

Difference of Resting State Functional Connectivity between Depressive Patients with and without Clinically Significant Insomnia

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Objective: Insomnia is a common symptom of major depressive disorder (MDD). However, the neural distinctions between MDD with and without insomnia remain unclear. This study investigated resting state functional connectivity (RSFC), specifically seeding the habenula and septal nuclei, to identify neurobiological abnormalities associated with insomnia in MDD.

Methods: Thirty-six patients with MDD and clinically significant insomnia (MDD_w/INS), 21 patients without insomnia (MDD_wo/INS), and 38 healthy controls underwent 3T resting-state fMRI. Seed-to-voxel RSFC analyses were conducted using the habenula and septal nuclei as seeds. Between-group comparisons and correlation analyses were adjusted for age, sex, years of education, and Hamilton Depression Rating Scale 17 items (HDRS-17) scores.

Results: The MDD cohort had a mean age of 35.6 years and mean illness duration of 7.3 years. Compared to the MDD_wo/INS group, the MDD_w/INS group exhibited significantly higher depressive symptom severity (HDRS-17: 19.4 ± 4.8 vs. 13.8 ± 4.3) but a shorter illness duration (6.4 ± 6.9 vs. 8.9 ± 6.8 years). RSFC was increased between the left habenula and the right Rolandic operculum and cuneus and between the right habenula and thalamic pulvinar in the MDD_w/INS group. In contrast, decreased RSFC was observed between the septal nuclei and right cerebellar Crus I. All altered RSFC patterns were significantly correlated with the severity of insomnia.

Conclusion: This study reveals distinct RSFC patterns associated with insomnia in MDD, emphasizing the role of the habenula and septal nuclei and their potential significance in modulating mood and sleep patterns in MDD.

KEY WORDS: Sleep initiation and maintenance disorders; Depressive disorder, major; Functional connectivity; Habenula; Septal nuclei.

INTRODUCTION

Major depressive disorder (MDD) is one of the most

common and disabling illnesses and is expected to become the primary leading cause of increased disability-adjusted life years by 2030 [1]. MDD is characterized by depressed mood and decreased interest and pleasure, lack of energy, feelings of guilt or worthlessness, poor concentration, appetite changes, psychomotor retardation or agitation, suicidal thoughts, and sleep disturbances [2,3]. Sleep disturbances such as insomnia or hypersomnia are common and relatively objective symptoms with an obvious phenotype compared to other symptoms of MDD [4]. However, the clinical effect of the presence or absence of insomnia in patients with MDD and the differences in brain function and activity between depression with and without insomnia remains unclear.

Previous functional MRI (fMRI) studies comparing func-

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tional connectivity between insomnia patients and healthy controls (HC) have yielded varying results. Cheng *et al.* [5] found increased resting state functional connectivity (RSFC) of the dorsal anterior cingulate cortex within the salience network and greater connectivity between the salience network and brain regions such as the dorsal lateral prefrontal cortex, superior frontal gyrus, sensorimotor area, and brainstem in insomnia patients compared with controls. Our previous study also reported increased RSFC between the thalamus and right medial superior frontal area, bilateral middle temporal area, left rectus and right parahippocampal area in insomnia patients compared with controls [6]. Leerssen *et al.* [7] reported that people with insomnia showed increased RSFC in the bilateral hippocampus and left middle frontal gyrus.

The habenula and septal nucleus, the two brain regions implicated in depression and sleep regulation, play crucial roles in modulating mood and sleep patterns, though their exact roles are still being studied. The habenula plays a role in the pathophysiology of depression. Research suggests that the habenula may be involved in processing negative stimuli, reward processing, and regulating stress responses [8]. Abnormal activation and increased connectivity of the lateral habenula have been observed in depressed individuals, suggesting its role in the pathophysiology of depression [9]. Emerging evidence suggests a relationship between the habenula and sleep regulation. Animal studies have demonstrated that habenular activity modulates sleep-wake transitions and regulates rapid eye movement (REM) sleep [10]. Dysfunctional habenular signaling has been associated with sleep disturbances, including insomnia and fragmented sleep patterns in depression [11]. The septal nucleus, a collection of nuclei in the brain's septal region, primarily comprising the septum pellucidum and the nucleus of the diagonal band [12], is interconnected with various brain regions involved in emotional and cognitive processing, including the hippocampus, amygdala, and prefrontal cortex [13]. Animal studies have shown that lesions or septal regional dysregulation could be related to depressive-like behaviors [14], and the septal nucleus alteration in humans has been associated with depressive symptoms [15,16]. In animals, the septal nucleus regulates sleep and wakefulness [17], and septal lesions can disrupt sleep patterns [18].

