Regional Brain Activation during Verbal Declarative Memory in Metastatic Breast Cancer

Shelli R. Kesler, F. Chris Bennett, Misty L. Mahaffey, and David Spiegel

Abstract

Purpose: To determine the neurofunctional basis of verbal memory dysfunction in women with metastatic breast cancer. This objective was based on previous research suggesting memory and other cognitive deficits in this population. We attempted to determine if verbal memory impairments were related to the most commonly studied disease parameters including adjuvant chemotherapy and chronic stress-related disruption of limbic system structures.

Experimental Design: We used functional magnetic resonance imaging to test our hypothesis that women with breast cancer would show significantly lower brain activation during verbal declarative memory tasks compared with age and education-matched healthy female controls. We also assessed several stress-related variables including diurnal cortisol levels to test our hypothesis that women with breast cancer would show higher stress and this would contribute to brain activation deficits during memory tasks.

Results: Women with breast cancer had significantly lower prefrontal cortex activation during the memory encoding condition compared with controls. However, the breast cancer group showed significantly greater activation than controls during the recall condition in multiple, diffuse brain regions. There were no significant differences between the groups in stress-related variables. Women who were treated with cyclophosphamide, methotrexate, and 5-fluorouracil chemotherapy showed lower prefrontal cortex activation during memory encoding.

Conclusions: These results suggest that women with metastatic breast cancer may be at risk for verbal memory impairments as a result of altered functional brain activation profiles. These findings may be associated with chemotherapy type and/or other aspects of the breast cancer disease process. (Clin Cancer Res 2009;15(21):OF1–9)

Women with breast cancer may have an increased risk for long-term cognitive-behavioral impairments including those of executive function and memory, likely due to neurotoxic side effects of chemotherapy (1–3). The incidence of these impairments is uncertain but has been reported to range from 28% to 75% among breast cancer patients receiving cyclophosphamide, methotrexate, and 5-fluorouracil chemotherapy (CMF; ref. 4). Cognitive-behavioral impairments significantly extend disease-related disability, affecting home, educational, and occupational activities. Additionally, the high prevalence of breast cancer and increasing survival rates contribute to a large and growing cohort of cognitively affected individuals (3). Currently, there are no specific treatments for these cognitive impairments and no preventive interventions are available.

Neuroimaging studies provide insight regarding the neurobiological mechanisms underlying cognitive impairment in various populations. To date, there have been only four such studies conducted in breast cancer to our knowledge. These reports indicate altered cerebral metabolism and decreased volume in executive function regions, including prefrontal cortex, basal ganglia, and the cingulate gyrus in women with breast cancer compared with controls (5, 6). Additionally, reduced corpus callosum genu white matter pathway integrity (i.e., fractional anisotropy) was noted in a small sample of women with breast cancer compared with controls and was correlated with lower processing speed (7). A case study of monozygotic twins discordant for breast cancer indicated increased white matter hyperintensities and altered frontal-parietal functional brain activation in the twin with breast cancer (8). This case study represents the only functional magnetic resonance imaging (fMRI) study conducted to date. fMRI allows in vivo assessment of brain function by detecting blood flow differences and is widely used to study brain-behavior relationships in children and adults (9–12). Group fMRI studies could significantly advance our understanding regarding the...
Translational Relevance

These findings provide neuroanatomically specific evidence of neurofunctional deficits associated with memory storage and retrieval in women with metastatic breast cancer, potentially specific to those treated with cyclophosphamide, methotrexate, and 5-fluorouracil chemotherapy. These results suggest that certain types of chemotherapy may induce verbal memory impairment as shown by altered functional brain activation profiles. They provide evidence regarding the oft cited but poorly understood syndrome of “chemobrain” among women with breast cancer, explaining their primary neuropsychological problem as difficulty in performing cognitive tasks rather than deficits in the outcome of their performance per se. They also indicate that one type of chemotherapy, cyclophosphamide, methotrexate, and 5-fluorouracil, was most strongly associated with these deficits, suggesting opportunities for adjusting chemotherapy to minimize neurocognitive impairment and/or targetting women who received this older regimen for cognitive interventions. These results have important implications not only for chemotherapy treatments in breast cancer, but also for the design of cognitive intervention programs for women with breast cancer who show verbal memory deficits.

neurobiological mechanisms underlying cognitive deficits in women with breast cancer. Additionally, fMRI studies are being increasingly used to guide and provide potential targets for cognitive interventions (13). This study aimed to implement an fMRI paradigm to investigate verbal memory function in women with breast cancer.

