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Thalamic–hippocampal–prefrontal disruption in relapsing–remitting multiple sclerosis

Kyle C. Kern[a], Stefan M. Gold[b], Brian Lee[a], Michael Montag[c], Jessica Horsfall[c], Mary-Frances O'Connor[d], Nancy L. Sicotte[a,c,*]

[a]Department of Neurology, Cedars-Sinai Medical Center, Los Angeles, CA, USA
[b]Institute for Neuroimmunology and Clinical Multiple Sclerosis Research, University Hospital Hamburg-Eppendorf, Hamburg, Germany
[c]Department of Neurology, University of California Los Angeles, Los Angeles, CA, USA
[d]Department of Psychology, University of Arizona, Tucson, AZ, USA

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ABSTRACT

Background: Cortical, thalamic and hippocampal gray matter atrophy in relapsing–remitting MS (RRMS) is associated cognitive deficits. However, the role of interconnecting white matter pathways including the fornix, cingulum, and uncinate fasciculus (UF) is less well studied.
Objective: To assess MS damage to a hippocampal–thalamic–prefrontal network and the relative contributions of its components to specific cognitive domains.
Methods: We calculated diffusion tensor fractional anisotropy (FA) in the fornix, cingulum and UF as well as thalamic and hippocampal volumes in 27 RRMS patients and 20 healthy controls. A neuropsychological battery was administered and 4 core tests known to be sensitive to MS changes were used to assess cognitive impairment. To determine the relationships between structure and cognition, all tests were grouped into 4 domains: attention/executive function, processing speed, verbal memory, and spatial memory. Univariate correlations with structural measures and depressive symptoms identified potential contributors to cognitive performance and subsequent linear regression determined their relative effects on performance in each domain. For significant predictors, we also explored the effects of laterality and axial versus radial diffusivity.
Results: RRMS patients had worse performance on the Symbol Digit Modalities Test, but no significant impairment in the 4 cognitive domains. RRMS had reduced mean FA of all 3 pathways and reduced thalamic and hippocampal volumes compared to controls. In RRMS we found that thalamic volume and BDI predicted attention/executive function, UF FA predicted processing speed, thalamic volume predicted verbal memory, and UF FA and BDI predicted spatial memory.
Conclusions: Hippocampal–thalamic–prefrontal disruption affects cognitive performance in early RRMS with mild to minimal cognitive impairment, confirming both white and gray matter involvement in MS and demonstrating utility in assessing functional networks to monitor cognition.

1. Background

Cognitive dysfunction in multiple sclerosis (MS) has a prevalence from 35% to 60% (Benedict and Zivadinov, 2011) and relates to disease progression (Amato et al., 2001), vocational status, and quality of life (Rao et al., 1991). However, conventional disease measures such as the Expanded Disability Status Scale (EDSS) and T2 lesion volume poorly predict cognitive decline. Advances in MRI and Diffusion Tensor Imaging (DTI) may identify better biomarkers for cognition.

MS is a demyelinating autoimmune disorder of focal inflammatory lesions in CNS white matter (WM) (Noseworthy et al., 2000). But imaging and pathology studies also demonstrate diffuse changes in both gray matter (GM) and WM contributing to cognitive dysfunction (Rovaris et al., 2002). Identifying early biomarkers predictive of subclinical

Abbreviations: AD, axial diffusivity; BDI, Beck Depression Inventory; BSRT, Buschke Selective Reminding Test; BVMT, Brief Visuospatial Memory Test; BVRT, Benton Visual Retention Test; CVLT-II, California Verbal Learning Test II; DTI, diffusion tensor imaging; EDSS, Expanded Disability Status Scale; FA, fractional anisotropy; FAST, FMRIB's Automated Segmentation Tool; FLAIR, Fluid Attenuated Inversion Recovery; FSL, Functional MRI of the Brain Software Library; FOV, field of view; GM, gray matter; MPRAGE, Magnetization Prepared Rapid Acquisition Gradient Echo; MRI, magnetic resonance image; NEX, number of excitations; PASAT, Paced Auditory Serial Addition Test; RAVLT, Rey Auditory Verbal Learning Test; RD, radial diffusivity; ROI, region of interest; RRMS, relapsing remitting multiple sclerosis; SDMT, Symbol Digit Modalities Test; TBSS, Tract-based Spatial Statistics; TE, echo time; TI, inversion time; TR, repetition time; UF, uncinate fasciculus; WAIS, Wechsler Adult Intelligence Scale; WM, white matter.

* Corresponding Author: Department of Neurology, Cedars-Sinai Medical Center, Rm 6414, 127 S. San Vicente Blvd., AHSP, Los Angeles, CA 90048, USA. Tel.: 1-310 423 1320.
E-mail address: Nancy.Sicotte@cshs.org (N.L. Sicotte).

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changes in cognitive networks allows for monitoring of both disease progression and efficacy of treatments such as anticholinesterases or cognitive therapy (Benedict and Zivadinov, 2011).

