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PAPER

Learning and memory-related brain activity dynamics are altered in systemic lupus erythematosus: a functional magnetic resonance imaging study

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Background: Memory impairment is prevalent in systemic lupus erythematosus (SLE); however, the pathogenesis is unknown. **Methods:** We studied 12 patients with SLE without clinically overt neuropsychiatric manifestations and 11 matched healthy controls, aiming to characterize neural correlates of memory impairment, using structural and functional magnetic resonance imaging (MRI). The paradigm consisted of three encoding and free-recall cycles, allowing characterization of dynamics along consecutive retrieval attempts. **Results:** During learning, patients with SLE and healthy controls showed brain activity changes in two principal networks, the default mode network (DMN) and the task-positive network (TPN). Patients with SLE demonstrated significantly less deactivation in the DMN and greater activation in the TPN, reflecting greater recruitment of both networks. The anterior medial prefrontal cortex (amPFC) of the DMN emerged as the only region where brain activity dynamics were altered both over the learning process ($p < 0.006$), and within free-recall period attempts ($p < 0.034$). Patients showed significant positive correlations between learning efficiency and hippocampal activity, and greater hippocampal functional connectivity, with pronounced connectivity to DMN structures. **Conclusions:** Increased brain activation in patients with SLE during learning may reflect compensatory mechanisms to overcome memory impairment. Our findings localize this impairment to the amPFC, consistent with the behavioral pattern seen in SLE. Altered networking of the hippocampal subsystem of the DMN is consistent with hippocampal neuronal damage seen in SLE, and may reflect compensatory cortical reorganization to cope with dysfunction in these regions pivotal to mnemonic functions. *Lupus* (2013) **22,** 562–573.

Key words: Systemic lupus erythematosus; magnetic resonance imaging; cognitive dysfunction

Introduction

Central nervous system (CNS) involvement in systemic lupus erythematosus (SLE) is common, affecting 14%–75% of patients.[1,2] Despite the revised standardization of neuropsychiatric SLE (NPSLE) manifestations,[3,4] the underlying pathogenetic mechanisms are not clear, leading to confusion regarding the choice of treatment. NPSLE manifestations range from overt neurologic dysfunction such as psychosis, seizure disorders, stroke and dementia to more subtle impairments, including cognitive dysfunction.[3] The reported prevalence of cognitive dysfunction in SLE ranges from 21% to 66%;[5–7] however, the etiology is unknown. Several studies have pointed to an association between cognitive abnormalities and other overt neuropsychiatric manifestations, but have not shown an association with active SLE, corticosteroid use or psychological stress.[8–10] The memory domain has been shown to be impaired frequently in patients with SLE when using standardized neuropsychological tests.[7,11–16] We have recently assessed behaviorally a cohort of 40 unselected patients with SLE without overt NPSLE as compared to 40 healthy matched controls using the

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Rey Auditory Verbal Learning Test (RAVLT), and found a poor and inefficient learning strategy reflected by an impaired learning curve, repeated omissions and decreased retrieval on free recall. This pattern of memory deficit resembles that seen in patients with frontal lobe damage. No correlation was found between this memory impairment and disease activity, disease duration, irreversible organ damage, corticosteroid use, other medications or depression.[17] The present functional magnetic resonance imaging (fMRI) study was performed in an attempt to localize and better characterize this learning and memory impairment. Numerous studies have demonstrated a significant role for a set of brain regions collectively termed the default mode network (DMN) in episodic memory[18–21] and in internally driven processes,[22,23] suggesting a unique role for the DMN in free-recall processes. Following our findings regarding the pattern of memory deficit in patients with SLE, we hypothesized that altered brain activity would be seen in the DMN when performing a learning and memory task involving free recall, and that altered activity would be localized to frontal regions. In view of the central role of the hippocampus in memory functions and reports of atrophic hippocampi in patients with SLE,[24] we further hypothesized that hippocampal activity and functional connectivity might also be altered in patients with SLE. To test these hypotheses, fMRI studies were conducted in patients with SLE and healthy controls, using an adaptation of the RAVLT for the fMRI setting.

Methods

Patients and healthy controls

Thirteen female patients with SLE without overt NPSLE and 11 healthy controls, matched for age and education, were recruited. Patients were recruited from the Lupus Clinic at the Tel-Aviv Sourasky Medical Center and fulfilled the revised American College of Rheumatology criteria[25] for the classification of SLE. All participants were right handed and native Hebrew speakers. An expert neuro-radiologist examined the structural MRI studies of all participants. One patient was excluded because of evidence of a previous stroke. The study was approved by the institutional ethical committee. After giving informed consent, all participants underwent fMRI scanning using a novel auditory verbal memory test paradigm as well as structural MRI studies. Data were collected regarding disease activity as assessed by the SLE Disease Activity Index (SLEDAI),[26] disease damage as assessed by the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) damage index score,[27] presence of antiphospholipid antibodies (aPL) including: anticardiolipin IgG and IgM; anti-β2 glycoprotein I IgG and IgM and lupus anticoagulant, history of thrombosis/pregnancy loss, history of NPSLE and current medication. The presence of depression was assessed by the Beck Depression Inventory (BDI-II).[28,29] A score of >13 was considered evidence of depression.

