Sleep Medicine 16 (2015) 785–791

Contents lists available at ScienceDirect

Sleep Medicine

journal homepage: www.elsevier.com/locate/sleep

Original Article

Mapping intrinsic functional brain changes and repetitive transcranial magnetic stimulation neuromodulation in idiopathic restless legs syndrome: a resting-state functional magnetic resonance imaging study

Chunyan Liu [a,b], Zhengjia Dai [c,d], Ruihua Zhang [e], Mo Zhang [f], Yue Hou [a], Zhigang Qi [f], Zhaoyang Huang [a], Yicong Lin [a], Shuqin Zhan [a], Yong He [c,d,*], Yuping Wang [a,b,**]

[a] Department of Neurology, Xuan Wu Hospital, Capital Medical University, Beijing, China
[b] Beijing Key Laboratory of Neuromodulation, Beijing, China
[c] State Key Laboratory of Cognitive Neuroscience and Learning & IDG/McGovern Institute for Brain Research, Beijing Normal University, Beijing, China
[d] Center for Collaboration and Innovation in Brain and Learning Sciences, Beijing Normal University, Beijing, China
[e] Department of Functional Neurology, Lu He Teaching Hospital, Capital Medical University, Beijing, China
[f] Department of Radiology, Xuan Wu Hospital, Capital Medical University, Beijing, China

ARTICLE INFO

Article history:
Received 1 October 2014
Received in revised form 11 December 2014
Accepted 29 December 2014
Available online 20 March 2015

Keywords:
RLS
ALFF
rTMS
Resting-state functional MRI
Pathophysiology
Neuromodulation

ABSTRACT

Objective: The objectives of this study were, first, to explore differences in brain activity between normal people and idiopathic restless legs syndrome (RLS) patients during asymptomatic periods; and, second, to determine whether administering repetitive transcranial magnetic stimulation (rTMS) to specific cortical regions would reverse any observed differences in brain activity and alleviate patient symptoms.
Methods: Fifteen idiopathic RLS patients (nine drug-naive patients) and 14 gender- and age-matched healthy controls were enrolled. Resting-state functional magnetic resonance imaging was used to measure the amplitude of low-frequency fluctuations (ALFF) in spontaneous brain activity during asymptomatic periods. Seven patients received high-frequency (5 Hz) rTMS directed toward the leg area of the primary motor cortex. Scores on the International Restless Legs Syndrome Study Group (IRLSSG) Rating Scale and ALFF values were measured before and after treatment.
Results: Compared with healthy controls, RLS patients showed lower ALFF in the sensorimotor and visual processing regions, and higher ALFF in the insula, parahippocampal and hippocampal gyri, left posterior parietal areas, and brainstem. These results were largely conserved when only drug-naive patients were considered. After rTMS treatment, ALFF in several sensorimotor and visual regions were significantly elevated and IRLSSG Rating Scale scores decreased, indicating improved RLS symptoms.
Conclusions: High-frequency rTMS delivered to the leg area of the primary motor cortex may raise functional activity in the sensorimotor and occipital regions, leading to improve symptoms in RLS patients. These results provide novel insight into RLS pathophysiology and suggest a potential mechanism for rTMS therapy in idiopathic RLS patients.

1. Introduction

Restless legs syndrome (RLS) is a sensorimotor disorder that consists of idiopathic RLS (without known cause) and secondary RLS, which is associated with iron deficiency, uremia, and peripheral neuropathy. Key features include an unpleasant sensation in the lower limbs that appears or worsens during the night and disappears or improves with movement [1]. Although the pathophysiology of idiopathic RLS remains incompletely understood, several studies suggest that it is related to central nervous system abnormalities [2–5]. Three self-evoked, event-related functional magnetic resonance imaging (fMRI) studies reported activation in the cerebellum, thalamus, brainstem, precentral gyrus, and primary somatosensory cortex during symptomatic periods [3–5]. The question arises as to whether or not patterns of functional activity change during asymptomatic periods. Resting-state fMRI, a promising neuroimaging technique that noninvasively measures spontaneous/intrinsic brain

Chunyan Liu and Zhengjia Dai contributed equally to this work.

* Corresponding author. State Key Laboratory of Cognitive Neuroscience and Learning & IDG/McGovern Institute for Brain Research, Beijing Normal University, Beijing 100875, China. Tel.: +10 5880 2036; fax: +10 5880 2036.
E-mail address: yong.he@bnu.edu.cn (Y. He).
** Corresponding author. Department of Neurology, Xuan Wu Hospital, Capital Medical University, Beijing 100053, China. Tel.: +10 8315 7841; fax: +10 8315 7841.
E-mail address: wangyuping01@sina.cn (Y. Wang).

http://dx.doi.org/10.1016/j.sleep.2014.12.029
1389-9457/

activity [6], has been widely used to study healthy and diseased brain function. Zang et al. [7] proposed using the amplitude of low-frequency fluctuations (ALFF; calculated as the square root of the power spectrum in a frequency range, usually 0.01–0.08 Hz) to assess the amplitude of resting-state spontaneous brain activity. By measuring the ALFF, researchers have found altered baseline brain activity in patients with attention-deficit/hyperactivity disorder [7] and post-traumatic stress disorder [8]. These recent studies indicate that the ALFF is physiologically meaningful and reflects intrinsic or spontaneous neuronal activity in the brain. Thus, measuring the ALFF during asymptomatic periods and comparing it to that of control subjects might reveal regions of altered functional activity in RLS that may be the basis for the development of symptoms.

