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Functional connectivity alterations in patients with chronic hepatitis C virus infection: A multimodal MRI study

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Summary

Chronic hepatitis C virus (HCV) infection is associated with fatigue and depression. Cognitive impairments are also reported in a smaller number of HCV-positive patients. Recent studies linked HCV to low-grade inflammation in brain. Here, we test the hypothesis that chronic HCV is associated with 3T-neuroimaging-derived grey matter volume (GMV) and functional connectivity alterations in a sample of chronic HCV (1b), without severe liver disease. Regional GMV and resting-state fMRI-derived eigenvector centrality (EC) were compared between 19 HCV-positive patients and 23 healthy controls (all females, 50-69 and 52-64 years, respectively), controlling for white matter hyperintensities and age. Standard tests were used to assess fatigue, depression and cognitive performance. Also, liver fibrosis stage and viral load were quantified among patients. In comparison with controls, HCV-positive patients had higher scores in fatigue and depression, and worse alertness scores. The groups performed similarly in other cognitive domains. We report higher EC in a cluster in the right anterior superior parietal lobule in patients, while no differences are found in GMV. Post hoc functional connectivity analysis showed increased connectivity of this cluster with primary and secondary somatosensory cortex, and temporal and occipital lobes in patients. Higher mean EC in the superior parietal cluster, adjusted for mean framewise displacement, was associated with better memory and attention performance, but not with fatigue, depression, viral load or level of liver fibrosis, among patients. These results suggest a compensatory mechanism in chronic hepatitis C and explain equivocal results in the literature about cognitive deficits in infected persons. Further studies should define the relation of these connectivity changes to the brain's inflammatory activity.

Abbreviations: AFNI, analysis of functional neuroimages; ARWMC, age-related white matter changes; AtoM, attention to memory; CNS, central nervous system; EC, eigenvector centrality; EPI, echo-planar imaging; FC, Functional connectivity; FD, framewise displacement; FIS, fatigue impact scale; fMRI, functional MRI; FSL, FMRIB Software Library; FWE, family-wise error; FWHM, full width at half maximum; GLM, general linear model; GMV, grey matter volume; HCV, hepatitis C virus; MNI, Montreal Neurological Institute; MPRAGE, magnetization-prepared rapid gradient-echo; MRS, magnetic resonance spectroscopy; PCA, principal component analysis; PCR, polymerase chain reaction; PET, positron emission tomography; rs-fMRI, resting-state functional MRI; SCL-90, Symptom Checklist-90; SSRI, selective serotonin reuptake inhibitors; sMRI, structural MRI; SPECT, single-photon emission computed tomography; SVR, sustained virological response; WMH, white matter hyperintensities.

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KEYWORDS
chronic hepatitis-C, cognitive performance, graph centrality, resting state fMRI, voxel-based morphometry

1 | INTRODUCTION

Hepatitis C is a contagious liver disease that affects 3-4 million people every year worldwide, and of which 55%-85% develop chronic infection.[1] It is also associated with multiple extrahepatic manifestations with the central nervous system (CNS) being one of the most studied loci in the recent decade. Up to 80% of patients with chronic hepatitis C virus (HCV) infection suffer from pathological fatigue, even in the absence of advanced liver dysfunction.[2-4] Depressive symptoms occur in up to 58%,[5,6] while a lower rate (~16%)[7] shows neurocognitive impairments mainly in domains of attention, memory and alertness.[8-10] A recent longitudinal study on more than 117 000 individuals (~58 000 HCV positive) has even proposed HCV infection as a risk factor for dementia.[11] Other studies, however, do not confirm a link between current HCV infection and cognitive impairments in the absence of other comorbidities.[12,13]

The first evidence of cerebral involvement of HCV was provided by Forton et al.,[14] reporting neurochemical abnormalities, which differed from changes due to hepatic encephalopathy. They found higher "choline-to-creatine ratios" in basal ganglia and frontal white matter in HCV-positive patients compared to controls. Further studies using proton magnetic resonance spectroscopy (MRS) and positron emission tomography (PET) in HCV-infected patients without cirrhosis confirmed and extended these findings beyond the basal ganglia and frontal lobe, to parietal and occipital white matter,[9,15-18] suggesting an inflammatory state within the brain, potentially as a result of a direct HCV invasion into cerebral tissue.

