Functional Magnetic Resonance Imaging Response to Increased Verbal Working Memory Demands Among Patients With Multiple Sclerosis

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Abstract: Multiple sclerosis (MS) patients frequently experience impaired verbal working memory (VWM). Functional magnetic resonance imaging (fMRI) may help identify neural mechanisms underlying these deficits. Neuroimaging studies of healthy adults have characterized responses associated with increased VWM demands during the n-Back task, suggesting that this experimental paradigm could help identify neural correlates of VWM deficits among MS patients. Fifteen MS patients and 15 matched control participants completed the n-Back during whole-brain fMRI. Mean signal during adjacent 0-Back blocks was subtracted, on a voxel-wise basis, from mean signal observed during n-Back blocks. Resulting difference scores for 1-, 2-, and 3-Back were compared across groups and difficulty levels. Signal intensity was positively related to difficulty level in anterior regions, including premotor, supplementary motor, and dorsolateral prefrontal cortices. MS patients exhibited significantly greater intensity in these areas compared to controls during the 1-Back, while portions of the left superior frontal gyrus, cingulate, and parahippocampal gyri were relatively less intense at more difficult levels. MS group responses were slower during the 1-Back and tended to be slower during the 3-Back; however, accuracy did not differ at any level. Lesion load was positively related to only 1-Back activity and unrelated to any performance measure. Results suggest that compensatory activity occurs among MS patients matched on performance accuracy. Furthermore, compensatory activity occurs predominantly in regions associated with VWM, and this may decline relative to controls as task demands increase. These findings may help to explain why MS patient performance decreases as a function of effort on neuropsychological tests. *Hum Brain Mapp* 27:28–36, 2006. © 2005 Wiley-Liss, Inc.

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INTRODUCTION

Functional neuroimaging studies have mapped a network of brain regions associated with verbal working memory

(VWM) [Smith and Jonides, 1997]. VWM-associated activity has been consistently reported within dorsolateral prefrontal cortices (DLPFC; anterior middle and inferior frontal gyri), supplementary motor area (SMA; medial superior frontal gyri), premotor cortices (PMC; posterior middle and superior frontal gyri), Broca's area (left inferior frontal gyrus), and posterior parietal cortices (PPC; inferior parietal lobule). In addition, increased VWM task difficulty has been related to increased activity in these brain regions [Braver et al., 1997; Smith and Jonides, 1997].

Approximately half of multiple sclerosis (MS) patients exhibit cognitive impairment [Beatty et al., 1989; McIntosh-

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Michaelis et al., 1991; Rao et al., 1991]. Deficits are most often reported on difficult measures of attention, memory, executive function, and cognitive processing speed. For instance, Piras et al. [2003] reported mild attention and short- and long-term memory impairments consistent with slowed processing speed in a recent longitudinal study. A consistent finding is that patients exhibit impairments on the Paced Auditory Serial Addition Test (PASAT), a difficult VWM task that taxes each of these domains [Beatty et al., 1989; Rao et al., 1991]. By contrast, MS patients tend to perform normally on tests of other intellectual functions, such as language and memory storage, despite learning impairments [Beatty et al., 1989; Gaudino et al., 2001; Rao et al., 1991].

Several studies have demonstrated that in addition to impairments in typical cognitive domains, tasks that require greater effort are more likely to yield lower MS group scores. For instance, D'Esposito et al. [1996] reported that MS participants exhibited greater decreases in individual vs. dual-task performance than control participants. It has been suggested that this effect of effort is related to slower reaction times (RTs) and greater cognitive fatigue [Chaudhuri et al., 2004]. Schwid et al. [2003] reported that MS patient accuracy on the PASAT declines over time, despite unchanging task demands.