Cognitive dysfunction in MS is also mediated by GM changes including cortical atrophy and cortical lesions found on MRI and histology (Benedict et al., 2002; Geurts et al., 2005). Subcortical atrophy in the thalamus (Cifelli et al., 2002; Houtchens et al., 2007) and hippocampus (Sicotte et al., 2008) is also related to cognitive deficits. The thalamus is affected early in MS(Cifelli et al., 2002), is associated with cognitive impairment (Houtchens et al., 2007), and predicts disease progression (Rocca et al., 2010). The hippocampus is implicated in MS-related memory impairment (Sicotte et al., 2008), but structural and functional changes are also seen with intact spatial memory (Roosendaal et al., 2010).

The anterior thalamic nuclei and the hippocampus interact bidirectionally via the cingulum bundle and the fornix to form the Papez circuit, which is critical to memory encoding and recall (Aggleton and Sahgal, 1993). MS-related damage has been shown *in vivo* in both the fornix (Kern et al., 2012; Roosendaal et al., 2009) and the cingulum (Mesaros et al., 2012). The uncinate fasciculus (UF) is also affected in MS(Fink et al., 2010) and connects the anterior temporal pole to the prefrontal cortex, completing a thalamic–hippocampal–prefrontal circuit (see Fig. 1). All 3 pathways affected in MS are linked to cognitive performance, although the relative contributions remain ill-defined.

White matter damage in MS is traditionally assessed using T2 lesion volume, but has shown only modest associations with cognition (Rovaris et al., 1998). However, DTI is sensitive to microstructural changes even in normal appearing tissue, and has been linked to cognition in MS(Dineen et al., 2009). DTI tractography allows identification of specific WM bundles and quantification of tissue changes *in vivo*(-Wakana et al., 2007). DTI tractography is suitable for investigating cognitive networks, such as a thalamic–hippocampal–prefrontal circuit that is likely disrupted in MS.

We hypothesize that microstructural damage in the thalamic–hippocampal–prefrontal circuit is associated with cognitive function, particularly memory domains, in relapsing–remitting MS (RRMS). Given the widely distributed connections of the thalamus and its known impact on cognition in MS(Houtchens et al., 2007), we expect that disrupting these circuits will also affect other cognitive domains frequently affected including processing speed, attention and executive function. We hypothesize that early structural changes predict cognitive performance in domains of attention and executive function, processing speed, verbal memory and spatial memory before significant impairment is evident. While pathways in this network have been implicated individually, here we assess the relative contributions of insult to the cingulum bundle, the fornix, the UF, the thalamus and the hippocampus to cognitive performance in RRMS.

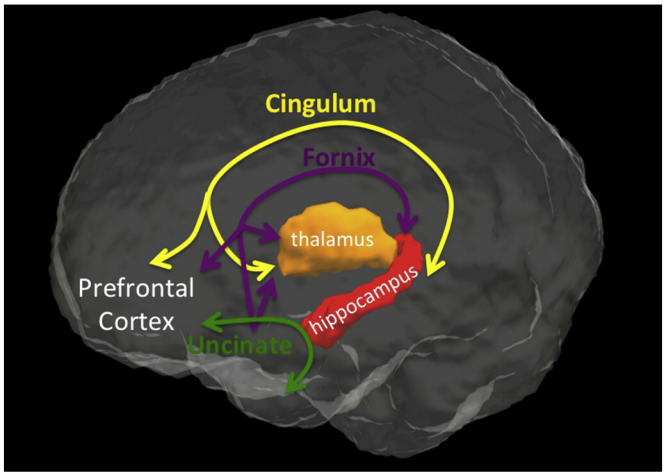

Fig. 1. Limbic pathways completing a thalamic–hippocampal–prefrontal circuit include the cingulum, fornix and the uncinate fasciculus.

2. Methods

2.1. Subjects

Subjects included 27 RRMS patients diagnosed by McDonald Criteria (McDonald et al., 2001) and 20 healthy controls matched for age, gender and education level. Participants were recruited from the University of California, Los Angeles multiple sclerosis clinic and from the community using flyers, advertisements and social media. Control subjects were free of any neurologic or medical conditions, were on no medications, and had normal neurologic examinations. Patients were excluded if they had a relapse or received steroids within the previous 3 months. Participants with a history of drug or alcohol abuse within the previous 3 years were also excluded. Patients were assessed with the Expanded Disability Status Scale (EDSS) and the Beck Depression Inventory II (BDI-II) (Beck et al., 1997) to assess the confounding factor of depression, which is associated with cognition and hippocampal volume (Gold et al., 2010; Gold et al., 2014; Heesen et al., 2010).

