Electroencephalography microstates as novel functional biomarkers for insomnia disorder

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ABSTRACT

Background Insomnia disorder (ID) is one of the most common mental disorders. Research on ID focuses on exploring its mechanism of disease, novel treatments and treatment outcome prediction. An emerging technique in this field is the use of electroencephalography (EEG) microstates, which offer a new method of EEG feature extraction that incorporates information from both temporal and spatial dimensions.

Aims To explore the electrophysiological mechanisms of repetitive transcranial magnetic stimulation (rTMS) for ID treatment and use baseline microstate metrics for the prediction of its efficacy.

Methods This study included 60 patients with ID and 40 age-matched and gender-matched good sleep controls (GSC). Their resting-state EEG microstates were analysed, and the Pittsburgh Sleep Quality Index (PSQI) and polysomnography (PSG) were collected to assess sleep quality. The 60 patients with ID were equally divided into active and sham groups to receive rTMS for 20 days to test whether rTMS had a moderating effect on abnormal microstates in patients with ID. Furthermore, in an independent group of 90 patients with ID who received rTMS treatment, patients were divided into optimal and suboptimal groups based on their median PSQI reduction rate. Baseline EEG microstates were used to build a machine-learning predictive model for the effects of rTMS treatment.

Results The class D microstate was less frequent and contribute in patients with ID, and these abnormalities were associated with sleep onset latency as measured by PSG. Additionally, the abnormalities were partially reversed to the levels observed in the GSC group following rTMS treatment. The baseline microstate characteristics could predict the therapeutic effect of ID after 20 days of rTMS, with an accuracy of 80.13%.

Conclusions Our study highlights the value of EEG microstates as functional biomarkers of ID and provides a new perspective for studying the neurophysiological mechanisms of ID. In addition, we predicted the therapeutic effect of rTMS on ID based on the baseline microstates of patients with ID. This finding carries great practical significance for the selection of therapeutic options for patients with ID.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Repetitive transcranial magnetic stimulation (rTMS) is an environmentally friendly and highly effective treatment for insomnia disorder, but its mechanism of action is not understood well.

WHAT THIS STUDY ADDS

⇒ There was a significant difference between the electroencephalography microstates of patients with insomnia disorder and good sleep controls.

⇒ rTMS modulated abnormal microstates, and the changes in microstates were significantly correlated with the improvement in objective sleep quality.

⇒ Baseline microstates could accurately predict the therapeutic effect of rTMS.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The rTMS efficacy prediction model has the potential for clinical application, offering valuable insights for making treatment decisions for patients with insomnia disorder.

INTRODUCTION

Insomnia disorder (ID) is one of the most common sleep disorders. Approximately one-third of adults in the general population experience at least one symptom of ID, with around 10% meeting the diagnostic criteria for ID.[1 2] ID includes nocturnal and daytime symptoms that severely impact an individual's quality of life and overall health, imposing a considerable economic burden on the patients' families as well as society.[3 4] Nocturnal symptoms of ID include prolonged sleep latency, difficulties in maintaining sleep and early morning awakening. Daytime symptoms include fatigue, reduced concentration, impaired cognitive function, irritability, anxiety and depressed mood.[5–8] However, the current understanding of the pathological mechanisms of ID is insufficient.[4] Therefore, there is an urgent need to understand its neural basis to improve treatment strategies.

Currently, electroencephalography (EEG) has been widely used for investigating psychiatric and neurological disorders as a non-invasive imaging technique that directly measures neuronal activity with high temporal resolution. Patients with ID exhibited increased beta-band power, resting-state EEG coherence abnormalities (eg, abnormally elevated coherence between F7 and O1 electrodes, T6 and O1 electrodes) and reduced prefrontal theta-gamma phase-amplitude coupling.[8–10] Repetitive transcranial magnetic stimulation (rTMS) is an emerging physical therapy modality that is widely used in psychiatric and neurological disorders.[11] Low frequency (1 Hz) rTMS can induce the inhibitory function of neurons and has achieved positive results in ID treatment.[12] One Hz rTMS over the left dorsolateral prefrontal cortex (DLPFC) significantly improved subjective and objective sleep quality in patients with ID and showed the potential to normalise the hyperexcited functional connections between the left DLPFC-right superior frontal gyrus. Furthermore, we observed disruption of cortico-hippocampal interactions in patients with ID, and rTMS may potentially rewire these impaired connections.[13] Meanwhile, rTMS has demonstrated the capacity to modulate abnormal EEG metrics in ID, including reversing the increased EEG coherence in the theta and alpha bands and improving the decreased theta-gamma phase-amplitude coupling.[8 10]

Microstates analysis leverages the spatiotemporal information of EEG, enabling the capture of the rapid dynamics of large-scale brain networks that are not available with traditional EEG analysis methods and limited temporal resolution brain imaging tools such as functional magnetic resonance imaging (MRI).[14 15] EEG microstates are defined as quasi-stable scalp voltage configurations that persist for an average of tens of milliseconds. Transitions between microstates are believed to reflect the dynamic activations of distributed brain networks at subsecond timescales.[16] Resting-state EEG microstates during eyes-closed wakefulness are most commonly categorised into four classes (conventionally labelled as microstate classes A, B, C and D) through topographical clustering techniques.[14] A microstate generally lasts for 60–120 ms before rapidly transitioning to another. The switching of microstates can describe the rapid changes in brain activity, which may be the basis of human cognitive functions. In healthy individuals, the four microstate maps during wakefulness remain unchanged during sleep with increased stability, and the attention-related microstates decreased during deep sleep.[17] These characteristics closely relate to functional brain networks during slow-wave sleep, providing an important electrophysiological basis underlying brain functional networks.[18]

