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ORIGINAL ARTICLE

Hippocampal atrophy and functional connectivity disruption in cirrhotic patients with minimal hepatic encephalopathy

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Abstract

The hippocampus is a crucial pathological node for minimal hepatic encephalopathy (MHE) and it is associated with various cognitive impairments. Investigations on alterations involving hippocampal morphology and functional connectivity (FC) in MHE are limited. This study aimed to simultaneously evaluate hippocampal volume and FC alterations and their association with cognitive decline in MHE. Twenty-two cirrhotic patients with MHE, 31 cirrhotic patients without MHE (NHE), and 43 healthy controls underwent high-resolution T1-weighted imaging, resting-state functional magnetic resonance imaging, and cognition assessment based on Psychometric Hepatic Encephalopathy Score (PHES). The structural images were preprocessed using a voxel-based morphometry method, during which hippocampal volume was measured. The hippocampal connectivity network was identified using seed-based correlation analysis. Hippocampal volume and FC strength were compared across the three groups and correlated against the PHES results of the cirrhotic patients. Compared to the controls, MHE patients exhibited a significantly lower bilateral hippocampal volume. A slight decrease in hippocampal volume was obtained from NHE to MHE, but it did not reach statistically significance. In addition, the average FC strength of the bilateral hippocampal connectivity network was significantly lower in the MHE patients. In particular, the MHE patients showed a decrease in FC involving the left hippocampus to bilateral posterior cingulate gyrus and left angular gyrus. The MHE patients also showed FC reduction between the right hippocampus and bilateral medial frontal cortex. A progressive reduction in hippocampal FC from NHE to MHE was also observed. The bilateral hippocampal FC strength (but not hippocampal volume) was positively correlated with the PHES results of the cirrhotic patients. Our assessment of MHE patients revealed decreased hippocampal volume, which suggests regional atrophy, and reduced hippocampal connectivity with regions that are primarily involved in the default-mode network, thereby suggesting a functional disconnection syndrome. These alterations reveal the mechanisms underlying cognitive deterioration with disease progression.

Keywords Minimal hepatic encephalopathy · Functional connectivity · Hippocampus · Atrophy · Resting state functional magnetic resonance imaging

Weiwen Lin, Xuhui Chen and Yong-Qing Gao contributed equally to this work.

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Abbreviations

MHE	minimal hepatic encephalopathy
HE	hepatic encephalopathy
FC	functional connectivity
PHES	Psychometric Hepatic Encephalopathy Score
DMN	default-mode network

Introduction

Minimal hepatic encephalopathy (MHE) is the mildest type of hepatic encephalopathy (HE) and it is characterized by a range of neurocognitive deficits, such as psychomotor slowing, shortened attention span and concentration,

dysfunctional executive abilities, and memory loss(Agrawal et al. 2015; Lee et al. 2016). MHE also impacts the quality of life and daily functions of an individual, including driving (Bajaj et al. 2008, 2009; Prasad et al. 2007). Furthermore, MHE has been utilized as a predictor for overt HE, which has been associated with the poor patient prognosis (e.g., higher mortality) (Agrawal et al. 2015; Ampuero et al. 2015; Romero-Gomez et al. 2001).

Previous neuropsychological studies have demonstrated that MHE involves various deficits involving the hippocampus, such as memory, learning ability, and navigation function (Blankenship et al. 2017; Eichenbaum and Cohen 2014; Lisman et al. 2017; Zeidman and Maguire 2016). For example, memory deficits and learning impairment have been well documented in MHE (Ciecko-Michalska et al. 2013; Ortiz et al. 2006). MHE results in the episodic memory impairment, with subsequent improvement after liver transplantation (Ciecko-Michalska et al. 2013; Lee et al. 2016; Ortiz et al. 2006). Liver failure and chronic hyperammonemia (the hallmark neuropathological feature that is related to HE) may result in the disruption of the glutamate-nitric oxide-cyclic guanine monophosphate (glutamate-NO-cGMP) pathway, which controls memory function as well as learning ability (Erceg et al. 2005). Furthermore, the recent studies have revealed that MHE patients have impaired navigation skills, which increases the risk for vehicular accidents (Bajaj et al. 2008). As a complex human ability, navigation ability involves functional integration of various component systems, such as episodic and spatial memory, as well as other processes (including visual attention, visuo-perceptual skills and judgment, short-term memory, decision-making processes, and response inhibition) (Bajaj et al. 2008). Previous studies have demonstrated that the hippocampus serves as a nucleating point for spatial navigation and episodic memory (Bird 2017; Lisman et al. 2017; Maguire et al. 1999; Sherrill et al. 2013).

The hippocampus is indeed one of the crucially pathological nodes for MHE. Both in vivo and ex vivo experimental data have demonstrated neuroinflammation (as indicated by microglial activation) involving the hippocampus due to hyperammonemia (Kong et al. 2016; Rodrigo et al. 2010). These neuroinflammation procedures lead to cognitive impairments such as learning disability in HE (Cauli et al. 2007; Shawcross et al. 2004; Srivastava et al. 2011; Zhang et al. 2014). In addition, the hyperammonemic model exhibits hippocampal astrocyte dysfunction, and a decrease in glutamate uptake as well as the levels of brain-derived neurotropic factor (BDNF), which contributes to synaptic plasticity and regulates long- and short-term memory (Galland et al. 2017). Furthermore, neuroimaging studies have revealed hippocampal structure vulnerability in cirrhotic patients without overt HE. Diffusion kurtosis imaging has revealed a reduction in kurtosis parameters in the hippocampus and other diffuse gray matter regions in cirrhotic patients, indicating decreased microstructural complexity that is associated with dendrites and spines, synaptic pruning and refinement, and cell packing density (Chen et al. 2017). Another study has also shown that hyperammonemia significantly decreases the dendritic spine density of hippocampal neurons (Chen et al. 2014b).