Some studies suggest that cognitive deficits in women with breast cancer are associated, at least in part, with illness-related stress (3, 14). Abnormal diurnal cortisol patterns have been observed among women with breast cancer (15, 16) and cortisol is known to play a vital role in cognitive and emotional function. In the present study, we obtained both psychological and physiological measures of stress including self-rating questionnaires, vital statistics, and salivary cortisol to use as covariates of nuisance in our analyses of brain activation.

Many studies of cognitive outcome in women with breast cancer do not include chemotherapy in the analyses due to the significant variation among patients in terms of chemotheraphy regimen. However, cognitive deficits in survivors of breast cancer have been associated with cytotoxic chemotherapy such as methotrexate (17) and hormonal blockade with tamoxifen (5, 18–20). Animal studies have suggested that certain chemotherapeutic agents including methotrexate, carmustine, cisplatin, 5-fluorouracil, and cytarabine may be more toxic to white matter progenitor and hippocampal stem cells than they are to actual cancer cells (21–23). We collected chemotherapy and tamoxifen treatment information to conduct exploratory analyses regarding the effects of particular treatment variables on brain activation during verbal memory.

Materials and Methods

Participants

Fourteen women (mean age, 55.1 ± 8.0 y; range, 43-65) with metastatic (n = 8) or locally advanced (n= 6) breast cancer and 14 age and education-matched healthy female controls (mean age, 54.2 ± 8.0 y; range, 40-65; P = 0.78; mean education rank, 5 ± 2; P = 0.83) participated in this study. Education data were gathered using the following ranking system: 1, less than high school; 2, graduated from high school/GED; 3, completed trade school; 4, some college; 5, bachelor's degree; 6, some graduate school; 7, master's degree; 8, PhD, M.D., and/or 1.D. All women with breast cancer had a history of adjuvant chemotherapy treatment and 11 had a history of radiation therapy to the neck, affected breast, axilla, and/or lumbar spine. Chemotherapy types included six cycles of CMF (n = 5) or four to eight cycles of Adriamycin, cyclophosphamide, and Taxol/taxotere (ACT; n = 9). All chemotherapy and radiation treatments had to be completed at least 6 mo before enrollment to be eligible for the study (mean time since last chemotherapy/radiation, 3.3 ± 3.3 y; range, 0.5-10.3). Eleven of the patients also had taken tamoxifen during their course of breast cancer treatment (mean time since tamoxifen, 5.3 ± 2.3 y; range, 2-9). Six of the 11 breast cancer/tamoxifen patients took tamoxifen after chemotherapy/radiation, 3 took it concurrently with chemotherapy/radiation, and 2 took tamoxifen before chemotherapy/radiation (these 2 were treated in 1991 and 1995, respectively). Information regarding anticancer treatments was obtained using both patient and physician report as well as medical records, when available.

Healthy female controls were recruited via community flyers and internet postings restricted to the same zip codes as the women with breast cancer to reduce differences in socioeconomic status. There were no significant differences between the groups in terms of minority status ($\chi^2 = 0.003, P = 0.74$).

All potential participants were excluded for MRI contraindications (e.g., metallic implants, biomedical devices) and were free from medications that affect cortisol (e.g., hydrocortisone, megestrol) and fMRI (e.g., antipsychotics, sedatives, MAO inhibitors, tricyclic antidepressants). Participants with breast cancer were excluded for active cancers within the past 10 y other than breast cancer, basal cell, or squamous cell carcinomas of the skin; in situ cancer of the cervix; positive supravacular lymph nodes as the only metastatic lesion at the time of initial diagnosis; history of neurologic conditions including metastases to the brain as well as history of learning or other developmental disability, preterm birth, and psychiatric or chronic medical conditions not related to breast cancer. Healthy controls were excluded for any history of learning or other developmental disability, preterm birth, and psychiatric, neurologic, or chronic medical condition. Their participation in the present study was approved by the Stanford University Institutional Review Board and all participants gave written informed consent before beginning the study.

