. J., Hammeke, T. A., Cunningham, J. M., Prieto, T. E., & Binder, J. R. (1999). Neural basis of endogenous and exogenous spatial orienting. A functional MRI study. *Journal of Cognitive Neuroscience, 11,* 135- 152.

Santos, J. F., & Montgomery, J. R. (1962). Stability of performance on the color- word test. *Perceptual Motor Skills, 15,* 397- 398.

Schoenbaum, G., Chiba, A. A., & Gallagher, M. (1998). Orbitofrontal cortex and basolateral amygdala encode expected outcomes during learning. *Nature Neuroscience, 1,* 155- 159.

Shulman, G. L., Corbetta, M., Buckner, R. L., Fiez, J. A., Miezen, F. M., Raichle, M. E., & Petersen, S. E. (1997). Common blood flow changes across visual tasks: I. Increases in subcortical structures and cerebellum but not in nonvisual cortex. *Journal of Cognitive Neuroscience, 9,* 624- 647.

Shulman, G. L., Fiez, J. A., Corbetta, M., Buckner, R. L., Miezen, F. M., Raichle, M. E., & Petersen, S. E. (1997). Common blood flow changes across visual tasks: II. Decreases in cerebral cortex. *Journal of Cognitive Neuroscience, 9,* 648- 663.

Smith, G. J., & Nyman, G. E. (1974). The validity of the serial color word test: A reply to Lennart Sjoberg. *Scandinavian Journal of Psychology, 15,* 238- 240.

Sobesky, W. E., Hull, C. E., & Hagerman, R. J. (1994). Symptoms of schizotypal personality disorder in fragile X women. *Journal of the American Academy of Child and Adolescent Psychiatry, 332,* 247- 255.

Stroop, J. R. (1935). Studies of interference in serial verbal reactions. *Journal of Experimental Psychology, 18,* 643- 662.

Sturm, W., de Simone, A., Krause, B. J., Specht, K., Hesselmann, V., Radermacher, I., Herzog, H., Tellmann, L., Muller-Gartner, H. W., & Willmes, K. (1999). Functional anatomy of intrinsic alertness: Evidence for a fronto-parietal-thalamic- brainstem network in the right hemisphere. *Neuropsychologia, 37,* 797- 805.

Talairach, J., & Tournoux, M. (1988). *Co-planar stereotaxic atlas of the human brain.* New York: Thieme.

Taylor, S. F., Kornblum, S., Lauber, E. J., Minoshima, S., & Koeppe, R. A. (1997). Isolation of specific interference processing in the Stroop task: PET activation studies. *Neuroimage, 6,* 81- 92.

Tremblay, L., & Schultz, W. (2000). Reward-related neuronal activity during go- nogo task performance in primate orbitofrontal cortex. *Journal of Neurophysiology, 83,* 1864- 1876.

Welch, J. L., & Williams, J. K. (1999). Fragile X syndrome. *Neonatal Network, 18,* 15- 22.

Zysset, S., Muller, K., Lohmann, G., & von Cramon, D. Y. (2000). *The Stroop tasks: Is it the anterior cingulate cortex?* Presented at Cognitive Neuroscience Meeting, San Francisco, CA.