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Dual roles of the amygdala–hippocampus circuit in the regulation of rapid eye movement sleep and depression symptoms by repetitive transcranial magnetic stimulation in patients with insomnia

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To the editor:

It is commonly reported that people with insomnia often experience comorbid emotional disorders, such as mood and anxiety disorders.[1 2] A study found that fragmented rapid eye movement (REM) sleep in individuals with insomnia is associated with higher Beck Depression Inventory (BDI) scores.[3] REM sleep architecture disruption is a typical symptom of insomnia.[4] Sleep homeostasis and plasticity interact with each other and jointly regulate sleep patterns and sleep quality. Sleep homeostasis is a complex neurobiological phenomenon involving molecular pathways, neurotransmitter release, synaptic activity and neural networks.[5] Repetitive transcranial magnetic stimulation (rTMS) is a commonly used non-invasive neuroregulation technique that regulates sleep by modulating synaptic plasticity and the strength of connections between brain regions.[6] While evidence suggests the potential of 1 Hz trams at the left dorsal lateral prefrontal cortex (DLPFC_L) to improve sleep quality for patients with insomnia,[7] the extent to which it influences REM sleep and the mechanisms involved remain unclear. REM sleep is initiated by basolateral amygdala dopamine signalling.[8] Restless REM sleep interferes with the adaptation of the amygdala circuits, consequently affecting emotion processing.[4] Individuals with insomnia are more likely to experience symptoms of depression or anxiety and they are closely connected by a bidirectional relationship.[9] However, the effect of rTMS on depression in patients with insomnia and the underlying correlation with REM remain unknown. A recent study revealed that diverse patterns of synchronic interaction between the amygdala, hippocampus and neocortex play crucial roles in emotional processes.[10] Thus, we hypothesised that 1 Hz rTMS at DLPFC_L may improve REM sleep and mood by modulating the amygdala–hippocampus circuits in patients with insomnia.

The inclusion criteria of this study for patients with insomnia are as follows: aged 18–65 years; right-handed; meeting the diagnostic criteria for insomnia disorders in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition; Pittsburgh Sleep Quality Index (PSQI) higher than 5 and the Insomnia Severity Index (ISI) higher than 8. Inclusion criteria for healthy controls are as follows: aged 18–65 years; right-handed; PSQI lower than 5 and ISI lower than 8; no symptoms or history of psychiatric disorders or sleep disorders, and not taking any psychotropic medications or hypnotics during their lifetime. Exclusion criteria for both groups are as follows: other comorbid mental disorders; serious neurological or medical conditions; other sleep disorders; body mass index score >30; frequent jet lag; contraindications for 3 Tesla magnetic resonance imaging (MRI) or transcranial magnetic stimulation.

60 patients with insomnia were enrolled (38 were female), alongside 30 age-matched, gender-matched and education-matched healthy controls. Written informed consent was obtained from all participants. Patients with insomnia were randomly assigned to the active (n=30) or sham groups (n=30). Active low-frequency rTMS was administered using a pulsed magnetic stimulation device (M-100 Ultimate; Shenzhen Yingchi Technology Co, Shenzhen, China) with the '5 cm rule' (move 3–5 cm to the left and 5 cm

