

Fornix damage limits verbal memory functional compensation in multiple sclerosis

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ABSTRACT

Selective atrophy of the hippocampus, in particular the left CA1 subregion, is detectable in relapsing-remitting MS (RRMS) and is correlated with verbal memory performance. We used novel high-resolution imaging techniques to assess the role that functional compensation and/or white matter integrity of mesial temporal lobe (MTL) structures may play in mediating verbal memory performance in RRMS. High-resolution cortical unfolding of structural MRI in conjunction with functional magnetic resonance imaging (fMRI) was used to localize MTL activity in 18 early RRMS patients and 16 healthy controls during an unrelated word-pairs memory task. Diffusion tensor imaging (DTI) and Tract-Based Spatial Statistics (TBSS) were used to assess the integrity of the fornix and the parahippocampal white matter (PHWM), the major efferents and afferents of the hippocampus. RRMS patients showed greater activity in hippocampal and extra-hippocampal areas during unrelated word-pair learning and recall. Increased hippocampal activity, particularly in the right anterior hippocampus and left anterior CA1 was associated with higher verbal memory scores. Furthermore, increased fractional anisotropy (FA) in the fornix was correlated with both greater fMRI activity in this region and better memory performance. Altered hippocampal fMRI activity in RRMS patients during verbal learning may result from both structural damage and compensatory mechanisms. Successful functional compensation for hippocampal involvement in RRMS may be limited in part by white matter damage to the fornix, consistent with the critical role of this pathway in the clinical expression of memory impairment in MS.

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Introduction

Multiple sclerosis (MS) is an autoimmune disorder causing demyelination of the central nervous system. Early symptoms often include sensory disturbances, limb weakness, and fatigue. A hallmark of MS-related imaging findings is focal, inflammatory, white matter plaques in the brain and spinal cord (Noseworthy et al., 2000). Although most clinical measures of disease severity reflect sensorimotor function, 40 to 65% of MS patients also demonstrate cognitive dysfunction (Amato et al., 2006).

Abbreviations: RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; MTL, mesial temporal lobe; fMRI, functional magnetic resonance imaging; DTI, diffusion tensor imaging; FA, fractional anisotropy; TBSS, tract-based spatial statistics; CA1, cornu ammonis 1; CA23DG, cornu ammonis 2–3 and dentate gyrus; AntCADG, Anterior CA1–3 and dentate gyrus; Sub, subiculum; PHC, parahippocampal cortex; PRC, perirhinal cortex; ERC, entorhinal cortex; Fus, fusiform gyrus; PHWM, parahippocampal white matter; TR, repetition time; TE, echo time; NEX, number of excitations; FOV, field of view; TI, inversion time; EPI, echo planar imaging; BOLD, blood oxygen level dependent.

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Cognitive deficits in MS vary, but affected domains can include memory, attention, processing speed, visuospatial and executive functions, but usually not intellectual or language functions (Bobholz and Rao, 2003). Among the earliest reported are memory deficits in both retrieval (Rao, 1986) and encoding (DeLuca et al., 1994) abilities. Although memory impairment in MS is progressive (Thornton and Raz, 1997), it develops independently of traditional disease markers such as relapse-rate or total lesion load (Duque et al., 2008). Recent studies have shown that cognitive impairment in MS is best predicted by degenerative changes beyond white matter damage such as global atrophy (Chard et al., 2002), cortical lesions (Geurts et al., 2005), cortical thinning (Sailer et al., 2003), and subcortical atrophy (Cifelli et al., 2002).

The hippocampus is a subcortical structure essential for memory function (Milner, 1958) and is impacted in MS as evidenced by decreased glucose metabolism (Paulesu et al., 1996), increased inositol levels suggesting gliosis (Geurts et al., 2006), and demyelination detected in vivo and verified on pathological sections (Geurts et al., 2005; Roosendaal et al., 2008). We recently demonstrated early selective hippocampal volume loss in the Cornu Ammonis 1 (CA1) subregion as well as overall hippocampal volume loss, both of which correlated with decreased performance on a verbal learning task,

but not information processing speed, in relapsing-remitting MS (RRMS) and secondary-progressive MS (SPMS) patients (Sicotte et al., 2008). Post-mortem histological analysis suggests that hippocampal atrophy may result from decreased neuronal numbers in the cornu ammonis subfields, as well as decreased neuron size in the CA1 region, although these studies were limited to SPMS patients (Papadopoulos et al., 2008).

White matter damage to hippocampal efferents and afferents may also contribute to memory impairment in MS. A voxel-based lesion-function study found that declarative verbal memory deficits in MS were associated with visible lesions in several frontal-temporal-limbic pathways (Sepulcre et al., 2008) and altered white matter diffusion metrics have been detected in the inferior longitudinal fasciculi and the fornix (Roosendaal et al., 2009). White matter lesions have also been detected in the fornix post-mortem in MS (Huitinga et al., 2001). The fornix is the major output of the hippocampus, but it also allows reciprocal connections with the septal nuclei, the anterior thalamic nucleus and the hypothalamus, which modulate hippocampal activity (Duvernoy, 2005). Fornix damage can cause severe memory deficits (Gaffan and Gaffan, 1991), though its role in MS-related memory impairment has not been studied in detail. The parahippocampal white matter (PHWM) consists of several hippocampal afferents such as the perforant pathway and the cingulum bundle in addition to reciprocal projections relaying multimodal sensory information primarily through the entorhinal cortex.

The presence of MS-related structural changes in the mesial temporal lobe (MTL) is evident, but the link between structure and function has not been fully elucidated. Functional magnetic resonance imaging (fMRI) can be used to localize changes in regional blood flow during cognitive tasks as a surrogate for neural activity. Traditional fMRI techniques are challenging to apply to the hippocampus given the convoluted neuroanatomical structure and susceptibility to signal distortion that occurs particularly in the anterior MTL. To overcome these limitations, we used high-resolution structural and functional imaging in conjunction with a novel cortical unfolding technique (Zeineh et al., 2000) to accurately detect subregional functional activity in the hippocampus and MTL in RRMS patients and controls. Furthermore, we evaluated the fornix and the PHWM using diffusion tensor imaging (DTI) to assess the relation between white matter damage to hippocampal efferents and afferents, MTL fMRI activity, and verbal memory performance in MS.

