Hindawi
Neural Plasticity
Volume 2020, Article ID 6389368, 9 pages
https://doi.org/10.1155/2020/6389368

Research Article

The Long-Term Effects of Acupuncture on Hippocampal Functional Connectivity in aMCI with Hippocampal Atrophy: A Randomized Longitudinal fMRI Study

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Received 15 October 2019; Revised 23 December 2019; Accepted 21 January 2020; Published 10 February 2020

Guest Editor: Marco Canevelli

Background. Acupuncture has been used to treat amnestic mild cognitive impairment (aMCI) for many years in China. However, the long-term effects of continuous acupuncture treatment remained unclear. *Objective*. We aimed to explore the long-term effects of continuous acupuncture treatment on hippocampal functional connectivity (FC) in aMCI. *Methods*. Fifty healthy control (HC) participants and 28 aMCI patients were recruited for resting-state functional magnetic resonance imaging (fMRI) at baseline. The 28 aMCI patients were then divided into the aMCI acupuncture group, which received acupuncture treatment for 6 months, and the aMCI control group, which received no intervention. All aMCI patients completed the second resting-state fMRI scanning after 6 months of acupuncture treatment. Analysis based on the region of interest and two-way analysis of covariance were both used to explore the long-term effects of acupuncture on cognition change and hippocampal FC in aMCI. *Results*. Compared to HC, aMCI showed decreased right hippocampal FC with the right inferior/middle temporal gyrus (ITG/MTG), left amygdala, and the right fusiform and increased FC with bilateral caudates at baseline. After acupuncture treatment, the right hippocampal FC with right ITG/MTG enhanced significantly in the aMCI acupuncture group, but continued to decrease in the aMCI control group. Whole brain FC analysis showed enhanced right hippocampal FC with the right ITG and the left MTG in the aMCI acupuncture group relative to the aMCI control group. Furthermore, FC strength of the right hippocampus with right ITG at baseline was negatively correlated with the changes in memory scores of aMCI acupuncture patients. *Conclusion*. Acupuncture treatment could alleviate the progression of cognitive decline and could enhance hippocampal FC with ITG and MTG in aMCI that may be associated with resilience to resistant against neurodegeneration. The findings provided a better understanding of the long-term effects of acupuncture treatment and confirmed the therapeutic role of acupuncture in aMCI.

1. Introduction

Amnestic mild cognitive impairment (aMCI) is considered as the prodromal stage of Alzheimer's disease (AD) [1]. It is reported that about 11.1% of the population between 70 and 89 years is affected by aMCI and among them about 16-41% develop AD every year [2, 3]. As dementia can severely affect quality of life and is a leading cause of death in elderly people, once the disease occurs, there is no cure for it; early diagnosis and treatment of AD at the prodromal stage is of great significance. As an important component of traditional Chinese medicine, acupuncture has been used to treat dementia in China for many years [4–6] and the potential mechanisms underlying its therapeutic efficacy have attracted increasing attention.

Functional magnetic resonance imaging (fMRI) provides a noninvasive means to investigate the neural mechanisms of the effects of acupuncture in different diseases, such as Parkinson's disease and insomnia disorder [7, 8]. Accumulating evidence has suggested acupuncture as a promising treatment for MCI by modulating cerebral functional connectivity (FC) [9–11] and intrinsic activity [12, 13]. Feng et al. reported that acupuncture enhanced functional correlations in memory-related brain regions, including the hippocampus (HP), thalamus, and fusiform gyrus [9] in MCI patients. In other studies, acupuncture could remediate the dysfunctional connections of the HP [10] and alleviate the loss of small world in MCI patients by enhancing the nodal centrality of the HP, postcentral cortex, and anterior cingulate cortex. [11] Further, as quantified by the amplitude of low-frequency fluctuation (ALFF), acupuncture modulated intrinsic activities of cognition-related brain regions, including the right superior temporal gyrus (STG) and HP, in MCI patients [12, 13].

In traditional Chinese medicine, a combination of several acupoints is often used in acupuncture as a treatment. In the theory of traditional Chinese medicine, the Si Guan (four gates) acupoints, composed of bilateral Hegu (LI4) and Taichong (Liv3), could harmonize yin and yang and regulate qi and blood to improve the cognitive capacity. With LI4 and Liv3 as the acupoints, previous studies have demonstrated increased or decreased activities of temporal and frontal brain regions in MCI and AD patients following acupuncture [14, 15]. Increased FC between the left HP and right middle frontal gyrus (MFG) and between the right HP and right inferior temporal gyrus (ITG) and STG have also been demonstrated in AD patients [5].