Due to the augmentation of RLS symptoms during long-term treatment with dopaminergic medications, we urgently need new therapeutic methods. Repetitive transcranial magnetic stimulation (rTMS) is a newly developed noninvasive technique that can modulate brain function by improving cortical plasticity and can be used to treat some neurological disorders [9–11]. Currently, the consensus is that high-frequency rTMS is excitatory, whereas low-frequency rTMS is inhibitory [12]. Here, we investigated whether or not excitatory rTMS could be used to therapeutically alter brain function in RLS patients. First, we used resting-state fMRI to search for brain regions for which the ALFF values differed between RLS patients and normal controls. Then we used rTMS to modulate one of the cortical regions with altered ALFF values, and we assessed the effect on RLS symptoms.

2. Methods

2.1. Subjects

Fifteen right-handed idiopathic RLS patients (12 females and three males; age range: 35–72 years; mean age: 56.53 ± 9.75 years; nine drug-naive patients) (Table 1) participated in the study. RLS was diagnosed through clinical interview by a neurologist with sleep medicine expertise (Y.H.) and according to the International Restless Legs Syndrome Study Group (IRLSSG) criteria [1]. Severity was scored on the IRLSSG Rating Scale and the Johns Hopkins Restless Legs Severity Scale. We also used the Pittsburgh Sleep Quality Index (PSQI) to assess sleep quality. We scored patients on the Hamilton Anxiety Rating Scale (HAM-A) and Hamilton Depression Rating Scale (HAM-D, respectively) to exclude patients with serious anxiety (score > 21) or depression (score > 20). In addition, we excluded patients with a history of alcohol or drug abuse, anemia, renal disease, spinal cord or nerve root injury, or other neuropathies or sleep disorders. All patients had normal results on general medical and neurological examinations. Routine laboratory test results (including serum levels of hemoglobin, iron/ferritin, urea, creatinine, vitamin

Table 1
Demographic and clinical data in idiopathic RLS patients and healthy controls.

Group	Sex	Age (y)	Disease course	Family history	Medication	Agree rTMS	IRLSSG	PSQI	HAM-A	HAM-D
Patients										
1	F	51	6	No			22	7	2	5
2	F	55	27	No			12	4	5	6
3	M	72	5	Yes		Yes	18	11	7	6
4	F	44	10	No			28	13	6	5
5	F	67	4	Yes		Yes	24	13	5	8
6	F	35	7	No			13	4	3	5
7	F	52	34	Yes		Yes	24	13	7	9
8	F	63	35	Yes		Yes	27	15	5	7
9	F	52	4	No		Yes	19	7	5	6
10	M	65	31	Yes	Trastal 50 mg		37	17	14	13
11	F	67	41	No	Madopar 187.5 mg Pramipexole 0.25 mg		29	11	5	8
12	F	54	15	Yes	Pramipexole 0.25 mg		20	5	8	5
13	F	61	39	Yes	Pramipexole 0.5 mg	Yes	28	10	8	7
14	F	51	21	No	Pramipexole 0.25 mg Clonazepam 1 mg		28	13	4	7
15	M	59	4	No	Estazolam 1 mg	Yes	22	12	6	13
Mean ± SD	12 F /3M	56.53 ± 9.75	18.87 ± 14.29				23.40 ± 6.52	10.33 ± 4.05	6.00 ± 2.78	7.33 ± 2.61
Controls										
1	M	62								
2	F	54								
3	M	71								
4	F	50								
5	F	65								
6	F	35								
7	M	71								
8	M	63								
9	M	65								
10	F	69								
11	F	53								
12	F	61								
13	F	52								
14	M	61								
Mean ± SD	8 F/6 M	59.43 ± 9.83								
p Value	0.18[a]	0.43[b]								

F, female; IRLSSG, International Restless Leg Study Group Severity Scale; HAM-D, Hamilton Depression Rating Scale; HAM-A, Hamilton Anxiety Rating Scale; M, male; PSQI, Pittsburgh Sleep Quality Index; RLS, restless legs syndrome; rTMS, repetitive transcranial magnetic stimulation; SD, standard deviation.

[a] *p* Value obtained by two-tailed Pearson χ^2 test, which was used for gender comparison between the idiopathic RLS patients and controls.

[b] *p* Value obtained by a two-sample, two-tailed t-test, which was used for age comparison between the idiopathic RLS patients and controls.

B_{12}, folic acid, thyroid hormone, and HbA_{1c}) were normal, and no patients were on psychotropic medications.

We also recruited 14 age- and gender-matched right-handed healthy controls (eight females; age range: 35–71 years; mean age: 59.43 ± 9.83 years) (Table 1). The controls had no history of neurological problems such as cognitive disorder, psychiatric illness, or a family history of RLS. Controls were recruited from the community and were evaluated with cognitive and psychiatric scales. All controls had a Mini-Mental State Examination (MMSE) score > 28, a Montreal Cognitive Assessment (MoCA) score ≥ 27, and a Neuropsychiatric Inventory (NPI) score of 0. This study was approved by the Medical Research Ethics Committee at Xuan Wu Hospital of Capital Medical University. Written informed consent was obtained from all subjects.