On the other hand, attempts to relate neurochemical changes to alterations of cognitive performance and/or psychiatric symptoms have produced inconclusive results. While some studies[15,17,19,20] have related HCV-associated brain changes to impaired cognitive performance (such as higher reaction times in working memory tasks, higher number of errors during tasks assessing attention or worse memory performance), Bokemeyer et al.[9] in their MRS study reported beneficial effects of the so-called inflammatory state within the brain. A neuroprotective effect of microglial activation has also been reported in a recent study[16] in chronic HCV patients without attention deficits, irrespective of their PCR status. Given these equivocal results, presently, no clear connection between viral load, cerebral abnormalities and cognitive performance can be made. In addition, depression and fatigue, as major complaints of patients, also suggest a more complicated interplay of the disease and the brain.[8,15]

Taken together, there is a considerable discrepancy regarding the type and spatial extent of cerebral involvement of chronic HCV, which could be due to different sample characteristics such as history of hepatic encephalopathy, drug abuse or other viral co-infections, diverse measurements and analysis routines and shortcomings of the techniques applied thus far.[21]

Despite a growing body of literature showing that grey matter structural alterations as well as changes in functional connectivity as assessed by structural MRI (sMRI) and resting-state functional MRI (rs-fMRI), respectively, correlate with neurocognitive features and psychiatric symptoms,[22] there have been only few attempts to identify cerebral involvement in patients with chronic HCV using these approaches (see[23] for a recent study).

In this study, we examined a unique sample of aetiologically homogeneous chronic HCV (1b) patients with no history of hepatic encephalopathy or drug abuse and without any other chronic viral coinfection. In these subjects, we used sMRI (for voxel-based morphometry) and rs-fMRI (to obtain graph-theoretic centrality measure of functional connectivity) to address the hypothesis that HCV is associated with cerebral involvement and that measures of neural involvement correlate with alterations in cognitive performance and/or psychiatric symptoms and virological parameters. Here, patients with chronic HCV were compared to a demographically matched control group.

2 | METHODS

2.1 | Participants

Patients were recruited from the German anti-D cohort, a large outbreak of HCV (1b) infections in young women that occurred in East Germany between 1978 and 1979 after legal administration of anti-D immunoglobulin after pregnancy. This cohort has been shown to have a very low rate of liver cirrhosis in the 35-year follow-up study.[24]

A total of 28 HCV RNA-positive (13 therapy naïve and 15 non-SVR (without sustained virological response) after antiviral therapy) as well as 25 demographically matched healthy control individuals underwent structured clinical interviews, neuropsychological tests, medical examinations including comprehensive laboratory tests (blood count, liver enzymes, bilirubin, c-reactive protein, albumin, alpha-fetoprotein, coagulation, creatinine; for healthy subjects liver enzymes only; Roche Diagnostics, Mannheim & Cell Dyn Abbott, Wiesbaden, Germany) and structural as well as functional (fMRI) magnetic resonance imaging (MRI) of the brain. Major brain pathology such as stroke or traumatic brain injury was excluded by a neurologist and a neuroradiologist at University Hospital Leipzig. One patient had to be discarded from further analysis due to tumour of the cerebellopontine angle. Additionally, 10 subjects (eight patients and two controls) were excluded due to current intake of selective serotonin reuptake inhibitors (SSRI). Further exclusion criteria were decompensated liver cirrhosis (according to transient elastography values, 13 patients without, three patients with fibrosis, but no one with cirrhosis[24]), hepatic encephalopathy (based on West Haven Criteria[25]), acute liver failure and other systemic diseases potentially impairing cognition or brain

function. White matter hyperintensities (WMH) were assessed independently by two experienced physicians, who were blind to clinical data, on 3D-FLAIR images according to the age-related white matter changes (ARWMC) scale[26] (see Supporting Information for details). All subjects signed an informed consent form. The study protocol was in accordance with the Declaration of Helsinki and was approved by the ethics committee of the University of Leipzig.

2.2 | Assessment of viral load

Patient status of the disease was defined by conducting quantitative polymerase chain reaction (HCV-PCR) to assess virus load (RNA). In healthy controls, disease-specific antibodies were investigated to exclude former HCV infection.

2.3 | Assessment of liver fibrosis stage

The estimate of liver fibrosis stage was assessed using transient elastography (fibroscan; Echosens, Paris, France) in all but one patient (due to individual anatomy).

2.4 | Assessment of cognitive functioning

All subjects completed neuropsychiatric questionnaires including fatigue impact scale (FIS),[27] Hamilton depression scale[28] and Symptom Checklist-90 (SCL-90),[29] which has been designed to evaluate a broad range of psychological problems and symptoms of psychopathology. In addition, they participated in a battery of cognitive assessment consisting of Regensburger (semantic and formal-lexical) word fluency test,[30] trial making tests A and B,[31] paced auditory serial addition test (the number of errors in 2.4-second version),[32] tonic and phasic alertness subtests of the test battery for the assessment of attention processing (TAP) [version 1.8][33] and the California verbal learning test[34] measuring learning, immediate and delayed recall as well as recognition performance.