However, these earlier studies focused on the instantaneous effects of acupuncture, and the long-term effects of acupuncture were rarely reported. Tan et al. performed acupuncture on MCI subjects for 4 consecutive weeks and reported that acupuncture could regulate the cognitive-related brain networks and improve the cognitive ability [4]. In the present study, we performed acupuncture treatment on aMCI patients for six months to explore its long-term effects and the neural processes for the long-term treatment. Considering that the HP is one of the earliest pathological sites of aMCI and HP activity appeared to be modulated by the instantaneous effects of acupuncture, we defined bilateral hippocampi as the regions of interest (ROIs). We hypothesized that acupuncture at the Si Guan acupoints would modulate the HP connectivity and alleviate the progression of cognitive decline in aMCI patients.

2. Materials and Methods

We used a randomized single-blinded clinical trial, with a 2×2 flexible factorial design, to investigate the long-term effects of acupuncture treatment on aMCI. The research protocol for the study was approved by the Ethics Committee of the Xuanwu Hospital (Clinical Research Review 2016 004).

2.1. Participants. Healthy control (HC) participants were recruited from community while aMCI patients were recruited from Xuanwu Hospital of Capital Medical University in Beijing. The objectives and procedures were explained in detail to all participants and written informed consents were collected before participating in this study.

The criteria for aMCI were as follows: (1) compliance with the core clinical criteria stipulated by the National Institute on Aging and the Alzheimer's Association [16]; (2) a Clinical Dementia Rating (CDR) score of 0.5, with a score of at least 0.5 on the memory domain; (3) hippocampal atrophy on MRI; and (4) regular use of rivastigmine (12 mg/day).

The criteria for HC were as follows: (1) no complaints of cognitive changes, (2) no neurological deficiencies in physical examinations, (3) no any abnormal findings on brain MRI, and (4) CDR score = 0.

Subjects were excluded when the following conditions occurred: (1) current or previous diagnosis of any neurological or psychiatric disorders; (2) major medical illness, severe visual or hearing loss; (3) contraindications for MRI such as use of cardiac pacemakers and claustrophobia; and (4) aMCI patients who did not complete the whole acupuncture treatment.

In this study, we defined the pattern of HP-FC and neuropsychological measurement scales as our primary outcome measures. At baseline, all participants underwent MRI scans and neuropsychological evaluation with the Mini Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), CDR, and Auditory Verb Learning Test (AVLT). Then, aMCI patients were randomly divided into two subgroups, namely the aMCI acupuncture group and the aMCI control group. The aMCI acupuncture group received acupuncture treatment for 6 months while the aMCI control group received no intervention. After acupuncture treatments were completed, all the aMCI patients completed a follow-up fMRI scan and neuropsychological evaluation that were identical to the baseline examination. The neuropsychological scale test was performed by a designated experienced neurologist, and the clinic was closed and quiet.

2.2. Acupuncture Intervention. The Si Guan acupoints were targeted in the study (Figure 1). The subjects laid down in the supine position and the skins over Si Guan acupoints were disinfected with 70% alcohol. Each needle (silver needle with a diameter of 0.30 mm and length of 25 mm) was inserted vertically at a depth of approximately 20 mm and manually twirled using mild reinforcing-reducing method to bring about the needling sensation. The needles would be retained for 20 minutes and be rotated 5 times. Needling sensation, known as "de qi" in Chinese and an indicator of effective needling, included numbness, swelling, tingling, and a feeling of muscle weakness. At the same time, acupuncturists also could feel local muscle contracted and wrapped the silver needle. Acupuncture treatment was given 3 times a week, 4 weeks as a course. The interval between the two courses was 2 weeks of rest, with a total of 4 courses in 6 months.

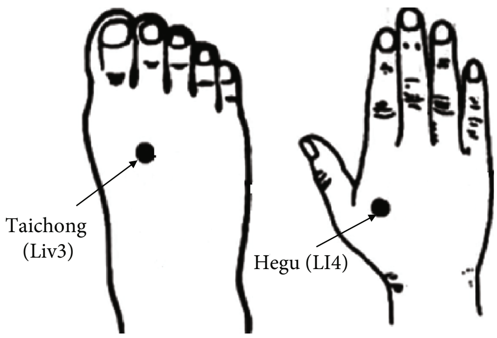

FIGURE 1: Location of acupoints used in the experiment.

2.3. MRI Data Acquisition. MRI data were obtained using a 3.0-Tesla scanner (Trio, Siemens, Erlangen, Germany). All participants were asked to lie supine with a headset to insulate scanner noise, hold still, and keep their eyes closed. Foam paddings were employed to reduce head motion. First, the routine head MRI scans were completed, including axial T1-weighted images scans, T2-weighted images scans, fluid-attenuated inversion recovery scans, diffusion-weighted imaging scans, and coronal and sagittal T1-weighted images scans. The parameters used to acquire 3D T1-weighted anatomical images and resting-state fMRI images were identical to those reported previously [17]. Resting-state functional MRI images were acquired using an echo-planar imaging (EPI) sequence: repetition time (TR) = 2000 ms, echo time (TE) = 40 ms, flip angle (FA) = 90°, field of view (FOV) = $256 \times 256\,mm^2$, matrix = 64×64, 28 axial slices, slice thickness/gap = 4/1 mm, bandwidth = 2232 Hz/pixel, and number of repetitions = 239. The 3D T1-weighted anatomical images were acquired with a magnetization-prepared rapid gradient echo (MPRAGE) method with the following parameters: TR = 1900 ms, TE = 2.2 ms, inversion time (TI) = 900 ms, FA = 9°, bandwidth = 199 Hz/pixel, matrix = 256×224, FOV = $256 \times 224\,mm^2$, 176 sagittal slices, and slice thickness = 1 mm.