Distress measures

Cortisol collection. As salivary cortisol has been found to be a reliable tool for investigations of HPA activity (24–26), saliva samples were collected on two consecutive baseline days, not including the day of the MRI. Samples were collected at awakening, 12, 5, and 9 p.m. on both days using cortisol-specific self-collection kits, according to a previously published protocol (15, 16). Participants were provided with both verbal and written collection instructions. Due to significant variance in the cortisol data, all cortisol variables were log transformed (see Table 1). Cortisol level could not be obtained for three women with breast cancer and two controls.

Cortisol questionnaire. Cortisol questionnaire is a 12-item self-rating measure designed to assess factors affecting stress and cortisol levels related to physical activity, stress level, sleep, and subjective sense of health.
Psychophysiology. Heart and respiratory rate were measured during the entire MRI scan.

Weinberger Adjustment Inventory. The Weinberger Adjustment Inventory (WAI) is an 84-item self-rating measure of typological stress coping strategy including anxiety, restraint, and repression (27). Although the WAI yields multiple subscale and composite scores, we examined only the three composite scores: Repressive-Defensive/Restraint, Distress, and Restraint, as well as the two subscales that we have previously shown to differentiate women with breast cancer and healthy female controls: Anxiety and Repressive-Defensiveness (15). This subset of WAI scores were used to reduce the number of comparisons and the amount of measurement error associated with multiple conditions; and smoothed to reduce the effects of noise. Images for each subject were then visually assessed for correct spatial normalization using in-house software that creates three-dimensional renders and slice conditions; and smoothed to reduce the effects of noise. Images for each subject were then visually assessed for correct spatial normalization using in-house software that creates three-dimensional renders and slice.

Beck Depression Inventory. The Beck Depression Inventory is a 21-item self-rating inventory of depressive symptomology including sadness, suicidal ideation, fatigue, loss of interest, and sleep disruption (28).

MRI acquisition protocols

We acquired structural scans using a protocol that provides a high level of anatomic resolution and tissue contrast: three-dimensional IR-prepared FSPGR; TR, minimum; TE, minimum; flip, 15 degrees; TI, 300 ms; BW: ±31.25 kHz; FOV, 22 cm; phase FOV, 0.80 FOV (22 × 15.4); slice thickness, 1.5 mm, 128 slices (to get 124 slice locations), 256 × 256 at 3 NEX.

Acquisition of fMRI data were based on a spiral-in/out blood oxygenation level–dependent protocol (29). This provides for improved blood oxygenation level–dependent signal in regions of interest such as the amygdala and hippocampus. Thirty-two axial slices (3-mm thick, 1 mm skip) parallel to the AC-PC line and covering the whole brain were imaged with a temporal resolution of 2 s using a T2*-weighted gradient echo spiral pulse sequence (TR, 2,000 ms; TE, 30 ms; flip angle, 80° and 1 interleave). The field of view was 200 × 200 mm², and the matrix size as 64 × 64, giving an in-plane spatial resolution of 3.125 mm. An automated high-order shimming method based on spiral acquisitions was used to reduce field heterogeneity.

fMRI tasks

Stimulus presentation. All stimuli were presented using E-Prime software (Psychology Software Tools), which also triggered the initiation of the scan. Stimuli were presented using a custom-adapted system that projects stimuli at high resolution (1,024 × 768) onto a screen attached to the head coil. Subjects look directly upward at a mirror to view the stimuli. Behavioral responses were recorded using a four-button fiber optic finger switch system. Task performance was acquired simultaneously while subjects did the tasks in the scanner.

Verbal declarative memory encoding. The encoding condition of the fMRI task required subjects to view visually presented nouns and make a semantic discrimination for each by pressing button 1 if the word represented something man-made and button 2 if the word represented something not man made.

Verbal declarative memory recall. During the recall task condition, subjects were again presented with single nouns and were asked to press button 1 if they recognized the word from the previous task and button 2 if they did not.

