Resting state functional connectivity in SLE patients and association with cognitive impairment and blood-brain barrier permeability

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Abstract

Objective

Extensive blood-brain barrier (BBB) leakage has been linked to cognitive impairment (CI) in systemic lupus erythematosus (SLE). This study aimed to examine the associations of brain functional connectivity (FC) with CI and BBB dysfunction among patients with SLE.

Methods

Cognitive function was assessed by neuropsychological testing (n=77). Resting-state FC (rsFC) between brain regions, measured by functional MRI (n=78), assessed coordinated neural activation in 131 regions across five canonical brain networks. BBB permeability was measured by dynamic contrast-enhanced MRI (DCE-MRI) (n=61). Differences in rsFC were compared between SLE patients with CI (SLE-CI) and those with normal cognition (SLE-NC), between SLE patients with and without extensive BBB leakage, and with healthy controls.

Results

A whole-brain rsFC comparison found significant differences in intra-network and inter-network FC in SLE-CI versus SLE-NC patients. The affected connections showed a reduced negative rsFC in SLE-CI compared to SLE-NC and healthy controls. Similarly, a reduced number of brain-wide connections was found in SLE-CI patients compared to SLE-NC (P=0.030) and healthy controls (P=0.006). Specific brain regions had a lower total number of brain-wide connections in association with extensive BBB leakage (P=0.011). Causal mediation analysis revealed that 64% of the association between BBB leakage and CI in SLE patients was mediated by alterations in FC.

Conclusion

SLE patients with CI had abnormalities in brain rsFC which accounted for most of the association between extensive BBB leakage and CI.

Key words: Systemic lupus erythematosus, Cognitive impairment, Blood-brain barrier, Functional connectivity, Neuroimaging.

Key Messages:

- Functional MRI demonstrated abnormalities in brain functional connectivity (FC) in SLE patients with cognitive impairment.
- Extensive blood-brain barrier (BBB) leakage is associated with cognitive impairment (CI) in SLE patients.
- FC accounted for 64% of the association between BBB leakage and CI.

Introduction

Cognitive impairment (CI) is one of the most common neuropsychiatric manifestations of SLE (NPSLE) (1), with a prevalence of 33–43% in unselected SLE patients (2). The majority of SLE patients with CI experience a fluctuating course of impairment (3), suggesting central nervous system dysfunction that is potentially reversible.

The neuropathological origins of CI in SLE are unclear. Structural brain abnormalities detected by magnetic resonance imaging (MRI) in patients with SLE have weak associations with CI (4). Brain activation assessed with functional MRI (fMRI) provides an indirect index of neuronal activity. Functional connectivity (FC) is calculated from correlation between simultaneous neuronal activity in different anatomical brain sites. Mutually synchronized activation in different regions, indicated by positive correlations, represents positive FC, while reciprocal de-activation between regions, indicated by negative correlations, represents negative FC. Synchronization of neuronal activity is the basis of integrating brain regions into networks that perform higher brain functions, including cognition.

Previous studies demonstrated differences in FC between SLE patients and healthy subjects (5-8) and an association between altered FC and CI (9, 10). We reported an association between extensive BBB leakage with CI in SLE patients (11) but no study has concurrently measured FC, BBB permeability and cognition. We hypothesized that in SLE patients the association between extensive BBB permeability and CI is mediated by alterations in FC.

We compared whole brain FC in SLE patients with and without CI to identify differences in patterns of connectivity between groups. We assessed the associations between FC patterns and extent of BBB leakage. Finally, we performed causal mediation analysis to determine whether the association between BBB leakage and CI is mediated by alterations in FC.

Participants and Methods

Participants

Seventy-eight patients fulfilling revised American College of Rheumatology (ACR) criteria for SLE (12) were consecutively recruited from the Dalhousie Lupus Clinic, Division of Rheumatology, Queen Elizabeth II Health Sciences Center, Halifax, Nova Scotia, Canada. Patients were not pre-screened for CI. Healthy controls (HC) recruited for fMRI scanning (n=71), were 18-75 years old with no history of neuropsychiatric disease, SLE, diabetes mellitus or chronic pain. The study was approved by the Nova Scotia Health Authority (NSHA) Research Ethics Board, and participants provided written informed consent.

For SLE patients, demographic variables, previous NP events and attribution (13), medications, lifestyle habits, and comorbidities (smoking, diabetes mellitus, and hypertension) were documented. Global SLE disease activity [Systemic Lupus Erythematosus Disease Activity Index-2000 (SLEDAI-2K) (14)] and cumulative organ damage [Systemic Lupus International Collaborating Clinics/ACR damage index (SDI) (15)] were recorded. Laboratory variables included complete blood count, serum

creatinine, urinalysis, anti-dsDNA, antiphospholipid (anticardiolipin, lupus anticoagulant) antibodies, C3, and C4 levels. Clinical and cognitive assessments, blood collection and MRI scanning were performed on the same day.