fMRI paradigm

All participants performed a block design paradigm based on an adaptation of the RAVLT for the fMRI setting (Figure 1), as reported previously.[19] The RAVLT[30,31] is a well-studied learning and recall task that includes five encoding-free-recall cycles an interference list followed by one immediate and one delayed free recall attempt and a recognition trial.[32] In the present adaptation we employed three encoding-free-recall cycles, excluding the interference list. The encoding periods (21 seconds (sec)) consisted of auditory presentation of a list of 15 concrete, frequently used and emotionally neutral Hebrew nouns presented at a rate of one word/1400 msec. Subjects were instructed to memorize the words, and press a button after hearing each word. The order of word presentation was consistent in the three consecutive cycles. Free-recall periods lasted 30 sec, during which participants were asked to retrieve the previously heard words and press a button when a word was retrieved. Covert retrieval was used to avoid interference of head and mouth movements during scanning, as used previously by Voets et al.[33] To overcome this limitation, performance during overt pre-scan free recall and covert free-recall during the scan was compared and found to be similar ($F_{(1,22)} = 0.078$, $p < 0.783$), validating the use of covert free-recall during scanning. During the recognition period (60 sec, two sec per word), participants were asked to identify the initial 15 words from a larger list of 30 words. The 15 additional distracting words were similar phonologically (six) or semantically (nine) to the initial 15 words. Experimental periods were separated by nine-sec baseline periods where participants were asked to perform a simple motor response to a tone. Auditory instructions prior to each period indicated the task to be performed. This protocol was repeated with three different lists. A training

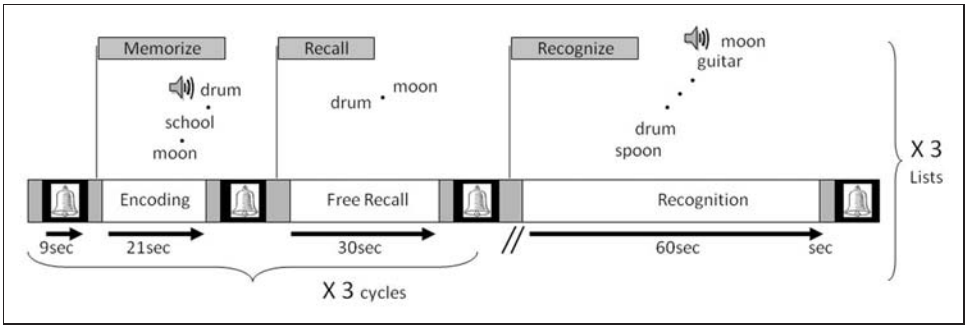

Figure 1 The experimental paradigm.
A functional magnetic resonance image (fMRI) adaptation of a word list learning and recall test was used. The paradigm included three encoding and free-recall cycles followed by a recognition trial.
Encoding: Participants were instructed to try to remember as many words as possible from a list of 15 words presented orally.
Free recall: Participants were instructed to covertly retrieve as many encoded words as possible, and press a button after each retrieved word.
Recognition: Participants were instructed to press a button when recognizing words from the initial list, out of a larger list of 30 words presented orally.

task with a similar list of words was performed before scanning. Auditory stimuli were presented through pneumatic headphones. Stimuli presentation and participants' responses were controlled by Presentation software (version 10.3). Participants' responses were recorded via a four-button response box (Current Designs, Philadelphia, PA, USA).

MRI data acquisition

Imaging was performed on a 3T GE Signa Horizon scanner (General Electric, Milwaukee, WI, USA). All images were acquired with a standard quadrature head coil. The scanning session included conventional anatomical MR images (T1-WI, T2-WI, T2-fluid-attenuated inversion recovery (FLAIR)), three-dimensional spoiled gradient echo sequence (3D-SPGR) (field of view (FOV), 250 mm; matrix size, 256×256; axial slices of 1-mm thickness, gap 0), and functional T2*-weighted images (FOV, 200 mm; matrix size, 64×64; repetition time (TR), three sec; echo time (TE), 35 ms; flip angle (FA), 90°; 45–46 axial slices of 3-mm thickness, gap 0).

Data analysis

Analysis of behavioral data

The total number of recall responses, measured by the number of button presses, was recorded per free-recall period. Learning efficiency was defined as the difference between the number of words retrieved in the first and third free-recall periods. SPSS software (version 12) was used for behavioral analyses. To compare learning effects between groups, a general linear model (GLM) with one between-subject factor (''group'') and one within-subject factor (''free-recall period,'' three levels: FR1, FR2, FR3) was constructed. For each participant, the average number of words retrieved across the three lists was entered as the dependent variable. A second GLM was constructed to compare overt and covert retrieval.

Structural data

Manual segmentation of hippocampi was performed by a blinded assessor (Y.G.) using the BrainVoyager QX analysis package (Brain Innovation, Maastricht, The Netherlands, version 2.1). Hippocampal boundaries were defined using validated anatomical landmarks,[34] allowing volumetric comparison between groups. The individually defined hippocampi were used as regions of interest (ROIs) in the functional analysis.

Analysis of fMRI data

Changes in blood oxygenation level-dependent (BOLD) signals as related to the cognitive memory task were analyzed using the BrainVoyager software Image. Preprocessing of functional scans (using BRAIN VOYAGER 2000) included: three-dimensional (3D) motion correction, slice scan time correction, spatial smoothing (a FWHM 4 mm Gaussian Kernel), linear trend removal and high-pass filtering (fast Fourier transform based with a cutoff of 2 cycles/time course). Head movements were minimal; however, when recorded movements exceeded 2 mm or 2 degrees the data were discarded. Functional images were superimposed on two-dimensional (2D) anatomical images and incorporated into the 3D data sets

through trilinear interpolation. The complete data set was transformed into Talairach space.[35] Statistical maps were prepared for each subject and list using BRAIN VOYAGER QX (Version 2.1).