In patients with ID, a resting-state EEG microstates study found a shorter mean duration of class C microstate and a higher frequency of class D microstate.[19] However, there are three key questions requiring further validation. First, it is necessary to determine whether there is a correlation between resting-state EEG microstates and objective sleep indicators (eg, polysomnography (PSG)) in patients with ID. Second, while rTMS has shown promise in improving abnormal microstate indicators in patients with schizophrenia and major depression, with the microstate changes potentially serving as an effective indicator of symptom improvement,[15–17] it remains uncertain whether these findings could extend to patients with ID. Currently, little is known about whether these changes are correlated to sleep improvement following rTMS treatment. Third, the effects of rTMS vary greatly among individuals, and whether EEG microstates could predict the therapeutic effect of rTMS for ID remains to be verified.[20] Such verification would be of great practical and economic value. Traditional univariable analyses offered preliminary insights into predicting treatment outcomes but have failed to recognise the specific pattern in rTMS treatment responses for patients with ID. A more advanced method is required for more precise predictions of changes in sleep quality. Furthermore, a robust and generalised model is needed to translate these electrophysiology findings into clinical applications. Machine learning has been used to predict antidepressant responses in major depression and could be a promising approach for these objectives. In this study, we investigated the abnormal properties of EEG microstates in 60 patients with ID and explored the efficacy of rTMS treatment in reversing the abnormal microstates. We further included another 90 patients with ID to assess the potential of baseline microstate characteristics for predicting the efficacy of rTMS for ID treatment.

METHODS
Participants
This study was conducted at the Second Hospital of Hebei Medical University in Shijiazhuang, China. Good sleep controls (GSC) and ID patients were recruited from outpatient clinics. Individuals aged between 18 and 65 years who were right-handed were screened based on the diagnostic criteria for ID in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition. A total score of ≥5 on the Pittsburgh Sleep Quality Index (PSQI) and ≥8 on the Insomnia Severity Index (ISI) was required for enrolment. Those with severe neurological or medical illness, other sleep disorders, restless leg syndrome and pregnancy (for females) were excluded. Additionally, individuals who received psychiatric medication, hypnotics or treatment with cognitive behavioural therapy for insomnia within the past 2 weeks were also excluded. For GSC, age-matched and gender-matched individuals were included based on the following criteria: no symptoms or history of psychiatric or sleep disorders, a total PSQI score of <5 and a total ISI score of <8 at the time of screening and no lifetime use of any psychotropic or hypnotic medication. For both groups, exclusion
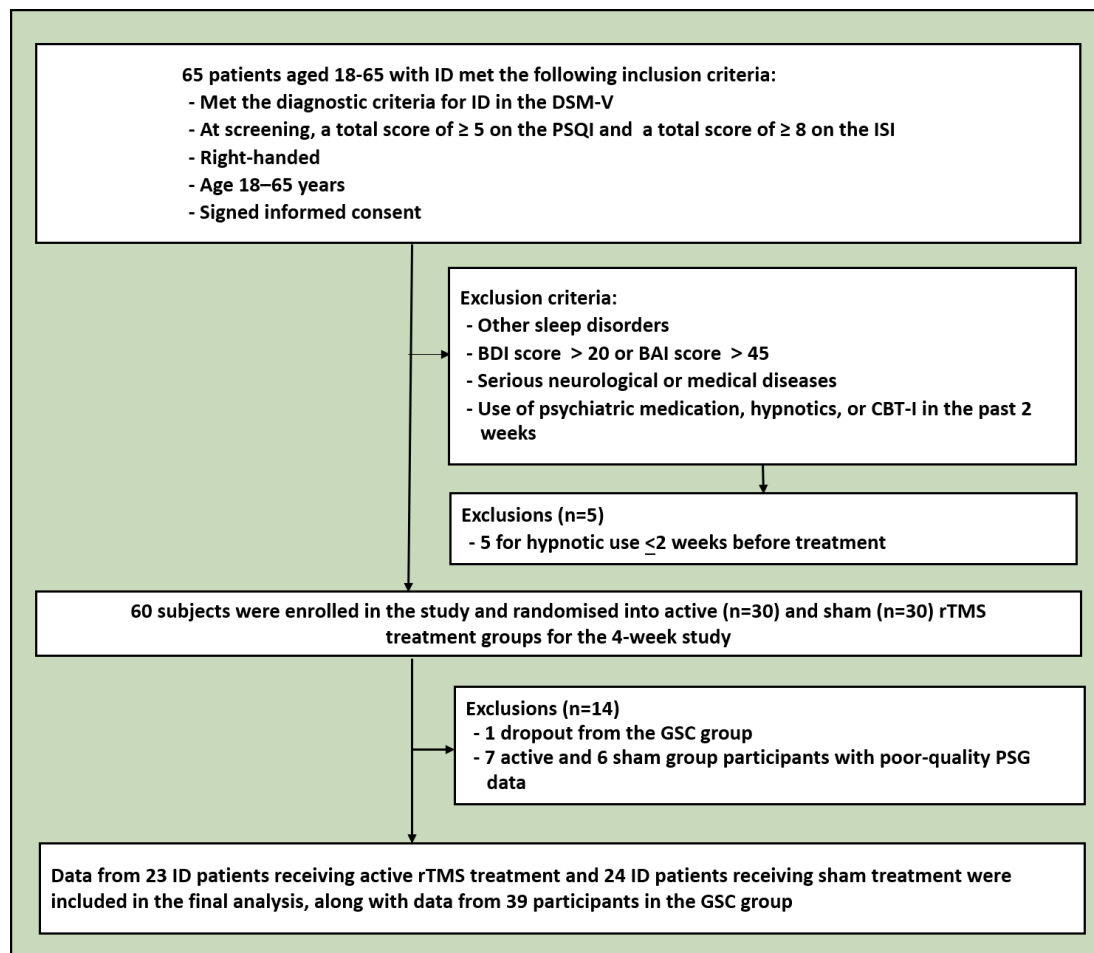
Figure 1 Flowchart of the study. BAI, Beck Anxiety Index; BDI, Beck Depression Index; CBT-I, cognitive behavioural therapy treatment for insomnia; DSM-V, Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition; GSC, good sleep controls; ID, insomnia disorder; ISI, Insomnia Severity Index; PSG, polysomnography; PSQI, Pittsburgh Sleep Quality Index; rTMS, repetitive transcranial magnetic stimulation.