Cognitive function

Neuropsychological tests based on ACR recommendations (16), were completed by 77 SLE patients (one non-native English speaker was omitted) to assess cognitive domains commonly affected in SLE (17). Information processing speed and executive abilities were assessed by the Symbol Digit Modalities Test (SDMT) (18) and Design Fluency Test (19), respectively. Components of the California Verbal Learning Test (CVLT-II) (20) provided indices of attention span, new learning, and delayed recall. Raw scores were standardized based on published normative data and converted to Z-scores (19): Z-scores ≤-1.5 in ≥1 domains indicated cognitive impairment (SLE-CI) with the remainder having normal cognition (SLE-NC).

Statistical analysis of clinical data

Summary group data were expressed as percentages, mean±SD, or median and interquartile ranges as appropriate. For categorical variables, comparisons between groups were performed with Fisher's exact test. For continuous variables that were normally distributed, t-tests and ANOVAs were used for group comparisons. For continuous variables not normally distributed, Wilcoxon rank sum tests were used.

Imaging

MRI acquisition

Neuroimaging used a 3T MRI scanner (Discovery MR750, GE Healthcare, Waukesha, WI), with a 32-channel head coil (MR Instruments, Inc., Minneapolis, MN, USA). T1-weighted images provided anatomical reference (specifications: 1x1x1 mm isometric voxels, 184x256x256 voxel resolution, echo time [TE] 1.33 ms, repetition time [TR] 4.0 ms, flip angle 9°), followed by resting state (rs) fMRI signals (3x3x3 mm voxels, 72x72x51 voxel resolution, TR 0.95s, 500 TRs), and dynamic contrast-enhanced T1-weighted images (1.25x1.25x6mm voxels, 192x192x34 voxel resolution, TE/TR=2/4ms, flip angle 15°, averages 1, Δt=20sec) acquired between 6-20 minutes after intravenous injection of gadobenate dimeglumine (0.1mmol/kg, MultiHance, Bracco Imaging Canada, Montreal, QC).

BBB leakage analysis

Dynamic contrast enhanced (DCE) MRI scanning for quantitative assessment of BBB permeability was available in 61/78 patients who had rsfMRI scans. Contraindication to contrast use, patient refusal or technical difficulties preventing interpretation accounted for missing scans. The analysis was performed as reported (11, 21, 22). In brief, this involved normalization to the Montreal Neurological Institute (MNI) standardized brain image, and identification of brain voxels with BBB leakage. The percentage of voxels with BBB leakage determined whole-brain BBB leakage.

Abnormal BBB leakage threshold was based on our previous study of DCE-MRI, which included 57/61 SLE patients in the current study, and 9 healthy controls. The prior study

identified extensive BBB leakage with an outlier analysis using the Median Absolute Deviation approach. Participants with >9.11% of brain tissue affected by BBB leakage were considered to have extensive BBB leakage (11).

Resting-state fMRI

Data from 78 SLE patients were pre-processed with FSL, AFNI, and FreeSurfer (23-25) based on scripts by the 1000 Functional Connectomes Project (26) to mitigate artefacts due to head motion and off-resonance fields, to filter out temporal and spatial noise, and to remove non-brain data.

Pre-processing involved cropping the first five time points to let the blood oxygen level-dependent (BOLD) signal reach steady state and motion correction to the mean of the remaining time series; this also allowed calculation of head motion parameters for later use. A sample frame was extracted from the mean-aligned data for image registration. Images were spatially smoothed with a Gaussian kernel (full width at half-maximum: 6 mm), intensity-normalized, temporally filtered with a band-pass filter (0.005-0.3 Hz cutoff frequencies), then detrended. Measures of motion outliers based on framewise displacement (FD) and the derivative of variability across voxels (DVARS) were calculated using previously published methodologies (27).

fMRI data were re-scaled, re-oriented, and registered to the MNI standardized brain (28). Images were segmented into grey matter, white matter (WM), and cerebrospinal fluid (CSF), and mean signals from the latter two were calculated. The WM and CSF signals, along with the global signal and head motion parameters, were regressed out of the BOLD signals for each voxel as nuisance data, leaving residuals that better represented rs

functional activity. Finally, motion outliers calculated previously were extracted for each participant: maximum FD≥3 mm and a motion outlier rate >30% in either FD or DVARS (29) were additional exclusion criteria, though no subjects met them .

Brain Network Analysis

From pre-processed data, BOLD signal time series were calculated for 131 regions of interest (ROIs), using an optimized version of the Harvard-Oxford anatomical parcellation (29-31). These ROIs were assigned to one of five canonical rs sub-networks (subcortical, sensory, default mode, attention/executive, and language/memory) using a meta-analytic framework (Neurosynth) described previously (31).

To calculate the rs functional connectivity (rsFC) between ROIs, a zero-lag Pearson correlation matrix was calculated between mean BOLD signals for each ROI and every other ROI. The correlation coefficient between BOLD signals in two ROIs is referred to as the *rsFC value* for that connection: large positive or negative rsFC values denote strong positive or negative connectivity between two ROIs, while small rsFC values denote weak to non-existent connectivity. ROIs were sorted by resting-state network (31) and the rsFC values for each connection within or between networks were summed and normalized based on the total number of possible connections for visualization purposes. rsFC values were compared between SLE-CI and SLE-NC using pairwise independent t-tests and corrected for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR) method at the ROI level (32); connections that survived correction were designated *connections of interest (COI)*.