Previous functional magnetic resonance imaging in a resting state has revealed that MHE is associated with a disruption in intrinsic functional connectivity (FC) of the brain (Zhang and Zhang 2018). MHE is characterized by FC disruption within multiple brain networks, such as default-mode network (DMN), attention network, and salience network (Chen et al. 2016; Chen et al. 2013, 2014a), and the unoptimizable topological organization of whole-brain network with a decrease in network efficiency (Hsu et al. 2012; Jao et al. 2015). In addition, extensive efforts have been made to investigate the abnormal pattern of FC of pivotal regions across brain networks in MHE patients, including the anterior cingulate gyrus (Zhang et al. 2013), posterior cingulate gyrus (Chen et al. 2012b), and basal ganglia and thalamus (Qi et al. 2013a, b). Among these investigations, a predominant decrease in seed-region FC alterations has been observed. Despite the above advances in investigating MHE-related FC abnormalities, studies on the correlation between resting-state hippocampal FC and MHE are limited.

In the above contexts, this study aimed to simultaneously evaluate hippocampal volume and FC alterations and their associations with cognitive decline in MHE, which may provide novels insights into neural substrates that cause hippocampal dysfunction in MHE.

Materials and methods

Participants

The Research Ethics Committee of the Fujian Medical University Union Hospital, China approved this study. Each subject has provided a written informed consent to participate in the study. Twenty-two cirrhotic patients with MHE, 31 cirrhotic patients without MHE (NHE), and 43 healthy controls (HCs) were included. Table 1 shows the demographic and clinical parameters of the participants. No significant differences in age, gender, or education level were observed across the three study groups. The following were used in the diagnosis of MHE: Psychometric Hepatic Encephalopathy Score (PHES) examination, which includes five subtests, namely, the digit symbol test, number connection tests A and B, serial dotting test, and line tracing. Details on the PHES tests and how to diagnose MHE have been previously described (Chen et al. 2015).

The following patient exclusion criteria for were used: (1) diagnosed with current overt HE or other neuropsychiatric

Table 1 Subjects' demographics and clinical characteristics

	Number of healthy controls ($n = 43$)	Number of NHE patients ($n = 31$)	Number of MHE patients ($n = 22$)	P value (ANOVA)
Age (year)	48.8 ± 9.9	51.9 ± 9.8	51.4 ± 8.9	0.343
Sex (Male/Female)	30/13	26/5	18/4	0.303 (χ^2-test)
Education level (year)	9.5 ± 3.4	8.2 ± 3.1	8.9 ± 2.8	0.173
Etiology of cirrhosis (HBV/alcoholism/ HBV + alcoholism/others)	–	22/4/2/3	13/4/2/3	–
Child-Pugh stage (A/B/C)	–	19/11/1	3/13/6	–
Final PHES score	0.63 ± 1.88	−0.74 ± 2.23†	−8.22 ± 3.04$^{*, \#}$	<0.001
Number connection test A (seconds)	35.4 ± 11.0	39.2 ± 10.6	57.4 ± 17.4$^{*, \#}$	<0.001
Number connection test B (seconds)	58.2 ± 25.6	73.6 ± 26.3	134.1 ± 62.2$^{*, \#}$	<0.001
Serial dotting test (seconds)	40.3 ± 7.1	46.9 ± 9.4†	65.2 ± 18.4$^{*, \#}$	<0.001
Digit symbol test (raw score)	48.2 ± 12.8	41.2 ± 12.6†	26.8 ± 8.5$^{*, \#}$	<0.001
Line tracing test (raw score)	113.0 ± 19.9	144.9 ± 36.8†	192.3 ± 47.4*	<0.001

MHE, minimal hepatic encephalopathy; NHE, patients not presenting with MHE; HCs, healthy controls; PHES, psychometric hepatic encephalopathy score. The markers *, †, and $^{\#}$ respectively represent significant neurological performance differences between MHE and HC, NHE and HC, as well as MHE and NHE

condition, (2) receiving psychotropic medications or drug abuse, (3) diagnosed with uncontrolled endocrine or metabolic disorder (e.g., thyroid dysfunction), (4) engaged in alcohol abuse in the past six months before the study, and (5) other MRI contraindications.

MRI data acquisition

Imaging data were collected using a 3.0 T MRI scanner (Siemens, Verio, Germany). Functional MR images in a resting state were obtained by echo planar imaging using the following settings: 35 contiguous axial slices, TR = 2,000 ms, TE = 25 ms, FOV = 240 mm × 240 mm, matrix = 64 × 64, flip angle = 90°, and slice thickness = 4 mm. The patients were advised to close their eyes closed, not to focus on any particular issue, and not to move their head. Three-dimensional T1-weighted sagittal images of magnetization-prepared rapid gradient echo (MPRAGE) were obtained using the following settings: TR = 1.9 ms, TE = 2.48 ms, FOV = 256 mm × 256 mm, matrix = 256 × 256, flip angle = 9°, slice thickness = 1.0 mm, 176 slices.