criteria included comorbid other psychiatric disorders and Beck Depression Inventory (BDI) scores >20 or Beck Anxiety Inventory (BAI) scores >45. To screen for other sleep disorders, participants were questioned in person about conditions including obstructive sleep apnoea syndrome, restless leg syndrome, etc. Also, the PSG data of the participants were collected and analysed. Individuals with other sleep disorders were excluded from the study.[10]

Two datasets were included in this study. The primary dataset included 40 GSC and 60 patients with ID. Patients with ID were randomly divided into active rTMS treatment (n=30) and sham rTMS treatment (n=30) groups. This dataset was used to explore abnormal brain microstates in patients with ID and to assess the effect of rTMS intervention on abnormal microstates in patients with ID. The second dataset included 90 patients who received active rTMS treatment; it was used to validate whether baseline microstates in patients with ID could predict the outcomes of 20-day active rTMS treatment. The inclusion and exclusion criteria for ID patients recruitment in the second dataset were consistent with those in the primary dataset. A flowchart of participant registration

and follow-up is shown in figure 1. The characteristics of the participants are summarised in online supplemental table S1 and online supplemental table S2.

Repetitive transcranial magnetic stimulation treatment
The rTMS therapy was carried out by using a pulsed magnetic stimulation device (M-100 Ultimate; Shenzhen Yingchi Technology, Shenzhen, China) with a figure-eight stimulation coil at the left DLPFC, as described in our previous study.[10] The specific parameters were as follows: stimulus frequency at 1 Hz, stimulus intensity at 80% of the motion threshold, stimulation number at 10 pulses per string and 150 strings, string interval at 2 s, and total stimulation pulses at 1500. For each rTMS session, the total stimulation time is 30 min, one session per day. Sham rTMS was performed by orienting the coil away from the skull at 90°. Twenty active or sham sessions of left DLPFC rTMS treatment were delivered over four consecutive weeks, with a frequency of five times per week.

Clinical assessment
All patients with ID received pre-treatment and post-treatment clinical assessments, including full-night

sleep PSG recordings using a Grael 4 K system (Compumedics, Victoria, Australia) and sleep-related scales. The Yet Another Spindles Algorithm (YASA) was used for sleep stage classification, and PSG metrics were calculated, including sleep onset latency (SOL), sleep efficiency (SE) and non-rapid eye movement sleep stage 3 (NREM 3) duration. The scales included the PSQI, ISI, Epworth Sleepiness Scale (ESS), BDI, BAI, Mini-Mental State Examination (MMSE) and the Montreal Cognitive Assessment (MoCA). GSC participants were subjected to the same assessment but only measured at baseline (ie, pre-treatment).

Resting-state electroencephalography data acquisition and preprocessing

The 10 min resting-state EEG recording was carried out in a shielded, sound-attenuated room. Participants were instructed to remain awake with their eyes closed, and relax without any active thinking. Resting-state EEG data were acquired at a sampling rate of 512 Hz (analogue bandpass filtering: 0.1 and 100 Hz), with a 19-channel Nicolet system (Cephalon, Denmark). The ground electrode was attached to the forehead, and reference electrodes were located on A1 and A2. For each electrode, the impedance was maintained below 10 kΩ. EEGLAB toolbox and custom MATLAB (MathWorks, Massachusetts, USA) scripts were employed for EEG data offline preprocessing.[21] By using a notch filter, we removed the 50 Hz AC line noise artefact. After filtering with a 2–20 Hz bandpass, the filtered EEG data were re-referenced to the common average. The bad channels and bad epochs were manually rejected and then interpolated from the EEG signals of adjacent channels. Finally, the eye movement and blink artefacts were removed using independent component analysis.

Microstates analysis

The microstate analysis was conducted using the functions from the EEGLAB plugin for microstates (http://www.thomaskoenig.ch/index.php/software/Microstates-in-eeglab) in MATLAB R2021b. The process of EEG microstates analysis is described in detail below (online supplemental figure S1).

First, the global field power (GFP) was calculated:

$$\text{GFP}\,(t) = \sqrt{\frac{1}{k}\sum_{i=1}^{k}(V_i\,(t)\text{-}V_{mean}\,(t)\,)^2}$$

Where k is the number of electrodes in the EEG data (19 in this case); $V_i(t)$ is the potential of the ith electrode at a certain time point and $V_{mean}(t)$ is the average value of the instantaneous potential across electrodes.