Given the substantial functional neuroimaging literature on the n-Back task and prominent VWM deficits among MS patients, this paradigm is an ideal method to examine neural correlates of these deficits. Several functional magnetic resonance imaging (fMRI) studies of VWM have been recently conducted with MS patients [Audoin et al., 2003; Mainiero et al., 2004; Penner et al., 2003; Staffen et al., 2002; Sweet et al., 2004; Wishart et al., 2004]. In order to examine possible functional correlates of MS-related VWM impairments, we administered the 2-Back task to 15 MS patients and 15 control participants during fMRI. We found that patients with matched cognitive performance exhibited more intense clusters of task-associated fMRI signal in the left DLPFC and dominant primary sensorimotor areas [Sweet et al., 2004]. Other research groups have reported greater volumes of activity within the regions normally associated with VWM during the n-Back and PASAT [Audoin et al., 2003; Mainiero et al., 2004; Penner et al., 2003; Staffen et al., 2002; Wishart et al., 2004]. In each of these studies, increased activity was interpreted as compensation for inefficient cognitive processing. Overall, these findings are consistent with the fMRI literature on localization of VWM function and provide support for compensatory activity among MS patients.

Although it is likely that MS-related neurocognitive deficits become more apparent as task difficulty increases, there is only one published report of brain activation as a function of changing task difficulty in this population [Wishart et al., 2004]. The authors presented the 1-Back and 2-Back to 10 mildly impaired MS patients. Less brain activity was observed in expected regions (associated with VWM in previous literature), while greater activity was observed in unexpected regions compared to 10 control participants. Both groups recruited larger volumes in response to greater

VWM demands; however, group differences by difficulty level were not significant.

In sum, fMRI studies of VWM among MS patients tend to provide evidence of shifting patterns of underactivity in expected VWM-related areas and overactivity in both expected and unexpected areas. Response to increasing difficulty level needs to be directly tested in larger, well-matched, and more impaired samples, with a larger range of task demands. We examined whether MS patients exhibit greater increases in brain activity than control participants as a function of greater effortful task demands in regions associated with VWM. MS patients were expected to respond more slowly and less accurately as VWM demands increased. Lesion load was expected to negatively impact performance and associated activity.

SUBJECTS AND METHODS

Participants

Fifteen right-handed relapsing-remitting MS patients (4 male and 11 female) were recruited from a group of 59 patients who completed an 8-year community-based longitudinal study of MS [Rao et al., 1991]. Duration of illness was at least 10 years for each patient at the time of this study (since symptom onset: mean = 21.36, SD = 4.60; since diagnosis: mean = 17.43, SD = 3.50). Mean Kurtzke Expanded Symptom Scale score was 2.27 (median and mode were 1.5) [Kurtzke, 1983]. Of nine out of 15 patients taking medication, immunomodulators ($n = 4$), replacement hormones ($n = 4$), corticosteroids ($n = 2$), analgesics ($n = 2$), and anti-incontinence medications ($n = 2$) were the most commonly prescribed. Fifteen right-handed control participants (4 male and 11 female) were recruited from a pool of 67 healthy control (HC) participants who completed the same longitudinal study. All participants included in this study were between the ages of 35 and 61. Potential participants were excluded if they refused to participate (7 MS; 9 HC), were unreachable (e.g., moved; 4 MS; 8 HC), were older than 65 years of age (21 MS; 32 HC), had a history of psychiatric or neurological disorder (other than MS; 1 HC), reported new metallic implants (1 MS), did not fit into the scanner (5 MS; 2 HC), or demonstrated poor performance on a pegboard task. Five of the 59 prospective MS participants were excluded because their performance on a modified pegboard task was more than 1 SD below the MS group mean assessed during the longitudinal MS study (no HC participant scored this low). Screening for motor impairment and age was done due to the sensitivity of the fMRI procedure to movement artifact and decreased reliability of fMRI signal known to occur among older adults [Huettel et al., 2001]. One additional MS patient was excluded during fMRI scanning due to equipment problems. Groups did not differ in age (MS mean = 47.27, SD = 6.76; HC mean = 48.07, SD = 6.28; $t(28) = -0.272$, $P = 0.713$), education (MS mean = 14.67, SD = 2.40; HC mean = 13.86, SD = 2.21; $t(28) = 0.508$, $P = 0.616$), or previously assessed verbal IQ (MS mean = 108.97, SD = 12.06; HC mean = 104.4, SD = 14.19; $t(28)$

$= -0.916, P = 0.368$). All 30 participants received at least one previous MR brain scan as part of the longitudinal study. More detailed participant recruitment information is described elsewhere [Rao et al., 1991].