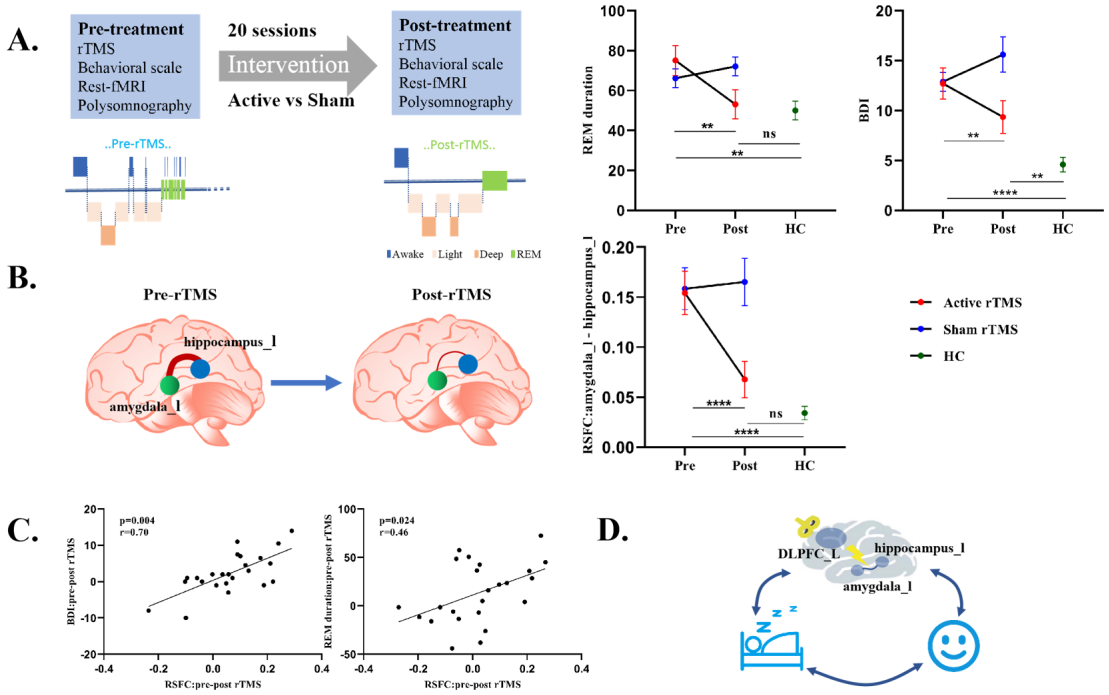

Figure 1 The effect of rTMS on REM sleep and mood in patients with insomnia and potential neural mechanisms. (A) Research procedure. A 2×2 factorial analysis of variance revealed significant interaction effects (treatment×time) for REM duration and BDI scores. (B) Changes in RSFC within the left amygdala–hippocampus circuit before and after rTMS. (C) Changes in RSFC within the left amygdala–hippocampus circuit were associated with BDI scores and REM duration changes in patients with insomnia. (D) Schematic diagram illustrating the dual roles of the left amygdala–hippocampus circuit in the regulation of REM sleep and depression by rTMS in patients with insomnia. BDI, Beck Depression Inventory; DLPFC_L, left dorsal lateral prefrontal cortex; fMRI, functional magnetic resonance imaging; HC, healthy control; ns, not significant; REM, rapid eye movement; RSFC, resting-state functional connectivity; rTMS, repetitive transcranial magnetic stimulation. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

forward from the Cz position) to locate the DLPFC_L. The rTMS was delivered at 1 Hz, and the stimulus intensity was set at 80% of the resting motor threshold (motion threshold was defined as the lowest stimulation power that can produce 5 finger movements out of 10 stimuli), stimulation number 10 pulses per string, a string interval of 2 s, a total of 150 strings and a total stimulation time of 30 min. Sessions were conducted once daily, 5 days per week for a 4-week treatment course (with 2 days off each weekend).[11] Sham rTMS sessions were conducted in the same pattern with the coil turned away from the skull at 90°. No adverse events were reported during or after brain stimulation.

Behavioural assessments (PSQI, ISI, BDI and Beck Anxiety Inventory (BAI)), all-night sleep polysomnography (PSG) recordings and MRI scans were collected before and after active or sham rTMS treatment. 30 healthy participants underwent the same assessment (figure 1A). PSG sleep monitoring was conducted using the Grael 4K system (Compumedics, Australia), including six-channel electroencephalography (EEG) placed based on the standard 10–20 system, as well as electromyography at a sampling rate of 512 Hz. Electrooculogram was recorded at the right and left outer canthi. EEG was referenced to bilaterally linked mastoids. Impedances were kept below 10 kΩ. 14 patients with insomnia withdrew from the treatment for personal reasons. The final statistical analyses included 24 participants in the active rTMS group and 22 in the sham group. PSG recorded sleep was staged using YASA algorithm[12] and the following parameters were calculated: (1) total sleep time (TST) (min); (2) sleep-onset latency: sleep onset to the first epoch of stage non-rapid eye movement (NREM) (min); (3) sleep efficiency: (TST/time in bed)×100; (4) REM sleep latency: sleep onset to the first epoch of stage REM (min); (5) REM sleep duration: time in stage REM; (6) the percentage of TST in stage REM: (time in stage REM/TST)×100; (7) percentage of TST in stage NREM: (time in stage NREM/TST)×100. The rules, terminology and technical specifications of the American Association for Sleep Research Manual of Interpretation of Sleep and Associated Events V.2.6 were used as the interpretation criteria to determine sleep staging and sleep events.[13]