2.2. Data acquisition

Imaging was performed during the daytime, starting from 12:00 when patients were asymptomatic and with empty stomachs. If a patient experienced leg discomfort during scanning, he or she was excluded (two patients were excluded for this reason). MRI data acquisition was performed on a Siemens Trio 3-Tesla scanner (Siemens, Erlangen, Germany). Foam padding and headphones were used to limit head motion and to reduce scanner noise. Subjects were instructed to move as little as possible, to relax their minds, and to keep their eyes closed without falling asleep. Functional images were collected axially using an echo-planar imaging (EPI) sequence with the following settings: repetition time (TR) = 2000 ms, echo time (TE) = 40 ms, flip angle (FA) = 90°, field of view (FOV) = 24 cm, resolution = 64 × 64 matrix, slices = 28, thickness = 4 mm, voxel size = 3.75 × 3.75 × 4 mm, gap = 1 mm, and band-width = 2232 Hz. The scan lasted for 478 s and thus included 239 functional volumes for each subject. A post-scan questionnaire showed that no subjects fell asleep during the scan. Three-dimensional T1-weighted magnetization-prepared rapid gradient echo (MPRAGE) sagittal images were collected using the following parameters: TR = 1900 ms, TE = 2.2 ms, inversion time (TI) = 900 ms, FA = 9°, resolution = 256 × 256 matrix, slices = 176, thickness =1.0 mm, and voxel size = 1 × 1 × 1 mm. For patients who received rTMS, a second MRI scan with the same parameters was performed after completing 14 rTMS sessions. The time interval between the last rTMS and the second MR scan was more than 24 hours. In principle, the RLS patients received rTMS treatment in the morning from 08:00 to 10:00 on 14 consecutive days. On the 15th day, they received the second MR scan starting from 12:00, which was the same time of day as the first MR scan.

2.3. Data preprocessing

Image preprocessing was carried out using Statistical Parametric Mapping (SPM8, http://www.fil.ion.ucl.ac.uk/spm) and Data Processing Assistant for Resting-State fMRI (DPARSF) [13]. The first 10 volumes were discarded to allow for scanner stabilization and participant adaptation to scanning. The remaining scans were first corrected for within-scan acquisition time differences between slices and further realigned to the first volume to correct for head motions. No participant was excluded for excessive head movements (more than 3 mm of translation or 3 degrees of rotation in any direction). Subsequently, each individual structural image (T1-weighted MPRAGE images) was co-registered to the mean functional image after motion correction using a linear transformation [14]. The transformed structural images were then segmented into gray matter (GM), white matter, and cerebrospinal fluid using a unified segmentation algorithm [15]. The motion-corrected functional volumes were spatially normalized to Montreal Neurological Institute (MNI) space and re-sampled to 3-mm isotropic voxels using the normalization parameters estimated during unified segmentation. The resultant normalized functional images were spatially smoothed with a 4-mm full-width-at-half-maximum (FWHM) Gaussian kernel, and linear trends were removed. Finally, all images were temporally filtered (0.01–0.08 Hz) to reduce the effects of low-frequency drift and high-frequency physiological noise.

2.4. Functional ALFF analyses

We used the Resting-State fMRI Data Analysis Toolkit (REST, http://rest.restfmri.net) to calculate the ALFF [7,16]. Briefly, for a given voxel, the time series was first converted to the frequency domain using a fast Fourier transform. The square root of the power spectrum was computed and then averaged across 0.01–0.08 Hz. This averaged square root was termed the ALFF. It was then divided by the global mean ALFF value for each subject to reduce global effects of variability.

2.5. Intervention: rTMS paradigm

Of the 15 idiopathic RLS patients, seven gave their informed consent to receive rTMS therapy (see Table 1). Focal rTMS was administered through a wind-cooled figure-eight coil (9-cm external diameter at each wing) connected to a magnetic stimulator, which gave a 2.0-Tesla pulse at maximal output (Magstim Super-Rapid; Magstim Co., Whitland, UK). The stimulation was directed to the leg area of primary motor cortex (M1) with the coil centered at the hot spot for the tibialis anterior (TA) muscle [17,18] (typically from 0 to 2 cm lateral to the vertex and from –1 to 2 cm posterior to the vertex). The stimulus intensity was fixed at 120% of active motor threshold (AMT) for the contralateral TA muscle. The AMT was defined as the minimum stimulation intensity that produced at least five motor-evoked potentials with amplitudes ≥ 100 μV over the course of 10 consecutive trials during voluntary contraction. For this measurement, the coil was centered over the leg motor area with the handle pointing laterally to induce a lateral-to-medial current flow in the cortex. Patients wore earplugs during the treatment and were seated in a comfortable chair in a reclined position. A head restraint was used to prevent movement. A coil holder kept the coil in a fixed position, and the coil was applied parallel to the vertex and tangentially to a participant's head surface. A daily session consisted of 20 rTMS trains, half delivered to the left leg area of M1 and half to the right. A single train consisted of 50 stimulations delivered at 5 Hz, and the intertrain interval was 50 seconds. All patients were treated daily for 14 consecutive days. A well-trained and qualified physical therapist delivered the rTMS to all patients. Patients who had already been taking dopamine agonists or benzodiazepines for at least one month continued to take their medication at the same dosage throughout the 2-week treatment. The IRLSSG, PSQI, HAM-A, and HAM-D scores were assessed before the first session and after the 14th session (Table 2). Any adverse effects related to the rTMS procedure were documented for each patient.