Cognitive Task

The n-Back task was administered to assess VWM. Stimulus presentation parameters were nearly identical to other functional neuroimaging studies of the n-Back [Braver et al., 1997; Smith and Jonides, 1997; Sweet et al., 2004]. During this task 18 series of individual consonants were presented visually—one letter every 3 s. Participants were asked to respond only if a presented consonant was the same as a previously presented reference consonant. A 0-Back control condition was alternated with one of three experimental conditions. The 0-Back task consisted of 18 series of nine consonants of random case, three of which (33%) were targets (case varied to encourage verbal encoding rather than visual matching). Participants were given a fixed reference consonant and asked to respond when it appeared. The 1, 2, and 3-Back were presented as three separate experimental conditions. Each of these tasks consisted of 15 consonants of random case, five (33%) of which were targets. In the experimental conditions, a consonant was considered a target if it was the same as the consonant presented three (3-Back), two (2-Back), or one (1-Back) earlier, depending on the condition (e.g., s, G, S, d, V, D, v during the 2-Back). In addition, each consonant list contained two foils from each of the other experimental condition lists (e.g., all 2-Back lists contained two 1-Back targets and two 3-Back targets). Six runs were presented. Each run included three n-Back blocks and three 0-Back blocks. Experimental conditions were arranged in pseudo-random order across runs, using all possible combinations of 1, 2, and 3-Back. The n-Back task has become a widely employed measure of VWM in functional neuroimaging studies [Awh et al., 1996; Braver et al., 1997; Smith and Jonides, 1997; Penner et al., 2003; Sweet et al., 2004; Wishart et al., 2004]. It requires buffering and updating consonants, matching, encoding, and responding. Typical activation patterns reported by these investigators include bilateral DLPFC, PPC, PMC, and SMA.

Apparatus

The n-Back task was presented using Superlab Pro for Windows (<http://www.superlab.com>) installed on a laptop computer. The computer video signal was back-projected onto a screen positioned 200 cm from the participant's nose using an LCD projector. Participants viewed the screen through prism glasses and corrective lenses, if needed, which were attached to the head coil. An MR-compatible piano-key response box was attached to the computer.

Procedure

After informed consent was obtained, each participant was instructed in and practiced the n-Back task. Practice consisted of nine 0-Back, two 1-Back, three 2-Back, and three

3-Back blocks, which lasted 10 min, 30 s, (excluding pauses between every block). After practice, participants applied earplugs and lay supine on the scanner table with their head in the head coil. Prism glasses were positioned and line of sight checked. High-resolution structural scans were acquired before the n-Back tasks were presented with concurrent functional echoplanar imaging. Finally, FLAIR, T2-weighted, and proton density scans were conducted on MS patients. There was an interval of at least 20 min between practice and testing.

During functional image acquisition, consonants were presented using bold 200 point Arial font. The actual size of the consonants was larger due to back-projection techniques, but remained constant between participants. Each consonant was presented for 500 ms with an interstimulus interval of 2,500 ms. Consonants were arranged in a pseudo-random order from a list of all consonants except "L" due to ambiguity in lower case form.

Imaging Methods and Measures

Whole-brain fMRI was conducted with a commercial GE 1.5 T scanner equipped with a three-axis local gradient head coil and elliptical endcapped quadrature radio frequency coil. Echoplanar images were collected using a single-shot, gradient-echo echoplanar pulse sequence (TR = 3000; TE = 40 ms; FOV = 240 × 240 mm; matrix size = 64 × 64 voxels). A sufficient number of contiguous 7-mm thick sagittal slices were selected to provide coverage of the entire brain. Minor anatomic distortions attributable to local field inhomogeneities were corrected using a shimming procedure. Prior to functional imaging, high-resolution (matrix size = 256 × 256) 1.2-mm thick spoiled gradient recall at steady state (SPGR) anatomical scans were obtained of the entire brain in the sagittal plane.