For each subject, a T1-weighted sequence was acquired with the parameters as follows: repetition time (TR)=6.3 ms; echo time (TE)=2.8 ms; inversion time=844.2 ms; data matrix=256×256; slices=176; field of view (FOV)=256×256 mm^2; slice thickness=1 mm. The resting-state functional images were acquired with a single echo using the following parameters: TR=2000 ms; TE=30 ms; flip angle=90°; FOV=240×240 mm^2; slice thickness=5 mm; slices=30; matrix size=64×64 and total volumes=185. AFNI (http://afni.nimh.nih.gov/) and FMRIB's Software Library (http://www.fmrib.ox.ac.uk/fsl/) were used for the preprocessing as described in our previous studies.[14] The resting-state functional

Table 1 Demographic and clinical characteristics of the participants

Characteristics	Active rTMS (n=30) Mean (SD)	Sham rTMS (n=30) Mean (SD)	HC (n=30) Mean (SD)	P value Active-sham	Active-HC	Sham-HC
Age (years)	43.31 (8.98)	43.15 (10.20)	41.50 (11.25)	ns	ns	ns
Gender (M/F)	12/18	10/20	12/18	ns	ns	ns
Education (years)	13.25 (3.85)	12.61 (3.88)	13.79 (4.22)	ns	ns	ns
PSQI	13.17 (2.78)	13.77 (3.23)	3.23 (1.46)	ns	<0.001	<0.001
ISI	16.53 (3.81)	17.31 (5.84)	1.40 (1.16)	ns	<0.001	<0.001
BDI	13.12 (2.62)	16.40 (5.14)	3.50 (4.11)	ns	<0.001	<0.001
BAI	35.22 (8.28)	36.72 (6.23)	26.10 (2.26)	ns	<0.001	<0.001
REM duration (min)	75.10 (36.58)	66.23 (21.92)	50.05 (25.72)	ns	0.005	0.021
RSFC (amy–hippo)	0.14 (0.11)	0.16 (0.10)	0.03 (0.03)	ns	<0.001	<0.001

amy-hippo, amygdala-hippocampus; BAI, Beck Anxiety Inventory; BDI, Beck Depression Inventory; HC, healthy control; ISI, Insomnia Severity Index; ns, not significant; PSQI, Pittsburgh Sleep Quality Index; REM, rapid eye movement; RSFC, resting-state functional connectivity; rTMS, repetitive transcranial magnetic stimulation; SD, standard deviation.

connectivity (RSFC) patterns were calculated for bilateral amygdala separately in patients with insomnia, which were defined according to Harvard-subcortical structural atlas (http://www.cma.mgh.harvard.edu/). The regional resting-state functional MRI time series was extracted for the bilateral amygdala by using the average functional time series of all voxels within each region. Pearson correlation was used to investigate the RSFC between the region of interest and the whole-brain regions, and then a Fisher's r-to-z transform was employed.

Paired t-test, two-sample t-test and two-way analysis of variance were used as two-sided tests, and p<0.05 was considered statistically significant. A series of Pearson correlation analyses were used to study the relationship between the bilateral amygdala RSFC and relevant parameter. In addition, a series of mediation analyses were carried out among REM duration, BDI/BAI and RSFC. For mediation analysis, we used the mediation package (http://cran.r-project.org/web/packages/mediation). Graphs were plotted using GraphPad Prism V.8.0.2 (GraphPad Software, California, USA).