In MDD, the presence or absence of insomnia is asso-

ciated with neurobiological differences in the brain; however, fMRI studies comparing MDD with and without insomnia are scarce. In particular, few studies have examined RSFC seeding the habenula and septal nuclei, which play crucial roles in mood and sleep. This study aimed to determine whether there are differences in RSFC of habenula and septal nuclei with other regions of interest (ROIs) between MDD with and without clinically significant insomnia.

METHODS

Participants and Clinical Measurement

The board-certified psychiatrist assessed the participants' eligibility for this study utilizing the Structured Clinical Interview for the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) [19]. The MDD group included patients who met DSM-5 diagnostic criteria for MDD and had a baseline Hamilton Depression Rating Scale 17 items (HDRS-17) [20] score of 10 or higher. Patients with MDD were categorized into MDD with significant insomnia (MDD_w/INS) group and MDD without significant insomnia (MDD_wo/INS) group. Patients with MDD were categorized into the MDD_w/INS group if their insomnia severity index (ISI) was 15 or higher on the day of brain imaging.

The common exclusion criteria for MDD and HC were as follows: age < 19 or > 65 years; left-handed use in the Edinburgh Handedness Test [21]; previous abnormal brain imaging findings; contraindications to MRI (e.g., metals in the body); pregnancy or lactation; current serious suicide risk; major or unstable medical and neurological disorders within the past year; history of head trauma; substance use disorder within the past year; intellectual disability; personality disorder; and neurocognitive disorders. Additional exclusion criteria for MDDs were comorbidities of major psychiatric disorders (i.e., schizophrenia spectrum disorders, obsessive-compulsive disorders, major anxiety disorders, and disruptive, impulse control, and conduct disorders). Further exclusion criteria for the HCs were as follows: psychiatric history; history of taking psychotropic medications; and first-degree relatives with schizophrenia, MDD, or bipolar disorder; HDRS-17 total score at baseline > 6.

All participants were interviewed for HDRS-17, and they were asked to complete the Korean version of the

Pittsburgh Sleep Quality Index (PSQI) [22] and ISI [23] on MRI scanning day. All procedures involving human subjects/patients were approved by the Institutional Review Board of the Gil Medical Center (IRB No. GDIRB2018-005 and GDIRB2020-207) and all the participants provided written informed consent to participate in the study.

MRI Data Acquisition

Resting-state (RS) functional and anatomical MRI data were acquired using a 3T MRI system (Siemens Verio or Vida) with a phased-array radiofrequency head coil. RS-fMRI data were obtained with a two-dimensional echo planar imaging sequence. The imaging parameters for RS-fMRI data were as follows: repetition time (TR) = 2,500 ms, echo time (TE) = 25 ms, acquisition time (TA) = 6 minutes 45 seconds, field of view (FOV) = 231 mm, flip angle (FA) = 90°, in-plane resolution = $3.5 \times 3.5 \text{ mm}^2$, slice thickness = 3.5 mm, slices = 42, matrix size = 66×66 , and measurements = 160. The brain anatomical images were obtained with a T1-weighted magnetization-prepared rapid acquisition gradient echo sequence. The parameters for the anatomical data were as follows: TR = 1,900 ms, TE = 3.3 ms, inversion time = 900 ms, TA = 3 minutes 40 seconds, FOV = 256 mm, FA = 9°, in-plane resolution = $1.0 \times 1.0 \text{ mm}^2$, matrix size = 256×256 , slice thickness = 1 mm, slices = 160.

MRI Data Processing

RS-fMRI data were preprocessed with the MATLAB-based CONN functional connectivity toolbox ver. 18b (www.nitrc.org/projects/conn) for the correlation analysis of functional connectivity. The first 5 (12.5 seconds) and the last 5 volumes were discarded because of the adaptation time for MRI scanning noise and accidental loss in participants, respectively. The remaining 150 volumes were utilized for preprocessing analysis. For each data, head motion was estimated by 6 motion parameters (x/y/z translation and rotation). The thresholds of head motion were defined as translational motion parameter $< 2.5 \text{ mm}$ and rotational motion parameter $< 2.5^\circ$ [24], and the framewise displacement (FD) had no significant difference between groups ($F = 2.399$, $p = 0.102$). The T1 anatomical image was coregistered with the functional images for spatial normalization. Next, the anatomical maps were segmented and normalized to the Montreal Neurological Institute with a resampling voxel size of $2 \times 2 \times$

2 mm^3 . Finally, all the functional images were smoothed using a Gaussian kernel with a full-width at half maximum of 8 mm.