The specific details of these tasks are described elsewhere (30).

fMRI preprocessing

Using Statistical Parametric Mapping 2 (SPM2) software images were realigned to correct for head movement using least square minimization, normalized to a standardized template to directly compare brain activation across subjects, stimulus types, and experimental conditions; and smoothed to reduce the effects of noise. Images for each subject were then visually assessed for correct spatial normalization using in-house software that creates three-dimensional renders and slice maps (axial, coronal, sagittal) of raw, normalized, and statistical images for comparison with template examples of correctly aligned images. We

| Table 1. Demographic, fMRI behavioral performance, and distress measures data |
|---|---|---|---|---|---|---|
| | Breast cancer | Control | t | F | U | P |
| Age (y) | 14 | 55.1 (8.0) | 14 | 54.2 (8.0) | 0.28 | 0.78 |
| Education (rank)* | 14 | 5 (2) | 14 | 5 (2) | 87 | 0.83 |
| Last chemo/radiation (y) | 14 | 3.3 (3.3) | 14 | 3.3 (3.3) | 9.3 | 0.005 |
| Last tamoxifen (y) | 14 | 5.3 (2.3) | 14 | 5.3 (2.3) | 3.125 | 0.04 |
| Encoding between group ROI | 14 | 0.21 (0.27) | 14 | 0.47 (0.23) | 3.125 | 0.04 |
| Encoding % correct | 14 | 0.91 (0.07) | 14 | 0.90 (0.08) | 0.26 | 0.79 |
| Encoding reaction time (ms) | 14 | 1,201 (177) | 14 | 1,337 (166) | -2.0 | 0.06 |
| Recall between group ROI | 14 | 0.34 (0.16) | 14 | 0.18 (0.20) | 4.9 | 0.04 |
| Recall % correct | 14 | 0.82 (0.14) | 14 | 0.76 (0.18) | 0.88 | 0.39 |
| Recall reaction time (ms) | 14 | 1,357 (139) | 14 | 1,124 (141) | 3.6 | 0.002 |
| Baseline cortisol slope† | 11 | -0.16 (0.06) | 12 | -0.15 (0.08) | -0.44 | 0.67 |
| Cortisol questionnaire | 11 | 3.3 (0.75) | 12 | 3.1 (0.73) | 0.78 | 0.44 |
| Average heart rate | 14 | 68.3 (5.4) | 14 | 64.7 (10.3) | 1.0 | 0.31 |
| Average respiratory rate | 14 | 19.6 (2.7) | 14 | 21.0 (4.6) | -0.86 | 0.40 |
| WAI Distress Composite | 14 | 57 (15) | 14 | 51 (9) | 1.3 | 0.28 |
| WAI Restraint Composite | 14 | 134 (6) | 14 | 130 (9) | 0.96 | 0.34 |
| WAI Repressive-Defensive/Restraint Composite | 14 | 80 (9) | 14 | 74 (8) | 2.6 | 0.12 |
| Repressive-Defensive Subscale | 14 | 35 (8) | 14 | 31 (6) | 1.9 | 0.18 |
| Anxiety Subscale | 14 | 19 (5) | 14 | 19 (6) | 0.16 | 0.69 |
| BDI total score | 14 | 7 (4) | 14 | 5 (6) | 1.2 | 0.21 |

NOTE: Data are shown as mean (SD).

*Education ranking key: 1, less than high school; 2, graduated from high school/GED; 3, completed trade school; 4, some college; 5, bachelor’s degree; 6, some graduate school; 7, master’s degree; 8, PhD, M.D., and/or J.D.; ROI, ROI representing the mean activation intensity value across all voxels in the areas of significant between-group difference; WAI, Weinberger Adjustment Inventory.

†Log transformed data.
also used our automated in-house software program, “ArtRepair”2 to automatically detect and repair motion and signal intensity outliers as well as other artifacts at the volume, slice, and voxel level (31).