Hippocampal volume measurement

The structural T1-weighted images were preprocessed using the VBM toolbox (VBM8, http://dbm.neuro.uni-jena.de/vbm) as implemented in the Statistical Parametric Mapping software (SPM8, http://www.fil.ion.ucl.ac.uk/spm). Segmentation of the structural images into gray matter (GM), white matter (WM), as well as cerebrospinal fluid (CSF) was performed using the SPM8 "unified segmentation" model. Then, a GM population template was generated using the entire image dataset with the Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL) technique (Ashburner 2007). Initial affine registration of the GM DARTEL template to the tissue probability map in the Montreal Neurological Institute (MNI) space was performed. Subsequently, non-linear warping of the GM images using the DARTEL GM template was conducted, at a 1.5-mm cubic resolution. Each voxel was assessed in terms of GM volume (GMV) by multiplying the GM concentration map with the non-linear determinants that were obtained using spatial normalization. Thus, the assessment of regional differences in the modulated data based on the brain absolute volume was then performed and confounding effects of various individual brain sizes were eliminated.

VBM analysis indicated that the bilateral hippocampus represents the nodes with significantly deceased volume in cirrhotic patients (see Supplementary Fig. 1). This primary finding prompted us to specifically investigate structural abnormalities involving the hippocampus during HE development (e.g., from NHE to MHE). In the current study, the hippocampal volume was extracted based on the Automated Anatomical Labeling (AAL) template, following the above VBM procedures, and compared among the three groups. Statistical significance was established using a $P < 0.05$.

Functional image preprocessing

Preprocessing of the functional data was performed using the SPM software as well as the Data Processing Assistant for Resting-State fMRI (DPARSF 3.0, http://www.restfmri.net/forum/DPARSF) tool. For every study participant, the first 10 volumes were removed to prevent initial MR imaging signal instability. Adjustment of slice-timing and correction of head-motion by

realignment were conducted. When a patient showed a translational movement >2 mm or a rotation >2.0°, this study participant was excluded from further analysis. Co-registration of individual structural images with the mean functional image was performed. Segmentation of the transformed structural images using a unified segmentation algorithm into GM, WM, and CSF was then performed. Subsequently, motion-corrected functional volumes were subjected to further normalization to the standard MNI space with normalization parameters that were estimated using the unified segmentation method and resampled to dimensions of 3 mm × 3 mm × 3 mm. Then, spatial smoothing of functional images was performed with a 4-mm full width at half maximum Gaussian kernel, followed by linear detrending. The generated functional data were then band-pass filtered (0.01–0.08 Hz) to decrease low-frequency drift as well as high-frequency physiological respiratory and cardiac noise. Considering that head motion as well as global WM and CSF signals may influence the results, various sources of spurious variance were removed using linear regression, which included six head motion parameters and the mean signals of the CSF, WM, and the entire brain.

Functional connectivity analysis

Further analysis of the functional data was performed using the seed-based FC method. The left and right hippocampal regions from the AAL template were utilized as seed regions, as previously described (Cao et al. 2012; Shu et al. 2014; Zhang et al. 2014). Voxel-wise FC analysis was conducted for every seed region. The average time series of all of the voxels within the every hippocampus was employed as seed reference time series. In addition, Pearson's correlation coefficient (time series between the seed reference and of every voxel in the brain) was calculated and expressed as the FC strength. Additional statistical analysis was performed by transforming the correlation coefficients into Z values via Fisher r-to-z transformation, generating a map that was representative of the whole-brain FC maps of both hippocampal regions (in terms of the Z values).

Group-level analysis of functional connectivity

The statistical module of the DPARSF software was applied for group-level voxel-wise analysis of hippocampal FC. (1) Within-group: To assess the FC patterns of both hippocampal regions in the three groups, we performed random effects analysis of the FC maps of each group with the one sample *t* test, using $P < 0.05$ for false discovery rate (FDR) correction during multiple comparisons. In addition, for every hippocampal seed, we integrated the within-group FC patterns of the three groups to generate a mask for subsequent between-group comparisons. (2) Between-groups: One-way ANOVA test was performed to detect hippocampal FC differences among the three groups, using age, gender, education level, and the voxel-wise GMV as covariates. The threshold of $P < 0.05$, according to the AlphaSim correction based on Monte Carlo simulation algorithm (voxel-wise $P < 0.001$ and cluster size >324 mm^3), was set to identify significance level. The Z values of the brain regions that showed statistical differences using ANOVA were extracted and then compared among the three groups. To prevent anatomical variations among groups to influence FC differences, the voxel-wise GMV was employed as a covariate during functional data analysis.

Mean Z values of each patient's hippocampal FC network (as indicated by one-sample t-test) were also