Subjects, materials and methods

Subjects

Subjects included 18 patients with clinically definite (Poser et al., 1983) RRMS and 17 healthy controls. The UCLA Human Subjects Protection Committee approved the protocol and informed consent was obtained. RRMS patients had disease duration less than 5 years from diagnosis and had not relapsed nor received steroids within the previous 3 months. No subject had a history of drug or alcohol abuse in the last 3 years. Controls did not suffer from any neurological conditions, were not taking any medications, and had normal neurological exams and normal (≥ 28) scores on the mini-mental status exam. All subjects were right handed, had no significant visual deficits, and were able to perform the verbal memory task. A single control subject was identified as an outlier due to very poor performance on the verbal memory task and was excluded from the analyses reported here, leaving a total of 16 controls and 18 patients. These subjects represent a subgroup of those reported previously (Sicotte et al., 2008).

Verbal memory task

To assess hippocampal function, subjects were tested with an unrelated word-pairs task known to engage the hippocampus and be

sensitive to hippocampal damage (Rausch and Babb, 1993). Seven different pairs of unrelated words (e.g. shelf: noisy) were presented one pair at a time in sequence for 30 s during the encoding phase. A 20 s rest period preceded the recall phase, in which one word from each pair was presented in sequence and the subject tried to remember the other word. The same 7 word-pairs were repeated over 6 trials. The number of words successfully recalled in each trial was recorded. The number of word-pairs successfully recalled in each of the 6 trials was added to create an overall performance score with a maximum of 42 (i.e. a subject who immediately recalls all 7 words in the first trial and subsequently in all the remaining trials receives a score of 42). This score reflects both speed and quantity of memory acquisition.

Before scanning, the test was administered and scored by a trained researcher to familiarize the subject with the task and accurately assess performance. In the scanner, a different set of 7 word-pairs was presented through earphones and displayed visually using MR compatible goggles and MacStim software (Darby, 2004). During the encoding phase, the first word of a pair was presented for approximately 1 s, and then the second word was presented adjacent to it for about 2 s, followed by a blank screen for 1 s before the next word pair presentation. During the recall phase, one word of each pair was presented for about 3 s and subjects were instructed to recall the second word silently (to avoid head motion) and press a button with their index finger to indicate if they remembered the second word, or to press a different button with their middle finger if they could not recall the second word. Word pair order was randomized across trials. The rest phase involved a distracter task in which subjects pressed a button whenever the presented symbol changed about every 6 s. All subjects were able to successfully perform the distracter task. A trained researcher assessed actual memory performance in the scanner with a post-test of a single recall block of the same 7 word-pairs presented in the scanner obtained immediately following the scan. The fMRI paradigm is shown in Fig. 1F.

MRI acquisition

Subjects were scanned on a Siemens Allegra 3.0 T MRI scanner. DTI was obtained with one b0 with no diffusion weighting and 12 non-collinear diffusion encoded spin echo EPI images with a single b-value of 900 s/mm². Seventy-five contiguous axial slices were acquired (TR = 10,200 ms, TE = 84 ms, Matrix = 128 × 128, FOV 256 mm, 2NEX, 2 mm³). The DTI scan was administered twice and the images were averaged to increase signal to noise. A sagittal localizer was used to identify the long axis of the hippocampus. All subsequent 3.0 T scans were aligned in the same oblique coronal plane perpendicular to the long axis of the hippocampus.

A high-resolution T2 SE pulse sequence (TR = 5200, TE = 105, 2 NEX, FOV = 200 mm, Matrix = 512 × 512, 3.00 mm, no gap) was acquired for structural hippocampal segmentation and unfolding. This scan (0.391 mm × 0.391 mm × 3 mm) encompassed the head, body, and most of the tail of the hippocampus. A functional high-resolution echo planar imaging (EPI) sequence (TR = 3000, TE 39, FOV = 200 mm, Matrix = 128 × 128, 1.6 mm × 1.6 mm × 3.0 mm slices, no gap), was used to detect blood-oxygen level dependent (BOLD) signal during the unrelated word-pairs task. An EPI matched bandwidth sequence (TR = 5000, TE = 66) was acquired to facilitate alignment of functional images to structural images. A GRE field-mapping scan (TR = 500, TE1 = 4.88, TE2 = 7.3) was acquired to correct for magnetic field inhomogeneities.

On the same scanning day, each subject underwent a second sequence in a Siemens 1.5T Sonata Scanner that was part of an ongoing longitudinal natural history study. A T1-weighted scan (TR = 1900, TE = 4.38, 1 mm³) was acquired to determine total brain volume. Brain volume percentage was calculated for each subject using SIENAX