2.6. Statistical analyses

2.6.1. Between-group differences

To examine between-group differences in ALFF, voxelwise general linear model (GLM) analysis was performed with age and gender as covariates. Statistical significance threshold was set at $p < 0.05$ (i.e., height threshold) and cluster size > 2214 mm^3 (i.e., extent threshold), which corresponded to a corrected $p < 0.05$. Correction for multiple comparisons was confined to a GM mask (size: 1,826,064 mm^3) that was generated by thresholding (a threshold of 0.2) an *a priori* gray matter probability map in SPM8 and performed by Monte Carlo simulations [19] using the AFNI Alpha

Table 2
Assessment of IRLSSG, PSQI, HAM-A, and HAM-D scores in idiopathic RLS patients (baseline and after 14 sessions of rTMS).

Patient	Sex	Age (y)	IRLSSG		PSQI		HAM-A		HAM-D	
			Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	F	61	28	15	10	6	8	6	7	5
2	M	72	18	9	11	11	7	4	6	5
3	F	67	24	18	13	11	5	5	8	6
4	M	59	22	14	12	8	6	5	13	7
5	F	52	24	12	13	8	7	5	9	7
6	F	63	27	19	15	12	5	5	7	6
7	F	52	19	12	7	6	5	5	6	6
Mean ± SD	5 F/2 M	60.86 ± 7.38	23.14 ± 3.76	14.14 ± 3.53	11.57 ± 2.57	8.86 ± 2.48	6.14 ± 1.22	5 ± 0.58	8 ± 2.45	6 ± 0.82
			$p < 0.0001$[a]		$p = 0.0072$[a]		$p = 0.0472$[a]		$p = 0.0327$[a]	

F, female; IRLSSG, International Restless Legs Syndrome Study Group Severity Scale; HAM-A, Hamilton Anxiety Scale; HAM-D, Hamilton Depression Scale; M, male; PSQI, Pittsburgh Sleep Quality Index; RLS, restless legs syndrome; rTMS, repetitive transcranial magnetic stimulation; SD, standard deviation.
[a] p Value obtained by paired t-test.

Sim program (http://afni.nimh.nih.gov/pub/dist/doc/manual/AlphaSim.pdf).

2.6.2. Within-group differences for the effect of rTMS

A paired t-test was performed to examine changes in the ALFF before and after rTMS treatment. Statistical significance threshold was set to $p < 0.05$ and cluster size > 2214 mm^3, which corresponded to a corrected $p < 0.05$. Correction for multiple comparisons was confined to the GM mask.

3. Results

3.1. Comparisons of the ALFF between RLS patient and control groups

Fig. 1A shows the differences in the ALFF between the group of 15 RLS patients and the group of healthy controls. RLS patients showed significantly lower ALFF values in the sensorimotor system, including the paracentral lobule, precuneus, superior parietal gyrus, supplementary motor area (SMA), right precentral gyrus, right postcentral gyrus, and visual processing system, including middle occipital gyrus, calcarine sulcus, cuneus, fusiform gyrus, and right inferior temporal gyrus. We also found that these patients had significantly higher ALFF values in the insula, parahippocampal and hippocampal gyri, inferior frontal gyrus, rectus, left inferior parietal gyrus, left superior parietal gyrus, left angular gyrus, and brainstem.

3.2. Comparisons of ALFF between the drug-naive RLS patient and control groups

Fig. 1B shows a similar pattern when comparing ALFF values between the nine drug-naive RLS patient group and the healthy control group. Lower ALFF values were again found in the paracentral lobule, precuneus, supplementary motor area, and occipital lobe. The lower ALFF values in the right precentral gyrus and higher ALFF values in the insula, hippocampus, and left posterior parietal area also survived the height threshold but not the extent threshold (1026 mm^3).

3.3. Comparisons of ALFF between RLS patients before rTMS treatment and healthy controls

Fig. 2A shows the differences in ALFF values between the healthy controls and the seven RLS patients who subsequently received rTMS therapy. We found lower ALFF values in the occipital lobe and higher ALFF values in the hippocampus, rectus, left insula, superior frontal gyrus, and left posterior parietal area. Lower ALFF in the SMA and right precentral gyrus also survived the height threshold but not the extent threshold (837 mm^3).