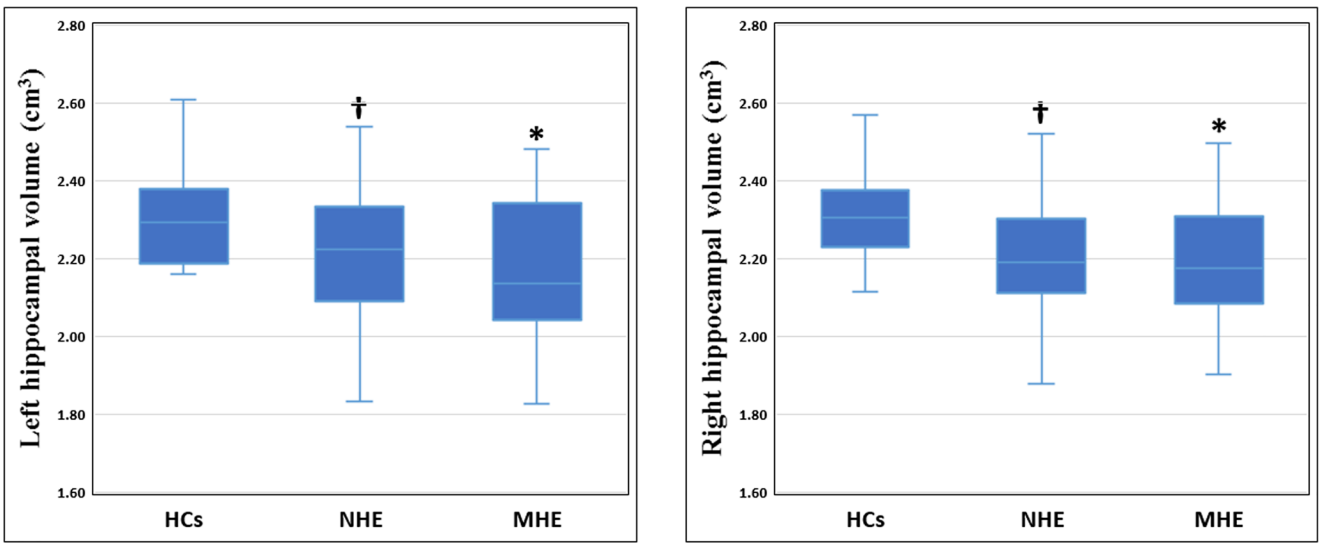


Fig. 1 The hippocampal volume of the three study groups. MHE, minimal hepatic encephalopathy; NHE, patients without MHE; HCs, healthy controls. The markers * and †respectively indicate the significant difference ($P < 0.05$) between MHE/NHE and HC

gathered and compared among the three groups by ANOVA and post hoc tests.

Correlation analysis

Pearson correlation analysis was used to examine the association between bilateral hippocampal volume and PHES results among the cirrhotic patients. In addition, the correlation of the Z values of brain regions showing significant FC differences among the three groups was assessed against the results of neuropsychological testing. Statistical significance was established at P values <0.05.

Results

Compared to the HCs, the NHE patients showed worse neurocognitive performance in several PHES subtests, namely, the serial dotting test, digit symbol test, and line tracing test; thereby, NHE patients had slightly lower PHES score than HCs. However, the lower PHES test scores of the MHE patients indicated poor performance compared those of the other two groups (Table 1).

Figure 1 shows the hippocampal volume across the three groups. MHE and NHE patients showed a significant reduction in bilateral hippocampal volume compared

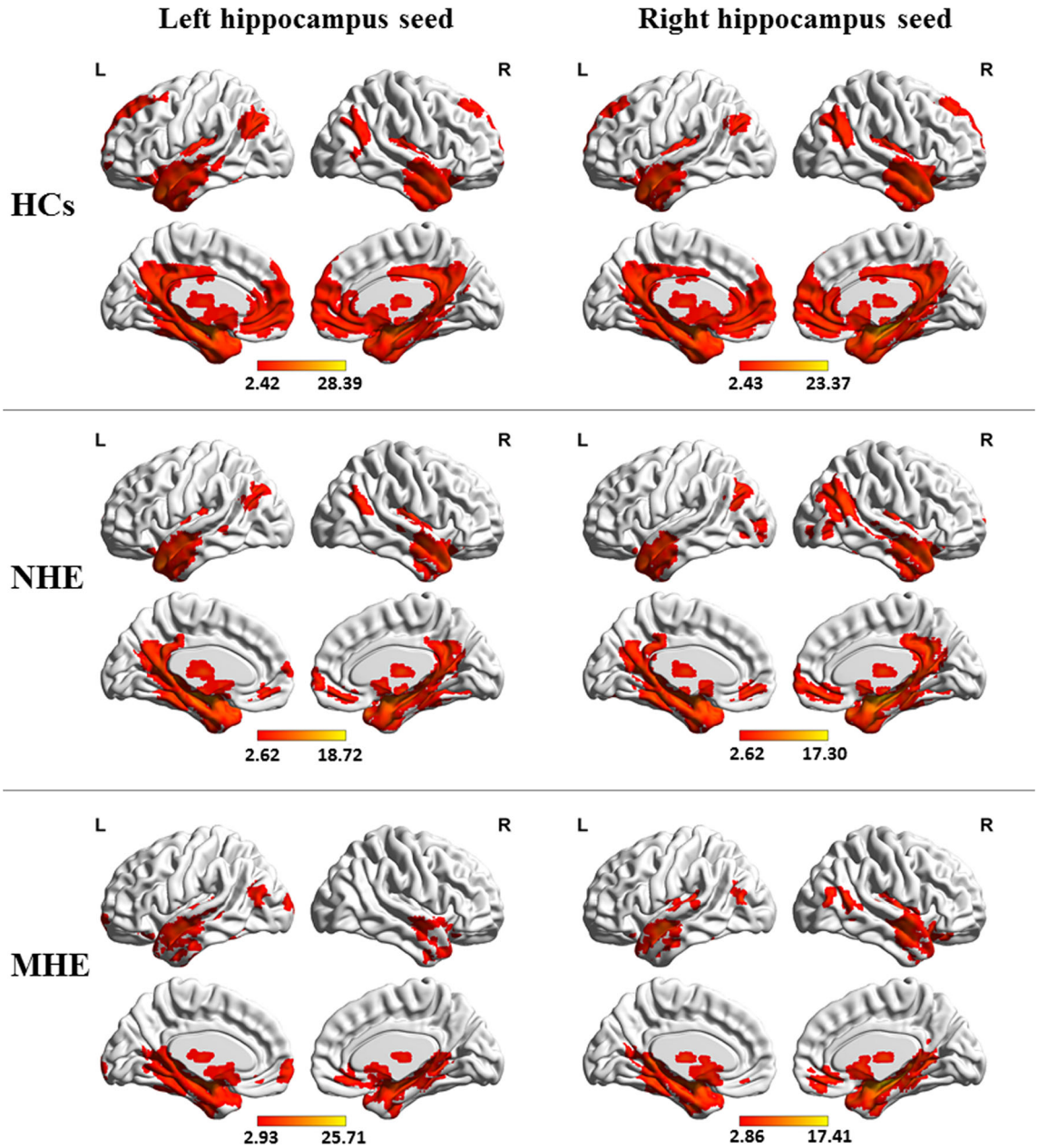


Fig. 2 The pattern of hippocampal functional connectivity among the three groups. MHE, minimal hepatic encephalopathy; NHE, patients without MHE; HCs, healthy controls

to the healthy controls. A slight trend of decreased hippocampal volume was noted from NHE to MHE, but did not reach statistical significance.