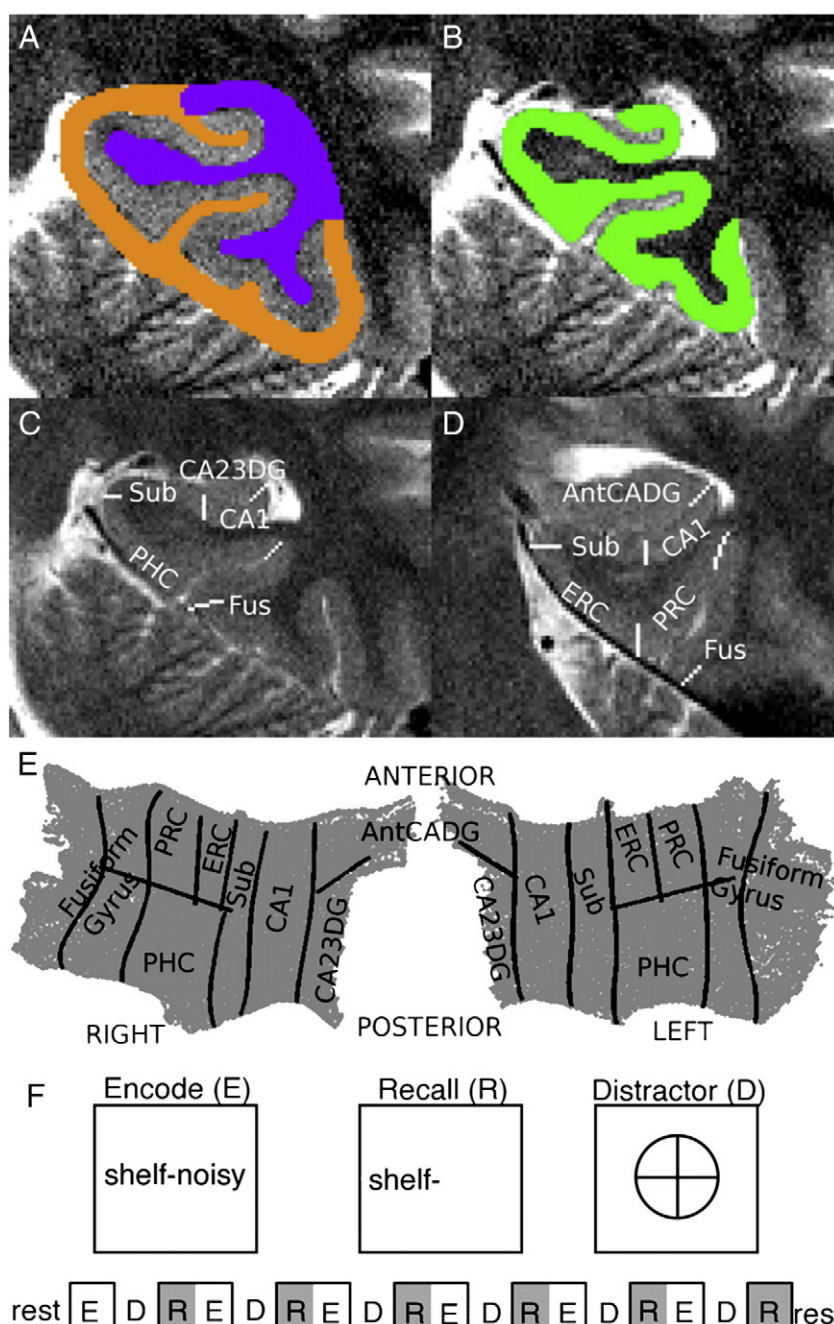


Fig. 1. Mesial temporal lobe unfolding procedure. A: White matter (purple) and CSF (orange) classifications. B: Gray matter expansion outward layer by layer from white matter classification. C–D: Subregion demarcations posteriorly (C) and anteriorly (D). E: Flattened template of hippocampus and medial temporal lobe with subregion boundaries. F: fMRI paradigm, each encode or recall block = 30 s, distraction block = 20 s. During the distraction block, subjects pressed a button when the center fixation point changed from a target to an open circle. CA23DG = cornu ammonis 2–3 and dentate gyrus, CA1 = cornu ammonis 1, Sub = subiculum, PHC = parahippocampal cortex, AntCADG = anterior cornu ammonis and dentate gyrus, ERC = entorhinal cortex, PRC = perirhinal cortex.

(Smith et al., 2002). A FLAIR scan ($TR=9000$, $TI=2400$, $TE=82$, $1.3 \times 0.9 \times 3.0$ mm) was acquired to determine T2 lesion volume.

Gray matter segmentation and unfolding

The hippocampal unfolding protocol has been described in detail previously (Ekstrom et al., 2009). First, the T2 high-resolution structural scans were used to segment and unfold the MTL region into white matter and CSF using mrGray software (Teo et al., 1997) (see Fig. 1A). Gray matter was grown between the white matter and CSF using a region expansion algorithm, resulting in a continuous volume of isotropic ~ 0.4 mm voxels that could be unfolded (Fig. 1B). Next,

mrUnfold software (Engel et al., 1997) was used to uncoil the MTL into a 2D flattened template.

Eight subregions in each hemisphere were identified in 3D space and demarcated as described previously (Zeineh et al., 2000). The subregions included the lateral and medial fusiform gyrus (Fus), the parahippocampal cortex (PHC), the perirhinal cortex (PRC), the entorhinal cortex (ERC), the subiculum (Sub), cornu ammonis 1 (CA1), a region combining cornu ammonis 2–3 and the dentate gyrus (CA23DG), and an anterior CA1–3 and dentate gyrus region (AntCADG). The CA2, CA3 and dentate gyrus were combined into one region because there are no distinguishing landmarks (Fig. 1C). In the hippocampal head, the CA1–3 and dentate gyrus were all

combined because the uncalled digitations make identifying these borders difficult (Fig. 1D) (Duvernoy, 2005). These boundaries were overlaid on the flattened 2D surface maps using the 3D to 2D transform (Fig. 1E).

Functional MRI analysis

All Individual fMRI analyses were performed using FSL (FMRIB Software Library) tools (Smith et al., 2004). After removing the first 2 volumes, motion correction and b0 unwarping were applied to the fMRI dataset. Non-brain material was removed and data was spatially smoothed with a 5 mm filter.

During the verbal memory task paradigm controls and patients learned at different rates as demonstrated in Fig. 3. The majority of new learning occurred during the first 3 blocks, although controls showed more learning in the first block. To account for differences in acquisition, individual performance curves were created based on reported learning and recall. For each subject, each block of the encoding and recall boxcar waveforms were multiplied by the number of new words that the subjects reported recalling in that block of scanning. The self-reported in-scanner memory score is a subjective indication of word-pair learning. The post test reflected an objective measure of successful word pair learning.

These weighted boxcar waveforms were smoothed and shifted with a double gamma hemodynamic response function and were separately regressed against the BOLD signal time-course at each voxel, creating functional activation images for the learning and recall tasks. Activation maps were first aligned to the matched bandwidth image, and then to the high-resolution structural image. These functional activations were then overlaid onto the 2D flat-maps using the 3D to 2D transform.

Group analysis

Individual 2D flat-maps were aligned by averaging subregion boundaries to create a group 2D space, and functional activations were warped to this common template. At each point in group space, a group t-statistic was calculated from individual activation intensities. Significant non-zero group activations with $t > 2.1$ (two-tailed $\alpha < 0.05$) were displayed on the group template (Ekstrom et al., 2009). Significant group differences were calculated using ANOVA. To correct for multiple comparisons, the alpha value was reduced based on the number of clusters (n) present at an alpha of 0.05 (corrected $\alpha = 0.05/n$). Functional activity was regressed against performance and DTI measures by multiplying the individual hemodynamic response function by the demeaned clinical data value. Within-group t-statistics were calculated from the weighted data and significantly non-zero regression coefficients were displayed on the group template.