Based on the above equation, a GFP curve that reflects the degree of change in the EEG potential between all electrodes at a given time can be obtained. GFP curve local maxima represented instants of the highest field strength.[22] EEG topographies tend to remain stable during high GFP periods and change rapidly around the

local minima of the GFP. Therefore, topographies at GFP peaks are representative of topographies at surrounding time points, and restricting the microstate analysis to these GFP peaks provided optimal topographic signal-to-noise ratios.[23]

Second, for the resting-state recording, all topographies at GFP peaks were selected and submitted to modified k-means clustering (despite spatial polarity) with 100 repetitions. The number of clusters was set to 4, and microstate maps (ie, cluster centres) were estimated. Third, a second cluster was performed among the subjects within each group. All microstate maps were submitted to modified k-means clustering (despite spatial polarity) with 100 repetitions. Finally, the group microstate maps were then fit back to the original data at GFP peaks, assigning each GFP peak to one microstate class based on the maximal spatial correlation between topographies. Microstate labels for data points between GFP peaks were interpolated with microstates starting and ending halfway between two GFP peaks. Potentially truncated microstates at the beginning and end of each epoch were excluded from the analysis.

After obtaining the microstates, the corresponding EEG microstates temporal parameters were extracted: (a) Duration_X: the mean duration of microstates of class X, in seconds; (b) Mean Duration: the mean microstate duration across all classes in seconds; (c) Occurrence_X: the mean frequency of observation of microstates of class X, per second; (d) Mean Occurrence: the mean frequency of observation of microstates across class X, per second; (e) Contribution_X: the proportion of the total time spent in microstates of class X; (f) Mean GFP_X: the mean GFP of microstates of class X in microvolts; (g) OrgTM_X->Y: the proportion of all observed microstate transitions from X to Y; (h) ExpTM_X->Y: the proportion of expected microstate transitions from X to Y, given only the observed occurrences; (i) DeltaTM_X->Y: the difference between the observed and the expected transition probabilities. Details are available in the online supplemental material.

Statistical analyses

The Shapiro-Wilk test was used to test for the normality of distribution. Independent samples t-test was used to compare the differences between the ID and GSC groups (basic information, scale scores, microstate indicators and PSG indicators). The Wilcoxon rank-sum test was performed on the gender of participants in the ID and GSC groups. Two-way repeated measure analysis of variance and post hoc Tukey tests were used for differences before and after active and sham rTMS (scale scores, PSG indicators and microstate indicators).

The topographies of the different microstate classes between the groups were compared using topographical analysis of variance (TANOVA) implemented in the Ragu software.[24] TANOVA was based on powerful and assumption-free randomisation statistics and could be

used for the statistical comparison of EEG scalp field maps between two or more conditions.

Pearson's correlation analysis was used to explore the potential significant correlation between abnormal microstate parameters and subjective and objective sleep quality in patients with ID. Data were analysed using SPSS V.26.0 (SPSS, Chicago, Illinois, USA). Graphs were plotted using GraphPad Prism V.8.0.2 (GraphPad Software, California, USA).

Construction of efficacy prediction model

PSQI scale scores were collected from patients with ID before and after treatment. Then, the percentage of improvement in the subjects' PSQI scale scores was calculated using the formula shown below:

$$Score\ reduction\ rate = \frac{PSQI(after\ treatment) - PSQI(before\ treatment)}{PSQI(before\ treatment)}$$

The percentages of improvement were sorted in ascending order, and the subjects were divided into optimal and suboptimal groups using the median 54 baselines. EEG microstate characteristics (Duration_X, Mean Duration, Occurrence, Mean Occurrence_X, Contribution_X, Mean GFP_X, OrgTM_X->Y, ExpTM_X->Y, DeltaTM_X->Y) were used as the features to build the dataset. Details of the characteristics are given in the 'microstate analysis' section. The dataset (10n) was divided into a training set (9n) and a test set (1n) using a hierarchical 10-fold cross-validation strategy. First, feature preprocessing of normalisation was performed for the features, and then principal component analysis dimensionality reduction was carried out to remove redundant features. The specific strategy retained 99% of the explainable variance. The remaining features were then input to a logistic regression classifier for model training. The test set was input to the trained model according to the same feature preprocessing and feature selection strategies for model evaluation. The performance metrics (accuracy, precision, recall, F1-score, area under the curve (AUC)) were calculated for each fold of the model, and the average performance metrics were averaged for each fold of the model to obtain the average performance metrics of the model.

RESULTS
Demographic information and behavioural results

Demographic information and behavioural results of the ID and GSC groups are presented in online supplemental table S1. There were no significant differences in age, gender, ESS or MoCA between the two groups (p>0.05). Significant differences were observed in PSQI, ISI, BDI, BAI and MMSE scores between the two groups (p<0.001).

Of the total 60 participants, one in the GSC group withdrew for personal reasons. PSG data from seven active and six sham group patients were excluded from the analyses due to poor data quality. The final PSG data used for analysis included 23 participants in the active treatment group and 24 in the sham treatment group. Significant impairments in both subjective sleep quality (PSQI: t_{97}=19.13, p<0.001; ISI: t_{97}=18.53, p<0.001) and objective sleep quality (SE: t_{84}=6.324, p<0.0001; NREM 3 duration: t_{84}=6.828, p<0.001; SOL: t_{84}=4.774, p<0.001) were observed in patients with ID when compared with the GSC group at baseline (online supplemental figure S2). No side effects were reported during or after brain stimulation.

Active rTMS treatment effectively enhanced sleep quality in patients with ID

Following active rTMS treatment, there was a noticeable improvement in subjective sleep quality in patients with ID, as indicated by reduced PSQI and ISI scores (figure 2A). Similarly, improvement in objective sleep quality was evidenced by increased SE, NREM 3 duration and shorter SOL (figure 2B). In contrast, no significant changes in subjective or objective sleep quality were observed in the sham group (figure 2). No significant differences were observed in MMSE, MoCA, BDI and BAI scores in patients with ID, both before and after treatment with active or sham rTMS.