The mean rsFC values of the COIs were compared groupwise between HC, SLE-NC, and SLE-CI using a one-way ANOVA with Bonferroni correction. This allowed comparisons of HC vs. SLE-NC and HC vs. SLE-CI with appropriate statistical rigor; the comparison of SLE-NC vs. SLE-CI was ignored, as a significant difference between them is intrinsic to the input variable.

Analysis of BBB leakage in relation to brain networks

For associations between rsFC and BBB leakage, the mean rsFC values for the COIs were compared between SLE patients with and without extensive BBB leakage using unpaired samples t-tests with FDR correction. Mean COI rsFC was also correlated with %BBB leakage using Spearman's rho. Then, %BBB leakage within each ROI was calculated using the same method used for the whole brain but restricted to voxels of each ROI.

We calculated the total number of brain wide connections between each ROI and every other ROI, termed degree (33), using the Brain Connectivity Toolbox for MATLAB (34). Correlation of the degree of each ROI with its %BBB leakage value was calculated using Spearman's rho to determine which areas of the brain were most affected by BBB dysfunction; P-values were corrected for multiple comparisons using FDR. For the ROIs in which degree was significantly associated with %BBB leakage, the average degree of affected regions was calculated and correlated with whole-brain BBB leakage using Spearman's rho, then compared groupwise between HC, SLE-NC, and SLE-CI using a one-way ANOVA with Bonferroni correction. In all cases, corrected $P<0.05$ was regarded as significant.

Causal mediation analysis

To determine if alterations in brain network connectivity mediate the association between extensive BBB permeability and CI among SLE patients, a causal mediation analysis was performed using the “medeff” package in STATA-IC Version 14 (StataCorp, TX, USA). Only SLE patients with complete data for mean rsFC, BBB permeability, and cognitive function were included. Of the 78 patients with rsfMRI scans, 17 were excluded due to missing DCE-MRI scans and one did not undergo cognitive testing, leaving 60 patientsfor this analysis. We fit regression models for the mediator and the outcome. The mediator model was a linear regression model for mean rsFC with BBB leakage (extensive vs. non-extensive) as the independent variable. The outcome model was a logistic regression model for cognitive status (CI vs. NC) with BBB leakage and mean rsFC as independent variables. Both models were adjusted for patient age as a covariate.

Using these models and a standard mediation approach (35), we estimated the total effect of BBB permeability on cognitive status, the average causal mediated effect (ACME) through mean rsFC, and the average direct effect (ADE) via pathways outside of the mediator of interest. Nonparametric bootstrapping (1000 replications) was used for the quasi-Bayesian approximation of 95% confidence intervals for the effect estimates. A 95% confidence interval for the ACME that did not cross zero was evidence that mean rsFC mediated the association between BBB permeability and cognitive status at $P<0.05$. The proportion of the total effect mediated by mean rsFC was reported as a percentage.

A key assumption underlying causal mediation analysis is sequential ignorability, which implies that there is no unmeasured confounding of the exposure-mediator, exposure-outcome, or mediator-outcome relationships. To evaluate the robustness of our results to

violations of this assumption, a sensitivity analysis was performed using the "medsens" package in STATA-IC. This sensitivity analysis is based on the correlation coefficient (ρ) between the error term in the mediator model and the error term in the outcome model. More extreme values of ρ represent larger departures from the sequential ignorability assumption and greater likelihood that unmeasured confounding will lead to a biased estimate of ACME. While the true value of ρ is unknown, it is possible to calculate the value of ρ for which the ACME would be zero: a large ρ at ACME=0 indicates a robust result.

Results

Demographic and clinical characteristics: The 78 SLE patients and 71 controls for rsfMRI differed in the proportion of females (89.7% vs. 69.0%; P=0.002), mean(±SD) age (49.4±14.3 vs. 38.9±12.9 years; U=3865, P<0.001), race/ethnicity (91.0% vs. 77.6% Caucasian; P=0.040), and years of education (15.5±3.6 vs.16.5±2.9 years; U=2100, P=0.014). The mean SLE disease duration was 15.8±11.0 years. Cumulative ACR classification criteria for the 78 SLE patients were malar rash (47%), discoid rash (6.4%), photosensitivity (53.8%), oral/nasal ulcers (56.4%), serositis (34.6%), arthritis (78.2%), renal disorder (28.2%), neurological disorder (10.3%), hematological disorder (89.7%), immunological disorder (83.3%) and antinuclear antibody (100%). Medication utilization and autoantibodies reflected that of a general lupus population (13) with low generalized disease activity and modest organ damage (Table 1).

Prior NP events (excluding CI) from all causes occurred in 46/78 (59.0%) SLE patients and NP events attributable to SLE were present in 18/78 (23.1%). The latter included transient ischemic attacks (N=4), stroke (N=3), cranial neuropathy (N=4), seizure disorder (N=4), acute confusional state (N=4), psychosis (N=1), and aseptic meningitis (N=1). All NP events had resolved by the time of study. Impairment in one or more cognitive tests occurred in 37/77 (48%) patients and included deficits in information processing speed (9%), attention span (22%), new learning (6%), delayed recall (14%), and executive abilities (27%). Patients with CI also had longer SLE disease duration (Table 1).