2.4. MRI Data Preprocessing and Functional Connectivity Analysis. Resting-state fMRI data were preprocessed with the statistical parametric mapping software SPM12 (https://www.fil.ion.ucl.ac.uk/spm), and seed-to-voxel correlation analysis was performed using the functional connectivity (CONN) toolbox v17f [18]. After discarding the first 10 functional images, the remaining 229 images of each individual subject underwent slice-timing correction and realignment. All subjects included in the present study exhibited head motion less than 3.0 mm in any of the x, y, or z directions and less than 3.0° of any angular dimension. The resulting images were spatially normalized to the Montreal Neurological Institute (MNI) space with a resampled resolution of $2 \times 2 \times 2\,mm^3$ and smoothed with a 4 mm full width at half maximum (FWHM) isotropic Gaussian kernel. After preprocessing, images were band-pass filtered to $0.008 \sim 0.09$ Hz to reduce the influence of noise. Further denoising steps included regression of the six motion parameters, regression of white matter, and cerebrospinal fluid (CSF) signals and a linear detrending.

The bilateral hippocampi were defined using the Automated Anatomical Labeling (AAL) templates. The correlation maps were generated by computing the Pearson correlation coefficients between the seed voxels and all other brain voxels. For group analyses, the correlation coefficients were converted to z value using Fisher's r-to-z transformation to improve normality. To consider the influence of volumetric difference in HP between aMCI and HC, we compared gray matter volume of the HP between the aMCI and HC groups.

Due to the particularity of acupuncture treatment, this study adopted a randomized, single-blind study. According to the previous study [15, 19], the estimated sample size was 30. The randomized digital table method combined with the random number remainder grouping method was used to randomly group the patients by a specialized statistical expert. The acupuncture treatment group and the blank control group were, respectively, 15 cases. Because the aMCI control group received no intervention, the patients in this study were not blind, but the functional magnetic resonance data analysts and the scale assessment experts that assessing outcomes were not aware of the treatment. Therefore, this study was a randomized, single-blind study.

2.5. Statistical Analysis. Demographic data and neuropsychological measures were analyzed using SPSS 22. Student's t-tests were conducted for continuous variables, including age, years of education, and neuropsychological test scores, and chi-squared tests were used to examine the differences in gender composition between groups.

In the random effect analysis of imaging data, we determined the voxel-wise FC patterns of bilateral hippocampi within each group, using one-sample t-test. Two-sample t-tests were performed to assess the differences in HP-FC between aMCI and HC subjects at the baseline. The significance threshold was set to $p < 0.05$ corrected for false discovery rate (FDR), with age, years of education, gender, and gray matter volume of the HP as covariates. Brain regions that showed significant differences in hippocampal FC were selected as ROIs and interregional FC values of the seeds were extracted. Two-sample t-tests were performed to investigate the differences of the FC changes between the aMCI acupuncture group and aMCI control group.

In addition, we employed a 2 (time: baseline versus follow-up) × 2 (group: aMCI acupuncture group versus aMCI control group) flexible factorial analysis, with 4 conditions—the aMCI acupuncture group at baseline (aMCI acupuncture1), the aMCI acupuncture group at follow-up (aMCI acupuncture2), the aMCI control group at baseline (aMCI control1), and the aMCI control group at follow-up (aMCI control2). The time by group interaction reflected the effects of acupuncture treatment on aMCI subject. The significance threshold was set to $p < 0.05$, FDR corrected, with age, years of education, and gender as covariates. Increased FC of HP in the aMCI acupuncture group after acupuncture relative to the aMCI control group was evaluated by [(aMCI acupuncture2 > aMCI acupuncture1) > (aMCI control2 > aMCI control1)] and decreased FC of HP in the aMCI acupuncture group after acupuncture

Table 1: Scoring results of neuropsychological scale.