DTI analysis of the fornix and parahippocampal white matter

DTI datasets of both averages were rigid-body aligned to the first b0 image to correct for head movement (Woods et al., 1998) and the diffusion-gradient tables were adjusted. A non-linear, 2D alignment of each slice to the first b0 corrected for eddy-current distortions (Woods et al., 1998). A tensor image and the eigenvalues, (L_1 , L_2 , and L_3) were calculated at each voxel using the 24 volume dataset and the corrected gradient tables. Fractional anisotropy (FA) was derived from these values.

Tract based spatial statistics (TBSS) (Smith et al., 2006) was applied to measure FA in the fornix and the parahippocampal white matter by constructing a group white matter skeleton using the 2nd and 3rd derivatives of FA images from all subjects non-linearly aligned to a common space (Fig. 2). The fornix was manually identified on the group FA skeleton from the tails of the hippocampi to

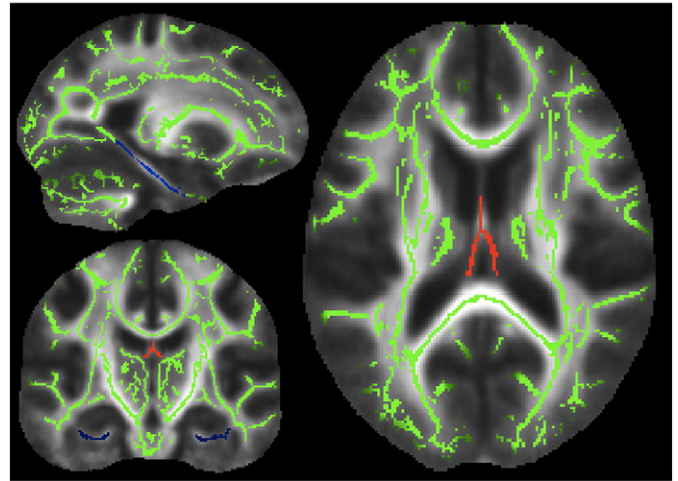


Fig. 2. DTI of the fornix and parahippocampal white matter using TBSS: TBSS was used to locate the centermost voxels of each white matter pathway for all subjects. The fornix (red) and the parahippocampal white matter (blue) were manually identified on the group template skeleton (green) and mean diffusion metrics within these regions were compared across subjects. TBSS minimizes the partial volume effects of CSF neighboring these structures.

the column of the fornix (Fig. 2), and the mean FA across this region was calculated for each subject. The PHWM was also identified on the group FA skeleton bilaterally from the hippocampal head anteriorly to the hippocampal tail posteriorly between the hippocampus and the collateral sulcus, just lateral to the parahippocampal and entorhinal cortices (Fig. 2). For each subject, the mean FA across both tracts was calculated. Student's t-test was used to compare metrics between groups, ANCOVA was used to compare group differences while correcting for age, Pearson's correlation coefficient was used to test associations between metrics and memory scores, and partial correlations were used to test these associations after correcting for age.

Results

Subjects and verbal memory performance

Table 1 shows the clinical characteristics of the subjects studied. The RRMS patients tended to be older than the control group, although not significantly. The gender ratio was similar in each group. Patients had mean disease duration of 3 years and mean Expanded Disability Status Score (EDSS) of 1.7. RRMS T2 lesion volumes averaged 7.3 cm³. Mean brain percentage fraction was reduced in patients, but not significantly.

The verbal memory pre-test score and self-reported score in the scanner were highly correlated as seen in Fig. 3. Similarly, the number of pairs learned by the last trial in the scanner was also highly correlated with the post-test administered immediately after scanning. MS patients tended to self-report higher scores in the scanner compared to the post-test results. Patients performed significantly worse on the verbal memory pre-test ($p = 0.02$), the self-reported in-scanner test ($p = 0.002$), and the post-test ($p = 0.03$).

Hippocampal functional activity during verbal learning

During new word-pair encoding, control subjects had significant functional activity confined to the hippocampal DG and CA regions. In contrast, RRMS patients showed more widespread activation of the right hippocampus, left anterior CA1, deactivations in the left CA1 posteriorly, and more change in functional activity throughout the MTL cortex beyond the hippocampus (Fig. 4). Group differences

Table 1
Subject characteristics.

	Controls n = 16	(Range)	RRs n = 18	(Range)	p-value*
Mean age in years	35.2	(24.0–50.3)	42.1	(23.0–54.5)	0.06
Females/males	14/2		14/4		0.45**
Mean years of education	17	(14–20)	16	(14–18)	0.10
Mean disease duration in years	NA		3	(1–5)	
Mean EDSS	NA		1.7	(1.0–3.0)	
Mean T2 lesion volume in cm ³	NA		7.32	(0.74–27.67)	
Mean brain percentage	0.866	(0.805–0.891)	0.852	(0.812–0.890)	0.06
Mean pre-test memory score (max = 42)	33	(21–41)	28	(14–38)	0.025
Mean in-scanner memory score (max = 42)	36	(20–41)	29	(16–39)	0.002
Mean fornix FA	0.35	(0.27–0.41)	0.31	(0.26–0.39)	0.03
Mean PHWM FA	0.43	(0.41–0.49)	0.43	(0.38–0.48)	0.98

*All tests calculated using student's 2-tailed *t*-test except **, calculated using chi-squared test.

showed that patients had significantly increased encoding activity in the left anterior CA1 and the bilateral ERC-PRC, but no significant reductions compared to controls.

During the recall phase of the learning task, controls had deactivations exclusively in the left AntCADG. RRMS patients had activation in the left CA1, subiculum, and ERC-PRC-PHC region as well as right CA1 and ERC-PRC activation. Group contrasts showed that patients had significantly greater ERC-PRC (extra-hippocampal) activity than controls but no areas of reduced activity (Fig. 5).