2.2. Cognitive assessment

Cognitive tests included: the Wechsler Adult Intelligence Scale (WAIS) and Memory Scale III(Wechsler, 1945), the Delis–Kaplan Executive Function System, the Symbol Digit Modalities Test (SDMT) (Smith, 2002), the Paced Auditory Serial Addition Test (PASAT) (Gronwall, 1977), the Buschke Selective Reminding Test (BSRT) (Buschke, 1973), and the 7/24 Spatial-Recall Task (Rao et al., 1991). For all tests, scores for subcomponents were converted into Z-scores based on the control group. Similar to the Rao Brief Repeatable Neuropsychological Battery (BRNB) (Rao et al., 1991; Sepulcre et al., 2006) and the MS-COG(Erlanger et al., 2014) we determined cognitive impairment using 4 core tests that have been shown to be reliable and sensitive to the effects of MS on cognition: SDMT, PASAT, BSRT learning, and 7/24 learning. These core tests were combined into a composite score and effect sizes *d* were calculated. To assess the relationships between structural, cognitive and clinical measures, individual tests were grouped into 4 cognitive domains. The attention/executive function subscore consisted of the WAIS digit span and spatial span, and Delis–Kaplan Executive Function system components: trailmaking, Stroop, and verbal fluency. Processing speed tests included the SDMT and the PASAT. Verbal memory was scored using Wechsler Memory Scale III components: verbal paired associates I and II scores, and BSRT components: total learning, consolidated long-term recall, and delayed recall. Finally, the spatial memory subscore comprised of the 7/24 spatial-recall tests A and B and delayed recall. Z-scores were averaged within each cognitive domain to create 4 domain Z-scores for each subject.

2.3. MRI acquisition

All subjects were scanned with a Siemens Trio 3T MRI scanner. Scans included: a T1 3D volume (MPRAGE, TR = 2200 ms, TE = 3.4 ms, TI = 900 ms, FOV = 256 mm, matrix = 256 × 256, 176 axial 1 mm slices, 1NEX, resolution = 1 mm isotropic); a hippocampal oriented, high resolution coronal T2 described previously (TR = 8290, TE = 64, FOV = 200 mm, matrix 512 × 512, in-plane resolution = 0.4 × 0.4 mm, 2NEX, coronal 3 mm slices, no gap); (Gold et al., 2010) a T2 FLAIR (TR = 11,760, TE = 88, TI = 2500, FOV = 256 mm, matrix = 256 × 256, axial 3 mm slices, no gap) and a DTI sequence (30 noncollinear directions, 2 averages, a single high b-value of 1000 mm/s^2, TR = 10,200 ms, TE = 84 ms, FOV = 256 mm, matrix = 128 × 128, 75 axial 2 mm slices, resolution = 2 mm^3).

2.4. Image processing

Images were processed using FMRIB Software Library (FSL: http://www.fmrib.ox.ac.uk/fsl) (Jenkinson et al., 2012) and Diffusion Toolkit and Trackvis (trackvis.org) (Wang, 2007).

T1 images were used to calculate brain parenchymal volumes using FSL's FAST. FSL's FIRST was used to segment the thalami bilaterally and calculate combined thalamic volume. FLAIR images were used to manually identify WM hyperintensities and calculate total lesion load. Hi-resolutionhippocampal-oriented T2 images were used to manually segment the hippocampi as described previously (Sicotte et al., 2008). While we previously described segmenting hippocampal subregions, here we use only mean total left and right hippocampal volumes without the entorhinal cortex.

DTI data were corrected for eddy current distortion and head motion, a diffusion tensor was calculated at each voxel, and eigenvalues