	aMCI control group			aMCI acupuncture group			Changes (follow-up vs. baseline)		
	Baseline	Follow-up	p value	Baseline	Follow-up	p value	aMCI control	aMCI acupuncture	p value
CDR	0.5	0.5		0.5	0.5				1
MMSE	25.4 ± 3.7	24.0 ± 4.0	0.01	25.0 ± 3.1	25.8 ± 2.9	0.29	−1.4 ± 1.7	0.8 ± 2.6	0.02
MoCA	22.1 ± 4.2	21.0 ± 5.2	0.09	20.5 ± 3.7	19.9 ± 3.6	0.31	−1.1 ± 2.2	−0.6 ± 2.1	0.59
AVLT-immediate	6.5 ± 1.8	5.6 ± 1.5	0.14	6.3 ± 0.9	6.0 ± 1.8	0.40	−0.8 ± 2.1	−0.3 ± 1.2	0.40
AVLT-delay	4.5 ± 3.0	2.6 ± 2.1	0.01	3.1 ± 3.4	3.8 ± 3.9	0.37	−1.9 ± 2.1	0.6 ± 2.4	0.01
AVLT-recognition	7.2 ± 4.1	7.9 ± 3.6	0.48	6.8 ± 2.8	7.2 ± 3.8	0.75	0.6 ± 3.4	0.4 ± 4.5	0.86

Values represent the means ± SD. aMCI: mild cognitive impairment due to Alzheimer's disease; Lt.: left; Rt.: right; HP: hippocampus; CDR: clinical dementia rate; MMSE: mini mental state examination; MoCA: Montreal Cognitive Assessment; AVLT: auditory verbal learning test.

relative to the aMCI control group was evaluated by [(aMCI acupuncture2 > aMCI acupuncture1) < (aMCI control2 > aMCI control1)].

The regions showing a significant interaction effect were defined as ROIs, and the FC values were extracted for *post hoc* pairwise comparisons and correlations analysis using SPSS 22. Independent-sample t-tests were applied to compute the group differences of FC between the aMCI acupuncture and aMCI control groups at baseline and follow-up separately. Paired-sample t-tests were performed to explore the longitudinal FC changes within each group at follow-up, as compared to baseline.

Finally, to identify the distinct FC biomarkers and predictor of treatment effects, partial correlations were computed between the FC strength at baseline and changes in neuropsychological scores (post- vs. pretreatment), with adjustment for age, gender, and years of education in the aMCI acupuncture group. In addition, Pearson's correlations were conducted between the FC alterations and neuropsychological score changes (post- vs. pretreatment) to evaluate the relationship between the effect of acupuncture and FC changes. Statistical significance was set at $p < 0.05$, Bonferroni corrected for multiple comparisons.

3. Results

3.1. Scoring Results of Neuropsychological Scale. Eighty including 30 aMCI and 50 HC participants were recruited. Two aMCI patients refused to complete the baseline examination after the numbering, so a total of 28 patients and 50 HC were enrolled at baseline. The data of one aMCI control patient and two aMCI acupuncture patients were discarded because of excessive head movements in the scanner at follow-up. There were no significant differences in age, gender, and years of education between the aMCI and HC groups. Compared to HC, aMCI exhibited significantly smaller HP volume, higher CDR scores, and lower MMSE, MoCA, and AVLT scores, in confirmation of episodic memory impairment (Supplementary Table 1). Randomly divided into two groups, the aMCI acupuncture group and aMCI control group did not show significant differences in age, gender, years of education, CDR, MMSE, MoCA, or AVLT score (Supplementary Table 1).

3.2. Effects of Acupuncture on Cognitive Assessment in aMCI. As shown in Table 1, as compared with baseline measures, MMSE scores increased in the aMCI acupuncture group after acupuncture treatment, whereas the scores of the aMCI control group have continuously declined. At the same time, the scores of AVLT delayed recall changed in the same pattern; AVLT delayed recall scores increased in the aMCI acupuncture group, whereas the scores of the aMCI control group declined. The comparison between the score changes was significant.

3.3. Hippocampal FC: Within-Group Analysis. In the HC group, there were significant FC between the bilateral hippocampi and several cognitive-related regions including the temporal lobe, precuneus, cingulate gyrus, frontal lobe, and some subcortical nuclei (Supplementary Figure 1). In the aMCI group, similar FC patterns of bilateral hippocampi were revealed with relatively smaller cluster sizes, mainly including the temporal lobe, precuneous cortex, cingulate gyrus, and some subcortical nuclei (Supplementary Figures 2–6).

3.4. Between-Group Hippocampal FC Analysis at Baseline. Compared to HC, the aMCI patients showed a significantly weakened FC of the right HP to the right inferior/middle temporal gyrus (ITG/MTG), the left amygdala, and the right fusiform. Significantly enhanced FC was found between the right HP and bilateral caudates (Table 2, Figure 2). On the contrary, no significant FC changes of the left HP were found.

3.5. Effects of Acupuncture on HP-FC in the aMCI Acupuncture Group: ROI Analysis. To explore the effect of acupuncture on HP-FC in the aMCI acupuncture group after the whole treatment course, the abovementioned brain regions were defined as seeds and the FC values were extracted. Two-sample t-tests were performed to investigate the differences in the longitudinal HP-FC changes between the two groups and a significant result was found in the right HP to the right ITG/MTG. The weakened right HP-FC to the right ITG/MTG significantly enhanced at follow-up in the aMCI acupuncture group, while that of the aMCI control group continued to decline (Figure 3(a)). On the contrary, the trends of the right HP-FC changes in the other brain regions were similar

Table 2: Significant between-group differences in functional connectivity of the right hippocampus.