3.4. Changes in ALFF values after rTMS treatment

Fig. 2B shows regions in which ALFF values changed after rTMS treatment in the seven RLS patients. Increased ALFF values were

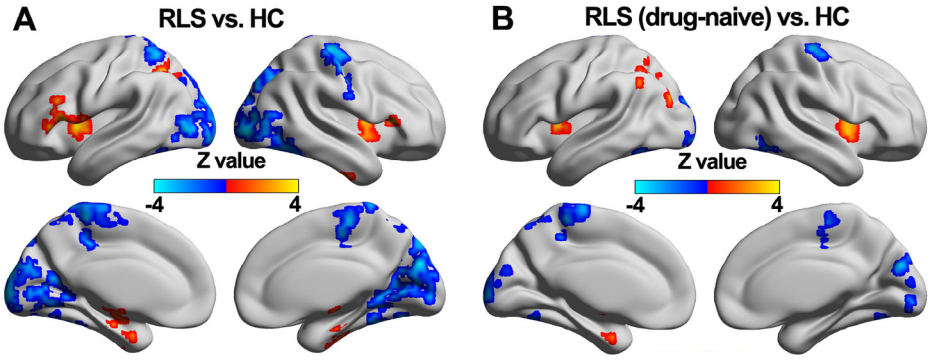

Fig. 1. Difference in amplitude of low-frequency fluctuations (ALFF) between the idiopathic restless legs syndrome (RLS) patients and the healthy control (HC) group. (A) Z-statistic difference map between the 15 RLS patients and the HC group. Statistical significance threshold was set at $p < 0.05$ (ie, height threshold) and cluster size >2214 mm^3 (ie, extent threshold), which corresponded to a corrected p value of <0.05. (B) Z-statistic difference map between the nine drug-naive RLS patients and the HC group. Lower ALFF values were again found in the paracentral lobule, precuneus, supplementary motor area, and occipital lobe. Lower ALFF in the right precentral gyrus and higher ALFF in the insula, hippocampus, and left posterior parietal area survived the height threshold ($p < 0.05$) but not the extent threshold (1026 mm^3).

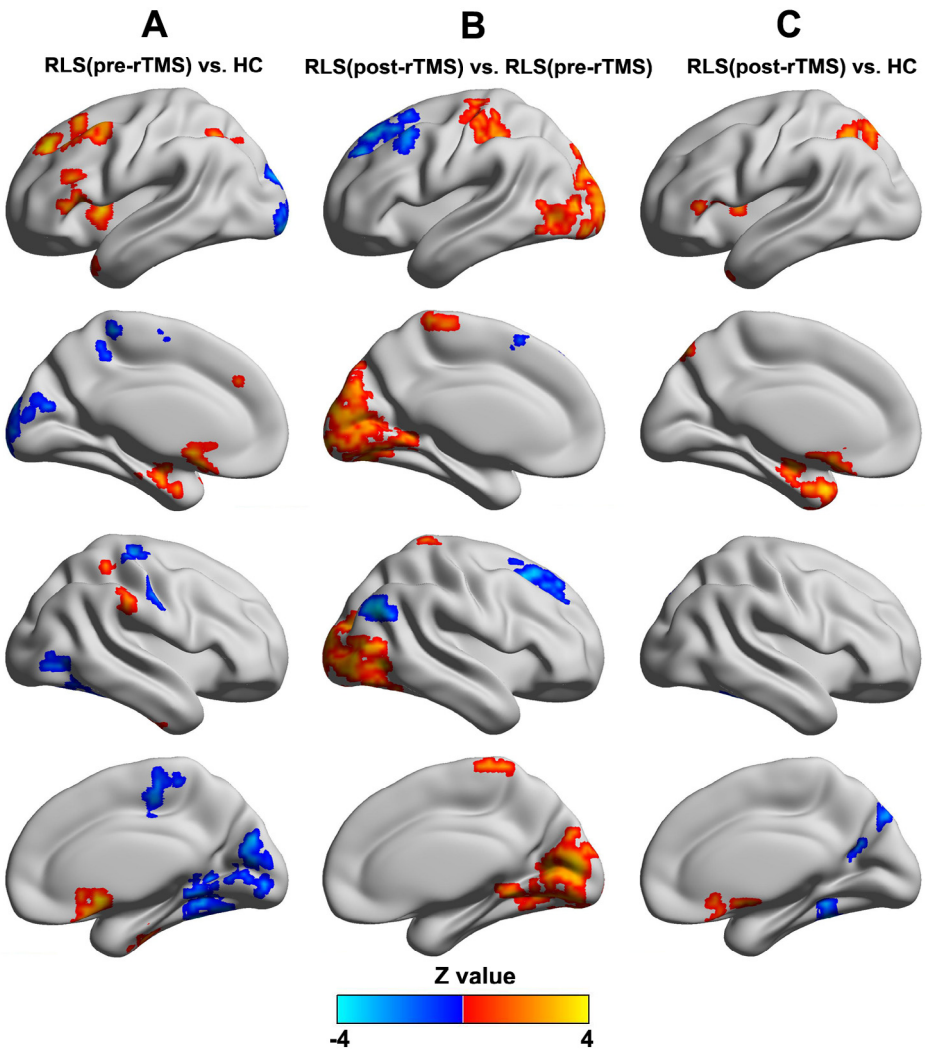

Fig. 2. (A) Difference in amplitude of low-frequency fluctuations (ALFF) between the seven restless legs syndrome (RLS) patients before repetitive transcranial magnetic stimulation (rTMS) treatment and the healthy control (HC) group. We found lower ALFF values in the occipital lobe and higher ALFF values in the hippocampus, rectus, left insula, superior frontal gyrus, and left posterior parietal area. Decreased ALFF in the SMA and right precentral gyrus survived the height threshold ($p < 0.05$) but not the extent threshold (837 mm^3). (B) Changes in ALFF after TMS treatment. Increased ALFF values were primarily in the sensorimotor cortex and the occipital lobe, and decreased values were primarily in the superior frontal gyrus, middle frontal gyrus, and right angular gyrus. (C) Difference in ALFF between the seven RLS patients after rTMS treatment and the HC group. Regions with higher ALFF in RLS patients included the rectus, left parahippocampal gyrus, hippocampal gyrus, left inferior parietal gyrus, and superior parietal gyrus. ALFF values in the sensorimotor cortex and occipital lobe did not differ between groups after rTMS treatment. Higher ALFF in the left insula and lower ALFF in right fusiform gyrus and right cuneus survived the height threshold but not the extent threshold (567 mm^3).