Brain networks in cognitively impaired and unimpaired SLE patients: Differences in rsFC between SLE-CI and SLE-NC are illustrated in Figure 1A and summarized in Supplementary Tables S1 and S2, available at *Rheumatology* online. The summed and normalized rsFC values by network are shown in Figure 1B. Significant intra-network rsFC differences were found in the sensory, attention/executive, and language/memory networks, while significant inter-network rsFC differences occurred primarily between other networks and the sensory and default-mode networks.

Post hoc comparison showed the mean rsFC of the COIs in HC differed significantly from both SLE-CI and SLE-NC ($F^{(2,145)}$=42.1, P<0.001, Figure 1C). In pairwise group comparisons, both SLE-NC and SLE-CI had significantly different mean rsFC values from HC. While mean rsFC values were negative for HC and SLE-NC, they were more frequently neutral or positive for SLE-CI.

The significant differences in rsFC between SLE patients and controls persisted after adjusting for age, sex, race/ethnicity and education using a one-way ANCOVA ($F^{(2,119)}$=35.353, P≤0.001); pairwise tests of HC vs. SLE-NC ($F^{(1,83)}$=13.175, P=0.001) and

HC vs. SLE-CI ($F^{(1,80)}$=7.063, P=0.028) remained significant. Disease duration was not a significant covariate of rsFC between SLE-CI and SLE-NC groups ($F^{(1,74)}$=1.935, P=0.168).

BBB leakage and association with brain networks: On DCE-MRI scanning, 16/61 (26.2%) SLE patients had extensive BBB leakage. The mean COI rsFC showed significantly reduced negative FC in SLE patients with extensive BBB leakage compared to those without (Figure 2A). There was also a significant correlation between extent of BBB leakage and mean rsFC in the total SLE group (Figure 2B). Figure 2C illustrates this association using sample BOLD signals for two ROIs in one HC subject and in four SLE patients with different BBB leakage levels.

The ROI-level BBB leakage analysis is depicted in Figure 3. Brain regions with significant correlations between degree and %BBB leakage are shown in Figure 3A: eight ROIs showed correlations that survived correction, of which seven had negative correlations (i.e., lower degree with greater BBB leakage) and one had a positive correlation. The mean degree for the seven negatively correlated ROIs showed a significant association with whole-brain %BBB leakage (Figure 3B). Mean degree also differed across HC, SLE-NC and SLE-CI groups ($F^{(2,123)}$=5.401, P=0.006; Figure 3C), with brain-wide rsFC of the seven ROIs significantly lower in SLE-CI group compared to SLE-NC and HC groups. Including the single positively-correlated region did not alter these findings, and repeating the analysis for the single positively-correlated region alone returned no significant results.

Causal mediation analysis: Analysis of mean rsFC as a potential mediator of the relationship between BBB permeability and cognitive status is summarized in Figure 4.

As we reported previously (11), a statistically significant total effect of BBB permeability on the probability of cognitive impairment was found after adjusting for age (total effect =0.352; 95% confidence interval: 0.079-0.571). The mediated effect by mean rsFC was also significant (ACME=0.235; 95% confidence interval: 0.049-0.405), accounting for approximately 64% of the total effect, while the direct effect of BBB permeability on cognitive status was not significant (ADE=0.118; 95% confidence interval: -0.065-0.319). Sensitivity analysis found that ρ would need to exceed 0.7 for the ACME of mean rsFC to become zero or negative, indicating robust findings.

Discussion

Nervous system disease in SLE is challenging due to heterogeneity of clinical manifestations, uncertain attribution of NP events to SLE or non-SLE causes, and low frequency of patients with more severe manifestations. Cognitive impairment is common, amenable to study, and may be viewed as an indicator of overall brain health (2). We report that CI in SLE is associated with reduced negative rsFC in several rs networks in brain regions associated with sensory functions, memory, and attention. Our findings suggest that the previously reported association between CI and increased BBB permeability in SLE (11) may be attributed, in part, to changes in rsFC.

Brain injury in patients with NPSLE may occur through two pathways (36). Ischemic injury results from intravascular thrombosis mediated by antiphospholipid antibodies, endothelial activation and complement activation. Inflammation-induced injury is mediated by enhanced BBB permeability that allows circulating proteins, autoantibodies

and immune cells to access brain tissue. These in turn bind to neuronal/glial cells or form immune complexes with downstream activation of astrocytes and plasmacytoid dendritic cells that trigger neuroinflammatory processes, and lead to neuronal-network reorganization (aberrant synaptogenesis/pruning). Both pathogenetic mechanisms can fluctuate in intensity over time in keeping with the evanescent clinical course of NPSLE.