Cognitive test scores were subsequently used to generate four composite scores representing episodic memory performance, attention, total alertness and word fluency (language), using an exploratory factor analysis (principal component analysis [PCA] followed by a varimax rotation).[35] A conventional threshold of one on the estimated eigenvalues defined the number of the determined cognitive components, resulting in four factors explaining 77% of variance of the whole dataset (see Figure 1A, for detailed definition of each cognitive factor). As it could be seen in Figure 1A, higher scores of the second and third factor represent lower attention and alertness scores.

2.5 | MRI acquisition parameters

All MRI examinations were performed on a 3-Tesla Magnetom Verio scanner (Siemens, Erlangen, Germany) equipped with a 32-channel head array coil. Anatomic T1-weighted images were acquired using a three-dimensional magnetization-prepared rapid gradient-echo (MPRAGE) sequence with the following parameters: TI 650 ms, TR 1300 ms, TE 3.5 ms, flip angle 10°, bandwidth 240 Hz/pixel, image matrix 256×240, 176 partitions, FOV 256×240×176 mm^3, sagittal orientation, voxel size 1×1×1 mm^3, no interpolation.

Resting-state functional MRI (rs-fMRI) data using a gradient-echo echo-planar imaging (EPI) sequence were acquired under eyes-closed condition. The following parameters were used: 300 whole brain volumes, acquisition matrix=64×64, slice thickness=4 mm (0.8-mm gap), 30 slices, TR=2000 ms, TE=30 ms, flip angle=90°, bandwidth=1954 Hz/pixel.

2.6 | Voxel-based morphometric assessments

T1-weighted MRIs were processed using VBM8 package (http://dbm.neuro.uni-jena.de/vbm/) integrated in the SPM8 software (www.fil.ion.ucl.ac.uk/spm) with default parameters. Whole brain images were bias-corrected, tissue classified and preregistered to standardized Montreal Neurological Institute (MNI) space using linear (12-parameter affine) transformations, within a unified model. The segmented images were then warped using high-dimensional deformations to a standard template. Grey matter segments were modulated by the Jacobian determinant of the deformations to account for local expansion and compression introduced by *nonlinear* transformation and then smoothed with an isotropic Gaussian kernel of 6-mm full width at half maximum (FWHM).[36]

2.7 | Processing of resting-state scans

Rs-fMRI data were preprocessed using FMRIB Software Library (FSL, http://www.fmrib.ax.ac.uk/fsl) and Analysis of Functional

(A)

Rotated component matrix[a]

		Component			
		1	2	3	4
Memory	Long dalay free recall	.948			
	Short dalay free recall	.899			
	Immediate recall	.897			
	Long dalay recognition	.817			
Attention	PASAT2.4s (number of errors)		.829		
	TMTB (time)		.734		
	TMTA (time)		.640		
Alterness	Phasic alertness			.922	
	Tonic alertness			.876	
Verbal fluency	RWT_semantic				.824
	RWT_phonemic				.801

(B)

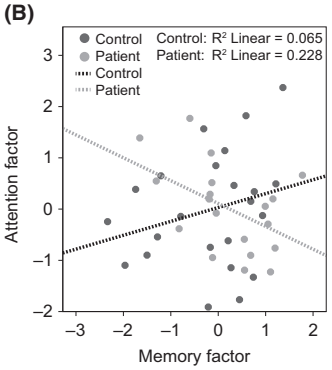

FIGURE 1 A, Identified component scores of cognitive factor (extraction method: PCA. Rotation method: varimax with Kaiser normalization). Cumulative explained variance 77%. B, Association between memory and attention factor, shown in each group separately. Lower attention factor scores represent better performance

NeuroImages (AFNI, http://afni.nimh.nih.gov/afni), based on the fcon1000 scripts (http://www.nitrc.org/projects/fcon_1000). The steps comprised the following: discarding the first ten volumes, slice time correction, motion correction, 6-mm FWHM spatial smoothing, four-dimensional mean-based intensity normalization, removing linear and quadratic trends, regressing out eight nuisance signals (white matter, cerebrospinal fluid and six motion parameters) and band-pass temporal filtering (0.005-0.1 Hz). Spatial linear normalization to MNI space was performed using individual skull-stripped T1 as a prior.