The detailed demographic and clinical characteristics of the participants are shown in table 1. A significant 'treatment×time' interaction effect was found in the left amygdala–hippocampus RSFC (F=4.87, p=0.030), REM duration (F=4.89, p=0.030) and BDI scores (F=4.04, p=0.050). For active rTMS, the BDI scores (t=3.22, p=0.004) were lower and the REM duration (t=3.69, p=0.001) was returned to a normal level (figure 1A). The hyper-RSFC within the left amygdala–hippocampus circuit (p<0.05, family-wise error corrected) in patients with insomnia was restored after active rTMS treatment (figure 1B). Moreover, the RSFC changes were significantly correlated with the improvement of REM duration (r=0.46, p=0.024) and BDI scores (r=0.70, p=0.004) (figure 1C). However, no mediation relationships were detected. No significant changes were observed after sham rTMS treatment.

Insomnia symptoms can increase the risk of developing depression, and depression symptoms exacerbate insomnia symptoms.[1] This inter-relationship is due to the common physiological and psychological mechanisms underlying both insomnia and depression.[2] Previous studies have verified that the association between poor sleep quality and depression severity was related to changes in functional connectivity among several brain regions, including the amygdala, hippocampus, parahippocampal gyrus, lateral orbitofrontal cortex, dorsolateral prefrontal cortex, anterior and posterior cingulate cortices, insula, temporal cortex and precuneus.[15] Given the association of REM sleep with emotion regulation, altered activity within amygdala circuits during REM sleep in patients with insomnia may be critical to understanding their characteristic difficulties in the regulation of emotion. In the present study, we observed that rTMS at DLPFC_L reversed the hyper-RSFC of the left amygdala–hippocampus circuit, which might contribute to the underlying neurobiological mechanisms of REM sleep normalisation and reduced depression in patients with insomnia. Moreover, the improvement of function connection was positively related to the improvement of BDI scores and REM sleep duration. We provided novel evidence for rTMS at DLPFC_L-generated remote effects on interactions among subcortical regions in patients with insomnia. The amygdala–hippocampus circuits might play dual roles in emotion processing and REM sleep regulation, since the RSFC changes were in accordance with changes in BDI scores and REM duration before and after rTMS treatment. Due to the implications of the dialogue of the neocortex with the amygdala and hippocampus in emotion and sleep,[10] we suggested a complicated relationship existed among them, as illustrated in figure 1D. This study was not able to confirm this through mediation analysis, which was possibly beyond linear correlations. Further studies involving more participants and advanced models are needed to further our understanding of this topic.

In conclusion, 1 Hz rTMS at DLPFC_L normalised the REM duration and reduced the depression symptoms for patients with insomnia, likely influenced by the modulation of hyper-RSFC within the left amygdala–hippocampus circuit. These findings provided novel evidence for the potential remote and even subcortical circuit effects of rTMS. Moreover, our

findings can serve as a valuable reference for developing targeted approaches for insomnia and mood disorders, contributing to the development of more effective interventions for improving sleep quality and alleviating depression in individuals with insomnia. Due to the intricate interplay of the neocortex with the amygdala and hippocampus in emotion and sleep, we propose a complicated relationship among them, potentially involving non-linear correlations. Future studies with a larger sample size and longer follow-ups are necessary for evaluating the complex interactions between REM and emotion changes induced by rTMS.

LIMITATIONS

We used the '5 cm rule' for locating the DLPFC_L. In the future, neuronavigated rTMS should be taken into consideration. Another limitation is the relatively small sample size in our study. Therefore, the findings should be interpreted and generalised with caution, and future studies should aim to increase the sample size for more robust results.

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Competing interests None declared.

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Ethics approval This study involves human participants and was approved by the Ethics Committee of Medical Research in the Second Hospital of Hebei Medical University, Shijiazhuang, China (approval letter no. 2022-R758). The experimental procedure was fully explained and informed written consent was obtained from all participants.

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