Previous studies using rsfMRI identified altered FC in SLE patients affecting multiple networks (5-10, 37-41), albeit with inconsistent findings (9, 40, 41). This may, in part, reflect highly variable methodologies for determining rsFC (9, 40, 41). Most studies have found differences in FC between SLE patients and healthy controls (5-8), but relatively few have reported an association with global SLE disease activity (5-7) or with CI (9, 10). In healthy participants the default-mode network (DMN) has negative rsFC with other networks (42). In the current study and in others (6, 8) reduction in negative rsFC was found in SLE patients, indicating DMN dysfunction. The DMN is associated with self-referential thinking and memory recall, and DMN function is positively correlated with cognitive performance (43). Thus, it is plausible that DMN dysfunction could directly relate to CI; indeed, one study has suggested that FC may be an early indicator of CI in SLE patients (37).

No previous studies of rsFC in SLE patients have included concurrent BBB imaging. Our study demonstrates reduced negative rsFC in SLE patients with CI compared to those without, and in SLE patients with extensive BBB leakage compared to those with normal BBB function. Causal mediation analysis found that 64% of the total effect of BBB permeability on cognitive status was mediated by changes in rsFC. Note, however, that pairwise group comparisons found that SLE-NC had significantly more negative mean

rsFC values compared to HC, while one of the eight significant correlations between degree and %BBB leakage was positive. This emphasizes the heterogenous nature of NPSLE and potentially a more complex relationship between BBB permeability and cognition.

Although the clinical phenotype of CI may look similar amongst SLE patients, there are likely to be different causal pathogenic mechanisms. Thus, the association of CI with objectively defined abnormalities in BBB permeability and FC could identify a specific clinical subset. This use of advanced, multimodal neuroimaging may be one pathway to personalized or precision medicine for diagnosis and treatment of NPSLE. Such an approach has resulted in advances in other fields including prediction of treatment responses in individual patients with depression (44) and in fibromyalgia (45).

One strength of the current study is the use of an unselected sample of adult SLE patients, representative of clinic-attending lupus patients, albeit predominantly Caucasian, with stable and generally quiescent SLE. Additionally, all clinical, cognitive, and neuroimaging assessments were performed on the same day, usually within 6 hours of each other, thereby minimizing temporal dissociations between CI and putative pathogenetic mechanisms detected by neuroimaging. Study limitations include a relatively small sample size that precluded identification of associations with subsets of CI. To avoid excessive demands on study participants, cognitive testing was constrained, and neuroimaging did not include task-based FC evaluations. The lack of information on other cardiovascular risk factors in HC limited the ability to fully investigate their potential contribution to group differences in FC and BBB function. Finally, we cannot exclude the possibility of unmeasured confounds in causal mediation analysis, although our sensitivity

analysis indicates a robust effect. Regardless, the reported associations will require independent confirmation and longitudinal study.

In summary, we have identified distinct changes in rsFC, in part associated with extensive BBB leakage, in SLE patients with CI compared to SLE patients without CI and healthy controls. Future studies should establish how these clinical and neuroimaging abnormalities evolve over time and the association, if any, with inflammatory mediators and lupus autoantibodies. Our study which combined multi-modal neuroimaging with comprehensive clinical evaluation of SLE patients demonstrates potential to unravel pathogenetic mechanisms underlying CI and other NP manifestations in SLE patients, thereby providing a pathway for the development and evaluation of novel therapeutic targets and strategies.

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Legends for figures:

Figure 1: The association between resting-state functional connectivity (rsFC) and cognitive impairment (CI) in patients with SLE.

A. Contrast maps showing brain regions (colored spheres) with significant reductions in functional connectivity (yellow lines) in SLE-CI compared to SLE-NC. Multiple comparisons were corrected using False Discovery Rate (FDR) at $q<0.05$. Node colors represent membership in each of the five canonical resting-state networks (color key legend at the left lower corner). Visualization created with BrainNet Viewer (46). For more information on these regions and connections, see Supplementary Tables 1 and 2, respectively. **B.** Summed and normalized rsFC contrast matrix depicting the relative strengths of the total significant functional connections between networks (SLE-CI<SLE-NC). Color heat map (right) represents the range of normalized sums; abbreviations represent resting-state networks as defined in the key below Panel A. **C.** Resting-state FC values of all significant connections identified in Panel A were averaged across connections and compared between HC, SLE-NC, and SLE-CI patients. Data is presented as a scatterplot over a boxplot; the mean is shown as a black line.

Figure 2: Reduced negative rsFC in patients with SLE is associated with extensive blood-brain barrier (BBB) leakage.

A. In individual SLE patients, the mean rsFC was significantly higher in those with extensive BBB leakage. Data is presented as a scatter plot over a boxplot; the mean is shown as a black line. Normal vs. extensive BBB leakage groups were defined using the

median absolute deviation approach in Kamintsky et al. (11). **B.** In individual SLE patients, there was a significant association between the mean of all significant functional connections with %BBB disruption. **C.** %BOLD signals (Z-scored) for two representative nodes are plotted for five different study participants in order of increasing %BBB disruption (#1 HC; #2 SLE-NC; #3-5 SLE-CI). The plots show a shift from negative (#1) or no connectivity (#2-3) to positive connectivity (#4-5) between the two nodes with increase in %BBB leakage in SLE. SMGp_L (orange line): left supramarginal gyrus posterior, part of the attention/executive network; MPFC_R (blue line): right medial prefrontal cortex, part of the default mode network.