primarily in the sensorimotor cortex and the occipital lobe, and decreased values were primarily in the superior frontal gyrus, middle frontal gyrus, and right angular gyrus.

3.5. Comparison of ALFF values between RLS patients after rTMS treatment and healthy controls

Fig. 2C shows the differences in ALFF values between the group of seven RLS patients after rTMS treatment and the group of healthy controls. Regions with higher ALFF values in RLS patients compared with healthy controls included the rectus, left parahippocampal gyrus, hippocampal gyrus, left inferior parietal gyrus, and superior parietal gyrus. ALFF values in the sensorimotor cortex and occipital lobe did not differ between the RLS patient and control groups after rTMS treatment. Higher ALFF in left insula and lower ALFF in right fusiform gyrus and right cuneus survived the height threshold but not the extent threshold (567 mm^3).

3.6. Changes in IRLSSG score after rTMS treatment

Fig. 3 shows IRLSSG Rating Scale scores before and after delivering rTMS to the leg area of M1 daily for 14 days. Data analysis

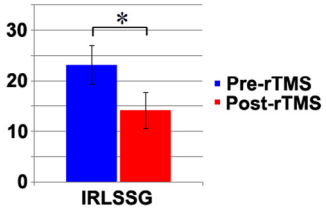

Fig. 3. International Restless Legs Syndrome Study Group (IRLSSG) Rating Scale scores before and after repetitive transcranial magnetic stimulation (rTMS). $^*p < 0.0001$.

revealed that scores significantly decreased after treatment (before: 23.14 ± 3.76; after: 14.14 ± 3.53; $p < 0.0001$).

4. Discussion

By measuring the ALFF, we found brain regions in which activity differed between the idiopathic RLS patient and the normal control groups, even when the patients were experiencing asymptomatic periods. The major findings included the following: (1) Patients showed lower ALFF in the sensorimotor areas and the occipital lobe, and higher ALFF values in the insula, parahippocampal and hippocampal gyri, left posterior parietal areas, and brainstem; and (2) after delivering high-frequency rTMS to M1 leg cortex, symptoms improved as assessed by the IRLSSG Rating Scale, and the ALFF values in the sensorimotor regions and occipital lobe significantly increased.

Clinical and pharmacological observations suggest that dopaminergic hypoactivity may play a role in the pathophysiology of RLS [20]. In our study, we found hypoactivation in M1 and SMA in idiopathic RLS patients. We speculate that this results from dopaminergic hypoactivity and cortical deafferentation of the basal ganglia–thalamo-cortical circuit [21]. The low activity in sensorimotor and occipital cortices during asymptomatic periods may contribute to abnormal somatosensory processing, leading to discomfort in the leg. Many studies regarding pain have found that exciting the primary motor cortex can have an antinociceptive role through the activation of a descending inhibitory pathway [22–25]. Because motor responses are closely linked to visual stimuli, visual information processing in the occipital lobe is an important part of the sensorimotor network. A positron emission tomography study conducted in resting fibromyalgic patients revealed regional cerebral blood flow that was lower in the occipital cortex (OC) than in that of controls [26]. Injection of glutamate into the OC also reduced pain in the rat tail-flick test [27]. Because glutamate excites neuronal cell bodies but not fibers, this suggests that the increased neuronal activity in the OC results in antinociception [26]. Thus, we speculate the low activity in motor and occipital cortices of idiopathic RLS patients may weaken the inhibitory effects of descending pathways, leading to the abnormal central somatosensory processing.

Regions with higher ALFF values were found in the insula, parahippocampal gyrus, hippocampal gyrus, and left posterior parietal areas. In RLS patients, changes have been found in the binding potential of dopamine D_2-receptors (D2Rs) in the insula [19]. Increased activation in the left pars opercularis was observed through fMRI during nighttime episodes of sensory leg discomfort and periodic limb movements [5]. Functionally, the insula has been postulated to play a key role in maintaining homeostasis by monitoring and integrating interoceptive visceral and somatic feelings, and translating these to conscious emotional perceptions [28,29]. Thus, it integrates sensory and visceral signals from peripheral receptors. The high activity of insular and posterior parietal areas may be related to abnormal central somatosensory processing. To date, most investigations of aversive conditioning have highlighted specific contributions from the amygdala and parahippocampal gyrus [30]. Findings show that patients with parahippocampal gyrus lesions do not habituate to mildly aversive somatosensory stimuli and are impaired in their ability to modify neural responses to them [31]. Thus, we speculate that in RLS patients, higher ALFF in the parahippocampal and hippocampal gyri may help habituation to unpleasant and aversive stimuli coming from the legs.