Figure 2 shows the pattern of hippocampal FC among the three groups. In the HCs, the bilateral hippocampus showed significant connectivity to a set of brain regions, particularly the bilateral limbic system (hippocampus, parahippocampal gyrus, amygdala, insula, and anterior and posterior cingulate gyrus), lateral temporal lobe, medial frontal cortex, medial and inferior parietal cortex, thalamus, and basal ganglia. NHE and MHE showed a similar pattern of hippocampal FC. However, visual inspection revealed that the extent and strength of bilateral hippocampal FC decreased stepwise from the NHE group to the MHE group.

Global ANOVA analysis revealed that the average FC of the left and right hippocampus significant differed among the three groups ($P = 0.010$ and $P = 0.036$, respectively). The average FC strength of global hippocampal connectivity network significantly decreased in MHE patients compared to the NHE patients and HCs. Moreover, a progressive decrease in hippocampal FC from NHE to MHE was observed (Fig. 3).

Figure 4 shows the between-group differences of the left hippocampal FC. ANOVA analysis showed significant differences in FC of the left hippocampus to bilateral posterior cingulate gyrus and left angular gyrus among the three groups (Table 2). Post hoc comparisons revealed that the NHE and MHE patients exhibited a significant reduction in FC between the left hippocampus and bilateral posterior cingulate gyrus and left angular gyrus compared to the HCs. Meanwhile, the FC between the left hippocampus and left angular gyrus was lower in the MHE patients than the NHE patients. Together, the reduction in the left hippocampal FC progressed from NHE to MHE.

Figure 5 shows the between-group differences of the right hippocampal FC. Significant FC differences between the right hippocampus and bilateral medial frontal cortex were observed among the three groups (Table 2). Post hoc comparisons showed that NHE and MHE patients exhibited a significant decrease in FC between the right hippocampus and bilateral medial frontal cortex compared to the HCs. In addition, MHE showed a significantly decrease in FC between the right hippocampus and right medial frontal cortex compared to the NHE patients. A progressive reduction in the right hippocampal FC from NHE to MHE was also observed.

Figure 6 presents the findings of correlation analysis. Among the cirrhotic patients, the neurocognitive performance (as indicated by PHES results) was positively correlated with FC strength between the left hippocampus and left angular gyrus and the FC strength between the right hippocampus and right medial frontal cortex. No significant correlation was observed between hippocampal volume and the PHES scores.

Discussion

The present study assessed hippocampal FC alterations in relation to regional volume, based on the fact that the hippocampus serves as the critical pathological node in MHE. The major findings follow. (i) Hippocampal volume is significantly lower in MHE, indicating regional atrophy. (ii) MHE patients exhibit a decrease in FC between the left hippocampus and bilateral posterior cingulate gyrus and left angular gyrus and between the right hippocampus and bilateral medial frontal cortex. Together, a disruption of hippocampal connectivity to