Figure 3: The total brain-wide connectivity of brain regions (degree) is linked with %BBB leakage and CI.

A. Nodes in brain regions with decreased (red) or increased (green) degree were correlated with %BBB leakage (Spearman ρ, corrected for multiple comparisons with FDR). Abbreviations: ACCrp_R, right anterior cingulate cortex rostral posterior, part of the default mode network; Amyg_L, left amygdala, part of the subcortical network; INSm_L, left middle insula, part of the sensory network; MTGp_R, right middle temporal gyrus, posterior division, part of the language/memory network; pHippp_R, right parahippocampal gyrus, posterior division, part of the language/memory network; PostC_R, right post central gyrus (S1), part of the sensory network; STGp_L, left superior temporal gyrus, part of the language/memory network; vMPFC_R, right ventral medial prefrontal cortex, part of the default mode network. **B.** Higher %BBB leakage is associated with lower average degree in the negatively affected nodes. **C.** The average

degree of affected nodes is significantly lower in SLE-CI compared to SLE-NC and HC. Data visualized as violin plot overlaid box and scatter plots; red horizontal line: median; white horizontal line: mean.

Figure 4: Association of blood-brain barrier permeability with cognitive impairment in SLE, mediated by altered functional connectivity.

Effect estimates (with 95% confidence intervals) represent the percentage-point change in the probability of cognitive impairment with extensive BBB leakage versus non-extensive BBB leakage via the total, mediated, or direct pathways. Approximately 64% of the total effect of BBB permeability on cognitive status is mediated by mean resting-state functional connectivity (rsFC). Both the mediator and outcome models are adjusted for patient age in years. Abbreviations: BBB, blood brain barrier; rsFC, resting state functional connectivity; ACME, average causal mediated effect; ADE, average direct effect; CI, cognitive impairment.