2.8 | Resting-state fMRI derivative

For each individual, as main derivate of rs-fMRI, eigenvector centrality (EC)[37] was calculated from the preprocessed rs-fMRI scans. EC not only quantifies how many connections each voxel has to all other voxels (a connection is defined as absolute pairwise nonself Pearson's correlation value higher than 0.2), but also additionally favours nodes that are connected to nodes that are themselves central within the network. Therefore, connections to regions which are themselves highly connected receive a higher weight and vice versa. This measure is computationally efficient, which enables centrality mapping on the voxel level. We used the EC implementation in LIPSIA.[38]

2.9 | Post hoc seed-based functional connectivity analysis

Functional connectivity (FC) analysis was subsequently assessed using Pearson's correlation between seeds defined based on group differences in the above-mentioned centrality measure (EC) and all other grey matter voxels for each individual. Correlation maps for each subject were then transformed using Fisher's r-to-z transformation. This exploratory step was added to investigate how the regions with altered centrality relate to other brain areas—essentially determining the location of regions that contributed to the observed difference in centrality scores.

2.10 | Statistical analysis

2.10.1 | Group differences on WMH and neuropsychological tests

Between-group differences on these measures were assessed using two-sample t-tests or Mann-Whitney U-test (in case of not-normally distributed measures), setting a two-sided *P*-value threshold .05.

2.10.2 | Grey matter volume and centrality group differences

Voxel-wise differences between patients and control subjects were assessed using independent sample t-test, implemented by means of the general linear model (GLM) in SPM with age and WMH as covariates of no interest. GLMs for EC and FC maps were additionally controlled for mean framewise displacement (FD).[39]

Correction for multiple testing was performed using the family-wise error (FWE) correction based on Gaussian random field theory[40] at cluster level ($P<.05$) combined with a primary uncorrected voxel-level threshold of $P<.001$.[41]

2.10.3 | Exploratory analyses

Next, we sought to determine whether centrality in brain regions showing difference between patients and control subjects was associated with measures of cognitive and neuropsychological performance or disease severity in the patient group. Therefore, mean EC of the significant clusters was extracted, for each individual and adjusted for mean FD using the following formula:

Adjusted mean-EC (at significant group difference cluster)=mean-EC (at significant group difference cluster) − *b*×mean FD. Association between cognitive factors and fatigue, depression, viral load and liver fibroscan values with the standardized adjusted mean EC, was evaluated using separate partial correlations controlling for age and WMH.

All variables were sufficiently normally distributed (|skewness|<1), except ratings of WM lesions and lacunes, fatigue (not when considering patients only), depression, alertness, fibroscan values and viral load. Statistics of these measures were tested using nonparametric tests.

These exploratory analyses were performed in SPSS 20 (PASW, SPSS, IBM), and significance level was set at $P<.05$ (two sided).

3 | RESULTS

In total, 19 patients (nine therapy naïve and ten non-SVR) and 23 healthy controls were included for group comparisons. For demographic characteristics see Table 1.

3.1 | Comparison of WMH between patients with HCV and healthy controls

Ratings of white matter lesions and lacunes did not show significant differences between patients and healthy controls (total lesion score: Mann-Whitney $U=189$, $P=.45$; total lacunes score: Mann-Whitney $U=202.5$, $P=.55$). The same result was obtained for all sub-regions (data not reported).

3.2 | Comparison of neuropsychiatric and cognitive measures between patients with HCV and healthy controls

In comparison with controls, patients showed significantly higher levels of fatigue (Mann-Whitney $U=86.5$, $P=.001$) and depression (Mann-Whitney $U=87.5$, $P=.001$). Within each group, depression and fatigue scores were highly correlated with each other (Spearman's $\rho=0.64$, $P=.001$, Spearman's $\rho=0.69$, $P=.001$, in controls and patients, respectively). Among patients, fatigue correlated marginally with alertness (Spaerman's $\rho=0.43$, $P=.06$). Depression score also followed the same