As described previously, functional activity for each individual was regressed against her or his novel word-pair learning curve as reported by the subject during scanning. Although this method accounts for differences in acquisition speed and reduces the influence of repeating previously-encoded information, it introduces potential errors in self-reporting of learning. We identified 3 RRMS subjects and no controls whose post-tests (assessed objectively by a researcher) were not consistent with the self-reported number of words recalled

in the final in-scanner recall block (difference > 2 word-pairs). One patient underreported her or his true learning while 2 patients over-reported their true learning. We removed these 3 subjects and reran the group analyses. For encoding and recall, the RRMS group analyses and RRMS > Controls group contrast remained largely unchanged (see Supplemental Fig. 1).

Relationship between fMRI activity and verbal memory performance

We then assessed the relationship between fMRI activity and verbal learning success to determine if involvement of bilateral and extra-hippocampal regions during learning predicted better performance. For each group we correlated fMRI activity during all learning trials with the overall performance score. In the patient group, there was a significant correlation of functional activity during encoding with overall verbal memory performance localized to the right

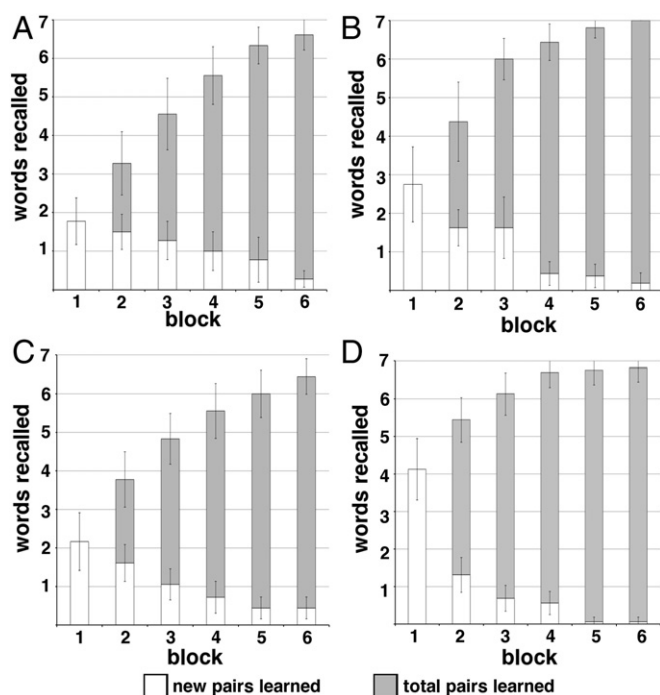


Fig. 3. Learning curves differed across groups. The number of new word-pairs learned in each block of the verbal memory pretest (white) as well as the total number of word-pairs learned (gray) are depicted for RRMS (A) and controls (B). New word pairs and total learned pairs during the in-scanner verbal memory test are also depicted for RRMS (C) and controls (D). Learning curves differed across groups and subjects, so individual learning curves were used to model the hemodynamic response of novel, successful word-pair encoding and recall in each subject.

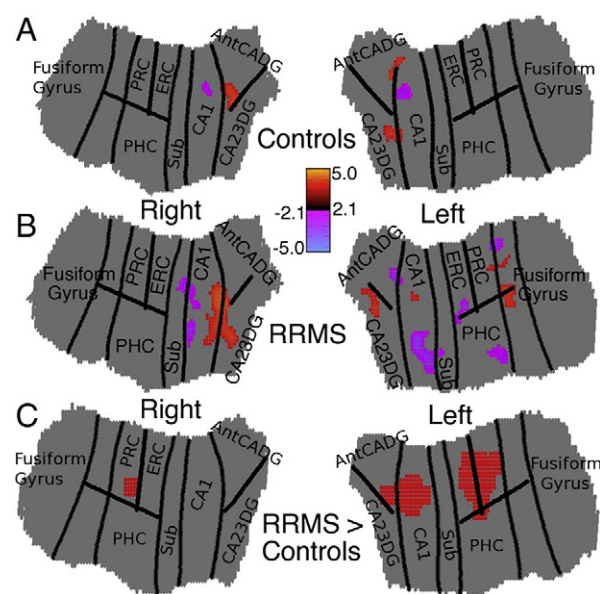


Fig. 4. Verbal Memory Encoding is characterized by greater left CA1 and bilateral MTL cortical fMRI activity in RRMS. Unfolded MTL maps depict activity significantly greater than baseline (red) and activity less than baseline (purple) during encoding in controls (A) and RRMS patients (B). Activity is weighted in each encoding trial by the number of new words successfully recalled. Activity is displayed as cluster corrected T-statistics thresholded for two-tailed $p = 0.05$. In (C), ANOVA was used to depict significant group differences during encoding, corrected for multiple comparisons. Patients had significantly greater activity in the left CA1 and bilateral ERC-PRC. AntCADG = anterior CA1–3 and dentate gyrus, CA23DG = CA2–3 and dentate gyrus, CA1 = cornu ammonis 1, Sub = subiculum, PHC = parahippocampal cortex, ERC = entorhinal cortex, PRC = perirhinal cortex.

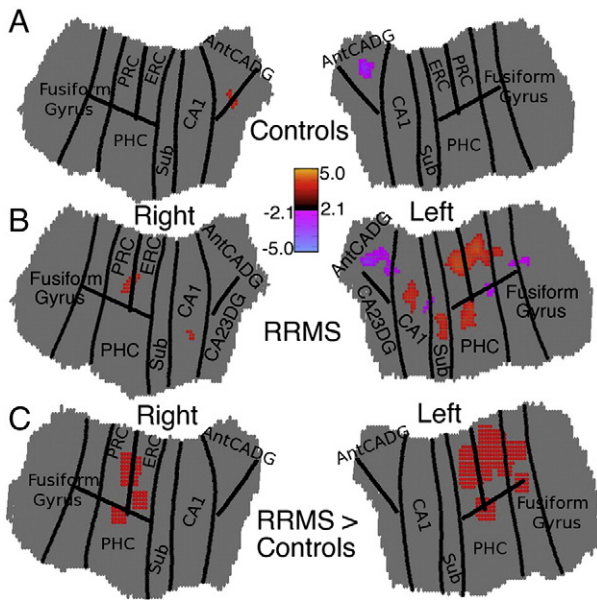


Fig. 5. Verbal Memory Recall is characterized by greater bilateral MTL cortical fMRI activity in RRMS. Unfolded MTL maps depict activity significantly greater than baseline (red) and activity less than baseline (purple) during recall in controls (A) and RRMS patients (B). Activity is weighted in each recall trial by the number of new words successfully recalled. Activity is displayed as cluster corrected T-statistics thresholded for two-tailed $p=0.05$. In (C), ANOVA was used to depict significant group differences during recall, corrected for multiple comparisons. Patients had significantly greater bilateral ERC-PRC fMRI activity. AntCADG = anterior CA1–3 and dentate gyrus, CA23DG = CA2–3 and dentate gyrus, CA1 = cornu ammonis 1, Sub = subiculum, PHC = parahippocampal cortex, ERC = entorhinal cortex, PRC = perirhinal cortex.