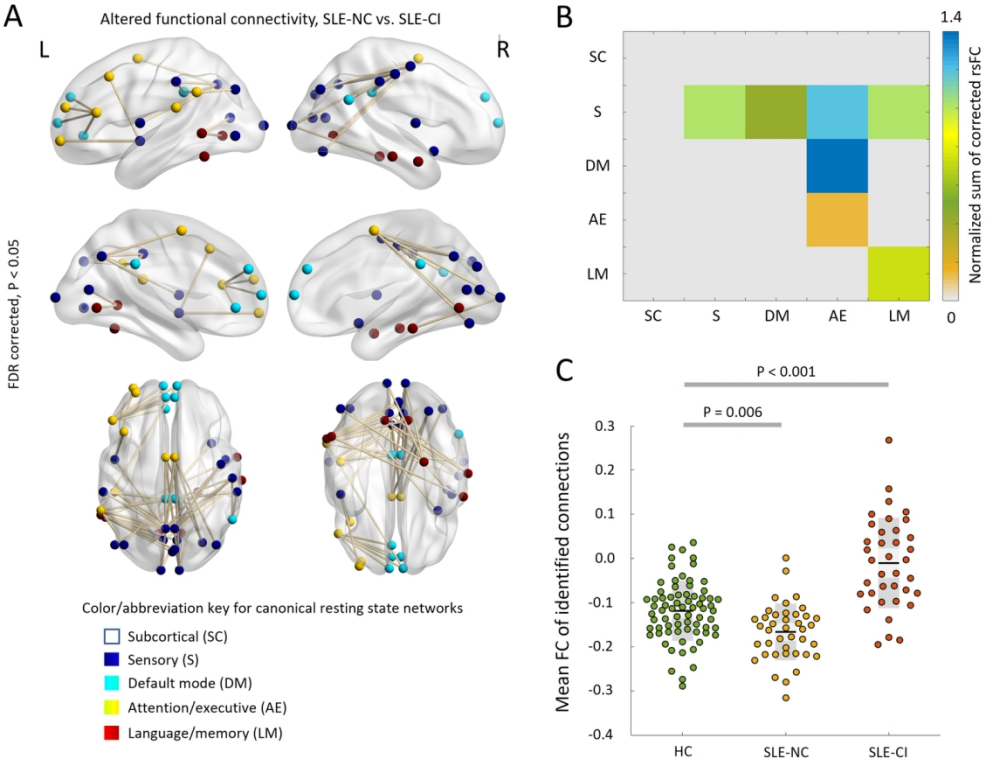

Fig 1

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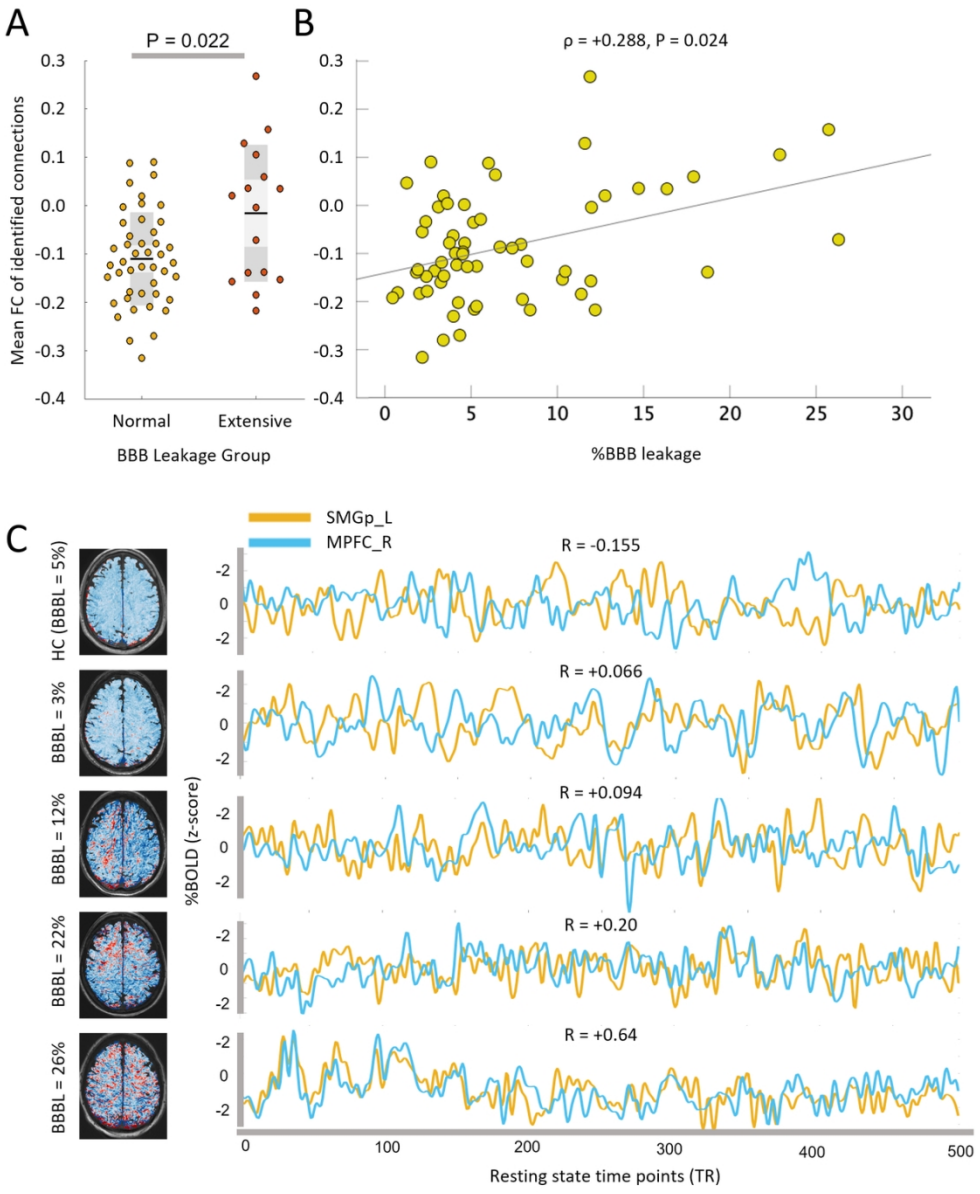

Fig 2

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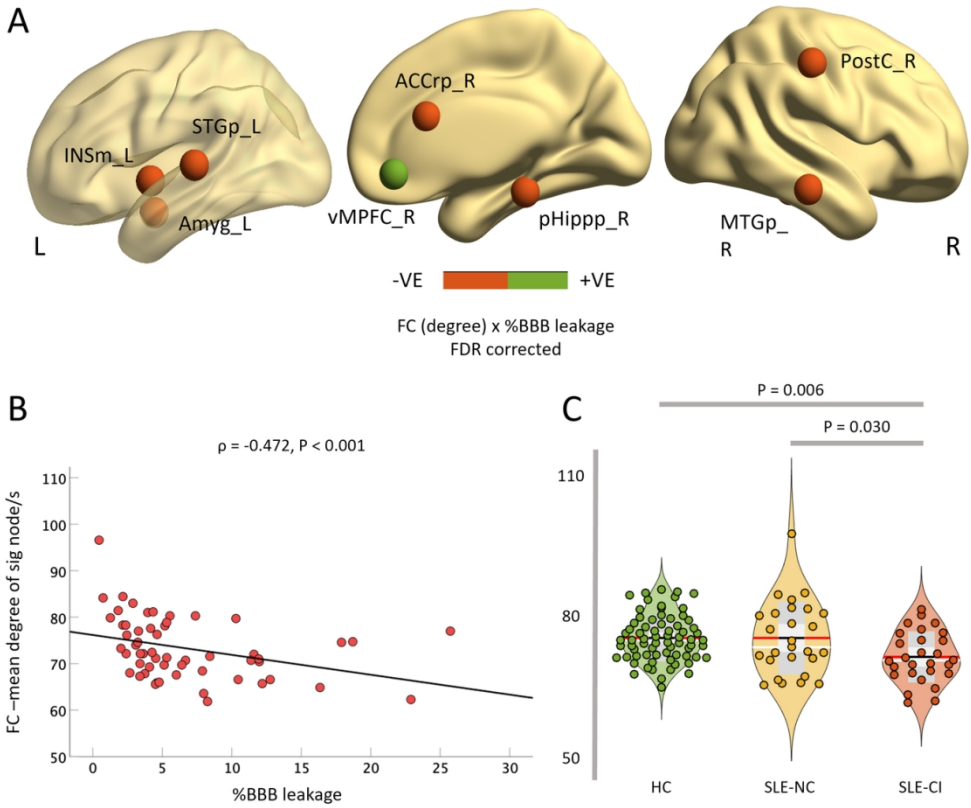