Contrast	Region	Cluster size (voxel)	MNI			T value
			x	y	z	
HC > aMCI	Rt. MTG/ITG	320	54	-12	-24	6.21
	Lt. amygdala	188	-32	2	-18	4.45
	Rt. fusiform	95	30	0	-48	4.39
HC < aMCI	Lt. caudate	90	-2	6	6	4.25
	Rt. caudate	100	16	20	8	4.04
(aMCI acupuncture2 > aMCI acupuncture1) > (aMCI control2 > aMCI control1)	Lt. MTG	71	-50	-18	-18	5.14
	Rt. ITG	66	54	-64	-12	4.86

Between-group results were thresholded at cluster-level $p < 0.05$ (FDR corrected). The anatomical localization was implemented by using xjView software. HC: healthy control; aMCI: amnestic mild cognitive impairment; aMCI acupuncture1: aMCI acupuncture group at baseline; aMCI acupuncture2: aMCI acupuncture group at follow-up; aMCI control1: aMCI control group at baseline; aMCI control2: aMCI control group at follow-up; MNI: Montreal Neurological Institute; Lt.: left; Rt.: right; MTG: middle temporal gyrus; ITG: inferior temporal gyrus.

between the aMCI acupuncture and aMCI control groups, which showed no significant difference.

3.6. Effects of Acupuncture on HP-FC in the aMCI Acupuncture Group: Whole Brain Analysis. For time by group interaction effect, the right HP showed significant enhanced FC with the right ITG and the left MTG in the aMCI acupuncture group in contrast to the aMCI control group. *Post hoc* pairwise comparisons showed that (1) at baseline, there was no significant between-group differences in FC of the right HP and right ITG ($p = 0.1618$) and the left MTG ($p = 0.1688$); (2) at follow-up, compared with the aMCI control group, the FC of the right HP and the right ITG ($p = 0.0126$) and the left MTG ($p < 0.0001$) were significantly increased in the aMCI acupuncture group; (3) compared with baseline, the FC between the right HP and the right ITG ($p = 0.0043$) and left MTG ($p = 0.0018$) were both significantly increased in the aMCI acupuncture at follow-up; (4) compared with baseline, the FC of the right HP and the left MTG ($p < 0.0001$) was significantly reduced in the aMCI control group at follow-up. These results showed that after acupuncture, the abnormal right HP-FC to the right ITG (Figure 3(b)) and the left MTG (Figure 3(c)) enhanced in the aMCI acupuncture group, while those of the aMCI control group continued to decline, verifying our hypothesis that abnormal HP-FC in the aMCI group would show a recovery after acupuncture therapy.

3.7. Correlations between HP-FC and Clinical Performance in the aMCI Acupuncture Subjects. In the aMCI acupuncture subjects, partial correlation analysis, adjusting for age, gender, and education years, revealed that the FC value of the right HP to the right ITG at baseline was negatively related with the longitudinal changes of MoCA scores ($p = 0.004$, $r = -0.767$), suggesting that patients with lower FC value at baseline will present stronger acupuncture treatment effect (Figure 3(d)). On the other hand, no significant relationship was found between FC values at baseline and other clinical changes except MoCA in the partial correlation analysis and there is no obvious correlation between FC changes and clinical alterations.

4. Discussion

We evaluated the long-term effects of acupuncture treatment on aMCI by investigating the longitudinal changes of neuropsychological tests and hippocampal FC. The results showed that acupuncture could slow down cognitive decline and enhance the FC of the right HP to the right ITG and the left MTG, which may associate with the efficacy of acupuncture treatment on aMCI. Further, predicting the longitudinal changes of MoCA scores, FC of the right HP-ITG might be a biomarker indicating acupuncture treatment for patients with aMCI.

Recently, a tau-positron emission tomography (PET) study revealed that individuals with preclinical AD and positive beta-amyloid presented higher tau-PET in the ITG, MTG, precuneus, amygdala, superior temporal sulcus, fusiform gyrus, entorhinal cortex, and inferior parietal cortex. [20] Gray matter atrophy and hypometabolism in the cognitive networks were also reported in MCI patients [21]. In functional network analysis, aMCI patients showed decreased connectivity between hippocampal subfields and the right fusiform gyrus [22]. An fMRI study also demonstrated significant alteration of connectivity patterns in a memory-related subnetwork, including part of the HP, amygdala, fusiform gyrus, ITG, and MTG at the late stage of MCI and the deficits propagated to the rest of the brain as disease progressed. [23] Granger causality analysis revealed that directed FC between the HP and the temporal lobe, frontal lobe, and cingulate cortex were significantly changed in MCI [24]. On the other hand, increased spontaneous brain activities and FC have also been revealed in the frontal and temporal cortex in aMCI patients [25, 26]. These enhancements were thought to function as compensatory mechanisms due to cognitive deficits in aMCI. The caudate nucleus has been reported to be larger in AD that possibly serving as a mechanism for temporary compensation [27]. In the present study, at baseline, aMCI patients as compared with HC showed significantly weakened FC of the right HP with the right ITG/MTG, the left amygdala, and right fusiform and increased FC with bilateral caudates. These findings were consistent with previous studies suggesting that neural impairment and compensation may coexist in aMCI.