Abnormal EEG microstate spatial topographies and temporal parameters in patients with ID

The microstate analysis results demonstrated that microstate classes A–D corresponded well to the canonical microstate maps reported in the literature (figure 3A).[25] No statistical difference was detected in the global explained variance between the ID group (mean (standard deviation, SD): 0.79 (0.06)) and the GSC group (mean (SD): 0.77 (0.06)) (t_{83}=1.713, p=0.090). The topographies of microstates A and C were significantly different between the ID and GSC groups (p=0.003, p=0.001). Additionally, at baseline, there were significant differences between patients with ID and the GSC in the occurrence (t_{83}=2.999, p=0.021) and contribution (t_{83}=0.0003, p=0.003) of class D microstate (figure 3B, online supplemental table S3). Furthermore, we found that the occurrence (n=23, p<0.0001, r=−0.74) and contribution (n=23, p<0.0001, r=−0.72) of class D microstate were negatively correlated with SOL (figure 4B). The differences of the other microstate features did not reach significance.

rTMS reversed the partial abnormal microstate parameters in patients with ID

The independent sample t-test result showed no statistical difference in the global explained variance between the ID group after active treatment (mean (SD): 0.77 (0.06)) and the GSC group (mean (SD): 0.77 (0.06)) (t_{62}=0.1723, p=0.860). After active rTMS treatment, the difference between patients with ID and the GSC on the microstate C

A

B
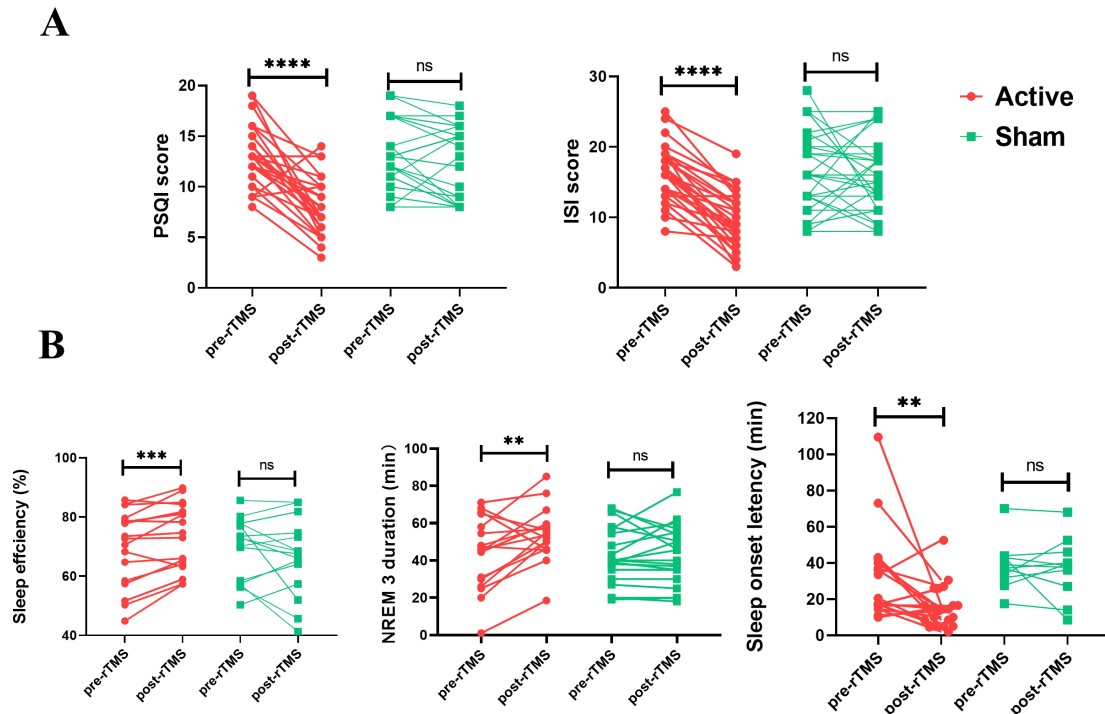
Figure 2 Improvement of subjective and objective sleep efficiency after active rTMS treatment. Two-way analysis of variance revealed a significant 'treatment×time' interaction effect in both subjective sleeping measurements (PSQI: F_{58}=25.17, p<0.0001; ISI: F_{58}=19.63, p<0.0001) and objective sleeping measurements (SE: F_{45}=7.265, p=0.010; NREM 3 duration: F_{45}=7.149, p=0.010; SOL: F_{45}=5.663, p=0.022). (A) Subjective sleep improvement was found by showing reduced PSQI (t_{29}=7.334, p<0.0001) and ISI (t_{29}=10.48, p<0.0001) in the active rTMS group. (B) Objective sleep improvement was found by showing increased sleep efficiency (t_{22}=4.412, p<0.001), NREM 3 duration (t_{22}=3.159, p=0.005) and shorter SOL (t_{22}=3.091, p=0.005) in the active rTMS group. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. ISI, Insomnia Severity Index; NREM, non-rapid eye movement; ns, not significant; PSQI, Pittsburgh Sleep Quality Index; rTMS, repetitive transcranial magnetic stimulation; SE, sleep efficiency; SOL, sleep onset latency.

topographic map became no longer significant (p=0.604, figure 3A). The occurrence and contribution of class D microstate in the ID group were improved, approaching the levels observed in the GSC group (t_{62}=0.1913, p=0.849 figure 4A). However, no such improvement was found in the sham group. The change in the occurrence of the class D microstate also showed a significant negative correlation with the improvement of SOL (n=23, p=0.012, r=−0.51, figure 4B) in the active rTMS group.