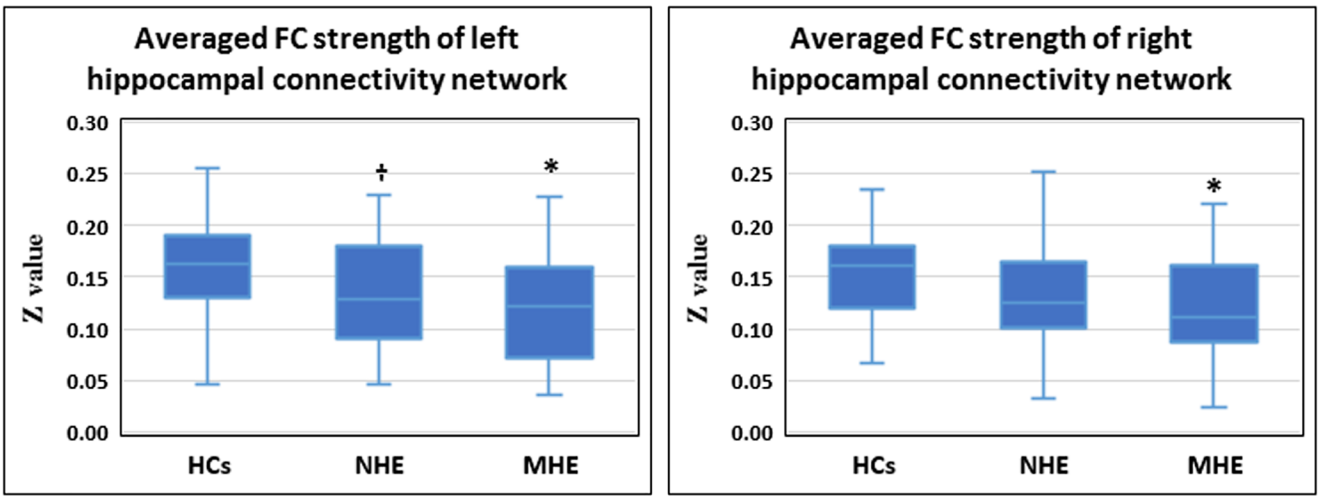
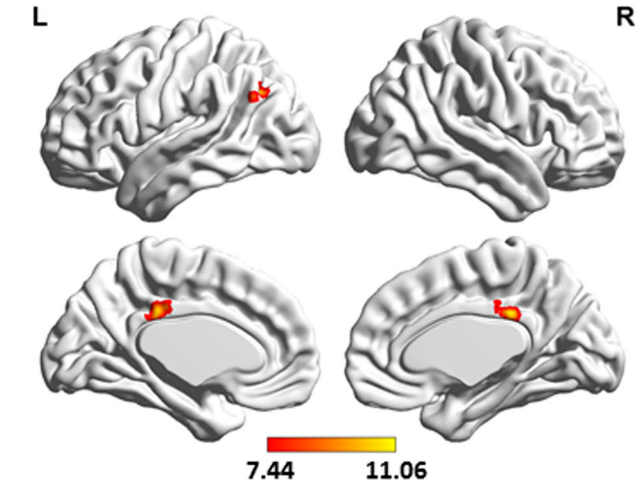
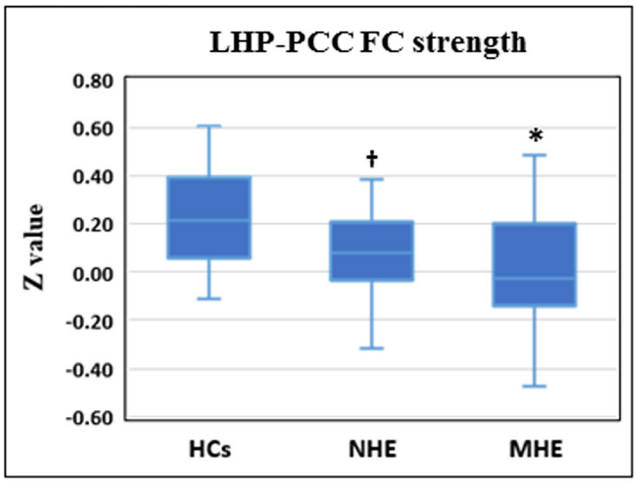


Fig. 3 The averaged functional connectivity strength (indicated by Z value) of global hippocampal connectivity network. One-way ANOVA and post hoc comparisons show a significantly lower mean Z value in MHE patients compared to the NHE patients and HCs. Moreover, a progressive decrease in hippocampal functional connectivity was observed from NHE to MHE. MHE, minimal hepatic encephalopathy; NHE, patients without MHE; HCs, healthy controls; FC, functional connectivity. The markers * and † indicate significant differences ($P < 0.05$) in FC strength between the MHE/NHE and HC groups





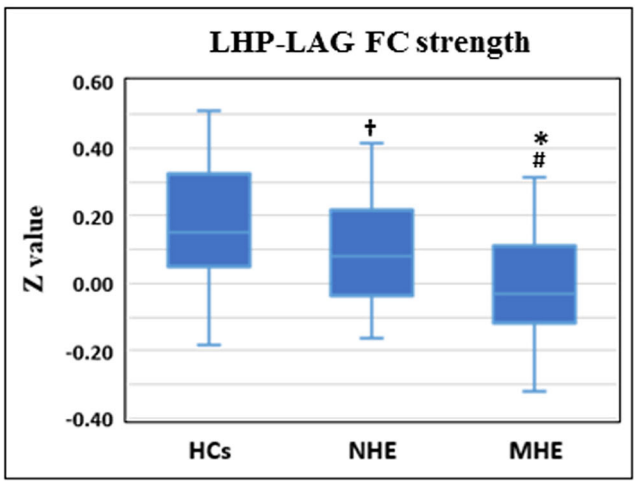


Fig. 4 Group differences in the left hippocampal functional connectivity (FC). ANOVA analysis shows significant differences in FC of the left hippocampus (LHP) to bilateral posterior cingulate gyrus (PCC) and left angular gyrus (LAG) among the three study groups. The bar graphs present the findings of post hoc comparisons between every two groups. A reduction in the left hippocampal FC was noted during progression from NHE to MHE. The markers *, †, and # respectively represent significant differences in FC strength between MHE vs. HC, NHE vs. HC, as well as MHE vs. NHE

Table 2 Brain regions exhibiting significant differences in the functional connectivity involving the hippocampus among the three study groups

Regions	Voxels	Brodmann area	MNI coordinates			Peak *F*-value
			x	y	z	
Connectivity to the left hippocampus						
Bilateral posterior cingulate gyrus	21	23	0	−33	33	11.06
Left angular gyrus	12	39	−48	−66	33	9.06
Connectivity to the right hippocampus						
Left medial frontal gyrus	22	10	−12	66	15	17.76
Right medial frontal gyrus	13	10	12	60	18	10.44

the default-mode network (including bilateral posterior cingulate gyrus, medial frontal cortex, and inferior parietal lobe) was observed in the MHE patients. (iii) The progressive decrease in hippocampal volume as well as FC was observed with disease progression from NHE to MHE. (iv) A correlation between hippocampal FC reduction and cirrhotic patients' worse cognitive performance was detected. The findings of the present study provide novel insights into structural and functional alterations involving the hippocampus due to MHE, which facilitates in the elucidating of the mechanisms underlying MHE.