All dataset processing and statistical analyses were conducted using Analysis of NeuroImage software (AFNI) [Cox, 1996]. Each time series was spatially registered to the third volume of the session using an iterative linear least-squares method to reduce the effects of head movement. This AFNI 3D registration program also yields a root-mean-square difference for each image, which was used to screen for uncorrected movement. No participants were excluded on this basis.

A local baseline subtraction method was used [e.g., Frost et al., 1999; Rao et al., 1997; Sweet et al., 2004]. For each voxel the mean blood oxygen level-dependent (BOLD) signal detected over 14 adjacent 0-Back task volumes (seven pre and seven post) were subtracted from BOLD signal averaged over 13 volumes in each n-Back task block. The first two brain volumes of every block were ignored to exclude hemodynamic transition periods. For each voxel, this technique yielded a set of six difference scores representing mean activation during each n-Back condition minus the mean local baseline (0-Back) activation. It also minimized error associated with linear drift.

The resulting six difference values for each participant were used to create summary statistical parametric maps

TABLE I. n-Back performance by group and difficulty level

	Multiple sclerosis	Healthy controls	Significance (<i>P</i>)
RT (ms)			
0-Back	546.53 (89.06)	502.43 (65.90)	ns
1-Back	649.40 (160.88)	543.33 (75.25)	<0.05
2-Back	776.41 (260.11)	639.37 (192.94)	ns
3-Back	816.14 (267.30)	649.67 (209.26)	<0.10
Accuracy (%)			
0-Back	96.59 (2.24)	97.65 (0.89)	ns
1-Back	88.04 (6.60)	85.96 (13.00)	ns
2-Back	73.40 (15.88)	74.49 (19.28)	ns
3-Back	66.27 (14.38)	64.63 (12.66)	ns
Commission errors (%)			
0-Back	0.85 (1.32)	0.37 (0.66)	ns
1-Back	8.11 (8.38)	5.78 (6.95)	ns
2-Back	9.33 (9.08)	7.33 (5.30)	ns
3-Back	7.67 (6.98)	5.78 (4.12)	ns

Values are expressed as mean (SD), unless otherwise indicated.

(SPMs) and as a dependent variable in tests of the hypotheses. In order to combine and compare datasets across participants, individual anatomical and functional volumes were linearly interpolated to volumes with isometric 1 mm³ voxels, coregistered, and transformed into standard stereotaxic space [Talairach and Tournoux, 1988]. To compensate for normal variation in anatomy across participants, the stereotactically resampled 3D datasets were spatially blurred over a 3-mm radius using a Gaussian kernel.

To create summary SPMs, six difference values per n-Back level were compared to a hypothetical mean of zero for each voxel of each participant using pooled-variance Student's *t*-tests. Positive values were averaged for each group and a one-tailed threshold ($P < 0.0005$) was used to reduce Type I error associated with multiple comparisons. This procedure resulted in mean summary SPMs of *t*-values for each n-Back level for each group.

To test hypotheses, a 2×3 (group by n-Back difficulty level) repeated-measures ANOVA was conducted for each voxel using individually averaged mean difference scores as the dependent measure of intensity. Main effects were examined among the full sample and separately for each group to identify brain regions in which activity changed as a function of difficulty level. Significance thresholds for these were set at $P < 0.0001$ to avoid Type I error associated with multiple comparisons. Since MS patients exhibited no regions of significant change in this test of our hypotheses, a lower threshold ($P < 0.005$) was applied to explore regions that might be associated with effort in this group.

Tests of hypotheses about group differences were performed using three voxel-wise between-group independent-samples *t*-tests. For these analyses we employed a two-tailed voxel threshold of $P < 0.001$ and cluster threshold of 347 μ l based on permutations of randomized group assignment to estimate Type I error across multiple comparisons. Clusters were identified using commercially available Talaraich Daemon software and confirmed by visual inspection.