TABLE 1 Sample characteristics

	HCV-positive patients (n=19)		Healthy controls (n=23)
	Therapy naïve (n=9)	Non-SVR (n= 10)	
Age (y)	55±3 (52-60)	58±6 (50-69)	57±4 (52-64)
BMI (kg/m^2)	27±4 (21-32)	28±5 (21-39)	25±4 (19-35)
Smoking (%yes)	11.1	0	13
Fibroscan (kPa)[a]	5.8±2.5 (2.3-9.6) [b]	5.7 [5-14.4][a]	N.A
Virus load (PCR titre; IU/mL)[a]	4×10^5[2.3×10^5-1×10^6]	12×10^5[4×10^5-4×10^6]	N.A.
Alanine aminotransferase (μkat/L; normal range: 0.17-0.60)	1±.66 (0.44-2.44)	1.11±.71 (0.41-2.86)	0.39±.29 (0.18-1.24)
Aspartate aminotransferase (μkat/L; normal range: 0.17-0.60)	0.85±0.44 (0.54-1.85)	0.91±.48 (0.4-2.86)	N.A
Fatigue impact scale (FIS; cut-off>50)	76.1±45.12 (5-140)	59.6±31.9 (17-127)	26 [16.5-37.5][c]
Hamilton depression scale (HAMD; cut-off>8)	6 [4-10][c]	6.6±4 (1-12)	1 [0-3][c]

NA, not available; PCR, polymerase chain reaction.
[a]Cut-off for cirrhosis >14.5 kPa, for fibrosis >8.8 kPa
[b]Fibroscan was not measured in one subject from the therapy naïve group.
[c]Data shown with median [interquartile range].

pattern as fatigue; however, correlations did not reach significance level (all *P*-values >.05—see Table 2).

Further, the alertness factor was significantly worse in patients in comparison with controls (Mann-Whitney *U*=123, *P*=.015). We did not find any significant difference in other cognitive domains between the two groups.

Among the patients, there was an association between higher memory factor scores and better attention (Pearson's *r*=−.48, *P*=.04). We did not find such significant association within controls (*P*>.05) (see Figure 1B).

3.3 | Association of viral load and fibroscan measure with demographic and neuropsychiatric measures

In our sample, older patients had lower viral load (Spearman's ρ=−0.55, *P*=.015). However, none of the cognitive factors as well as measures of fatigue or depression showed significant association with PCR values, even after controlling for age (all *P*-values >.05). Patients' liver fibroscan results were neither associated with viral load nor with any of cognitive factors, level of fatigue or depression score (all *P*-values>.05—see Table 2).

3.4 | Comparison of grey matter volume between patients with HCV and healthy controls

Whole-brain voxel-wise comparison of GMV between HCV-positive patients and controls, corrected for confounding effects of age, did not result in any significant differences.

3.5 | Comparison of rs-fMRI-derived centrality and subsequent FC differences between patients with HCV and healthy controls

In group comparison of resting-state centrality maps (EC), patients in comparison with controls showed higher EC in right postcentral sulcus, which extended to the (anterior) superior parietal lobule (Figure 2A).

Between-group difference of the post hoc functional connectivity analysis using the seed at the significant EC cluster revealed significant increased connectivity of this region with primary and secondary (parietal operculum) somatosensory cortex, paracentral lobule, superior temporal gyrus and occipital lobe in patients with HCV in comparison with controls (Figure 2B).

3.6 | Association between neuropsychiatric measures and adjusted mean EC postcentral cluster

When considering patients and controls together, depression and fatigue were positively associated with adjusted mean EC of the significant group difference cluster (Table 2). However, within each group, these associations were not statistically significant (all *P*-values >.05).

Among cognitive factors, only higher memory factor, indicating better memory performance, was associated significantly with higher adjusted mean EC in postcentral cluster, when not separating the groups (Pearson's *r*=.4, *P*=.005).

This association was significant within the patients, even after considering the effects of age and WMH (partial *r*: .57, *P*=.02). In this group, a higher adjusted mean EC of postcentral cluster was also associated with better scores of attention (partial *r*=−.59, *P*=.01) (see Figure 3). Other cognitive factors were not associated with adjusted mean EC of this cluster (patient group). Also, adjusted postcentral mean EC in the healthy control group was not associated with cognitive factors (trend: attention factor partial *r*=−.4, *P*=.05).

3.7 | Association of viral load and fibroscan measure with adjusted mean EC in postcentral cluster

Using nonparametric partial correlation, controlling for age and WMH, we did not find statistically significant evidence of an association between viral load or fibroscan values and adjusted mean EC in postcentral cluster among patients.