Previous studies have demonstrated the structural abnormalities in MHE, such as GM atrophy and WM microstructural integrity impairment (Guevara et al. 2011; Montoliu et al. 2013). The decreased microstructural complexity of the hippocampus has also been revealed by a recent diffusion kurtosis imaging study (Chen et al. 2017). Our finding of decreased hippocampal volume agrees with the existing reports. The FC reduction in hippocampal connectivity during the progression from NHE to MHE also coincides with the findings of a previous report, in which graph theory-based analysis on whole-brain network revealed the development of HE induces a gradual reduction in hippocampal FC with all of the other brain regions and the hippocampal regional efficiency (Hsu et al. 2012). Various pathological procedures associated with an increase in brain ammonia metabolism could induce hippocampal atrophy and disrupt FC, such as neuroinflammation (characterized by microglial activation (Kong et al. 2016; Rodrigo et al. 2010)), disruption in astrocyte function (as indicated by loss of immunoreactivity (Hiba et al. 2016)), deficiency in the activation of glutamatergic neurotransmission (Galland et al. 2017), and abnormal metabolism of energy (as indicated by activity impairment of enzymes that are related to the mitochondrial respiratory chain (Dhanda et al. 2018)).

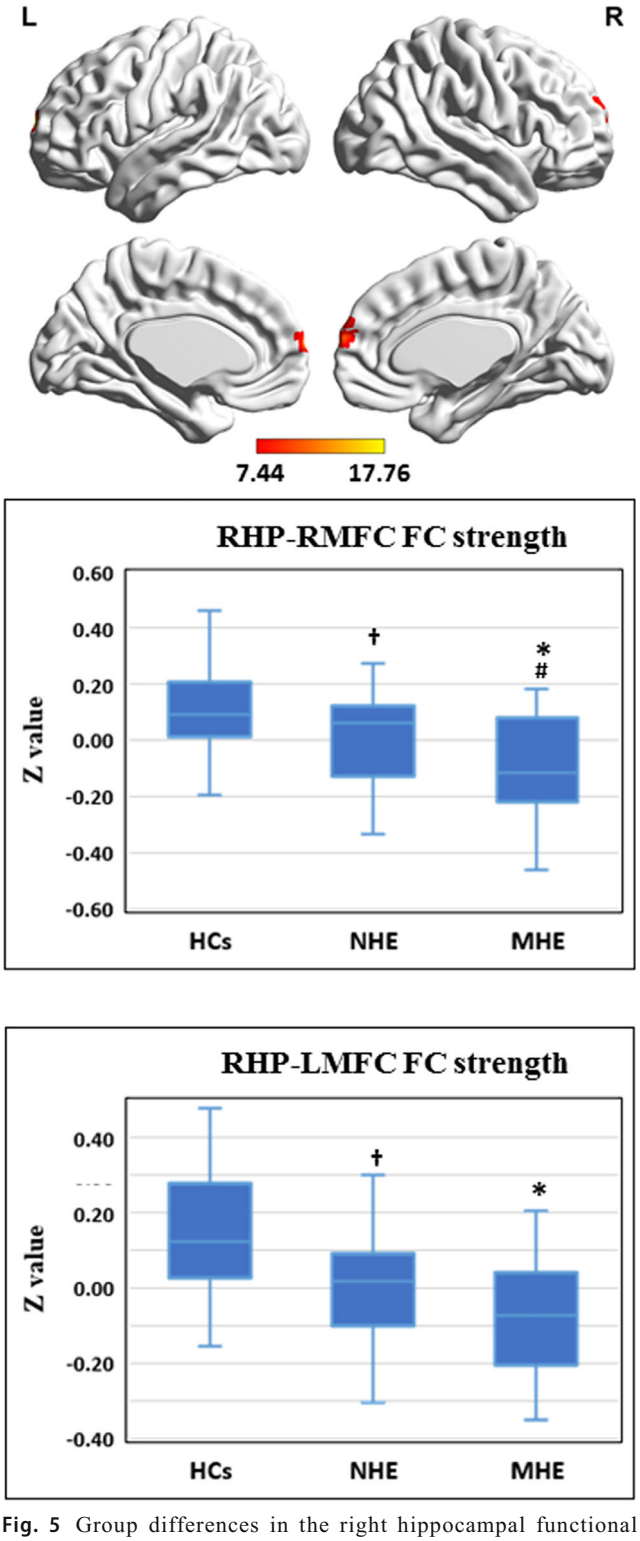


Fig. 5 Group differences in the right hippocampal functional connectivity (FC). ANOVA analysis shows significant differences in FC of the right hippocampus (RHP) to bilateral medial frontal cortex (MFC) among the three study groups. The bar graphs present the findings of post hoc comparisons between every two groups. A reduction in the right hippocampal FC was noted during progression from NHE to MHE. The markers *, †, and # respectively represent the significant difference in FC strength in MHE vs. HC, NHE vs. HC, and MHE vs. NHE

In addition, we observed a positive correlation between hippocampal FC alterations and cognitive decline in the patient group, which is discordant to the results of a previous report, in which voxel-wise FC analysis shows that the FC density of the right hippocampus is negatively correlated with cognitive ability and blood ammonia level in cirrhosis (Zhang et al. 2017). This discrepancy may be derived from the difference in sample collection between our study and the previous study. The existing studies have demonstrated that the distinct etiologies of cirrhosis and history of prior overt HE episode are associated with various degrees of brain functional and structural abnormalities (Chen et al. 2012a, 2013; Guevara et al. 2011).