The constructed GLM included eight regressors: three representing each of the encoding blocks, three representing each of the free-recall blocks, one for the recognition task and one for instructions. Regressors were modeled as boxcar functions convolved with the hemodynamic response function, assuming a hemodynamic lag of six sec. Single-subject analysis was followed by a multi-run, multi-subject analysis computed with random effects. This model was designed to identify regions that respond to free-recall in a learning-dependent way using a conjunction analysis: main effect for free-recall in conjunction with higher brain activity in the third as compared to the first free-recall period. This analysis was performed for each group separately. Regions that emerged when performing the same analysis across groups were used as ROIs in which brain responsiveness patterns of patients were directly compared to those of controls. The percent of signal change relative to baseline was calculated for each participant and list using Microsoft Excel. Each baseline signal was computed by averaging the activity level in the last three sec (three sec = TR) of all baseline periods for each participant and word list, allowing the signal to decay. A delay of two TRs (six sec) was incorporated. For each participant, the data obtained in the three lists were averaged. Percentage signal changes were calculated for each TR and each free-recall period separately, allowing the examination of the main effects and interaction of learning as well as time along the free-recall period. These effects were examined in SPSS, using repeated-measures GLM with one between-subject factor (''group,'' two levels: SLE, healthy controls) and two within-subject factors (''free-recall period,'' three levels: FR1, FR2, FR3 and ''time within free-recall periods,'' three levels: TRs 1–3, TRs 4–7, TRs 8–10). To adjust for any non-homogeneity of covariance for the within-subject effects, we used p values that were adjusted using the Huynh-Feldt method.

Correlations between learning efficiency and hippocampal volumetry or percentage signal changes in brain activity in the hippocampus were calculated.

Functional connectivity analysis was applied to identify regions in which brain activity response was similar to that seen in the hippocampus when performing the task, using the normalized time courses obtained in the hippocampus for each subject and list as predictors in a new GLM.

For each participant, the averaged whole-brain activity was orthogonalized with respect to the hippocampal signal and included as a confound predictor in the statistical model, to reduce noise and non-specific effects. Separate models were created for the right and left hippocampus, for each group separately, as well as across groups, allowing direct comparison of the groups using analysis of covariance (ANCOVA).

Results

Demographic and disease characteristics

Twelve female patients with SLE and 11 female healthy controls were studied. The groups were similar with respect to age (median age: 30 years in the patient group; 29 years in the control group) and years of education (median 15 years in both groups).

In the SLE group, disease duration ranged from 1.5 to 14 years (median 9.5 years). The disease activity was mild to moderate in most patients (median SLEDAI 4, range of 0–20), median damage according to SLICC/ACR damage index score was 1 (range 0–3). Of the 12 patients with SLE, three patients reported cognitive complaints of difficulty in remembering names and words. Corticosteroid treatment was prevalent in the patients (58%), 57% of whom were treated with a low dose of prednisone (0–12.5 mg/d, median 5 mg/d, range 0–50 mg/d). All patients were treated with hydroxychloroquine; seven were also treated with immunosuppressive drugs. Three patients had antiphospholipid syndrome (APS), none of which involved the CNS. One patient had a history of arterial thrombosis, two had a history of venous thrombosis and none had a history of obstetrical APS. Five patients had antiphospholipid antibodies (aPL) without APS, and four patients were aPL negative. Mild depression (BDI-II score: 15) was seen in one of the 12 patients. There was no other history of NPSLE manifestations except for one patient with a history of peripheral neuropathy. The disease characteristics of the 12 patients with SLE are shown in Table 1.

Behavioral assessment of free-recall

The ability to freely recall and learn new word lists was assessed over several acquisition trials using a

Table 1 Demographic and disease characteristics of the patients with SLE

Patient no.	*Age*	*Disease duration (years)*	*SLEDAI*	*SLICC*	*Major organ involvement due to SLE active/ inactive*	*Prednisone dose*	*Additional medication*	*NPSLE with CNS involvement-ever*	*NPSLE non CNS*	*aPL/APS*	*Additional medical condition*
1	24	12	6	1	Lupus nephritis—inactive	20	HCQ MMF	No	Depressed mood	aPL negative	None
2	25	8	2	0	Lupus nephritis—inactive	0	HCQ	No	Depressed mood	aPL negative	None
3	32	11	2	3	Lupus nephritis—inactive	5	HCQ AZA Warfarin	No	No	APS—s/p renal vein thrombosis	None
4	35	13	2	1	Polyarthritis—active	12.5	HCQ MTX Warfarin	No	No	APS—s/p splenic infarcts	None
5	47.5	8	8	1	Polyarthritis —inactive peripheral neuropathy—inactive	0	HCQ	No	Peripheral neuropathy	aPL positive	None
6	36	1.5	4	1	Lupus nephritis—inactive	0	HCQ MMF	No	No	aPL positive	None
7	29.5	14	4	3	Polyarthritis-active, SCLE rash	5	HCQ AZA	No	No	aPL positive	None
8	30	8	16	0	Lupus nephritis—active	50	HCQ CYC Euro-Lupus protocol Warfarin	No	No	APS—s/p DVT	None
9	30	8	0	0	Inactive	0	HCQ	No	No	aPL negative	None
10	35	7	2	0	Inactive	0	HCQ	No	No	aPL positive	None
11	26	12	20	1	Lupus nephritis-active	20	HCQ MMF	No	No	aPL negative	None
12	28	12	4	0	Lupus nephritis—inactive, SCLE rash	10	HCQ	No	No	aPL positive	None