Total lesion area (TLA) was quantified using University of Texas Image Tool software (<http://ddsdx.uthscsa.edu>),

which yielded lesion areas in cm² per slice. Tracings were performed on axial fluid-attenuated inversion recovery (FLAIR) images (TR = 10,000; TE = 2600 ms; FOV = 220 × 220 mm; matrix size = 256 × 256, in 5-mm thick slices with 2-mm gaps). The ventricles and precentral and post-central gyri, with underlying white matter, were excluded from tracings to produce a more sensitive measure to relate to higher cognitive function. Volume and intensity of brain activity was also individually averaged in this region during 1-, 2-, and 3-Back blocks in order to relate lesion load to volume and intensity of activity. Volume was the brain area that exceeded the significance threshold (as above) and intensity was the average mean difference score (as above) within these volumes. TLA and volume of activity were expressed as ratios to brain area (minus ventricles and sensorimotor regions) in order to account for individual differences in brain size. To avoid experimenter bias, all image files were randomly assigned a subject number before tracing began. Correlation coefficients were inspected for relationships of lesion load to behavioral and functional imaging measures.

Behavioral Data

Analyses of behavioral data included three 2×4 (group × n-Back level) repeated-measures ANOVAs. The first was calculated using percent accuracy as the dependent variable (DV), the second used median RT as the DV, and the third used percent (of total possible) commission errors as the DV. Significance levels were set at $P < 0.05$ using Fisher's protected *t*-test procedure to avoid Type I Error associated with multiple comparisons.

RESULTS

Verbal Working Memory Task Performance

All participants performed above chance during each condition (Table I). ANOVAs revealed slowed median RTs among the patients ($F(1) = 4.355$, $P = 0.046$), but no signif-

Figure 1.

Brain activity associated with n-Back tasks by group and difficulty level. **A:** Overlay of group statistical parametric maps showing significant activity compared to the 0-Back task ($P < 0.0005$). Corresponds to Table II, top. **B:** Regions that exhibited significant change across n-Back levels. Insert shows regions of decreasing activity. Significance thresholds were more stringent due to larger effect sizes. Corresponds to Table II, middle. **C:** Regions that exhibited significant group differences ($P < 0.001$). Corresponds to Table II, bottom.

icant group differences in accuracy ($P = 0.858$) and neither RT nor accuracy group by n-Back level interactions were significant ($P > 0.285$). Group contrasts of RT were significant during the 1-Back ($t(28) = 2.285$, $P = 0.030$), while the 3-Back difference was considered a trend ($t(28) = 1.922$, $P = 0.065$) and the 0-Back and 2-Back differences were not significant ($P > 0.112$). Main effects for n-Back level were significant for RT ($F(2.022) = 21.269$, $P < 0.001$) and accuracy ($F(3) = 62.546$, $P < 0.001$). Differences were significant between every level ($P < 0.036$), except 2-Back compared to 3-Back RT ($P < 0.275$), such that accuracy declined and RT increased as difficulty level increased. Commission error rates did not significantly differ between groups ($F = 0.884$,

$df = 1$, $P = 0.355$). The main effect of difficulty level was significant ($F = 19.916$, $df = 3$, $P < 0.001$) and there was no group by n-Back level interaction ($F = 0.284$, $df = 3$, $P = 0.837$). Commission rates significantly increased with each increase in n-Back level ($P < 0.046$), except from 1-Back to 2-Back ($P = 0.209$).