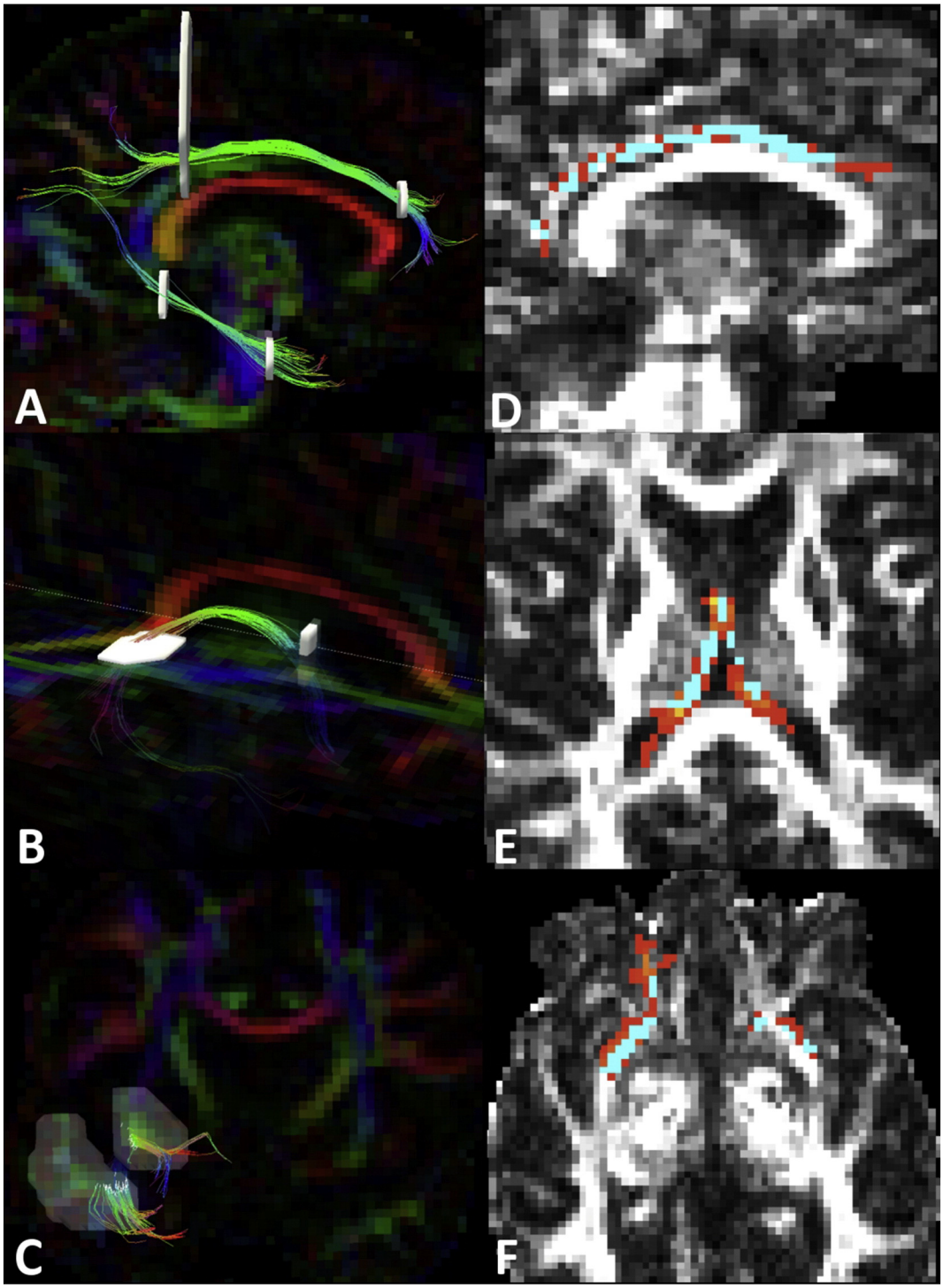

Fig. 2. Tractography of the upper and lower cingulum (A), the fornix (B), and the uncinate (C) was achieved using multiple regions of interest (white) according to published tractography protocols {Concha, 2005; Wakana, 2007}. Cingulum (D), fornix (E) and uncinate (F) tractography (Red) is overlaid on the TBSS white matter skeleton (blue) to acquire diffusion metrics from only the centermost voxels of each tract in native space.

were used to calculate fractional anisotropy (FA), radial diffusivity (RD) and axial diffusivity maps (AD). FA images were skeletonized using FSL's Tract-Based Spatial Statistics (TBSS), which identifies the centermost voxels with maximal FA values of each white matter bundle for each subject. Tractography results were overlaid on these FA TBSS skeletons to derive statistics from the most robust voxels at the center of each tract. By using only TBSS skeleton voxels in statistical analyses, we minimize the direct effect of very low FA values within lesions and limit the effects of differences in trackability or anatomic variation by comparing an anatomically equivalent region of each tract across individuals. FA skeleton projections were transformed to RD and AD images to calculate tract statistics for these measures as well.

2.5. Tractography

Trackvis's Diffusion Toolkit uses deterministic fiber-tracking to identify all WM pathways in the brain, and individual pathways are manually selected using multiple regions of interest (ROIs). The 3 pathways were identified bilaterally on blinded FA images using a priori anatomical knowledge and established tractography protocols (Concha et al., 2005; Wakana et al., 2007). The cingulum was identified in two parts: the superior and the hippocampal portions, each selected with an anterior and posterior coronal ROI (see Fig. 2A). For the fornix, the first ROI selected the body of the fornix coronally while the second ROI axially selected a unilateral hippocampal tail. Anteriorly, the columns of the fornix were terminated at the level of the anterior commissure (see Fig. 2B). We identified the UF with 2 coronal ROIs in the anterior temporal and frontal lobes (see Fig. 2C). We assessed reproducibility by tracking 10 subjects twice and calculating a Kappa score of voxel-wise overlap. Mean FA was derived from each tract, and bilateral tracts were averaged for each subject.

2.6. Statistical analyses

T-tests compared groups for the clinical variables of age, BDI-II, and years of education while a chi-squared test compared gender distribution. To assess cognitive impairment we used ANOVA to compare the raw scores for the PASAT, SDMT, BSRT learning, 7/24 learning, and the standardized composite score. Group differences in cognitive domain *Z*-scores were also assessed using ANOVA. Similarly, structural measures including mean FA of each pathway and GM volumes were compared across groups using ANOVA. Effect sizes were calculated using Cohen's *d*. Associations between structural or clinical measures and cognitive scores were tested first with univariate correlations. Predictors considered were: mean FA of each pathway, thalamic volume, hippocampal volume, and BDI-II score. Structural associations with each domain Z-score found to have Pearson's $R > 0.30$ were considered as predictors in a step-wise linear regression model to identify only significant, independent predictors of cognitive performance.

For significant associations, we went on to compare left vs right structures as well as axial (AD) vs radial diffusivities (RD) as predictors of cognitive function for descriptive purposes. For each significant association, we calculated bivariate correlations with the cognitive domain Z-score and the four diffusion metrics (left RD, left AD, right RD, right AD) or two volumes (left, right).