SLE: systemic lupus erythematosus; SLEDAI: systemic lupus erythematosus disease activity index; SLICC: Systemic Lupus International Collaborating Clinics; NPSLE: neuropsychiatric systemic lupus erythematosus; CNS: central nervous system; aPL: antiphospholipid antibodies; APS: antiphospholipid syndrome; HCQ: hydroxychloroquine; MMF: mycophenolate mofetil; AZA: azathioprine; CYC: cyclophosphamide; DVT: deep vein thrombosis; SCLE: subacute cutaneous lupus erythematosus.

modified version of the RAVLT. Free-recall was impaired in patients with SLE across all three free-recall periods (main effect for "group," $F_{(1,21)}=4.436$, $p<0.047$), similar to our previous findings in a larger group of patients.[17]

Neural correlates of free-recall

Learning-associated dynamics

During the learning process, both groups showed similar changes in activation in regions associated with two principal networks (Figure 2): In the DMN deactivation increased over learning, and in the TPN activation increased over learning. However, the magnitude of change was different, as patients demonstrated more pronounced increase in activation in the TPN, and a lesser degree of deactivation in the DMN, particularly in the anterior medial prefrontal cortex (amPFC) (Figure 2). To statistically examine this effect, we repeated the same conjunction analysis across all study participants using a single GLM for the whole group. Five regions showed an increase in activation (p value of 0.01 false discovery rate (FDR), cluster size $10*3^3$), and two regions showed an increase in deactivation (p value of 0.05 FDR, cluster size $10*3^3$) (Table s1 in Supplementary Appendix). The identified regions were used as ROIs within each of which the two groups were directly compared. Within each ROI, percent signal change was computed for each free-recall period and compared between groups (Figure 3). Significant interaction between group and free-recall period was evident in several areas: in the amPFC of the DMN ($F_{(2,36)}=5.911$, $p<0.006$), and in the TPN: in the left premotor cortex ($F_{(2,36)}=4.424$, $p<0.019$) and supplementary motor area ($F_{(2,36)}=3.470$, $p<0.042$), as well as a trend in the left caudate body ($F_{(2,36)}=2.850$, $p<0.071$). This interaction indicates a significant difference between patients with SLE and controls in the pattern of activation changes during the learning process. Post hoc comparisons using the Bonferroni procedure were used to clarify these differences. Healthy controls showed a significant increase in deactivation from the first to the second ($p<0.001$) and from the second to the third ($p<0.001$) free-recall period in the amPFC. Patients with SLE failed to show these increases in deactivation (Figure 3(a)). Within the premotor cortex and the supplementary motor area (SMA),

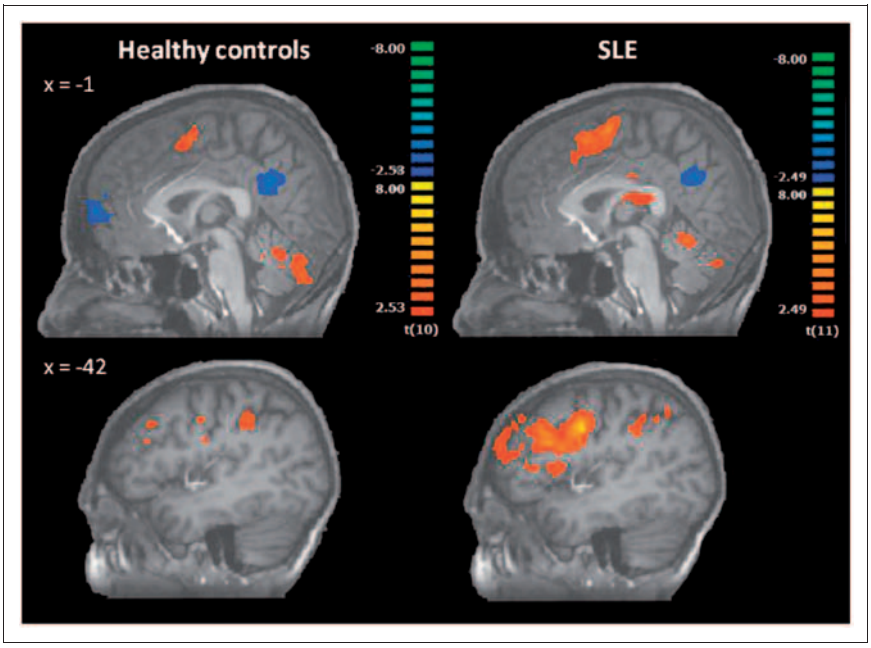

Figure 2 Learning-associated brain activity changes.
Whole-brain activation maps show regions where brain activity changed during free recall as learning proceeded. For the purpose of demonstration maps were constructed separately for patients with systemic lupus erythematosus (SLE) and healthy controls. Learning-associated decrease in brain activity is shown in blue. This was seen particularly in the default mode network (DMN). Learning-associated increase in brain activity is shown in red. This was seen particularly in the task-positive network (TPN). Random effect, $n=12$ (SLE), $n=11$ (healthy controls). For the purpose of illustration, activations are shown at $p<0.03$ (uncorrected), cluster size $10*3^3$.