Functional Imaging Data

Group summary SPMs are presented in Figure 1A and Table II. As expected, the n-Back task elicited greater intensity in bilateral SMA (1), PMC (2), DLPFC (3), and PPC (4), and near Broca's (8) area during each difficulty level com-

TABLE II. Brain activity associated with the n-Back by group and difficulty level

Clusters of activity	x, y, z	Region
Associated with the n-Back tasks*	$\pm 00, -13, 48$ $\pm 29, 02, 51$ $\pm 45, -10, 28$ $\pm 36, 54, 40$ $\pm 34, -47, 10$ $\pm 33, 15, 10$ $\pm 11, 19, 14$ $52, -10, 25$ $\pm 17, 50, -23$	Bilateral medial and superior frontal gyri (1) Bilateral middle frontal gyri (2) Bilateral middle and inferior frontal gyri (3) Bilateral inferior and superior parietal lobule (4) Bilateral anterior middle frontal gyri (5) Bilateral insula (6) Bilateral dorsal thalamus (7) Left inferior frontal gyrus (8) Bilateral dentate (not shown in figure 1)
Associated with increasing n-Back difficulty level**	$\pm 00, -12, 44$ $\pm 29, 02, 51$ $-45, 39, 44$ $43, 30, 15$ $\pm 00, -04, 46$ $-42, -30, 38$ $\pm 47, 03, 03$ $\pm 00, 13, 11$ $11, -14, -03$ $42, 25, 57$ $\pm 17, 50, -23$	Bilateral medial and superior frontal gyri (1) Bilateral middle and inferior frontal gyri (2) Right inferior parietal lobule (3) Left superior temporal gyrus (4) Bilateral anterior cingulate gyri (5) Left anterior middle frontal gyrus (6) Bilateral insula (7, not completely shown) Bilateral thalamus (8) Left anterior caudate nucleus (9, not completely shown) Left precentral and postcentral gyri (10) Bilateral dentate (not shown)
In which groups differed during the n-Back tasks***	$42, 25, 57$ $05, 05, 55$ $\pm 00, -10, 25$ $-20, 37, 32$ $-23, -40, 43$ $-48, -22, 39$ $50, 35, 17$ $-45, 39, 44$ $36, 18, 22$ $-33, 17, -14$	Left precentral and postcentral gyri (1) Left medial frontal gyrus (2) Bilateral anterior cingulate gyri (3) Right posterior cingulate gyrus (4) Right superior frontal gyrus (5) Right middle frontal gyrus (6) Left superior temporal gyrus (7) Right inferior parietal lobule (8) Left insula (9) Right parahippocampal gyrus (10)

*Reference in Figure 1A.

**Reference in Figure 1B.

***Reference in Figure 1C.

pared to the 0-Back control task. Additional areas of activity compared to baseline included bilateral dorsal thalamus (7), insula (6), and cerebellum (not shown). Qualitative comparisons of group summary SPMs suggest several regions in which recruitment may differ between groups. Although these regions were significantly active compared to baseline in both groups, larger regions of recruitment were evident among the MS group during all n-Back levels in the anterior right hemisphere. Larger regions of PPC activity were apparent among the HC group at every n-Back level. Significant right dorsal thalamic activation was noted only among the MS group during the 1-Back and only among the HC group during 2-Back. Bilateral thalamic activity was noted in both groups during the 3-Back; however, it was more evident among the HC group.

Brain response to increased VWM demands is shown in Figure 1B. Voxels are highlighted that exhibited significant change in mean intensity as n-Back difficulty level increased. The largest clusters of increased intensity among all 30 par-

ticipants were located in anterior regions. As expected, these regions also included bilateral SMA and PMC (1 and 2), right DLPFC (6), anterior PPC (3), and clusters near Broca's area (2 and 7). Additional regions of increased activation were detected in the bilateral cerebellum (not shown) and dominant primary motor cortex (10).

It appears that the HC group activity primarily influenced these full sample results because the HC-only main effects (Fig. 1B) were similar to the full sample main effects, except thalamic change was also evident and clusters appeared larger, more intense, and more symmetrical. In contrast, no clusters remained significant following the MS-only voxel-wise ANOVA (Fig. 1B). Thus, the one-tailed threshold of the MS-only main effects was set at $P < 0.005$ to explore sub-threshold activation trends. The SPM of MS-only main effects for difficulty level shares only three clusters in common with the full sample main effects, and only the SMA/superior anterior cingulate (5) increased in intensity. Since F-values are shown, the direction of intensity change was

determined by follow-up *t*-tests. Among the HC group, the intensity of all clusters significantly increased. However, among the MS group left insula (posterior to 7), left superior temporal gyrus (4), left anterior caudate (9), and bilateral anterior cingulate (anterior to 5) declined in intensity as difficulty level increased.