Results after comparing ALFF between drug-naive patients and controls followed a similar pattern. Although significant changes were not seen in exactly the same regions, this was likely due to the small sample size (nine RLS patients). Indeed, after reducing the significance threshold, we did find the same pattern of changes in the same brain regions. It appears that *hypo*activity in the sensorimotor regions and the occipital lobe and *hyper*activity in the insula and posterior parietal areas both contribute to RLS pathogenesis.

In our study, patient compliance was high; no adverse effects were observed; and high-frequency rTMS stimulation over leg motor-cortex relieved RLS symptoms. However, the mechanism underlying the therapeutic effects is still unclear. This could be explained by changes in the dopaminergic system. High-frequency rTMS over M1 was reported to induce a focal release of endogenous dopamine within the ipsilateral dorsal striatum (putamen, caudate nucleus), probably by activating cortico-striatal projections [32]. In addition, dopamine acting on D2Rs plays a role in descending inhibitory control at several central sites, including dorsal striatum, hypothalamus A11-cell group, and spinal cord [33]. The increased release of endogenous dopamine could act on these sites to enhance descending inhibition and to prevent abnormal somatosensory processing. Furthermore, the antinociceptive effect induced by stimulation of the OC likely also results from the activation of a descending inhibitory pathway [34]. In this way, normalizing M1 and occipital cortical activity could be a way to relieve unpleasant sensation in the legs, whereas abnormal function in these brain regions likely has a pathophysiological significance in idiopathic RLS patients.

The abnormally high ALFF values in the rectus, left parahippocampal and hippocampal gyri, and posterior parietal cortex did not change significantly after rTMS. This could have been because the stimulation did not penetrate to deeper brain regions such as the rectus and the parahippocampal and hippocampal gyri. Another reason could be that the duration of stimulation was not long enough for normalization of functional activities in these regions to occur. Further studies that increase the number of rTMS sessions should be conducted to further elevate activity in the motor cortex, and thus increase descending inhibition and help prevent abnormal somatosensory processing.

Several limitations of our study should be addressed. First, only seven patients received stimulation, and no sham stimulation control was included. Although the improvement of IRLSSG Rating Scale scores in RLS patients was between 25% and 50%, we cannot exclude the possibility that this was a placebo effect. Moreover, several patients were medicated while receiving rTMS treatment, and thus a potential cumulative effect of magnetic stimulation and medication cannot be excluded. Second, most patients had abnormal PSQI scores, implying sleep deprivation [35,36]. Because PSQI scores were not obtained from controls, we cannot rule out the possibility that the lower ALFF in the visual processing system was in part related to sleep deprivation in RLS patients. Third, the current dataset is cross-sectional and does not allow us to examine ALFF-related dynamic changes with RLS circadian variations; future follow-up studies are warranted to examine RLS circadian variations. Finally, the sample size was small, and further validation using a large sample is therefore necessary. This project is still ongoing, and we are planning to make these adjustments and to observe the cumulative effect of increasing the number of repetitive sessions.

In conclusion, abnormally low spontaneous activity in the sensorimotor cortex and the occipital lobe may be involved in the pathogenesis of idiopathic RLS. High-frequency (5-Hz) rTMS directed toward M1 (leg area) can mitigate this problem and relieve some symptoms of idiopathic RLS. rTMS may promote the release of dopamine and, in turn, enhance the descending inhibitory pathway and prevent abnormal central somatosensory processing.

Conflict of interest

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: http://dx.doi.org/10.1016/j.sleep.2014.12.029.

Acknowledgement

This work was supported by the National Natural Science Foundation of China, Grant Nos. 81271494, 81301138, 81030028, and 81225012.