TABLE 2 Bivariate associations shown as correlation value and *P*-value

	Fatigue	Depression	Fibroscan[b]	PCR	Memory	Attention	Alertness	Fluency	Adjusted postcentral mean EC	WMH
Fatigue	–	**0.7, 10^{-3}*[a]**	–	–	−0.05, 0.75[a]	−0.02, 0.9[a]	0.22, 0.16[a]	−0.3, 0.05[a]	**0.4, 0.005*[a]**	−0.003, 0.9[a]
Depression	**0.7, 0.001*[a]**	–	–	–	−0.003, 0.99[a]	−0.03, 0.85[a]	0.13, 0.4[a]	−0.14, 0.36[a]	**0.5, 0.001*[a]**	−0.01, 0.94[a]
Fibroscan[b]	−0.13, 0.6[a]	0.025, 0.9[a]	–	–	–	–	–	–	–	–
PCR value	−0.23, 0.34[a]	−0.12, 0.6[a]	−0.3, 0.19[a]	–	–	–	–	–	–	–
Memory	0.07, 0.78[a]	0.13, 0.6[a]	−0.32, 0.19[a]	−0.14, 0.6[a]	–	0.000, 1.0	0.04, 0.8[a]	0.000, 1.0	**0.4, 0.005***	−0.07, 0.66[a]
Attention	0.24, 0.33[a]	0.08, 0.74[a]	0.13, 0.62[a]	0.04, 0.85[a]	**−0.48, 0.04***	–	−0.02, 0.9[a]	0.000, 1.0	−0.05, 0.8	0.12, 0.46[a]
Alertness	0.43, 0.06[a]	0.33, 0.17[a]	0.01, 0.97[a]	0.004, 0.99[a]	−0.04, 0.88[a]	0.06, 0.82[a]	–	0.025, 0.87[a]	0.2, 0.2[a]	−0.19, 0.22[a]
Fluency	0.011, 0.9[a]	0.19, 0.45[a]	−0.16, 0.53[a]	−0.28, 0.24[a]	−0.29, 0.22	−0.05, 0.84	0.3, 0.21[a]	–	−0.179, 0.26	−0.05, 0.77[a]
Adjusted postcentral mean EC	0.1, 0.7[a]	0.19, 0.43[a]	−0.4, 0.1[a]	−0.3, 0.21[a]	**0.6, 0.005***	**−0.55, 0.02***	0.13, 0.6[a]	0.02, 0.93	–	−0.08, 0.62[a]
WMH	−0.17, 0.48[a]	−0.25, 0.3[a]	0.27, 0.28[a]	−0.056, 0.82[a]	−0.26, 0.28[a]	−0.09, 0.71[a]	−0.38, 0.1[a]	−0.1, 0.71[a]	−0.4, 0.1[a]	–

The upper triangle shows the correlations in the whole sample, and the lower triangle shows the same associations only among the patients.

[a]Spearman's rank correlation.

[b]n = 1 missing.

*Significant at $P < .05$ (two-sided).

4 | DISCUSSION

This study investigated the alterations of the GMV and functional connectivity and their associations with clinical symptoms, in a unique sample of aetiologically very homogeneous chronic HCV (1b) patients with only mild liver disease, with no history of drug abuse and without any other chronic viral co-infection.

Several previous studies have identified fatigue, depression, poorer memory and concentration ("brain fog") as common neurological symptoms in chronic HCV patients without severe liver disease.[8,10,18,42] In line with these reports, chronic HCV in our study was also associated with significantly higher scores in fatigue and depression questionnaires. Among the cognitive domains, alertness was the only measure in which patients had worse scores in comparison with controls. Also, in agreement with previous reports, neither the grade of liver disease nor virus replication rate was associated with psychometric measures.[43,44]

Over the past 10 years, several groups have studied possible cerebral effects of HCV using MRS, positron emission tomography (PET) and single-photon emission computed tomography (SPECT). The majority of these studies in patients without advanced liver disease have demonstrated metabolite and neurochemical abnormalities, suggestive of an inflammatory state within the brain[9,10,14,20,45] and alterations in serotonergic and dopaminergic neurotransmission.[46] Yet, studies investigating the association of this mild inflammatory state with neuropsychiatric symptoms of HCV have produced conflicting results. While Forton et al.[14] and Grove et al [17] showed increased microglial activation in association with neurocognitive dysfunction, Bokemeyer et al.,[9] Pflugrad et al.[16] and Weissenborn et al.,[8] based on their MRS and PET results, proposed a beneficial effect of the inflammatory state on cognitive performance and lower level of fatigue, among patients with chronic HCV. Further, although a chronic neural degeneration cannot be regarded as a consequence of HCV mono-infection,[47] some but not all studies have shown decreased NAA (N-accetylaspartate), which is a marker of neuronal and axonal viability,[48] in frontal and parietal white matter[8,15,18,45] as well as parietal grey matter[8] (but see[9] for studies showing increased NAA in patients with HCV). In agreement with neural alterations (in noncirrhotic HCV-positive patients), another study found decreased cortical thickness in bilateral occipital cortex and left frontal lobe. In our study, however, we found no significant differences in grey matter volume between HCV-positive patients and demographically matched healthy controls. Differences in sample sizes, morphometric measures[49] as well as patient characteristics (e.g. inclusion of HCV-positive patients with various genotypes in the study by Hjerrild and colleagues in contrast to the exclusive inclusion of HCV genotype 1-infected patients in our study) may account for the differences in our results.[23] Also, 28% of HCV-positive patients in[23] had a history of substance abuse, and 6% were taking antidepressants, which might have resulted in more severe cortical thinning in their sample.[50]