3. Results

There were no significant differences between the 27 RRMS and 20 controls in age, years of education, or gender distribution (see Table 1). RRMS had significantly higher mean BDI-II. 4 RRMS had BDI-II scores consistent with moderate to severe depressive symptoms (BDI-II > 19) while 2 controls met this cutoff. 3 additional RRMS had BDI-II scores between 14 and 19, consistent with mild depressive symptoms.

RRMS patients performed worse on the SDMT, (mean RRMS: 53.4 ± 10.3 vs mean control: 61.2 ± 10.3; effect size = 0.71; $p = 0.014$) but differences were not significant for the PASAT,BSRT learning, 7/24 learning, or the composite score for these 4 tests. In RRMS patients, T2 lesion volumes were significantly associated with performance on the BSRT learning ($R = -0.54$; $p = 0.004$), but not with the PASAT, SDMT, or 7/24 learning scores. EDSS was associated with the performance on the PASAT, ($R = -0.42$; $p = 0.03$) the SDMT, ($R = -0.51$; $p = 0.001$) the 7/24 learning ($R = -0.43$; $p = 0.02$) and the composite score ($R = -0.58$; $p = 0.001$). There were no group differences in the 4 cognitive domain Z-scores, though patients tended to perform worse. Cognitive domain scores were not associated with T2 lesion volumes, although they were each associated with EDSS (Attention/Executive $R = -0.57$, $p = 0.002$; Processing Speed $R = -0.54$, $p = 0.003$; Verbal Memory $R = -0.46$, $p = 0.016$; Spatial Memory $R = -0.39$, $p = 0.04$).

DTI tracking was reproducible as determined by a high Kappa. Tracks were reliably reconstructed in all participants and there were no differences in tractography image volumes between groups. Patients had reduced FA in each of the 3 WM pathways (see Table 2; ANOVA $p = 0.003$). Mean FA ± standard deviation for the cingulum was 0.484 ± .030 in controls and 0.450 ± .052 in RRMS ($p = 0.008$). Mean FA for the UF was 0.411 ± 0.012 for controls and 0.386 ± 0.012 for RRMS ($p = 0.005$). Mean FA for the fornix was 0.318 ± 0.036 in controls and 0.263 ± 0.061 in RRMS ($p = 0.0004$).

Mean total left and right subcortical GM volumes were compared across groups after correcting for head size. RRMS had decreased mean total hippocampal volume as reported previously (Gold et al., 2010). RRMS also had decreased mean total thalamic volume compared to healthy controls (see Table 2).

To identify the most likely predictors of cognitive performance in RRMS patients, we assessed univariate associations with cognitive domain Z-score and the following structural and clinical variables: cingulum FA, fornix FA,UFFA, thalamic volume, hippocampal volume and BDI-II. See Supplemental Table 1 for univariate correlation matrix in RRMS. Associations with $R > 0.30$ were considered in multivariate regression models for each cognitive domain.

Table 1
Clinical characteristics.

	Controls mean ± std dev	Patients mean ± std dev	Effect size *d*	*p*-Value
N	20	27		
Age	34.1 ± 9.4	37.9 ± 8.2	−0.43	0.15
Female/male	18/2	23/4		0.63
Years of education	16.3 ± .4	16.2 ± .5	0.21	0.92
EDSS	N/A	2.5 ± 1.1		
Lesion volume (cm^3)	N/A	7.1 ± 2.0		
Beck depression-II	3.5 ± 7.4	9.4 ± 8.9	−0.66	**0.02**
PASAT raw score	45 ± 12	46 ± 12	−0.08	0.41
SDMT raw score	61 ± 10	53 ± 11	0.71	**0.02**
BSRT learning raw score	46 ± 11	46 ± 11	−0.02	0.94
7/24 learning raw score	32 ± 3	30 ± 5	0.33	0.23
Composite cognitive score	0 ± 0.77	−0.34 ± 0.78	0.43	0.14
Attention/executive Z-score	0 ± 0.62	−0.28 ± 0.44	0.48	0.11
Processing Speed *Z*-score	0 ± 0.90	−0.34 ± 0.86	0.39	0.20
Verbal memory Z-score	0 ± 0.91	−0.18 ± 0.44	0.19	0.52
Spatial memory Z-score	0 ± 0.77	−0.50 ± 1.1	0.52	0.16

Bold values indicate significance at $p < 0.05$.

Table 2
Group differences in WM FA and GM volume.

	Mean controls ± std dev	Mean RRMS ± std dev	Effect size *d*	*p*-Value
Cingulum FA	0.48 ± .03	0.45 ± .05	0.72	0.008
Fornix FA	0.32 ± .036	0.26 ± .06	0.94	0.0004
Uncinate FA	0.41 ± .03	0.39 ± .03	0.79	0.005
Thalamic volume (mm^3)	20,945 ± 1286	18,872 ± 2049	1.02	0.0001
Hippocampal volume (mm^3)[a]	8819 ± 1090	8304 ± 689	0.57	0.01

[a] Hippocampal volumes reported previously (Gold et al., 2010).