References

[1] Allen RP, Picchietti D, Hening WA, et al. Restless legs syndrome: diagnostic criteria, special considerations, and epidemiology: a report from the restless legs syndrome diagnosis and epidemiology workshop at the National Institutes of Health. Sleep Med 2003;4:101–19.
[2] Trenkwalder C, Paulus W, Walters AS. The restless legs syndrome. Lancet Neurol 2005;4:465–75.
[3] Bucher SF, Seelos KC, Oertel WH, et al. Cerebral generators involved in the pathogenesis of the restless legs syndrome. Ann Neurol 1997;41:639–45.
[4] Margariti PN, Astrakas LGT, Souli SG, et al. Investigation of unmedicated early onset restless legs syndrome by voxel-based morphometry, T2 relaxometry, and functional MR imaging during the night-time hours. AJNR Am J Neuroradiol 2012;33:667–72.
[5] Spiegelhalder K, Feige B, Paul D, et al. Cerebral correlates of muscle tone fluctuations in restless legs syndrome: a pilot study with combined functional magnetic resonance imaging and anterior tibial muscle electromyography. Sleep Med 2008;9:177–83.
[6] Biswal B, Yetkin FZ, Haughton VM, et al. Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. Magn Reson Med 1995;34:537–41.
[7] Zang YF, He Y, Zhu CZ, et al. Altered baseline brain activity in children with ADHD revealed by resting-state functional MRI. Brain Dev 2007;29:83–91.
[8] Lui S, Huang X, Chen L, et al. High-field MRI reveals an acute impact on brain function in survivors of the magnitude 8.0 earthquake in China. Proc Natl Acad Sci U S A 2009;106:15412–17.
[9] Chen R, Classen J, Gerloff C, et al. Depression of motor complex excitability by low-frequency transcranial magnetic stimulation. Neurology 1997;48:1398–403.
[10] Boroojerdi B, Prager A, Muellbacher W, et al. Reduction of human visual cortex excitability using 1 Hz transcranial magnetic stimulation. Neurology 2000;54:1529–31.
[11] Rotenberg A, Muller P, Birnbaum D, et al. Seizure suppression by EEG-guided repetitive transcranial magnetic stimulation in the rat. Clin Neurophysiol 2008;119:2697–702.
[12] Fitzgerald PB, Fountain S, Daskalakis ZJ. A comprehensive review of the effects of rTMS on motor cortical excitability and inhibition. Clin Neurophysiol 2006;117:2584–96.
[13] Yan CG, Zang YF. DPARSF: a MATLAB toolbox for “pipeline” data analysis of resting-state fMRI. Front Syst Neurosci 2010;4.
[14] Collignon A, Maes F, Delaere D, et al. Automated multi-modality image registration based on information theory. Inf Process Med Imag 1995;263–74.
[15] Ashburner J, Friston KJ. Unified segmentation. Neuroimage 2005;26:839–51.
[16] Song XW, Dong ZY, Long XY, et al. REST: a toolkit for resting-state functional magnetic resonance imaging data processing. PLoS ONE 2011;6:e25031.
[17] Terao Y, Furubayashi T, Okabe S, et al. Interhemispheric transmission of visuomotor information for motor implementation. Cereb Cortex 2005;15:1025–36.
[18] Terao Y, Furubayashi T, Okabe S, et al. Modifying the cortical processing for motor preparation by repetitive transcranial magnetic stimulation. J Cogn Neurosci 2007;19:1556–73.
[19] Ledberg A, Akerman S, Roland PE. Estimation of the probabilities of 3D clusters in functional brain images. Neuroimage 1998;8:113–28.
[20] Simon C, Sven EP, Robert A, et al. Support for dopaminergic hypoactivity in restless legs syndrome: a PET study on D2-receptor binding. Brain 2006;129:2017–28.
[21] Buhmann C, Glauche V, Stuèrenburg HJ, et al. Pharmacologically modulated fMRI-cortical responsiveness to levodopa in drug-naive hemiparkinsonian patients. Brain 2003;126:451–61.
[22] Reidler JS, Mendonca ME, Santana MB, et al. Effects of motor cortex modulation and descending inhibitory systems on pain thresholds in healthy subjects. J Pain 2012;13:450–8.
[23] Fregni F, Boggio PS, Lima MC, et al. A sham-controlled, phase II trial of transcranial direct current stimulation for the treatment of central pain in traumatic spinal cord injury. Pain 2006;122:197–209.
[24] Mendonca ME, Santana MB, Baptista AF, et al. Transcranial DC stimulation in fibromyalgia: optimized cortical target supported by high resolution computational models. J Pain 2011;12:610–17.
[25] Fenton BW, Palmieri PA, Boggio P, et al. A preliminary study of transcranial direct current stimulation for the treatment of refractory chronic pelvic pain. Brain Stimul 2009;2:103–7.
[26] Wik G, Fischer H, Bragée B, et al. Retrosplenial cortical activation in the fibromyalgia syndrome. Neuroreport 2003;14:619–21.
[27] Fries W, Zieglgansberger W. A method to discriminate axonal from cell body activity and to analyze ‘silent cells’. Exp Brain Res 1974;21:441–5.
[28] Craig AD. The sentient self. Brain Struct Funct 2010;214:563–77.
[29] Craig AD. Significance of the insula for the evolution of human awareness of feelings from the body. Ann NY Acad Sci 2011;1225:72–82.
[30] Andreea OD, Mahesh M, Jimmy J, et al. Dopamine-induced changes in neural network patterns supporting aversive conditioning. Brain Res 2010;1313:143–61.
[31] Ousdal OT, Jensen J, Server A, et al. The human amygdala is involved in general behavioral relevance detection: evidence from an event-related functional magnetic resonance imaging Go–NoGo task. Neuroscience 2008;156:450–5.
[32] Strafella AP, Ko JH, Grant J, et al. Corticostriatal functional interactions in Parkinson’s disease: a rTMS/[11C]raclopride PET study. Eur J Neurosci 2005;22:2946–52.
[33] Wood PB. Role of central dopamine in pain and analgesia. Expert Rev Neurother 2008;8:781–97.
[34] Zhang JH, Cao XD, Lie J, et al. Neuronal specificity of needling acupoints at same meridian: a control functional magnetic resonance imaging study with electroacupuncture. Acupunct Electrother Res 2007;32:179–93.
[35] Habecka C, Rakitina BC, Moeller J, et al. An event-related fMRI study of the neurobehavioral impact of sleep deprivation on performance of a delayed-match-to-sample task. Cogn Brain Res 2004;18:306–21.
[36] Anna SU, Antti H, Sami H, et al. Preliminary findings of proton magnetic resonance spectroscopy in occipital cortex during sleep deprivation. Psychiatry Res Neuroimaging 2006;147:41–6.