Group contrasts are presented in Figure 1C. MS patients exhibit greater activation in dominant primary motor and somatosensory cortices (1) at every level. MS patients also demonstrated greater activity in the right SMA (2) during the 1-Back, greater activity in the left SMA during the 2-Back, and less intensity in left SMA during the 3-Back. Clusters of greater MS-group activity were evident within right anterior regions (DLPFC and PMC; 5 and 6) left insula (9), and left superior temporal gyrus (7) during the 1-Back. Differences during the 2-Back included greater HC-group intensity in the right anterior cingulate (3), right posterior cingulate (4), and bilateral right parahippocampal gyri (10). In addition to the noted differences in primary sensorimotor cortices and SMA, MS patients exhibited greater activity in left PPC during the 3-Back.

Lesion Load

Mean TLA was 21.41 cm² (median = 21.67, SD = 17.74). TLA ratio was unrelated to RT or accuracy at any n-Back level ($P > 0.136$). Larger TLA ratio was significantly related larger volumes of 1-Back activity ($r = 0.63$, $P = 0.013$) and tended to be associated with larger volumes during the 3-Back ($r = 0.473$, $P = 0.075$). Larger TLA ratio also tended to be associated with more intense activity during the 1-Back. No other intensity measures were related to TLA ratio ($P > 0.354$).

DISCUSSION

This study extends our knowledge of VWM among MS patients by describing relationships between varying task demands, behavioral performance, lesion load, and concurrent brain function. Previously, we reported that MS patients matched on 2-Back performance exhibited overactivity in left prefrontal and dominant sensorimotor regions and underactivity in regions not typically associated with VWM [Sweet et al., 2004]. In the current study we found that MS patients did not differ from control participants in VWM performance, although they responded slower in some conditions. We also confirmed that abnormal brain activity occurred in regions typically associated with VWM. The current results extend previous findings by demonstrating that MS patients exhibited different brain activity in response to increasing difficulty levels compared to matched control participants. Specifically, MS patients exhibited normal patterns of VWM-related recruitment, with greater volume and intensity compared to controls, and a diminished response to increased VWM demands. Recruitment of unexpected brain regions (e.g., reorganization) was not observed. Our results suggest a bilateral compensatory response in expected regions during low VWM demands and less additional recruitment in response to greater VWM demands

Patients utilized expected cortical regions, but with larger and more intense volumes during the 1-Back, particularly in right anterior regions. Bilateral recruitment has been reported as a normal compensatory mechanism in response to increased difficulty level in previous n-Back neuroimaging studies [Awh, 1996; Smith and Jonides, 1997], and was evident in our control group. Several studies of other neurological patient groups and older adults have also reported increased bilateralization of activity, especially when groups were matched for performance [see Cabeza, 2002; Cohen et al., 2004]. Group contrasts also support this conclusion. Right anterior activity was significantly greater among MS patients during the 1-Back, but not the 2-Back. According to the group summary maps, these right hemisphere areas are recruited in both groups. Therefore, a lack of group differences in performance during the 2-Back suggests that the HC group began to recruit these contralateral regions at an intensity first evident among the MS patients during the 1-Back.

Greater activity in dominant primary somatosensory and motor areas may reflect a compensatory mechanism in a brain region associated with known physical impairments among MS patients. This has been previously reported in primary motor regions among MS patients [Reddy et al., 2002; Rocca et al., 2003] and supports the notion that greater activity in association cortices might be a more sensitive measure of deficit than behavioral performance.