Statistical analyses
Distress measures. Group differences in baseline 2-d cortisol slope, stress questionnaire, heart rate, and respiration rate were conducted using independent t tests, and WAI scores were measured using MANOVA in SPSS 16.0. As there were no between group differences in distress measures, we did not include them as covariates in further analyses.

fMRI task performance data. fMRI task performance data (percent correct, reaction time) were compared between groups using independent t tests.

fMRI whole brain activation data. Statistical analyses were done with SPM2 (32). Any images that represented movement, signal, and/or rotation outliers as indicated by the ArtDetect’s procedure were excluded from the statistical analyses. The number of outlier images excluded did not total >1% of the total number of images for any scan. A within-subjects procedure was used to model all the effects of interest for each participant. 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 s) was applied to the fMRI time series at each voxel. A temporal smoothing function (Gaussian kernel corresponding to dispersion of 8 s) was applied to the fMRI time series at each voxel.

Brain activation and task performance differences. For the breast cancer group only, ROI values were also entered as dependent variables in a multiple regression with tamoxifen (1, yes; 0, no) and chemotherapy type (0, ACT; 1, CMF) as independent variables. ROI values were correlated (two-tailed Pearson) with task response data that were significantly different between the groups to explore the associations between brain activation and task performance differences.

Results
Distress measures. There were no between group differences in any of the distress-related variables (Table 1).

Memory encoding fMRI. The two groups performed the encoding condition similarly in terms of accuracy (P = 0.79) but there was a nonsignificant trend for the women with breast cancer to show faster reaction times (P = 0.06). The breast cancer group showed significant brain activation in bilateral inferior occipital gyrus extending into the left fusiform gyrus, left cerebellum,

Table 2. Within and between group results of fMRI analyses indicating significant brain activation during the encoding task (experimental minus control contrast)

<table>
<thead>
<tr>
<th>P (FDR corrected)</th>
<th>Cluster size</th>
<th>T score</th>
<th>MNI coordinates</th>
<th>Location description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer group</td>
<td>0.04</td>
<td>1,048</td>
<td>7.33</td>
<td>-44, -86, -12</td>
</tr>
<tr>
<td>0.04</td>
<td>1,535</td>
<td>6.65</td>
<td>-40, 28, 12</td>
<td>Left inferior frontal gyrus extending into left insula</td>
</tr>
<tr>
<td>0.05</td>
<td>541</td>
<td>6.34</td>
<td>36, -94, -8</td>
<td>Right inferior occipital gyrus extending into right middle occipital gyrus</td>
</tr>
<tr>
<td>Control group</td>
<td>0.003</td>
<td>50,983</td>
<td>10.25</td>
<td>-8, 6, 52</td>
</tr>
<tr>
<td>Control &gt; breast cancer</td>
<td>0.01</td>
<td>6,448</td>
<td>3.92</td>
<td>-14, -8, 78</td>
</tr>
</tbody>
</table>

Breast cancer > control = none

NOTE: Height and extent threshold: P < 0.05, FDR.
and right middle occipital gyrus and the left inferior frontal gyrus extending into the left insula (Table 2). The control group showed significant activation in the left superior frontal gyrus extending into bilateral anterior cingulate, right superior frontal, bilateral middle frontal, left inferior frontal, and bilateral superior temporal gyri as well as bilateral basal ganglia (Table 2).

The control group showed significantly greater activation in the left superior frontal gyrus extending into the right superior frontal gyrus, bilateral middle frontal gyri, and left postcentral gyrus, compared with the breast cancer group (Table 2; Fig. 1A). The breast cancer group showed no regions of significantly greater activation compared with controls. These between group results were irrespective of age, confirmed by ROI GLM analysis (F = 9.25; P = 0.005; Table 1).

Chemotherapy type, but not tamoxifen, predicted activation in the between group difference ROI (i.e., the regions illustrated in Fig. 1A; adjusted R² = 0.52; F = 7.4; P = 0.01) in the breast cancer group. Given the negative coefficient values for chemotherapy type (B = -0.40; t = -3.2; P = 0.009), women with breast cancer who received CMF chemotherapy showed significantly lower activation in the ROI. A post hoc GLM examining encoding ROI activation between controls, CMF, and ACT groups indicated significant differences between CMF and controls (P < 0.0001) as well as CMF and ACT (P = 0.001) but no significant difference between controls and ACT (P = 0.25). This finding is illustrated graphically in Fig. 2. There were no significant differences between ACT and CMF groups in terms of age, ethnicity, education, stress variables, time since tamoxifen, or fMRI task performance. Time since chemotherapy/radiation treatment was significantly greater in women treated with CMF compared with ACT (P = 0.006). However, the difference in encoding ROI activation between the CMF and ACT groups remained even after covarying for time since chemotherapy/radiation treatment (P < 0.0001).