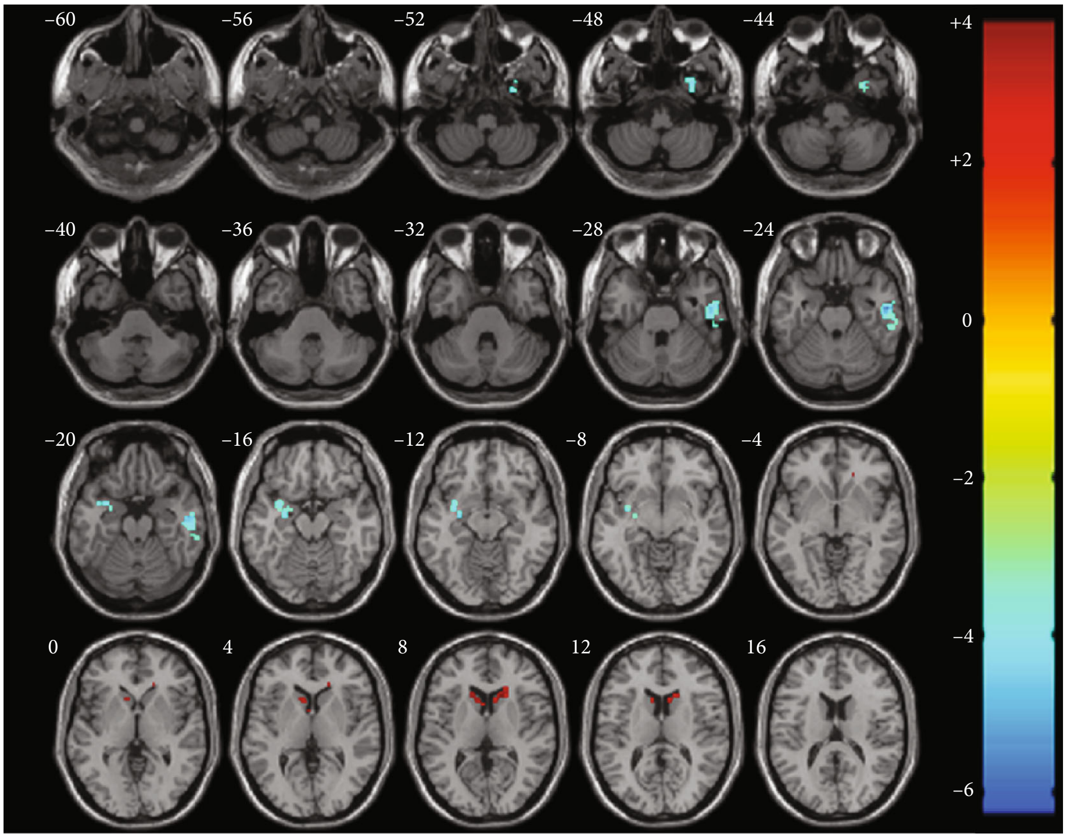

Figure 2: Brain regions showing altered connectivity to the right hippocampus in the aMCI group comparing to HC at baseline. Numbers in the figure indicate the Z coordinate in MNI space. The color scale represents t values. Warm color represents increased connectivity; cold color represents decreased connectivity.

Acupuncture is growing in popularity worldwide for its therapeutic effects on age-related cognitive decline [28]. Since the theory of traditional Chinese medicine cannot be explained by contemporary science, we regard its traditional Chinese medicine therapeutic mechanism as a black box. The key points are to find objective evidences of its efficacy and potential imaging markers to predict the curative effects. Acupuncture at the four gate acupoints is widely used in China to treat dementia. Jiang et al. reported in a rodent model of AD that acupuncture treatment on four gate acupoints improved learning and memory, along with decrease in the hippocampal amyloid-beta 42 immunoactivity and interleukin-1 (IL-1) beta content and upregulation of the hippocampal IL-2 [29]. In our previous study, we investigated the instantaneous effects of acupuncture at the four gate acupoints and found that acupuncture could activate cognition-related regions, including bilateral fusiform gyrus, right MTG, right parahippocampus gyrus, and frontal cortex in MCI and AD subjects [14, 30]. Moreover, bilateral MTG, left ITG, and fusiform gyrus showed enhanced hippocampal connectivity after acupuncture [5]. These results revealed that acupuncture at four gate acupoints modulate intrinsic brain activity and functional connectivity in MCI and AD patients.