Fig 3

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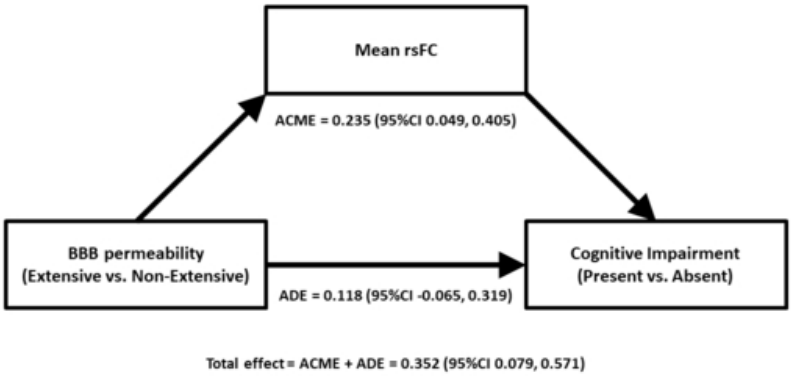

Fig 4

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Table 1: Total SLE patients and comparison of those with and without cognitive impairment.

	SLE (N=78)	Cognitive Impairment (N=37)	Normal Cognition (N=40)	P
Female N (%)	70 (89.7%)	34 (91.9%)	36 (90.0%)	1.0
Age (years), mean ± SD	49.4 ± 14.3	50.2 ± 14.4	48.7 ± 14.4	0.658
Race/Ethnicity, N (%)				
Caucasian	71 (91.0%)	31 (83.8%)	39 (97.5%)	0.051
Other	7 (9.0%)	6 (16.2%)	1 (2.5%)	
Years of education, mean ± SD	15.5 ± 3.6	14.7 ± 2.9	16.2 ± 4.0	0.093
Current smokers, N (%)	8 (10.3%)	6 (16.2%)	2 (5.0%)	0.144
Ever smokers, N (%)	29 (37.2%)	17 (45.9%)	11 (27.5%)	0.104
SLE disease duration (years) mean ± SD	15.8 ± 11.0	18.7 ± 11.8	12.8 ± 9.3	**0.027**
Prior NP events (excluding CI), N (%)	46 (59.0%)	22 (59.5%)	24 (60.0%)	1.0
Prior NP events attributed to SLE (excluding CI), N (%)	18 (23.1%)	11 (29.7%)	7 (17.5%)	0.282
SLEDAI-2K score, median (IQR)	2 (0-4)	2 (1-4)	2 (0-3.5)	0.131
SLEDAI-2K score without NP variables, median (IQR)	2 (0-4)	2 (1-4)	2 (0-3.5)	0.131
SLICC/ACR Damage Index, median (IQR)	1 (0-2)	1 (0-2)	0 (0-1)	0.105
SLICC/ACR Damage Index without NP variables, median (IQR)	0 (0-2)	1 (0-2)	0 (0-1)	0.180
Medications, N (%)				
Corticosteroids	8 (10.3%)	5 (13.5%)	3 (7.5%)	0.470
Antimalarials	56 (71.8%)	23 (62.2%)	33 (82.5%)	0.072

Immunosuppressive drugs	36 (46.2%)	18 (48.6%)	17 (42.5%)	0.651
ASA/clopidogrel	9 (11.5%)	4 (10.8%)	5 (12.5%)	1.0
Warfarin	7 (9.0%)	5 (13.5%)	2 (5.0%)	0.251
Psychoactive drugs	33 (42.3%)	18 (48.6%)	15 (37.5%)	0.363
Autoantibody positivity N (%)				
Lupus anticoagulant (LAC)	17/76 (22.4%)	10/36 (27.8%)	7 (17.5%)	0.409
Anticardiolipin (aCL)	9/76 (11.8%)	5/36 (13.9%)	4 (10.0%)	0.728
Anti-β2 glycoprotein I (anti-β2 GPI)	9 (11.7%)	5 (13.5%)	4 (10.0%)	0.731
LAC or aCL or anti-β2 GPI	23/76 (30.3%)	13/36 (36.1%)	10 (25.0%)	0.326
Co-morbidities, N (%):				
Hypertension	11 (14.1%)	7 (18.9%)	4 (10.0%)	0.336
Diabetes	4 (5.1%)	3 (8.1%)	1 (2.5%)	0.346

P values refer to the comparison between SLE patients with and without cognitive impairment (CI). NP, Neuropsychiatric; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000; SLICC/ACR, Systemic Lupus International Collaborating Clinics/American College of Rheumatology; *Immunosuppressive drugs: methotrexate, azathioprine, cyclophosphamide, leflunomide, mycophenolate mofetil and intravenous gamma globulin.*