The hippocampus serves as a critical hub for a number of cognitive processes, including learning, memory, and navigation (Blankenship et al. 2017; Eichenbaum and Cohen 2014; Lisman et al. 2017). Previous reports (Bai et al. 2011; Brueggen et al. 2016; Shu et al. 2014) have shown that weaker global hippocampal connectivity always indicates impaired functional integration, which in turn may contribute to deficits involving the above hippocampal functions in MHE patients. For instance, it has been demonstrated that lower FC between posterior hippocampus and retrosplenial complex (including posterior cingulate gyrus, retrosplenial cortex proper, and the nearby ventral parietal-occipital sulcus and anterior calcarine sulcus) predicts poorer navigational ability. In addition, the activation of the inferior parietal cortex has been associated with successful navigation (Maguire et al. 1999). Thus, these findings imply that the decrease in FC between the left hippocampus and bilateral posterior cingulate gyrus and left angular gyrus may be one of the major neural substrates for MHE-related navigation deficit. In addition, the medial frontal cortex plays an essential role in decision-making and learning in cognitive context (Szczepanski and Knight 2014). A recent FC study has demonstrated the role of the medial frontal cortex-thalamus-hippocampus network in memory function (Thielen et al. 2018). Thus, the weaker hippocampal connectivity to medial frontal cortex may account for the observed memory dysfunction in MHE.

The present study has a number of limitations. First, this investigation involved a relatively small study population, which possibly restricts our findings. Second, the cross-sectional design of our study does not allow for the evaluation of the causal effect of MHE on hippocampal structure and function. A longitudinal study clarifying the value of hippocampal volume and FC alterations as the biomarkers for detection of MHE and monitoring HE development is thus warranted. Third, both structural and functional analyses were performed, with the whole hippocampus as the region of interest, whereas structural and functional segmentations of the hippocampus have recently been investigated (Zarei et al. 2013; Zeidman and Maguire 2016).

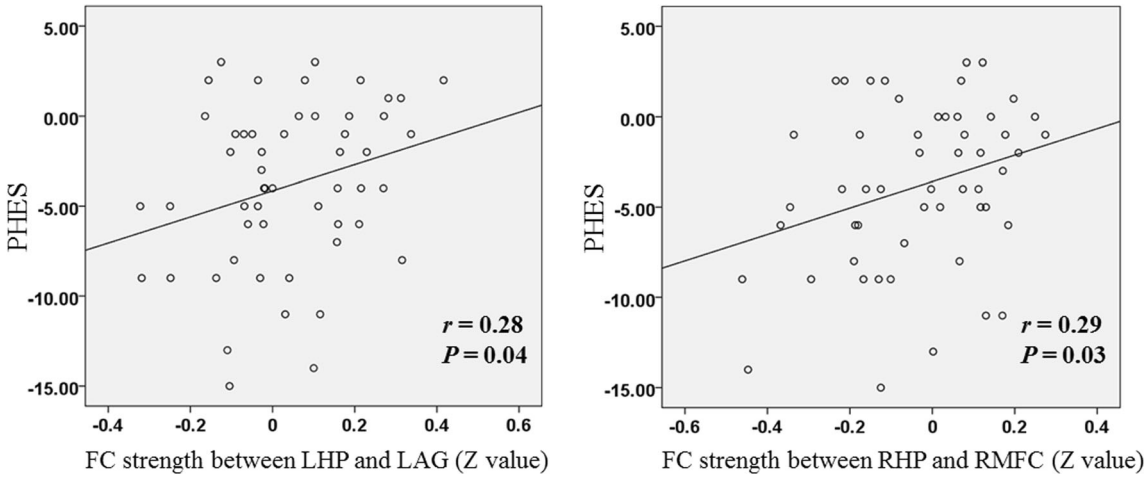


Fig. 6 Scatterplots of the extracted Z values (that represent the hippocampal connectivity strength) and neurocognitive performances (PHES result) of the cirrhotic patients. LHP, left hippocampus; LAG, left angular gyrus; RHP, right hippocampus; RMFC, right medial frontal cortex; PHES, psychometric hepatic encephalopathy score; FC, functional connectivity

Additional studies investigating the volume and FC alterations of distinct hippocampal subregions in MHE are thus warranted. Fourth, cognition assessment was performed using the PHES battery tests, which can generally reflect cirrhotic patients' cognitive status. However, our cognition assessment did not include specific tests for evaluating hippocampal functions, such as memory and navigation ability; and that may contribute to the relatively weak correlation (see Fig. 6) between hippocampal FC and PHES result. Thus, other associations between hippocampal volume and FC and cognitive performance may have been obscured. Fifth, we applied functional connectivity analyses, but which don't consider causal interactions within the hippocampal network and can't evaluate how information propagates through brain regions. The analysis of effective connectivity, quantifying the directed functional coupling among brain regions (Deshpande and Hu 2012), may be contributed to better understand how different brain areas interact in MHE and is recommended in the future study. Finally, the more sufficient laboratory data (e.g. venous blood ammonia level) should be also recorded to further evaluate the relationship between imaging findings and clinical variables, which may clarify the potential of these imaging features serving as the biomarkers for the disease.

In sum, our results have revealed a decrease in hippocampal volume in MHE patients, suggesting regional atrophy, and a reduction in hippocampal connectivity with regions that are primarily involved in the default-mode network, suggesting a functional disconnection syndrome. Meanwhile, hippocampal FC alterations are correlated with worse cognitive performance in cirrhosis and aggravate with HE progression. Both structural and functional abnormalities of the hippocampus are involved in the mechanism underlying cognitive deterioration with disease progression.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval The Research Ethics Committee of the Fujian Medical University Union Hospital, China approved this study.

Informed consent Each subject has provided a written informed consent to participate in the study.

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