AntCADG region and left anterior CA1 as shown in Fig. 6A. Note that the posterior deactivation of the left CA1 during encoding (Fig. 4B) was not correlated with performance. In the control group, overall performance was not correlated with fMRI activity.

Patient fMRI activity during the recall phase was similarly correlated with memory performance. Once again, greater functional

activity in the right AntCADG and left CA1 was positively correlated with successful recall. In addition, fMRI activity in the right subiculum was also associated with better performance (Fig. 6B). However, controls showed no correlation between recall activity and memory performance.

Because in-scanner verbal memory performance was self-reported and therefore susceptible to over or underestimation of true learning, we also examined the relationship between functional activity and performance on the objective verbal memory post-test. For each group we correlated fMRI activity during all learning trials with the post-test performance score (maximum score = 7). For RRMS patients, greater activity in the right anterior hippocampus and left extra-hippocampal areas during both encoding and recall were also associated with more word-pairs recalled on the administered post-test. No significant associations were found in the control group (see Supplemental Fig. 2).

Hippocampal connectivity and verbal learning

Patients had significantly reduced FA within the fornix ($p=0.01$) and higher FA in patients was correlated with better performance on the verbal memory pre-test ($r=0.47$, $p=0.04$) and post-test ($r=0.47$, $p=0.04$, Fig. 7), but not the self-reported in-scanner memory performance. After correcting for age, group differences in FA ($p=0.02$) and associations between FA and memory performance ($p=0.04$) remained significant. PHWM FA, on the other hand, was not significantly different in RRMS patients, nor was it associated with any of the memory scores. When we separated left and right PHWM FA measures, there were still no significant associations with verbal memory scores. Neither of these white matter pathways was significantly correlated with verbal memory performance in control subjects. To investigate the role of the fornix in mediating hippocampal function, we correlated fornix FA with fMRI activity during learning and recall. In RRMS patients, fMRI activity in the left and right anterior hippocampus during successful encoding was positively correlated with fornix FA (Fig. 8A). During recall, increased functional activity in the right anterior hippocampus was also positively correlated with fornix FA in patients (Fig. 8B). Fornix FA was not significantly correlated with fMRI activity or memory performance in controls.

Role of gray matter atrophy

To determine if a relationship exists between hippocampal atrophy and functional activity, we correlated fMRI activity with averaged left and right volumes for the CA1 region and the whole hippocampus. However, neither measure showed a significant correlation with functional activity. These volumes have been reported previously and have been shown to be associated with verbal memory but not working memory in RRMS patients (Sicotte et al., 2008).

Discussion

Altered functional activity in hippocampal subregions during verbal learning was detectable in RRMS patients with disease duration less than 5 years. In comparison to healthy controls, RRMS patients had more widespread and bilateral hippocampal activity during both encoding and recall during an unrelated word-pairs learning task. In RRMS patients, increased activity in the right anterior hippocampus and engagement of the left CA1 was associated with better verbal memory performance suggesting that at least in the earliest phases of RRMS, increased recruitment of right hippocampal areas may compensate for ongoing pathological damage, thereby limiting detectable memory impairment. However, this compensatory activity appears to rely on hippocampal connectivity to other brain regions, since damage to the fornix, as assessed by DTI, was associated with

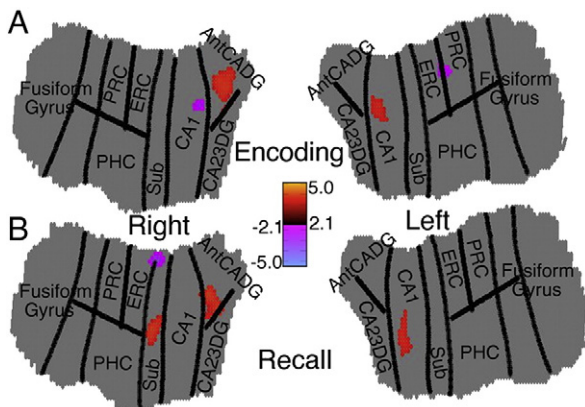


Fig. 6. Functional Activity is correlated with memory performance in RRMS. Figure depicts functional activity during all blocks of the encoding phase (A) and recall phase (B) that was significantly correlated with verbal memory performance in left and right hippocampi of RRMS patients. Red-yellow indicates T-statistic for positive correlations while blue-purple indicates T-statistic for negative correlations. Better performance was correlated with increased activity in the anterior left CA1 and right AntCADG during encoding and retrieval. No significant correlations were found in control subjects (not shown). AntCADG = anterior CA1–3 and dentate gyrus, CA23DG = cornu ammonis 2–3 and dentate gyrus, CA1 = cornu ammonis 1, Sub = subiculum, PHC = parahippocampal cortex, ERC = entorhinal cortex, PRC = perirhinal cortex.