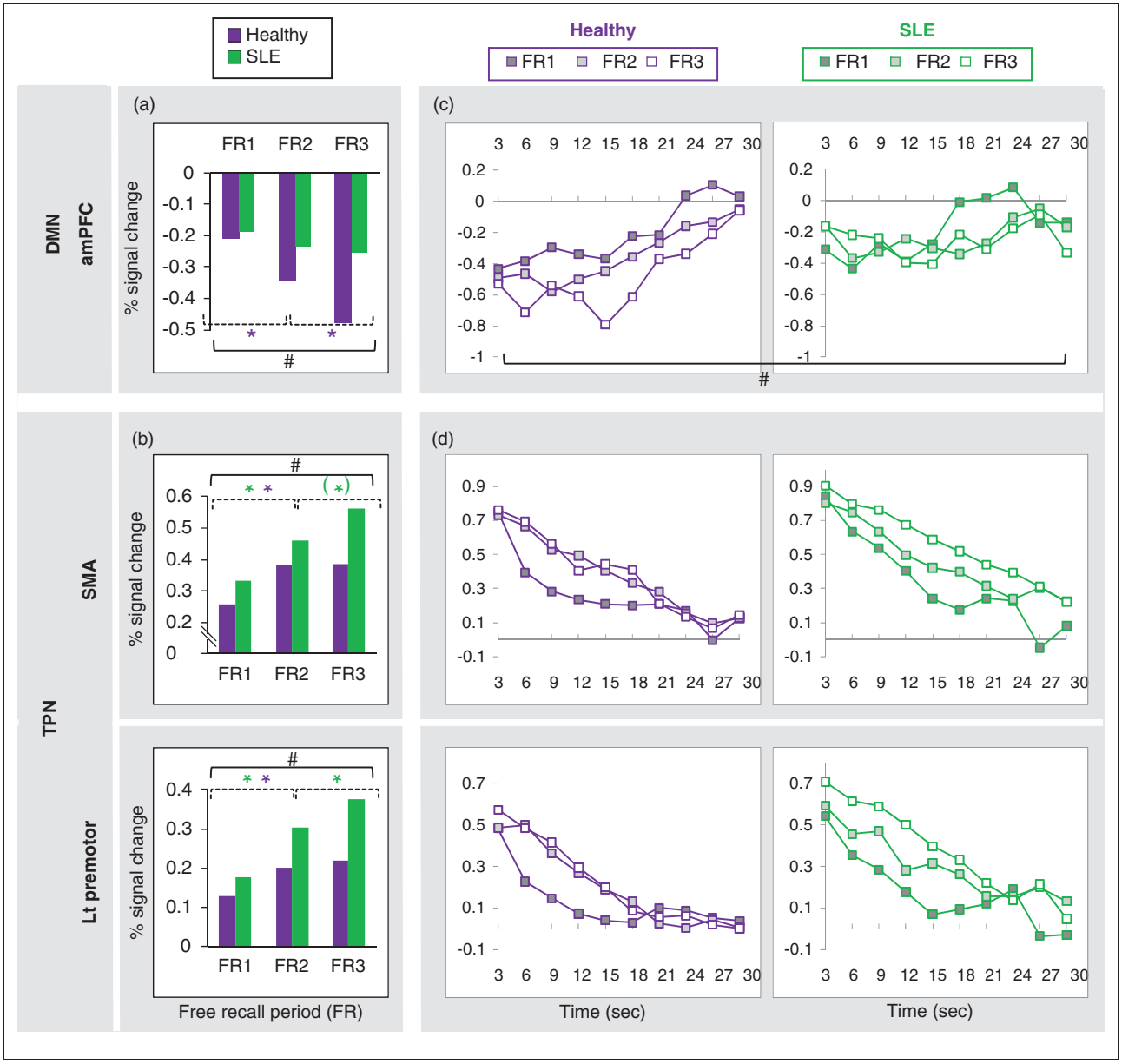

Figure 3 Percentage of signal changes in brain activity during free-recall periods, in regions of interest of the default mode (DMN) and task-positive networks (TPN).

Averaged activity in each free-recall period ((a),(b)) **3(a)** demonstrates the magnitude of deactivation in each free-recall period during the learning process within the anterior medial prefrontal cortex (amPFC) region of the DMN. In systemic lupus erythematosus (SLE) patients as compared to healthy controls, less increase in deactivation was seen as learning proceeded from FR1 to FR3 (interaction between group and learning. $F_{(2,36)} = 5.911$, $p < 0.006$).

3**(b)** demonstrates the magnitude of activation in each free-recall period during the learning process within regions of interest in the TPN. In patients with SLE, a greater increase in activation was seen as learning proceeded from FR1 to FR3, as compared to healthy controls (interaction between group and learning. Supplementary motor area (SMA): $F_{(2,36)} = 3.470$, $p < 0.042$; Lt premotor: $F_{(2,36)} = 4.424$, $p < 0.019$).

#Statistically significant interaction effects. *Significant differences in brain activity between the first and second free-recall periods, as well as the second and third free-recall periods, in each group (post hoc comparisons).

Dynamics of brain activity along each fre- recall period ((c), (d)) **3(c)** demonstrates changes in brain activation within each 30-second period allocated for free recall, within the amPFC region of the DMN. When looking at the dynamics of brain activation within each 30-second period, patients with SLE showed less change in brain activity, as reflected by the blunted slopes (interaction between group and dynamics within the 30-second period: $F_{(2,36)} = 3.715$, $p < 0.034$).

3(d) demonstrates changes in brain activation within each 30-second period allocated for free-recall within regions of interest of the TPN. No interaction was found between group and the dynamics of brain activation within each 30-second period.