As predictors of attention/executive function Z-score, the regression model considered thalamic volume (R = 0.56), cingulum FA (R = 0.41), BDI-II (R = –0.40), uncinate FA (R = 0.40) and fornix FA (R = 0.39). Significant independent predictors included thalamic volume and BDI-II (std B1 = 0.539, std B2 = −0.350, adj R^2 = 0.360, p = 0.002; see Fig. 3A). The association with thalamic volume was marginally stronger on the left, although significance was bilateral (Supplemental Table 2).

For the processing speed domain, uncinate FA (R = 0.40), thalamic volume (R = 0.37) and cingulum FA (R = 0.32) were considered in the regression model. The only significant independent predictor was UF FA (std B = 0.40, adj R2 = 0.129, p = 0.037; see Fig. 3B). The strength of the association was similar bilaterally but driven by RD (Supplemental Table 2).

For verbal memory performance, thalamic volume (R = 0.41) and fornix FA (R = 0.39) were considered as predictors in the regression model. Thalamic volume remained the only significant independent predictor (B = 0.387, adj R^2 = 0.116, p = 0.046; see Fig. 3C). The univariate correlation only reached threshold on the left.

The model for spatial memory Z-score considered uncinate FA (R = 0.42) and BDI-II (R = –0.34) as predictors, and both were independently associated (std B1 = 0.513, std B2 = −0.393, adj R^2 = 0.382, p = 0.001; see Fig. 3D). The UF association was driven by right RD (Supplemental Table 2).

4. Discussion

4.1. Interpretation

This study evaluated MS-related pathology in a limbic circuit *in vivo* and its role in cognition in a group of RRMS patients with mild to minimal cognitive impairment, as indicated by reduced SDMT performance only. We hypothesized that damage to a thalamic–hippocampal–prefrontal circuit contributes to cognitive changes in RRMS. We demonstrated both reduced WM FA and subcortical GM atrophy in this circuit. Using linear regression we determined the relative contributions of each component to 4 cognitive domains within the MS group: attention/executive function, processing speed, verbal memory, and spatial memory. Attention/executive function was most closely associated with thalamic volume and depressive symptoms, processing speed was associated with UFFA, verbal memory was associated with thalamic volume and spatial memory was associated with UF FA and depressive symptoms.

Our cohort of early RRMS patients had mild to minimal cognitive impairment despite significant structural changes in each of the biomarkers we studied. While patients had no significant group differences in cognitive domains, they had lower SDMT scores, a test which has been shown to reflect cerebral dysfunction early on and is the most sensitive to MS pathology (Parmenter et al., 2007; Strober et al., 2009). The strong associations found between imaging structural biomarkers and cognitive performance, despite only mild impairment should prompt future longitudinal studies to assess their utility in predicting disease progression and monitoring treatment efficacy.

4.2. Thalamus

While each structure investigated is affected in MS, thalamic volume is a strong predictor of cognitive performance, particularly attention/executive function and verbal memory. Thalamic volume has previously been linked to multiple tests including the Controlled Word Association Test, Judgment Of Line Orientation, CVLT, the Brief Visuospatial Memory Test, PASAT, and the SDMT(Batista et al., 2012; Benedict et al., 2009; Houtchens et al., 2007). Although we did not find thalamic volume to independently predict processing speed (PASAT and SDMT) when included in the full regression, there was a moderate association (R = 0.37) in univariate analysis. This reflects the strong association between thalamic volume and brain white matter integrity (UF FA vs Thalamic Volume R = 0.47) due to its widespread connections, but also suggests that with a larger *N* this association may have reached significance. The thalamus communicates widely and reciprocally with both cortical and subcortical areas, thus thalamic atrophy contributes to multiple realms of cognitive deficits. Thalamic atrophy has been attributed to neuronal loss (Cifelli et al., 2002), however, whether this neuronal loss is due to direct immunological insult or secondary to axonal transection is unclear (Minagar et al., 2013).

4.3. Uncinate fasciculus

FA in the UF predicted both spatial memory Z-score and processing speed Z-score in RRMS. The UF communicates between medial temporal memory encoding structures and the frontal cortex. Mesaros et al. (2012) also showed spatial memory deficits linked to lesions in the UF using a random forest approach classifying cognitively impaired MS patients. They also demonstrated a role for the UF in visual processing speed, sustained attention, and verbal memory. Prior to this study, the UF has been identified as important for episodic *verbal* memory function on the CVLT across a cohort of MS patients and controls (Fink et al., 2010). Diehl et al. (2008) identified laterality in UF function in temporal lobe epilepsy, demonstrating left UF DTI metrics associated with verbal memory function and right UF DTI metrics associated with spatial memory function. In investigating laterality, we also found spatial memory to be more associated with the right UF.