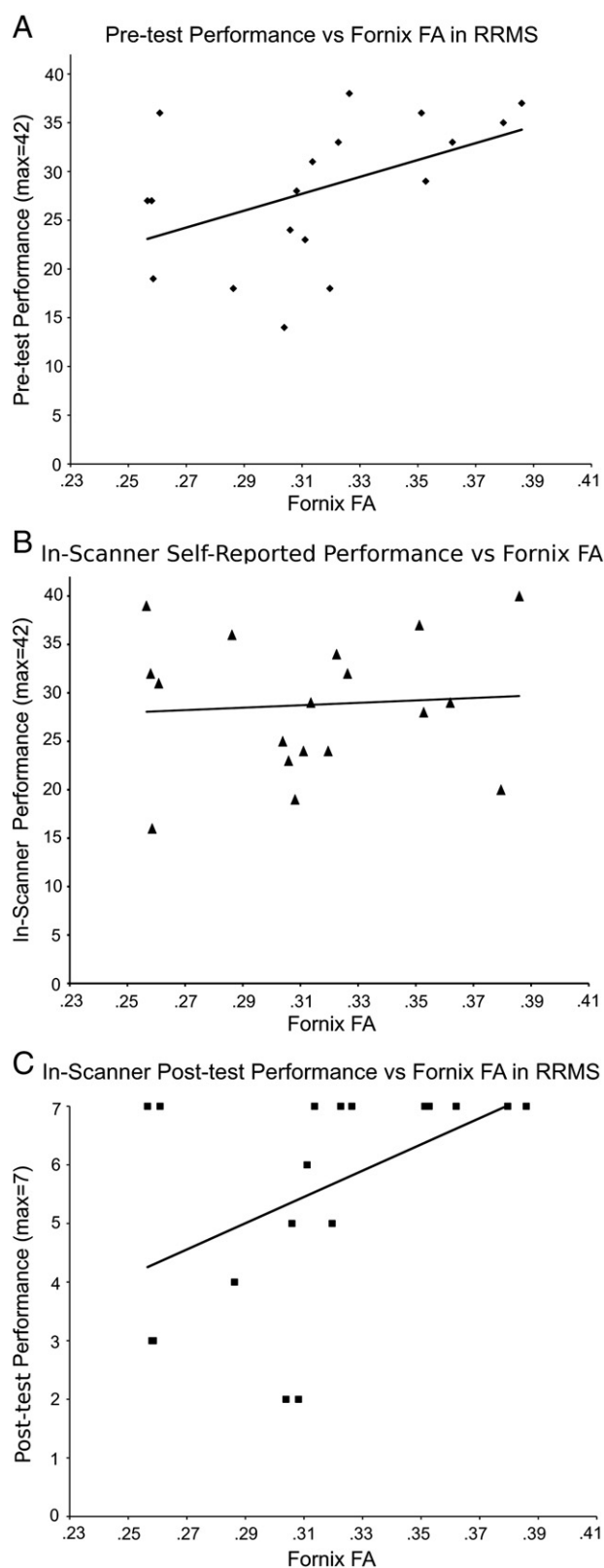


Fig. 7. Fornix FA is associated with verbal memory performance in RRMS. (A) scatterplot showing memory performance on the pre-test vs fornix FA in RRMS patients, which was administered before scanning and scored by a researcher (max score = 42). (B) Scatterplot depicts memory performance on the post-test vs fornix FA, in which a researcher asked the subject to recall the 7 unrelated word-pairs learned during scanning immediately after the fMRI sequence (max score = 7). Fornix FA was not significantly correlated with the self-reported score during the scanning session (not shown). PHWM FA was not correlated with any of the verbal memory tests and no significant associations were found between verbal memory and white matter integrity in control subjects.

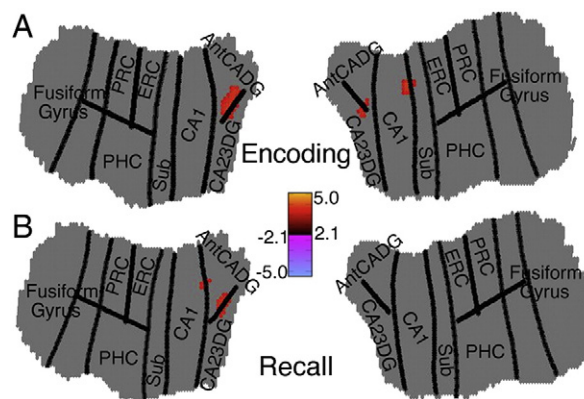


Fig. 8. Fornix FA was correlated with fMRI activity during successful memory performance in RRMS patients. Figure depicts location of functional activity that occurred during successful learning in the encoding phase (A) and recall phase (B) that was significantly correlated with the fornix FA of RRMS patients. Higher fornix FA values were associated with bilateral hippocampal activations during encoding (right AntCADG, left AntCADG, CA1) and recall (right AntCADG). Red-yellow indicates T-statistic for positive correlations while blue-purple indicates T-statistic for negative correlations. No significant correlations were found in control subjects (not shown). AntCADG = anterior CA1–3 and dentate gyrus, CA23DG = cornu ammonis 2–3 and dentate gyrus, CA1 = cornu ammonis 1, Sub = subiculum, PHC = parahippocampal cortex, ERC = entorhinal cortex, PRC = perirhinal cortex.

reductions in right anterior hippocampal fMRI activity and poorer performance on a verbal memory task.

This study demonstrates hippocampal functional changes in RRMS that may reflect adaptation. Similar patterns of cortical activation have been identified in patient groups with a variety of neurological insults such as during cognitive tasks of attention and memory (Mainiero et al., 2004) and motor tasks (Lee et al., 2000) in MS, and in individuals at risk for Alzheimer's disease (Bookheimer et al., 2000). But increased or bilateral activity could reflect a variety of aberrant processes including the loss of inhibitory input as seen in the motor system, in which the degree of ipsilateral motor cortex activity is not associated with improved function (Lenzi et al., 2007; Sicotte et al., 2010). In this study, the detection of more right hippocampal activation was associated with better memory performance in the patient group consistent with a compensatory process.

However, RRMS patients also had more functional activity in extra-hippocampal regions of the MTL compared to controls, including the ERC-PRC regions bilaterally during encoding and recall. The PRC receives multimodal sensory input from several cortical regions and feeds into the hippocampus via the ERC (Lavenex and Amaral, 2000). In our previous study of MTL volumetry in MS (Sicotte et al., 2008), the ERC was least affected in MS patients (PRC volume was not measured), and was not significantly associated with verbal memory performance. During the functional task in this study, unlike the hippocampal changes, increased ERC-PRC activity was either associated with poorer performance (encoding) or not at all (recall). Such activity may reflect non-adaptive physiological processes related to disease such as disinhibition of reciprocal hippocampal-ERC pathways or even interference from competing, aberrant sensory input signals. Disruptive ERC-PRC input to the hippocampus may also be indirectly affecting memory function. On the other hand, this activity could instead reflect increased perceptual load in MS patients, or could reflect compensation for other components of cognition not directly measured by the verbal memory score.