Treatment outcome prediction from baseline EEG microstates

Here, the EEG microstate parameters (Duration, Incidence, Contribution, MeanGFP, OrgTM, ExpTM, DeltaTM) of 86 ID patients (the EEG data of 4 patients were excluded due to quality issues) were downscaled using PCA, and then a ten-fold cross-validation strategy was implemented to partition the dataset, which was then fed into a logistic regression classifier for training and testing.

The final average prediction accuracy was 80.13% (figure 5A). The top 10 most important features for the prediction model are shown in figure 5B. The average AUC area was 0.81, and the average F1 score was 0.62. The specific performance of each fold of the efficacy prediction model (accuracy, precision, recall, F1 score and AUC) is shown in online supplemental table S4.

DISCUSSION
Main findings

The findings of this study have several important implications. First, we observed that the baseline occurrence and contribution of class D microstate were significantly lower in the ID group compared with the GSC group. The topographic maps of microstates A and C in the ID group were significantly different from those in the GSC group, potentially serving as a novel biomarker for SOL for patients with ID. Second, we found that active rTMS treatment improved the occurrence and contribution of class D microstate and partially reversed the topography of microstate class C in patients with ID. Finally, our findings supported that the baseline microstate characteristics of patients with ID could predict the improvement of sleep quality following rTMS treatment.

The four canonical microstate classes exhibited spatial patterns of well-known resting-state networks.[26] Specifically, intra-individual fluctuations of class A, B, C and D microstates were linked to the activation of the auditory, visual, salience and attention networks, respectively. Abnormal EEG microstates have been detected in various mental and neurological disorders, such as depression, schizophrenia and Lewy body dementia.[27–29] These abnormalities might reflect a disruption of the intricate
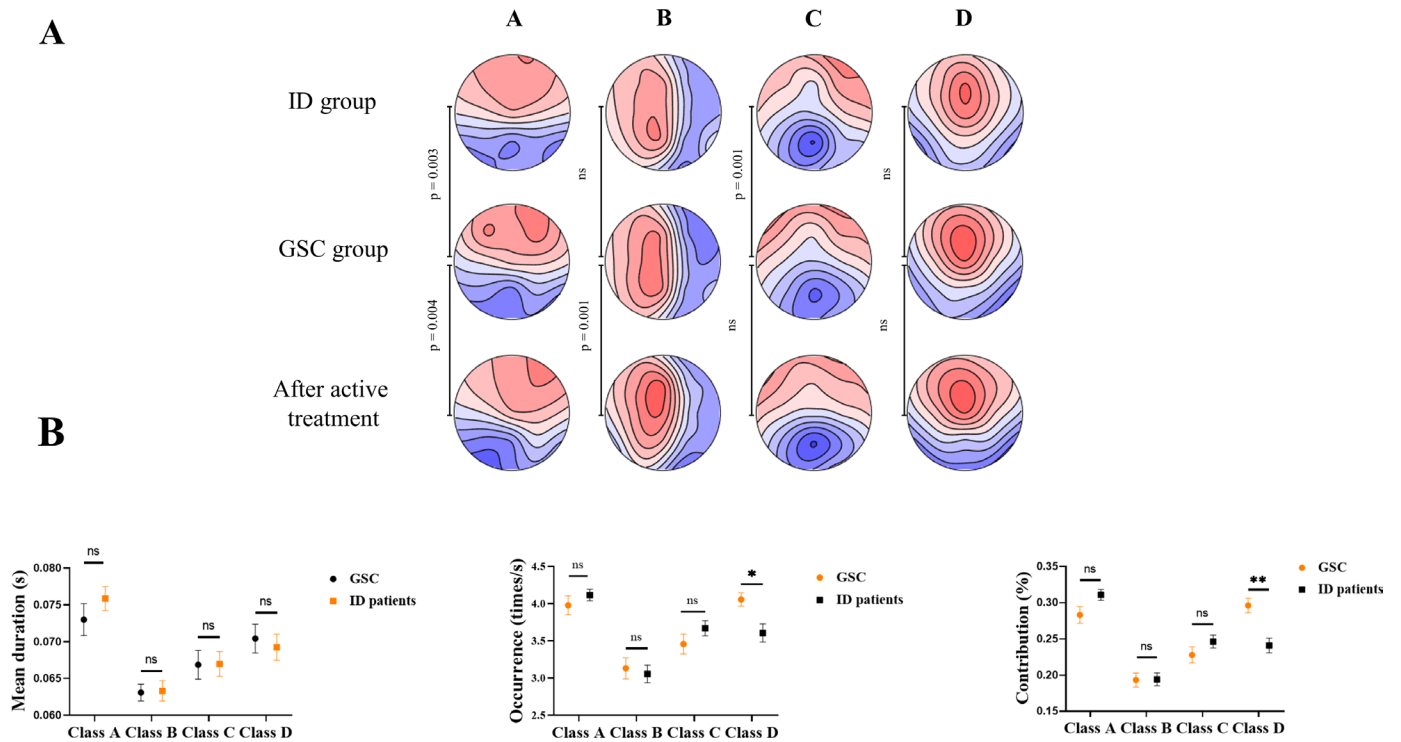
Figure 3 Spatiotemporal properties of microstates. (A) P values result from comparing the group topographies between groups using topographical analysis of variance. (B) Group comparison of microstate duration and occurrence per second overall and contribution for each microstate class separately. *p<0.05, **p<0.01. GSC, good sleeper controls; ID, insomnia disorder; ns, not significant.
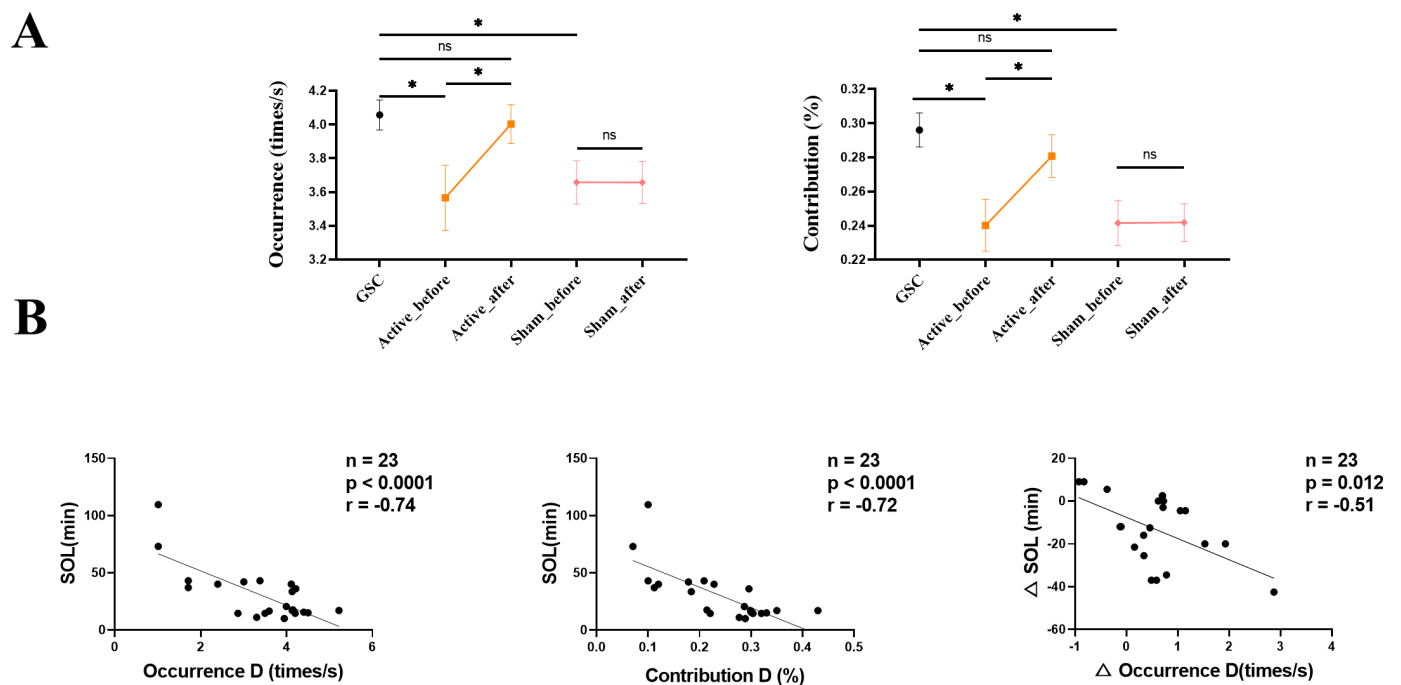
Figure 4 Improvement of microstate indicators after active rTMS treatment and its correlation with objective sleep quality. (A) Abnormal class D microstate occurrence and contribution improved after active treatment, while these metrics did not improve after sham treatment. (B) At baseline, SOL showed a significant negative correlation with the contribution and the occurrence of class D microstate. After treatment, improvement in occurrence showed a significant negative correlation with improvement in SOL. *p<0.05. ns, not significant; rTMS, repetitive transcranial magnetic stimulation; SOL, sleep onset latency.