4.4. Cingulum

Cingulum FA was associated with attention/executive function, but this relationship was not independent of thalamic volume or depressive symptoms. The indirect association may be due to its widespread cortical connections in distributed attentional networks. It has also been implicated in several other realms of cognitive performance in MS. Dineen et al. (2009) showed associations between cingulum FA and the PASAT, Benton Visual Retention Test (BVRT) and the California Verbal Learning Test II (CVLT-II). Yu et al. (2012) found reduced FA in the cingulum associated with the Rey Auditory Verbal Learning Test (RAVLT) and the SDMT. Mesaros et al. (2012) used a random forest analysis to demonstrate that cingulum DTI metrics were the best classifiers across numerous tests: PASAT, SDMT, 10/36 Spatial-Recall Test, BSRT, and Word List Generation. In contrast, we did not find cingulum FA to be an independent predictor of verbal or spatial memory, as these domains were better explained by thalamic volume, fornix FA,UFFA, and BDI-II score. We included the PASAT and SDMT in the processing speed domain in our study since these tests are both sensitive to cognitive changes in MS, and although cingulum FA showed a moderate correlation with the processing speed Z-score (R = 0.32), it was not independently predictive.

4.5. Fornix

The fornix is the primary hippocampal efferent and we previously found fornix insult to be associated with poor performance on an unrelated word-pairs task and adaptive increases in fMRI BOLD signal during the task in RRMS patients (Kern et al., 2012). In other studies the fornix has also been associated with MS differences on verbal memory tests including the CVLT-II(Dineen et al., 2009; Fink et al., 2010), the BVRT(Dineen et al., 2012) and the RAVLT(Yu et al., 2011). In this study we identified a link between fornix FA and verbal memory performance, but this relationship was not independent of thalamic volume. Interestingly, thalamic volume and fornix FA were strongly associated (R = 0.72), as has been shown previously (Dineen et al., 2012), likely due to both direct and indirect hippocampal–anterior thalamic connections via the fornix, as well as their proximity within the 3rd ventricle. Third ventricular width has been previously identified as a marker for cognition and is associated with neocortical volume (Benedict et al., 2004).

4.6. Hippocampus

Similar to thalamic atrophy, hippocampal atrophy in MS is driven by neuronal loss, but demyelination is also evident (Papadopoulos et al., 2009). We previously found that subregional hippocampal volume correlates with verbal memory using an unrelated word-pairs task in a group of patients with verbal memory impairment (Sicotte et al., 2008), while others have found lateralized hippocampal associations with verbal memory on the left (Koenig et al., 2014; Pardini et al., 2014), and spatial memory on the right in MS(Pardini et al., 2014). But in this study we did not find an association independent of fornix FA or thalamic volume. However, in this study no patient had impaired verbal memory function. Thalamic atrophy and disruption of the fornix, a hippocampal–thalamic connection, may be earlier manifestations of verbal memory alterations in patients who are not yet impaired. Previous studies have shown a strong association between fornix DTI metrics and hippocampal volume (Koenig et al., 2014). Furthermore, altered hippocampal function may precede volume loss, also contributing to verbal memory deficits. We previously showed altered hippocampal fMRI activity during a verbal learning task that was associated with verbal memory performance and fornix FA, but independent of hippocampal volume (Kern et al., 2012), while Roosendaal et al. showed reduced interhemispheric hippocampal connectivity (Roosendaal et al., 2010).

4.7. Depression

We considered BDI-II score in our analysis as a confounder since depressive symptoms are common in MS and affect cognition (Heesen et al., 2010). This is evident in our sample since BDI-II scores were higher in RRMS patients. As reported previously from this sample, depressive symptoms did not correlate with total hippocampal volume but were significantly associated with smaller volumes in the hippocampal subregions CA2, CA3 and dentate gyrus (Gold et al., 2010). We recently replicated the association between depressive symptoms and hippocampal subregions in a larger sample (Gold et al., 2014). Depressive symptoms were not associated with other structural measures in this study, but were independently associated with lower scores in spatial memory and attention/executive function. These results highlight the importance of including measures of depressive symptoms when assessing cognitive performance. Associations between depressive symptoms and limbic circuit structural biomarkers may be more evident in patients diagnosed with major depressive disorder (MDD), as has been shown previously in patients without MS but with MDD who have reduced gray matter in the subgenual anterior cingulate cortex (Drevets et al., 2008). Future studies should include MS patients with and without MDD to better distinguish MS limbic pathology from MDD limbic pathology.

4.8. White matter lesions

RRMS patients had a relatively low average T2 lesion volume in this study, and we minimized the direct effects of WM lesions by deriving diffusion metrics from a WM skeleton of only the centermost voxels of each pathway. Lesion volume was associated with BSRT learning performance confirming the sensitivity of this test to MS pathology. However the global effect of lesions was not predictive of performance in specific cognitive domains, which may be more sensitive to localized insult to circuits such as a thalamic–hippocampal–prefrontal circuit. Identifying tract-specific insult in eloquent areas may better predict domain-specific cognitive changes than global measures such as T2 lesion volume.