Encoding ROI activation was significantly correlated with reaction time in controls (r = 0.58, P = 0.04) but not in the breast cancer group (r = 0.04, P = 0.90). The difference in these correlations (Fisher r-z transformation) was nearly significant (z = -1.46, P = 0.07). Encoding ROI activation in the breast cancer group was not correlated with time since treatment.

Memory recall fMRI. The two groups showed similar accuracy on the recall task (P = 0.39) but the breast cancer group showed significantly slower reaction time (P = 0.002). Women with breast cancer showed significant brain activation in the left inferior occipital gyrus extending into right inferior and bilateral middle and superior occipital gyri, bilateral cerebellum, bilateral cuneus and precuneus, bilateral inferior and superior parietal lobe, bilateral fusiform gyr, bilateral superior and inferior temporal gyri, left middle temporal gyrus, bilateral hippocampus, bilateral dorsolateral prefrontal cortex, bilateral cingulate, bilateral medial frontal gyrus, bilateral basal ganglia, and bilateral lateral orbital gyr (Table 3). Controls did not show any regions of greater activation compared with women with breast cancer.

Compared with controls, the breast cancer group showed significantly greater activation in right superior temporal gyrus extending into bilateral fusiform, bilateral lingual gyr, left hippocampus, bilateral basal ganglia, right precentral gyrus, right superior and inferior frontal gyri, right middle frontal gyrus, bilateral inferior frontal gyrus, right cingulate gyrus, bilateral insula, bilateral parahippocampal gyrus, bilateral cuneus, bilateral precuneus, bilateral superior parietal lobe, and cerebellum (Table 3; Fig. 1B). Controls did not show any regions of greater activation compared with women with breast cancer during memory encoding.

Verbal Memory in Breast Cancer

Fig. 1. A, axial SPM maps showing regions of significantly greater functional brain activation in controls compared with women with breast cancer during memory encoding. B, regions of significantly increased brain activation in women with breast cancer compared with controls during memory recall. Color bar, T score range. Slice labels, MNI z coordinate.
Discussion

To our knowledge, this is the first cohort study of functional brain activation in women with breast cancer. We showed reduced prefrontal cortex activation in women with breast cancer compared with controls during the semantic encoding phase of a verbal declarative memory task. During the recall condition of the task, women with breast cancer showed significantly increased spatial extent of cortical activation compared with controls. Regions of increased activation included those less commonly involved in declarative verbal memory retrieval (e.g., orbital prefrontal, right superior temporal gyrus, inferior temporal gyrus, occipital gyri) as indicated by studies using a similar fMRI task (34). These findings suggest overactivation of brain regions during this memory task. These results were irrespective of age and therefore not likely explained by aging or menopause. Women with breast cancer were similar to controls in performance accuracy during both task conditions but showed a tendency to respond more quickly during encoding and significantly more slowly during recall.

Previous studies have shown abnormalities of the prefrontal cortex (5, 6) in women with breast cancer who have undergone adjuvant chemotherapy. The prefrontal cortex is known to play a critical role in declarative memory (35). Our present results offer increased insight into and more specific neural correlates of verbal memory difficulties in certain women with breast cancer. Specifically, during memory encoding, women with breast cancer showed lower prefrontal activation as well as quicker, perhaps more impulsive, response time compared with controls. Reduced prefrontal resources may indicate lower attention and/or organizational skills associated with learning and memory. For example, women treated with CMF chemotherapy for breast cancer may have more difficulty attending to stimuli and/or engaging in organizational or mnemonic strategies for memorization. Increased prefrontal activation was associated with increased reaction time in controls but not in the breast cancer group. It seems that the controls were able to use more prefrontal resources and took more time to encode the stimuli. Because the stimuli were not properly encoded, the recall condition required a significant increase in neural effort in the breast cancer group. These findings have implications for potential treatments for verbal memory impairments in women with breast cancer. For example, focusing cognitive interventions on executive, prefrontal skills could provide rehabilitation that will improve encoding efficiency and reduce recall effort.