However, while we did not find any significant GMV differences between the groups, we detected an increased centrality of a cluster

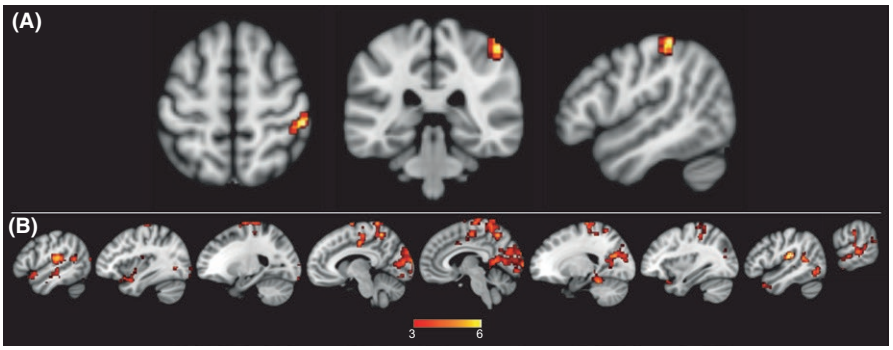

FIGURE 2 A, Statistical t-map of the significant cluster in the right postcentral sulcus extending to the superior parietal lobule (peak voxel: 51, −33, 57 mm), showing significant higher eigenvector centrality in patients compared to controls. B, Regions showing significantly higher functional connectivity to cluster in right postcentral sulcus (shown in A) in patients compared to controls (significant clusters, surviving a voxel-level threshold of $P<.001$ (uncorrected) and a cluster-level threshold of $P<.05$ (FWE corrected))

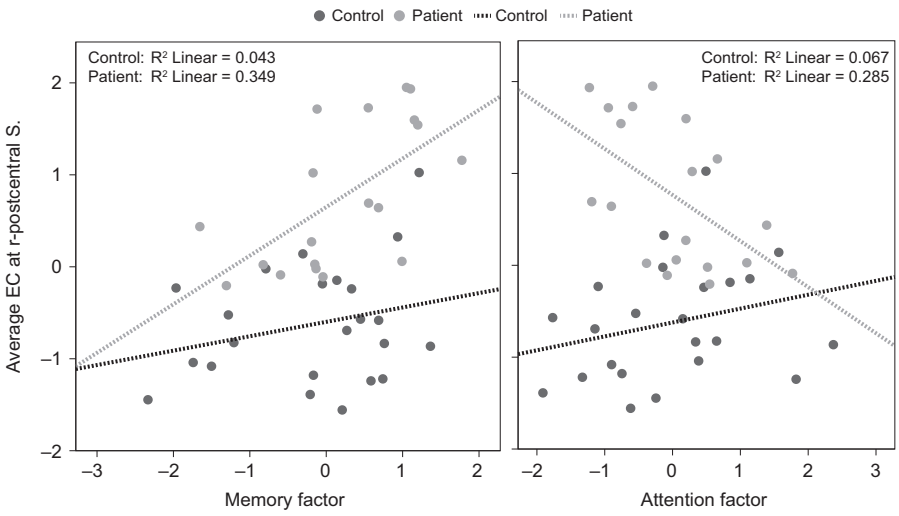

FIGURE 3 Association of mean EC in right postcentral sulcus with memory and attention factor scores in both groups. Higher score in memory factor means better performance, while lower scores are better in the attention factor

in the right postcentral sulcus extending to anterior superior parietal lobule in patients. Higher EC in this region was associated with better episodic memory and better performance in attention tests among patients but not in the healthy control group. However, within each group, neither fatigue and depression scores, nor the viral load and level of liver fibrosis explained significantly the variance seen in the centrality of this cluster.