We previously showed early atrophy in the CA1 region in these subjects that was associated with poor performance on the unrelated word-pairs learning task, in particular the left CA1 (Sicotte et al., 2008). In the healthy control group, successful learning was characterized more by activity in anterior CADG regions and deactivation of the left CA1. Overall, controls showed relatively small, circumscribed

changes during learning as compared to patients, consistent with previously described neuronal “efficiency” (Landau et al., 2004; Reichle et al., 2000). The RRMS group demonstrated activity in both CA1 regions during learning and recall, with anterior activations associated with better performance suggesting that the amount of atrophy detected in these early patients (12% in the CA1) (Sicotte et al., 2008) did not directly affect BOLD activations. Additionally, hippocampal volumes were not correlated with fMRI activity in either phase of the task.

The three-way relationship between white matter integrity in the fornix, functional activity in the hippocampus, and memory performance reflects the role of hippocampal connectivity in cognitive compensation. Bilateral fornix damage can cause amnesia (Gaffan and Gaffan, 1991), and previous studies have shown focal lesions (Huitinga et al., 2001) and reduced FA (Roosendaal et al., 2009) in this pathway in MS. The hippocampus has reciprocal connections with both the anterior thalamic nucleus and the mammillary bodies via the fornix, and damage to either region can lead to severe memory impairment (Aggleton and Sahgal, 1993; Kopelman, 1995). Animal studies have shown that fornix input can modulate hippocampal excitability (Toth et al., 1997), and our findings suggest that functional reorganization during memory processes may be possible as long as this white matter pathway remains intact. However, while we chose to investigate only the fornix and the PHWM because they are the major efferents and afferents of the hippocampus, it is likely that several other white matter pathways are also involved in mechanisms of verbal memory compensation, such as fronto-temporal pathways, temporoparietal pathways, and interhemispheric pathways. Future studies should assess the role of these connections as well.

The difference in memory performance between MS patients and controls must also be considered when interpreting altered functional activity. Although the individual functional analyses only weighted task trials in which active learning occurred, the patient group, who performed worse on average, might have perceived greater task difficulty. Therefore, increased activation may reflect increased effort that is not specific to MS pathology as opposed to an adaptive hemodynamic response to structural damage. Future studies should include a group of MS patients with intact memory performance as well to test whether these hemodynamic changes are detectable before functional memory impairment is evident. There was also a trend for a difference in age ($p=0.06$) between patients and healthy controls that may have contributed to altered functional activity. However, the marginally older MS patients generally showed greater MTL activity than controls, while previous studies have shown that hippocampal activation during memory tasks is often decreased in older individuals (Beeri et al., 2011; Mitchell et al., 2000; Tsukiura et al., 2011).

Another limitation of this study is that we used a subjective measure of learning for the in-scanner memory performance. Patients self-reported the word-pairs they remembered, leaving open the possibility of under or overreporting true learning. The fact that the in-scanner memory performance was not associated with the FA of the fornix while the other objective memory scores were associated may be a result of this limitation. However, we performed 2 additional analyses to further investigate this inconsistency and found that the three-way relationship between verbal memory performance, fornix FA and hippocampal functional activity remained intact.

Since the new word-pair learning curve was dictated by the subject's own reporting, we first used the objective post-test measure to identify any subjects who may have reported in the scanner inaccurately. After removing 3 RRMS patients who under or overreported their in-scanner learning, we reassessed the functional activity during new word-pair learning and found the results during encoding and recall to be very similar (see Supplemental Fig. 1).

While better self-reported in-scanner memory performance was associated with greater right anterior hippocampal fMRI activity during encoding and recall (across all trials), we also regressed group functional activity against the objective post-test measure and found the same localized association in the right anterior hippocampus (see Supplemental Fig. 2).

These results suggest that although the self-reported performance does not truly reflect the underlying structural damage, functional activity may be similar between perceived learning and true learning. Another possibility is that some form of true learning occurred in the scanner but either patients forgot during the gap between the end of the scan and the objective post-test (about 1 min duration) or could not verbalize this learning when confronted with an auditory instead of visual stimulus. This could reflect performance anxiety when tested by an examiner rather than self-testing, or may reflect preferences to recall modalities. Further studies are needed to parse these subtle differences between perceived learning and true learning.

While the high-resolution unfolding technique provides a unique, detailed look at a brain region critical to memory function, it comes at a loss of the overall brain activation patterns, which may be useful in further elucidating the patterns of cortical reorganization during memory tasks. Previous studies have shown that whole networks may be involved during cognitively adaptive reorganization (Rocca et al., 2010). The effect of fornix damage on memory performance supports this idea. Further studies of memory impairment in MS might also investigate functional activity in the anterior nucleus of the thalamus, cingulate cortex, and frontal lobes for evidence of functional compensation.

The complex and evolving relationship between structural and functional changes in MS suggests that simple measures such as regional brain volumes in cross-sectional studies may not be informative. Longitudinal studies assessing both structural and functional parameters may shed light on brain compensatory processes and provide insight into the relationship between detectable changes and clinical status. Assessing very early MS may be particularly useful in understanding how the nervous system adapts to ongoing disease. In addition, monitoring these structural–functional correlations holds promise as future readouts of remyelination or neuroprotective treatments.

Conclusions

Using a cortical unfolding technique, we demonstrated MS-related changes in hippocampal functional activity on a subregional level during a verbal memory task. Greater bilateral hippocampal activation in patients was associated with better memory performance, a pattern suggesting adaptive functional compensation. Poor performance was associated with less bilateral activation and greater damage to the fornix as reflected by DTI, implicating an important role for this pathway in mediating compensatory mechanisms. These results demonstrate how structural brain damage to relevant connective pathways can limit functional reorganization in early RRMS.

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