A

ROC curve (Logistic regression)

B

Top 10 feature contributions
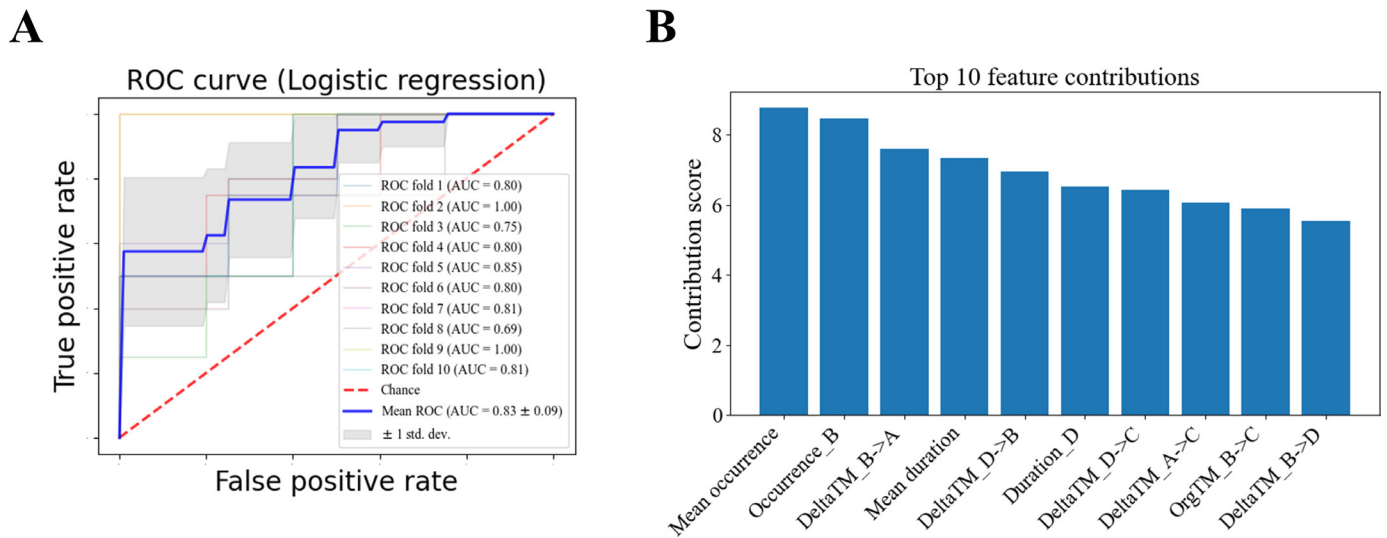
Figure 5 Baseline microstates to predict the improvement in sleep quality in patients with ID after rTMS treatment. (A) The average accuracy of the final prediction is 80.13%. (B) The 10 most important features for prediction models are shown. AUC, area under the curve; ID, insomnia disorder; ROC, receiver operating characteristic; rTMS, repetitive transcranial magnetic stimulation.