#Statistically significant interaction effects.

controls showed an increase in activation from the first to the second free-recall period ($p < 0.039$, $p < 0.001$, respectively) with no further increase in activation in the third free-recall period. In contrast, patients with SLE showed a progressive and greater increase in activation in all three free-recall periods (premotor cortex, FR1-to-FR2: $p < 0.004$, FR2-to-FR3: $p < 0.028$; SMA, FR1-to-FR2: $p < 0.008$, FR2-to-FR3: $p < 0.057$) (Figure 3(b)).

Dynamics within free-recall periods

We further analyzed brain activations within the 30 sec of each free-recall period. Healthy participants' amPFC in the DMN showed a progressive decrease in deactivation within the 30-sec period, while patients showed an attenuated change in deactivation during this period (an interaction between group and TR, $F_{(2,36)} = 3.715$, $p < 0.034$) (Figure 3(c)). This effect was not demonstrated in the precuneus. In the TPN a similar pattern of brain activation dynamics was seen in both groups within free-recall periods (Figure 3(d)).

Dynamics in the hippocampus

Although the hippocampus did not emerge as a brain region demonstrating brain activity changes associated with the learning process in our paradigm, we further examined hippocampal activity during learning due to its important role in episodic memory and previous reports of hippocampal atrophy in SLE.[24]

Hippocampal volume and learning efficiency

The left hippocampal volume was significantly smaller in patients with SLE as compared to healthy controls (controls: 2524.2 ± 255.1 voxels, SLE: 2319 ± 186.7 voxels, $t_{(21)} = 2.21$, $p = 0.038$). A similar trend was seen in the right hippocampus (controls: 2682.5 ± 359.2 voxels, SLE: 2423.9 ± 260 voxels, $t_{(18.1)} = 1.96$, $p = 0.065$). To examine whether hippocampal atrophy underlies the learning and memory difficulties of patients with SLE, we looked at the correlation between hippocampal volume and learning efficiency (i.e. the number of learned words). No correlation was found (SLE: left hippocampus FR3–FR1: $r = 0.09$, $p < 0.76$, right hippocampus FR3–FR1: $r = -0.02$, $p < 0.94$).

Hippocampal activity and learning efficiency

In each group, the behavioral measure of learning efficiency was correlated with percent signal change in activity from baseline during each component of the learning task: encoding, free-recall and recognition. The percent signal change was averaged across the three encoding and three free-recall periods. Among patients with SLE, activity changes during the three components of the learning task correlated with learning efficiency (left hippocampus activity during encoding: $r_{(12)} = 0.61$, $p < 0.034$, free-recall: $r_{(12)} = 0.65$, $p < 0.023$ recognition: $r_{(12)} = 0.58$, $p < 0.049$; right hippocampus activity during encoding: $r_{(12)} = 0.68$, $p < 0.016$, free-recall: $r_{(12)} = 0.65$, $p < 0.024$ and recognition: $r_{(12)} = 0.64$, $p < 0.024$) (Figure 4). This correlation was not seen in healthy controls.

Functional connectivity of the hippocampus

In view of the unique correlation of hippocampal activity with learning efficiency in patients with SLE, we looked at its functional connectivity with other brain regions. In patients with SLE, hippocampal connectivity was more widely distributed throughout the brain as compared to healthy controls, with pronounced connectivity to DMN structures (Figure 5(a), (b)).

Correlation of dynamics in brain activity with clinical measures

To further appreciate the clinical relevance of our results, we examined the activation patterns of patients with and without aPL and with and without APS. Patients with APS ($n =$ three, two with venous thrombosis, one with arterial thrombosis, average age 32 years, average education 16 years) were compared to patients without APS or aPL ($n =$ three, average age 27 years, average education 15 years). aPL-negative patients ($n =$ four, average age 26 years, average education 15 years) were compared to aPL-positive patients ($n =$ four, average age 32 years, average education 14.5 years). aPL-positive patients and APS patients showed a greater increase in brain activity in the TPN as compared to aPL-negative and non-APS patients, respectively (Figure 6). The number of patients in these subgroups is too small to allow for meaningful statistics; however, the pattern of brain activity was markedly different, with a greater increase in brain activity in the more affected group as compared to the less affected group, respectively.

Discussion

In this fMRI study, utilizing a learning and memory task paradigm, we have demonstrated significantly different brain activation dynamics in patients with SLE as compared to healthy controls. During the learning process patients with SLE showed a significantly lesser degree of deactivation