Cognitive difficulties in chemotherapy-treated breast cancer have been somewhat controversial because neuropsychological testing scores are often within normal limits (2) or not different from controls (18). There may be significant discrepancies between subjective and objective measures of cognitive ability as well as associations between subject cognitive complaints and psychological distress (18) leading some to conclude that treatment-related cognitive deficits in breast cancer are merely based on anxiety about performance. Our results show that, although some women with breast cancer might perform as accurately as controls on certain memory tasks, they have brain-based deficits related to memory encoding and thus require a great deal more neural effort when attempting to recall information. This likely results in increased cognitive fatigue and frustration, resulting in negative subjective evaluation of cognitive ability.

Some studies suggest cognitive deficits associated with breast cancer and its treatment tend to resolve over time, at least in some patients (36–38). However, our sample of women with breast cancer were, on average, 3.3 years posttreatment and we showed no relationship between increased time since treatment and improved brain function during memory tasks. Some women with breast cancer may be able to compensate for neurofunctional changes associated with chemotherapy whereas others cannot. For example, one study suggested that women with breast cancer who have the E4 variant of the APOE gene, which is believed to affect memory function and elevate risk for Alzheimer’s dementia (39), might be at higher risk for cognitive difficulties than women with breast cancer who carry other APOE alleles (40). Further studies are required to determine if there are other variables that predict cognitive outcome in women with breast cancer.

Contrary to our hypotheses, we did not find that our sample of women with breast cancer was more distressed than controls. It is possible that only those women who do not have significant distress agreed to an MRI, which can be a stressful and intimidating procedure. Sampling bias may thus influence our findings. However, our results show that neurofunctional deficits exist even in seemingly nondistressed women with breast cancer.
cancer during memory tasks. Therefore verbal memory impairments in some women with breast cancer cannot be explained by illness-related stress.

In fact, verbal memory impairments might be related to the type of chemotherapy regimen. Our results suggested that women with breast cancer who underwent adjuvant CMF chemotherapy showed significantly lower prefrontal cortex activation during memory encoding than women treated with ACT chemotherapy. As shown in Fig. 2, women with breast cancer who received ACT chemotherapy were more similar to controls in terms of prefrontal activation. Additionally, three of the five women treated with CMF actually showed deactivation of the prefrontal cortex (negative mean ROI value) during encoding. CMF rather than ACT was associated with greater brain activation impairment likely because of methotrexate. Previous studies implicate an association between cognitive deficits and methotrexate in women with breast cancer (17), an animal model suggested negative effects of methotrexate on the frontal lobe functions in rats (23), and one study showed abnormal prefrontal cortex morphology in children with leukemia treated with methotrexate (41). However, our results should be considered preliminary given the small sample size, particularly of the CMF group. Additionally, CMF is an older chemotherapy regimen that is no longer as commonly used as other regimens (women in our sample treated with CMF underwent chemotherapy in the late 1990s). Therefore, this finding may have clinical relevance only for women who underwent chemotherapy in the more remote past.

It is unclear why CMF chemotherapy was associated with brain activation during encoding but not recall. Between-group activation differences during recall were much more diffuse than during encoding, which may have reduced the power of the recall ROI analysis. As indicated above, methotrexate has been associated with prefrontal cortex abnormalities. Although both encoding and recall tasks were associated with significant between-group differences in prefrontal cortex, encoding differences were more in the left hemisphere, whereas recall differences were more in the right. This is consistent with previous studies demonstrating a left-sided bias for encoding and a right-sided bias for retrieval (42). Children with leukemia who received methotrexate showed more significant left prefrontal abnormalities (41). Thus, left prefrontal cortex regions may have increased vulnerability to methotrexate-related toxicity. This potential leftward vulnerability may stem from the numerous well-documented left-right asymmetries of the prefrontal cortex including lower white matter volume (43) and coherence (44) of the left frontal hemisphere. Additionally, the left frontal cortex has significantly lower N-acetylaspartate.