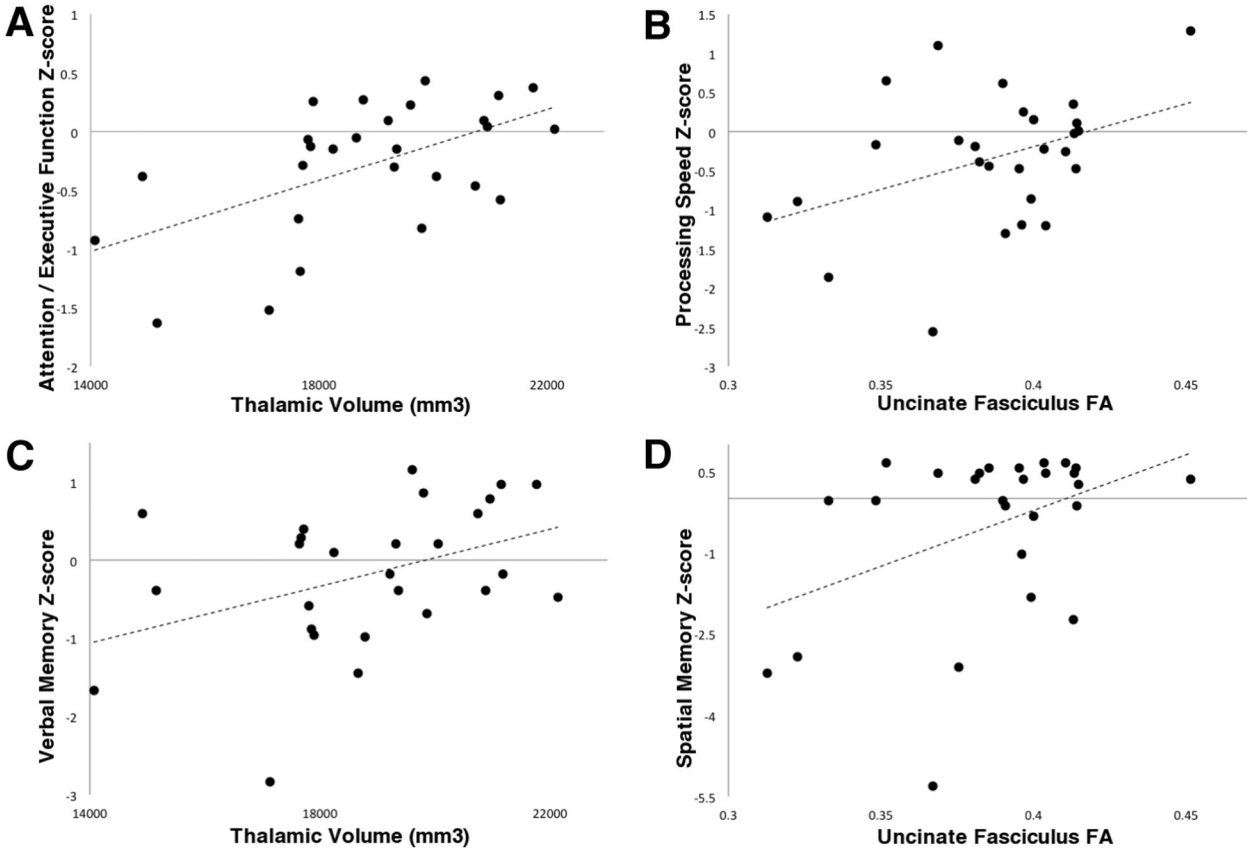

Fig. 3. Structure–cognition scatterplots. Attention–executive function Z-score in RRMS patients is associated with thalamic volume (A). Processing speed Z-score is associated with uncinate FA (B). Verbal memory Z-score is associated thalamic volume (C). Spatial memory Z-score is associated with uncinate FA (D).

4.9. Limitations

Our study has several limitations but also prompts future investigations. Unlike other DTI studies using non-hypothesis driven approaches or assessing brain-wideWM pathways, we limited our study to a thalamic–hippocampal–prefrontal circuit to investigate the relative contributions of each component in a group of early RRMS with mild to minimal cognitive impairment. In doing so we likely omitted important contributions from other affected structures in MS that also relate to cognitive dysfunction such as the corpus callosum (Yu et al., 2012), basal ganglia (Batista et al., 2012), and neocortex (Benedict et al., 2002). Another limitation is that we did not include a measure of fatigue, which is common in MS and known to affect cognition (Heesen et al., 2010).

Furthermore, while we identified components predictive of cognitive function, a better understanding of the temporal dynamics is needed. Thalamic atrophy is strongly linked to cognition, but perhaps FA changes can be detected earlier. We predict that thalamic atrophy occurs early in the disease but cannot say whether atrophy is due to primary GM insult or secondary to WM insult and Wallerian degeneration. Hippocampal atrophy may also occur secondary to thalamic insult or secondary to loss of hippocampal–thalamic connections. Longitudinal studies could distinguish these hypotheses.

4.10. Conclusions

Hippocampal–thalamic–prefrontal disruption affects cognitive function in RRMS before significant functional impairment is evident, confirming both white and gray matter involvement in MS and demonstrating utility in assessing functional networks to monitor cognition.

Uncited document objects

The following figures were not cited: Fig. 1; Fig. 3.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nicl.2014.12.015.

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