After 6 months of acupuncture treatment, ROI analysis revealed that the weakened FC between the right HP and the right MTG/ITG were upregulated in the aMCI acupuncture group while the aMCI control group continued to decline. Whole brain analysis with bilateral hippocampi as the seeds confirmed this result. Compared with previous studies focused on instantaneous effects of acupuncture, the brain regions showing increased FC were much smaller in the present study. This discrepancy may be related with difference between instantaneous effects and the long-term effects of acupuncture. The latter has excluded the effects of pain and irritation during acupuncture that cannot been ignored in the instantaneous effect studies. The ITG and MTG are important components of the default mode network [31]. Amyloid deposits, hypometabolism, and atrophy have been consistently reported in these brain regions in MCI [32–34]. Furthermore, metabolic levels in the right ITG and MTG are significantly correlated with the speed of conversion to dementia [35], and 18F 3H-2-fluoro-2-deoxy-D-glucose (FDG) uptake reduction in ITG and MTG could significantly predict overall cognition impairment as assessed by MMSE score [36]. In resting-state fMRI, aMCI patients showed disrupted connectivity between the right HP subregion and the MTG, which is closely correlated with reduced capacity of episodic memory [37]. All the abovementioned studies suggested that destroyed ITG and MTG functions play an important role in cognition impairment. On the other hand, a recent study revealed less extended