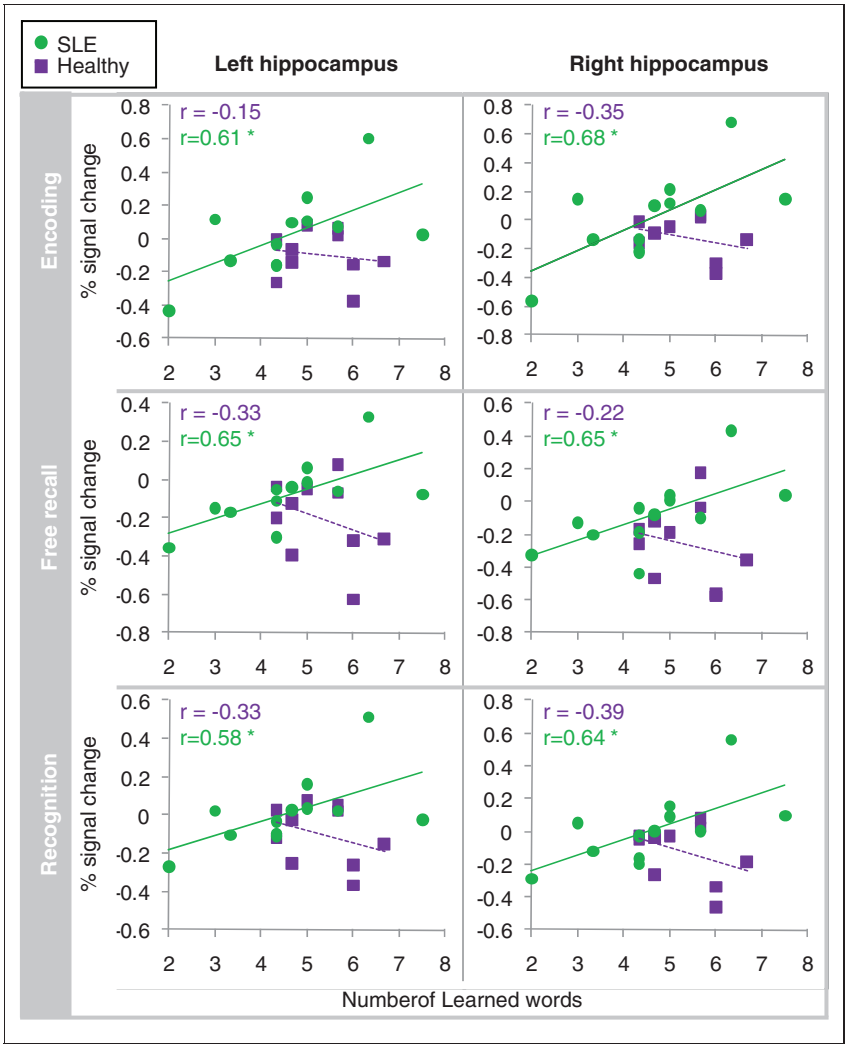

Figure 4 Correlation between learning efficiency and percent signal changes in brain activity within the hippocampus.
In systemic lupus erythematosus (SLE) patients, changes in brain activity in the hippocampus during the encoding, free-recall and recognition periods correlated with learning efficiency (i.e. number of learned words). In healthy controls, no correlation was seen.
*Statistically significant correlation.

in the amPFC component of the DMN across the three free-recall periods. Abnormal activity in the DMN during rest and on performance of cognitive tasks has been demonstrated in several neurological disorders, including Alzheimer's disease[36] and multiple sclerosis.[37] The failure to downregulate activity in the amPFC, despite repeated encoding of the same list of words, may underlie the poor and inefficient learning strategy, previously shown on RAVLT testing in patients with SLE.[17] In addition, when looking at the dynamics within the 30-sec periods of free-recall attempts, patients with SLE showed a lesser degree of deactivation of the amPFC in the DMN throughout all of the 30-sec period, while controls showed increased recruitment only toward the end of this period. This increased recruitment of the DMN within the free-recall period possibly reflects an increase in memory search that is typically seen during free-recall attempts.[38,39] Early recruitment of the amPFC in patients with SLE from the beginning of the free-recall period may suggest that these patients rely more on memory search strategies than healthy controls. Furthermore, the amPFC emerged as the only region showing abnormal dynamics in the two time scales examined, both within the 30-sec periods of free-recall attempts as well as over the free-recall cycles of the learning process. These findings localize the learning and memory impairment to the frontal region, and more specifically to the anterior medial part of it. The marked impairment in the amPFC is consistent

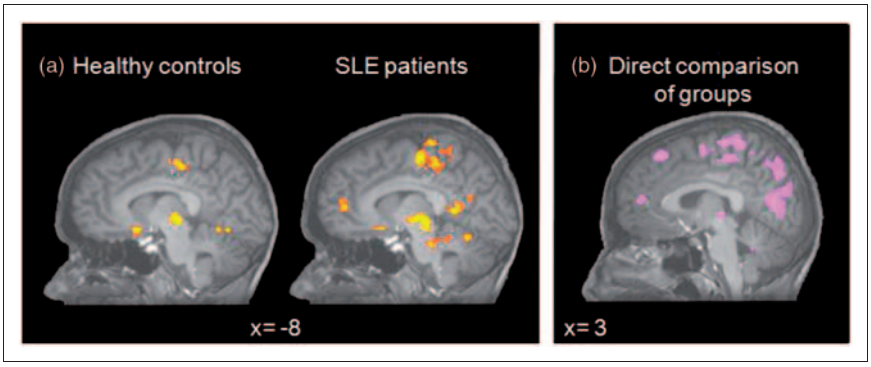

Figure 5 Functional connectivity of the hippocampus.
5**(a)** In systemic lupus erythematosus (SLE) patients, hippocampal connectivity was more widely distributed throughout the brain as compared to healthy controls, with pronounced connectivity to default mode network (DMN) structures. For the purpose of illustration, activations are shown at $p < 0.01$ with Bonferroni correction, cluster size 10×3^3.
5**(b)** Direct comparison of functional connectivity between the two groups demonstrates areas where hippocampal connectivity was significantly different between the groups.
Analysis of covariance (ANCOVA), activations are shown at $p < 0.01$ false discovery rate (FDR), cluster size 10×3^3.