As WM difficulty increased, a cortical-limbic interaction was evident. In response to higher VWM demands, the HC group recruited larger and more intense volumes in anterior regions already utilized among the MS group during the less difficult 1-Back. Meanwhile, MS patients may have begun to abandon nonessential neural processing in limbic regions order to focus. Given reported functions associated with the anterior cingulate and parahippocampal gyrus, this might represent less conflict monitoring and consolidation of episodic information into long-term memory [Garavan et al., 2002; van Veen and Carter, 2002]. A lack of group differences in most of these limbic cortices during the 3-Back suggests that the HC group might also have employed this strategy during the most difficult VWM challenge. These regions have not been consistently associated with this task and relationships to VWM processing remain to be identified.

Greater right PPC and less left SMA intensity were observed among the MS group during the 3-Back. We speculate that the reversal of direction of group differences in the SMA from 2-Back to 3-Back may reflect fatigue among the MS group, as RT also tended to slow at this level relative to the HC group. Greater right PPC activity might be explained as compensatory bilateral activity. These possibilities need further study.

PPC activity was clearly associated with the n-Back performance in each group at each difficulty level; however, only right PPC significantly changed as the difficulty level increased among the full sample. This finding is different from previous n-Back studies of HC groups, which show

bilateral, or left-lateralized increases [Smith and Jonides, 1997]. These unexpected activation patterns were attributed to a diminished response to difficulty among the MS patients. Despite a lack of group differences in the left PPC at any level, a separate repeated-measures ANOVA including only HC participants revealed significant intensity increases in bilateral PPC, while the MS-only analyses lacked these main effects, even at a less stringent exploratory threshold. This suggests that MS patients did not normally recruit this region at more difficult levels, or that it had already been recruited to maximal capacity during the 1-Back.

Greater lesion load was not related to any measure of performance; however, it was associated with larger volume of significant activity and a tendency for greater intensity in these volumes during the 1-Back. This is interesting because this is the condition in which MS patients responded significantly slower and exhibited greater brain activity compared to controls. Therefore, under low task demands, greater activity was related to greater lesion load and slower RT. The tendency for a positive relationship between lesion load and volume of activity during the 3-Back is intriguing because patients also tended to respond slower during this condition. Thus, it appears that lesion load and brain activity were most related when performance slowed compared to controls.

Our results differ from previous fMRI studies of VWM processing among MS patients in that we did not find significant reorganization of function. Our method was a comprehensive assessment of group comparisons, as we examined recruitment and changes in signal intensity among larger and well-matched community-based samples. More importantly, we directly compared activity across groups and conditions without first thresholding. In some previous studies investigators have tallied voxels that were significantly related to the task paradigm before comparing tallies between groups. Thus, studies employing these methods are unable to conclude if groups actually differ in those voxels that they have tallied. Moreover, significance thresholds are typically set extremely high in fMRI experiments to avoid Type I error associated with multiple comparisons. By definition, this substantially increases Type II error. Type II error is particularly important when trying to determine if reorganization has taken place because "real" task-related activity is very likely ignored when the voxels are labeled active or not. A second problem is that categorization of a voxel as task-related or not results in a loss of information about variance, range is restricted, and parametric statistical assumptions are violated.

The lack of expected performance deficits was probably due to the high functional status of the MS group and sample sizes that may have been too small to detect differences in RT. Each patient had completed an 8-year study and was screened for severe motor impairment. Furthermore, our subsamples did not differ on PASAT scores ($P > 0.421$) during that study. On the other hand, a lack of accuracy differences and modest differences in RT were taken as evidence that different brain activity was less likely due to

unequal cognitive performance. Other limitations to this study include uncertainty about equity of the baseline. It is possible that MS patients began the experiment more fatigued than controls. Future experiments might carefully design baseline tasks to allow matching for fatigue level and potential differences in baseline activity. In this study the lack of performance differences during the 0-Back lends some support for its use as a baseline. Finally, the TLA results were disappointing. Future work might use smaller and more specific lesion load measures for comparison to behavior and brain activity.

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