dynamic properties of the brain, leading to a loss of flexibility and adaptability that was crucial for normal brain functioning. Our study found a decreased occurrence and contribution of microstate class D in patients with ID when compared with the GSC group. This suggests that patients with ID may not get sufficient rest during the night, which can affect daytime functioning and impair both attention and cognitive control. It is worth noting that a previous study reported a shorter mean duration of class C microstate and a higher frequency of class D microstate in patients with ID.[19] However, these results require further validation, considering the differences in the timing of resting-state EEG acquisition and the number of clusters. In addition, we employed PSG to assess the objective sleep quality in patients with ID, which was not included in the previous insomnia disorder EEG microstates research. Our correlation analysis showed that the baseline occurrence and contribution of class D microstate were correlated with the SOL in patients with ID (figure 4B). In summary, we extended this microstates analysis to reveal the pathology of ID and found that EEG microstates may serve as a biomarker for the sleep quality of patients with ID, especially for the SOL. These hypotheses need to be tested by including cognitive assessments in future studies.

In this study, we observed significant improvements in the sleep quality of patients with ID following rTMS treatment in both subjective and objective dimensions, which is in line with the remarkable efficacy of rTMS in the treatment of ID reported previously.[30–33] However, the mechanism of rTMS in ID treatment requires further investigation. Our group previously reported that active rTMS may re-establish the disrupted cortico-hippocampal interactions in patients with ID.[13] Meanwhile, we observed normalisation of the hyper EEG coherence in theta and alpha bands after rTMS treatment, with changes in theta

band coherence correlating with changes in SE.[8] The hyperarousal model hypothesised that the brains of patients with ID exhibited hyperactivity during both day and night,[34] which may disrupt the transitions of EEG microstates. However, it remains unknown whether rTMS can improve the pattern of microstate abnormalities. EEG microstates can respond to the transformation of the overall collaborative pattern of functional brain activity. This study provided scientific evidence that the occurrence and contribution of abnormal class D microstate in patients with ID improved after active rTMS (figure 4A). Moreover, the change in the occurrence of microstate class D showed a significant negative correlation with the shortening of SOL, which may serve as an indicator of SOL changes induced by active rTMS in patients with ID.

Although rTMS can effectively improve sleep quality in patients with ID, the effectiveness of rTMS treatment varies largely between individuals.[35] Therefore, the distinction between high-responders and low-responders to rTMS is a crucial consideration for subsequent treatment strategies. We used a machine learning method to predict efficacy in an independent dataset and found that baseline microstate indicators in patients with ID could accurately predict whether their sleep quality would significantly be improved after 1 month of rTMS treatment (figure 5). For low-responders to rTMS, changing treatment modality or adopting rTMS combination therapy (eg, rTMS in combination with medication or cognitive-behavioural therapy) may improve treatment outcomes.[36] The top 10 most important features demonstrated that the transitions among different microstates contributed to the prediction model, suggesting that more attention should be paid to other microstate indicators in future studies, such as DeltaTM_X->Y and OrgTM_X->Y (figure 5B).

Limitations

This study had several limitations. First, we did not conduct a follow-up for patients with ID in this study, preventing us from assessing the long-term effects of rTMS on ID. Second, we did not fully explore the higher-order indexes of PSG or the relationship between the coupling of various frequency bands of PSG and the EEG microstates. Lastly, we used the MMSE scale for clinical assessment, which is not a comprehensive cognitive assessment.[37] In future studies, we aim to address these limitations and further investigate the pathogenesis of ID as well as the mechanism of action of rTMS to provide additional insights and improve ID treatment strategies.

Conclusion

In conclusion, this study is the first to investigate the temporal dynamics and spatial topography of four typical EEG microstates in patients with ID before and after rTMS treatment. We also explored the relationship between microstates and objective sleep indicators in patients with ID using PSG. Our findings revealed the abnormal occurrence and contribution of class D microstate in patients with ID, which correlated with SOL. 20 days of 1 Hz rTMS over L_DLPFC could modulate the occurrence and contribution of the EEG class D microstate. Moreover, baseline microstate temporal indexes (eg, the transitions among different microstates) aided in selecting potential high-responders to rTMS treatment. These results provided new electrophysiological insights on the pathology of ID and the possible mechanisms of rTMS for ID treatment. They highlighted the clinical significance of EEG in guiding rTMS treatment for ID.

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