Post hoc functional connectivity analysis revealed that increased centrality in patients was due to significantly increased connectivity of this parietal cluster with primary and secondary (parietal operculum) somatosensory cortex, paracentral lobule, superior temporal gyrus and occipital lobe in patients. Generally speaking, the finding of an increased centrality may indicate a compensatory underlying mechanism, even more so as the increased centrality positively relates to better cognitive function in the patient group.

The parietal lobes are traditionally known to support both bottom-up and top-down attention processes.[51] Functional imaging experiments showing activations in parietal lobules (more stably in the left parietal lobule) during memory-related tasks have extended our view regarding their function. Based on these task-based activation as well as rs-fMRI patterns, ventral and dorsal dissociations have been identified within the parietal cortex.[52-56] In particular, dorsal parietal lobule is suggested to mediate top-down attention and support strategic adjustments to cue processing, when the memory search is effortful and the recovery is poor (attention to memory (AtoM) model).[52,54,57,58]

Many HCV-infected patients are reported to have difficulties in both domains of attention and memory. Such worse performance in memory and attention tasks was not observed in our cohort of patients with HCV, and rather they had comparable memory and attention performance to controls. Given that the patients had a (compensatory) centrality increase in a region, presumably related to attention, and given that there is a significant correlation between memory performance and attention scores among our patient group, one may speculate that the increased connectivity in regions supporting top-down attention may help to sustain memory performance in this cohort.

Mechanisms of more demanding memory retrieval in patients with chronic HCV may be related to destructive long-term low-grade liver disease. Grey and white matter volume reductions in cirrhotic patients with and without hepatic encephalopathy have been reported in midline regions, temporal, parietal and occipital lobes, parahippocampal

gyrus and cerebellum.[59,60] Also, functional connectivity alterations in dorsal attention, visual, auditory and default mode network,[61] have been proposed as good markers of disease progression.

Higher connectivity of the same regions, which are affected primarily by liver disease, namely the temporal and parietal and occipital lobes, to the right anterior superior parietal lobule in our cohort of subjects with noncirrhotic chronic HCV might hint towards neuronal reorganization to compensate for the detrimental effects of long-term low-grade liver dysfunction. As mentioned earlier, top-down support of parietal lobule on memory performance has been reported more stably in the left hemisphere. Yet, reorganization of connectivity in the right parietal lobe in our sample of patients with HCV, which was associated with better memory performance, is in line with models of compensation. Such compensatory reorganization has been also reported and discussed in older adults ("HAROLD" model, "Hemispheric asymmetry reduction in older adults").[62] Whether chronic inflammation, which has been associated with beneficial as well as detrimental consequences in patients with chronic HCV, could underlie these possibly compensatory functional connectome alterations[9,10,16,17] may be clarified by further studies combining the measurements of brain inflammatory activity and the functional connectome. Several limitations should be considered when interpreting our findings. First, the limited number of subjects in each group, although comparable to similar studies in the field, might have resulted in low power to detect small effects. Second, the cross-sectional nature of this work only provides correlational but no causal associations. Third, the lack of markers of the brain's inflammatory state limits the interpretability regarding a potential link of our findings to brain inflammation.

However, strength of our study is the inclusion of patients solely from the anti-D group of eastern Germany with a known route and time point of infection. This cohort has been shown to have a very low rate of liver cirrhosis in the 35-year follow-up study.[24] We further followed strict exclusion criteria and discarded participants taking SSRIs or with any systemic disease potentially impairing cognition or brain function. Also, all participants underwent high-resolution neuroimaging at 3T and provided information on a comprehensive set of potential confounders, accompanied by an assessment of cognitive tests in all major cognitive domains.

5 | CONCLUSION

In chronic HCV patients without overt liver disease and no other comorbidities (such as HIV co-infection or drug abuse), we did not find changes in brain structure (no changes in grey matter volumes); however, we found signs of increased functional connectivity in right posterior parietal regions. As these changes were associated with better episodic memory and better performance in attention tests, we speculate that these functional reorganizations serve as a compensatory mechanism to provide additional attentional resources for better memory performance, in patients. Future studies combining the assessment of microglial activity with resting-state fMRI may clarify whether these findings are linked to brain inflammation.

STANDARD PROTOCOL APPROVALS, REGISTRATIONS AND PATIENT CONSENTS

As mentioned in the Methods section, all subjects signed an informed consent form and received a small financial compensation. The study protocol was in accordance with the Declaration of Helsinki and approved by the ethics committee of the University of Leipzig.

TRANSPARENCY

The authors affirm that the manuscript is an honest, accurate and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained. Also, the authors take full responsibility for the data, the analyses and interpretation, and the conduct of the research; full access to all of the data; and the right to publish any and all data.

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