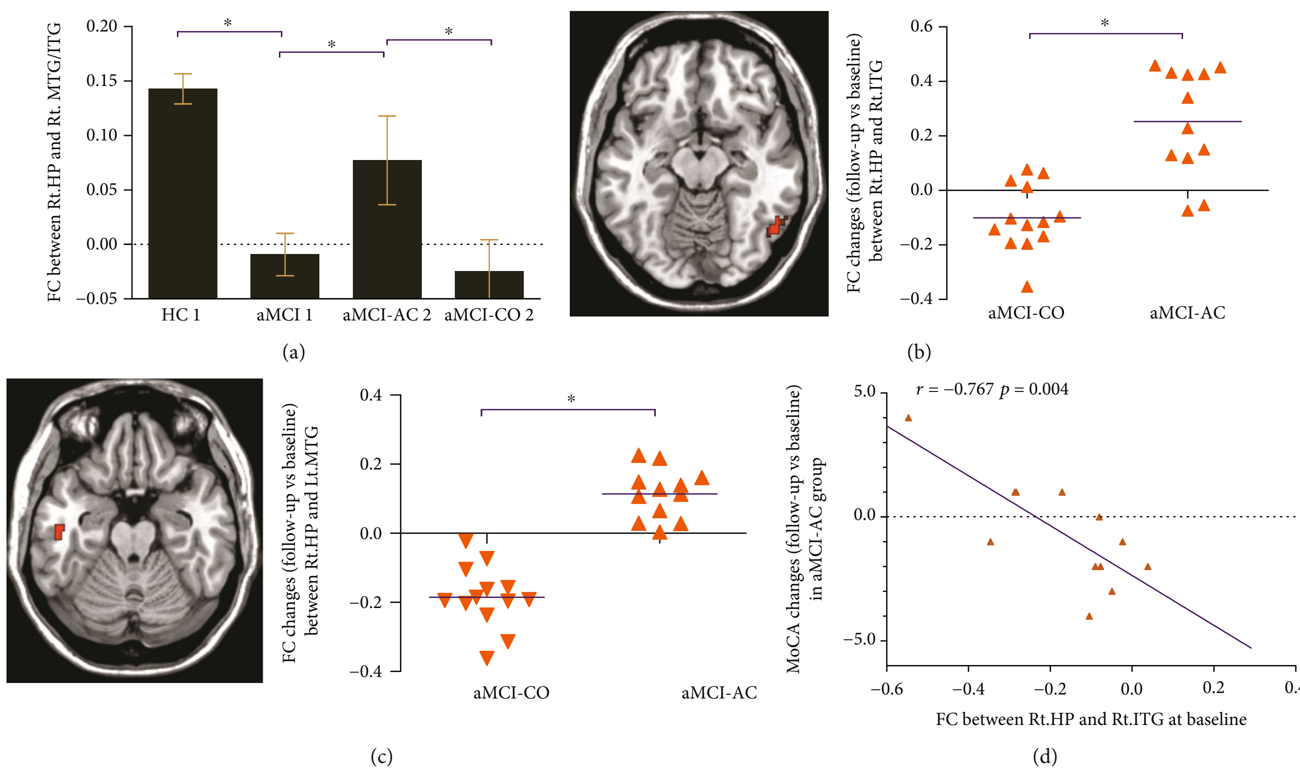

FIGURE 3: (a) The value of FC between the right HP and right MTG/ITG in HC at baseline, aMCI at baseline, aMCI acupuncture at follow-up, and aMCI control at follow-up. (b) Increased FC between the right HP and right ITG in aMCI acupuncture subjects as compared to aMCI control at follow-up vs. baseline (interaction effect). (c) Increased FC between the right HP and left MTG in aMCI acupuncture subjects as compared to aMCI control at follow-up vs. baseline (interaction effect). (d) Linear correlation of longitudinal changes in MoCA scores and the FC between the right HP and the right ITG at baseline in aMCI acupuncture subjects. HC 1: healthy control participates at baseline; aMCI: amnestic mild cognitive impairment; aMCI 1: all aMCI subjects at baseline; aMCI-AC 2: aMCI acupuncture group at follow-up; aMCI-CO 2: aMCI control group at follow-up; FC: functional connectivity; HP: hippocampus; Lt.: left; Rt.: right; MTG: middle temporal gyrus; ITG: inferior temporal gyrus; ∗ indicates cluster-level $p < 0.05$, FDR corrected.

and severe hypometabolism in ITG and MTG in late-converter MCI patients as compared with those with typically progressing MCI, suggesting that ITG and MTG may represent sites of resilience to slow the disease progression despite harboring the primary risk factors [38]. In the present study, the aMCI acupuncture group showed cognitive improvement and increased HP-MTG/ITG FC after continuous regular acupuncture treatment. We suggested that acupuncture probably could enhance the resilience in ITG and MTG to resistant against neurodegeneration and alleviate disease progression.

We also found that the changes in neuropsychological performances in the aMCI acupuncture group were significantly different from those of the aMCI control group and the longitudinal changes of MoCA scores were negatively correlated with the FC between the right HP and the right ITG at baseline in the aMCI acupuncture group, suggesting that subjects with lower FC values would present stronger acupuncture therapeutic effect at the aMCI stage. This similarly negative relationship has been reported in healthy cognitive aging and participants who were worse at baseline benefit from physostigmine treatment, while those with better baseline performance either did not show such benefits or showed poorer memory [39]. At the same time, no significant correlation was found between the FC changes and the neuropsychological assessment changes, which might suggest that acupuncture played a therapeutic role by regulating the whole brain FC of the hippocampus rather than a separate brain region-related FC.

Several limitations need to be considered. First, the sample size of the study was relatively small, and studies with a larger sample will be needed to confirm the current findings and elucidate the long-term effects of acupuncture on aMCI. Second, without access to PET examination and cerebrospinal fluid analysis [40], the aMCI patients have been chosen to include only those with hippocampal atrophy on MRI. It is well known that these patients with morphological changes will have very high chance of converting to AD. Based on this, the criteria used in the present study suggested an intermediate level of certainty that the aMCI syndrome is due to AD and the effect of acupuncture on AD-related pathological status is explored. Third, sham acupoint acupuncture intervention was not used in the aMCI control group and should be included in the future study.

5. Conclusions

In summary, acupuncture treatment at four gate acupoints significantly alleviated the progression of cognitive decline and enhanced the resilience to resistant against neurodegeneration by modulating the FC strength of the HP-MTG/ITG. The FC strength of right HP-ITG at baseline was significantly associated with neuropsychological changes. The findings confirmed the therapeutic roles of acupuncture treatment and provided objective evidences with the application of fMRI.

Abbreviations

aMCI: Amnestic mild cognitive impairment
AD: Alzheimer's disease
HC: Healthy control
aMCI-AC: aMCI acupuncture group
MCI-CO: aMCI control group
fMRI: Functional magnetic resonance imaging
FC: Functional connectivity
HP: Hippocampus
ITG: Inferior temporal gyrus
MTG: Middle temporal gyrus.

Data Availability

The datasets analyzed during the current study are not publicly available due to the unfinished study of the whole project but are available from the corresponding author on reasonable request.

Disclosure

The abstract was presented at Alzheimer's Association International Conference Monday Poster Abstract (https://alz-journals.onlinelibrary.wiley.com/doi/10.1016/j.jalz.2019.06.1241).

Conflicts of Interest

All authors declare no conflict of interest.

Acknowledgments

This work was supported partly by grants from the National Natural Science Foundation of China (81471649, 81571648, 81370037, and 81873892), the Clinical Medicine Development Special Funding from the Beijing Municipal Administration of Hospital (ZYLX201609), the Key Projects in the National Science and Technology Pillar Program during the Twelfth Five-Year Plan Period (2012BAI10B04), the Beijing Municipal Science and Technology Commission (Z171100000117001), and the NIH (United States) grant R21MH113134.

Supplementary Materials

All the figures are the results of one-sample T-tests of each group at baseline or at follow-up and the table is the clinical characteristics of subjects with amnestic mild cognitive impairment (aMCI) and healthy control (HC) at baseline. Supplementary Table 1: clinical characteristics of subjects with amnestic mild cognitive impairment (aMCI) and healthy control (HC). *(Supplementary Materials)*

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