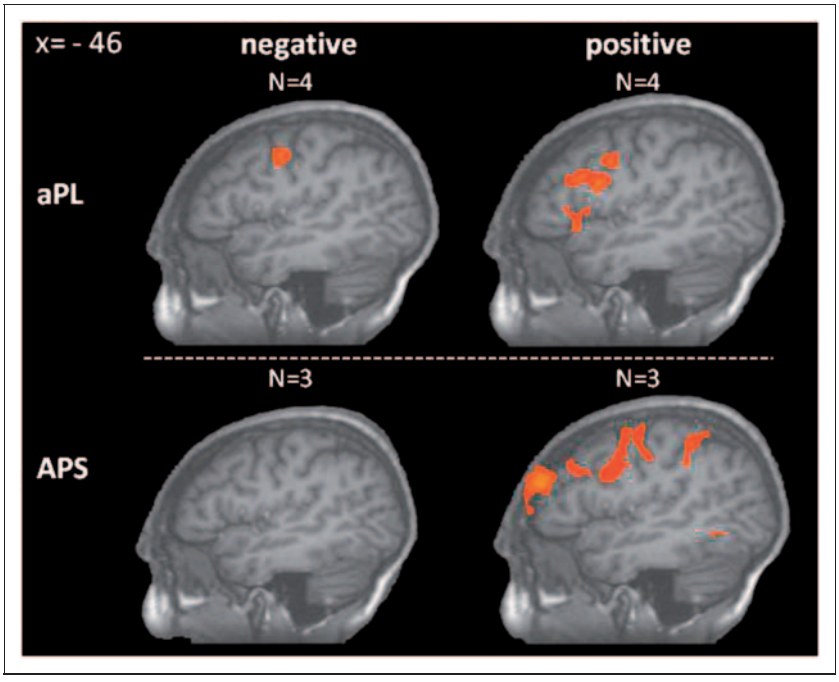

Figure 6 Clinical correlates.
Antiphospholipid antibody (aPL)-positive patients and antiphospholipid syndrome (APS) patients showed a greater increase in brain activity in the task-positive network (TPN) as compared to aPL-negative and non-APS patients, respectively. For the purpose of illustration, activations are shown at $p < 0.005$ (uncorrected), cluster size 5×3^3.

with our hypothesis predicting frontal damage in patients with SLE. Abnormal brain activity in SLE may reflect white matter connectivity, as suggested by DiFrancesco et al.[40] Interestingly, the corpus callosum has recently been reported to associate with the DMN,[37] suggesting that atrophy of this tract, which is seen in SLE,[41,42] may contribute to the altered recruitment of the DMN that we have shown here. In the present study patients with SLE also demonstrated increased activation of the TPN during the learning process. Unlike healthy controls, in which TPN activity increased between the first and second free-recall periods but remained at the same level in the second and third free-recall periods, activity in patients with SLE continued to increase across all three free-recall periods.

The learning-associated brain activity abnormalities demonstrated in patients with SLE in the DMN and TPN represent a greater increase in recruitment of both networks. Similarly, DiFrancesco et al.[40] have shown increased brain activity on fMRI studies of childhood SLE. Activation in selected cortical areas was found to correlate negatively with results of a subset of individual neuropsychological test scores. Fitzgibbon et al.[43] assessed working memory in patients with NPSLE and similarly found that NPSLE patients showed greater fronto-parietal activation than healthy controls or rheumatoid arthritis patients during the memory task, suggesting a greater need to recruit extra-cortical pathways, possibly to supplement impaired function of standard pathways. Similarly, Rocca et al.[44] reported increased fMRI activation in multiple sclerosis patients affecting multiple regions. The same group also studied motor function in patients with NPSLE and found evidence for increased activation in standard motor areas as well as in other less-classical regions, within the frontal and parietal lobes.[45]

We also found increased activation in our group of patients with SLE, seen mainly within the same networks recruited by healthy controls. This increased recruitment was seen in patients without evidence of overt NPSLE, suggesting that this impairment may be an early and frequent compensatory phenomenon and may serve as a mechanism to overcome subtle neuronal damage. Neuronal damage may be due to ischemia mediated by aPL.[46] In the present study the number of patients with or without aPL or APS was too small to allow for statistically significant conclusions; however, the pattern of brain activity was markedly different with a greater increase in brain activity in the TPN in aPL-positive patients and APS patients as compared to aPL-negative and non-APS patients, respectively, suggesting that aPL may have a role in the altered brain activity dynamics seen here. Alternatively, neuronal damage may be due to anti-N-methyl-d-aspartate (anti-NMDA) receptor antibodies, shown to be toxic to neuronal cells of the hippocampus on breach of the blood-brain barrier.[47] Similarly, Katzav et al.[48] have shown that anti-ribosomal P antibodies specifically bind to neurons in the hippocampus when directly injected into the brain in a rat model. Hippocampal volume was smaller in our patients with SLE as reported previously by Appenzeller et al.[41] Hippocampal atrophy in SLE is consistent with our findings of DMN dysfunction in SLE, since the hippocampus is functionally connected with this network.[23] Activity changes in the hippocampus while performing the memory task correlated with learning efficiency in patients but not in controls. In addition, in patients with SLE, hippocampal functional connectivity was more widely distributed with pronounced connectivity to DMN structures. These findings suggest that patients with SLE rely more on hippocampal mechanisms during a memory and learning task. Altered functionality and connectivity of the hippocampal subsystem of the DMN may reflect compensatory cortical reorganization to cope with hippocampal atrophy and dysfunction. Taken together, these findings are consistent with the traditional role of the hippocampus[49] as well as the recent role attributed to the DMN[19–21] in memory processes.

Our findings of altered brain activity patterns in areas shown to be atrophied as well as in areas where structural abnormalities have not been demonstrated suggest that fMRI studies may play an important role in localizing affected areas to be studied further. A better understanding of the pathogenic mechanisms leading to altered brain activity, and possibly to impaired cognition in SLE, may guide targeted treatment for this common impairment seen even in young patients with no history of NPSLE.

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Conflict of interest

None declared.

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