### Table 3. Within and between group results of fMRI analyses indicating significant brain activation during the recall task (experimental minus control contrast)

<table>
<thead>
<tr>
<th>Location description</th>
<th>P (FDR corrected)</th>
<th>Cluster size</th>
<th>T score</th>
<th>MNI coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left inferior occipital gyrus extending into right inferior and superior occipital gyri, bilateral cerebellum, bilateral cuneus and precuneus, bilateral inferior and superior parietal lobe, bilateral fusiform gyri, bilateral superior and inferior temporal gyri, right middle temporal gyrus, bilateral hippocampus, bilateral dorsolateral prefrontal cortex, bilateral cingulate, bilateral medial frontal gyri, bilateral basal ganglia, and bilateral lateral orbital gyri</td>
<td>&lt;0.0001</td>
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<td>Right inferior frontal gyrus</td>
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<td>Right anterior cingulate extending into left anterior cingulate and left superior frontal gyrus</td>
<td>0.006</td>
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<td>6, 20, 48</td>
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<td>Left cuneus extending into left lingual and fusiform gyri</td>
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<td>-26, -94, -4</td>
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<td>Right inferior frontal gyrus extending into right middle frontal gyrus</td>
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<td>815</td>
<td>7.90</td>
<td>58, 12, 30</td>
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<td>Right middle occipital gyrus extending into right cuneus</td>
<td>0.01</td>
<td>490</td>
<td>6.32</td>
<td>30, -92, -2</td>
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<td>Right inferior parietal lobe extending into right superior parietal lobe and right precuneus</td>
<td>0.01</td>
<td>473</td>
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<td>36, -64, 44</td>
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<td>Right cerebellum and left superior parietal lobe</td>
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</tr>
<tr>
<td>Right superior temporal gyrus extending into bilateral fusiform, bilateral lingual gyri, left hippocampus, bilateral basal ganglia, right precentral gyrus, right superior and inferior frontal gyri, right middle frontal gyrus, bilateral inferior frontal gyrus, right cingulate gyrus, bilateral insula, bilateral parahippocampal gyrus, bilateral cuneus, bilateral precuneus, bilateral superior parietal lobe and cerebellum</td>
<td>Breast cancer &gt; control &lt;0.0001</td>
<td>20,056</td>
<td>5.20</td>
<td>46, 10, -14</td>
</tr>
</tbody>
</table>

Control > breast cancer = none

NOTE: Height and extent threshold: P < 0.05, FDR.
levels than the right. N-acetyl aspartate is believed to play a role in glial cell–specific signaling and myelination (45). Methotrexate is known to cause white matter abnormalities (46, 47). The left frontal cortex may have less white matter “reserves” to deal with neurotoxic injury than the right due to the asymmetries described above.

Tamoxifen did not predict brain activation in regions of between-group difference but there was not enough variance to truly explore this relationship given that only 3 women did not receive tamoxifen. Future studies should include a second comparison group of women with breast cancer treated only with surgery as many of these receive tamoxifen or other similar hormonal therapies, but not chemotherapy. The addition of this “no chemotherapy” group would also help address the weakness of the present study inherent in cross-sectional designs. Specifically, based on the present results, it is difficult to determine if the altered brain activation profiles in the breast cancer group stem from chemotherapy effects, additional aspects of breast cancer (e.g., immunosuppression, inflammatory responses), or a combination of these factors. In fact, some studies have suggested that a percentage of women with breast cancer may have cognitive impairments—including verbal memory deficits—before beginning adjuvant chemotherapy (14).

In conclusion, our present findings provide preliminary but compelling evidence of neurofunctional deficits associated with verbal declarative memory in women with metastatic breast cancer, potentially specific to those treated with CMF chemotherapy. Our results have important implications not only for chemotherapy treatments in breast cancer but also for potential cognitive interventions in women with breast cancer who show verbal memory deficits. However, small sample sizes and a cross-sectional design limit the interpretation of findings. Larger studies using neuroimaging techniques with appropriate comparison groups and/or longitudinal designs are necessary to further elucidate the neurobiological status associated with cognitive deficits in women with breast cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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