For analysis in the ROI, such as bilateral habenula and septal nuclei, were pre-segmented from single-subject high-resolution T1 volume images as in a previous study (Jung *et al.* [16]) and were imported as seeds for analysis.

Functional Connectivity Analysis and Statistical Analysis

Potential confounding effects were removed utilizing the denoising process and linear regression, and temporal band-pass filtering was performed to minimize the influence of physiological, head-motion, and other noise sources. The potential confounding effects, such as noise components from white matter and cerebrospinal fluid, were identified using the anatomical component-based noise correction strategy. After regression, temporal frequencies below 0.008 Hz and above 0.09 Hz were removed from all blood-oxygen-level-dependent (BOLD) signals.

Seed-to-voxel analysis was performed to identify the connectivity pattern of segmented regions to calculate RSFC patterns between pre-defined seeds (habenula and septal nuclei) and other brain regions. Seed-based connectivity maps were computed using the Fisher-transformed bivariate correlation between the BOLD time series in the seed and each voxel.

One-way ANCOVA covariate control was used to compare differences in RSFC between MDD_w/INS and MDD_wo/INS groups, adjusting for potential confounders (i.e., age, sex, education years, and total score of HDRS-17) to identify insomnia effects in the MDD group. To further validate the reliability of the between-group findings, an additional seed-to-voxel analysis was conducted within the entire MDD group using the same seed regions. Pearson's partial correlation analysis controlling for the same potential confounders was performed using Fisher-transformed correlation coefficients and ISI and PSQI scores to examine the relationship between RSFC and clinical sleep questionnaires (i.e., ISI and PSQI) in the participants with MDD.

In addition, an ANCOVA analysis was performed, controlling for age, sex, and education years to compare the difference in RSFC between MDD and HC. The result of functional connectivity was thresholded at a false discovery rate (FDR) corrected cluster level of $p < 0.05$ and an uncorrected peak level of $p < 0.001$ for each seed.

RESULTS

The analysis in this study included 36 participants in the MDD_w/INS group, 21 in the MDD_wo/INS group and 38 in the HC group. Table 1 shows the demographic and clinical characteristics of the participants. Age, sex ratio, years of education, and duration of depression were

not different between MDD with and without insomnia, but there were differences in HDRS-17, ISI, and PSQI total scores.

In comparing the differences in RSFC between MDD_wo/INS and MDD_w/INS groups, significant differences in RSFC of septal nuclei and habenula with several areas were found (Tables 2, 3, Figs. 1, 2). The significant differ-

Table 1. Demographic and clinical characteristics of the participants

Clinical variables	MDD (n = 57)	MDD_w/INS (n = 36)	MDD_wo/INS (n = 21)	HC (n = 38)	Statistics	
					MDD_w/INS vs. MDD_wo/INS	MDD vs. HC
Age at scan, yr	35.6 ± 12.4	36.8 ± 12.5	33.5 ± 12.2	35.9 ± 13.3	$t = 0.98$, $p = 0.331^a$	$t = -0.12$, $p = 0.906^a$
Sex (male:female)	11:46	6:30	5:16	11:27	$\chi^2 = 0.44$, $p = 0.510^b$	$\chi^2 = 1.19$, $p = 0.275^b$
Education, yr	13.6 ± 3.0	13.4 ± 2.9	13.9 ± 3.2	14.6 ± 2.1	$t = -0.63$, $p = 0.530^a$	$t = -1.98$, $p = 0.051^a$
Duration of illness, yr	7.3 ± 6.9	6.4 ± 6.9	8.9 ± 6.8	N/A	$t = -1.34$, $p = 0.186^a$	N/A
Clinical scales at MRI scanning						
HDRS-17 score	17.3 ± 5.3	19.4 ± 4.8	13.8 ± 4.3	2.6 ± 2.4	$t = 4.43$, $p < 0.001^a$	$t = 18.34$, $p < 0.001^a$
BDI score	27.7 ± 11.9	30.9 ± 10.9	22.1 ± 11.8	3.6 ± 3.6	$t = 2.87$, $p = 0.006^a$	$t = 14.33$, $p < 0.001^a$
BHS score	11.6 ± 5.5	11.2 ± 5.7	12.2 ± 5.2	2.4 ± 1.8	$t = -0.69$, $p = 0.491^a$	$t = 11.75$, $p < 0.001^a$
ISI score	16.0 ± 7.5	20.8 ± 4.0	7.8 ± 3.9	3.1 ± 3.4	$t = 11.88$, $p < 0.001^a$	$t = 11.47$, $p < 0.001^a$
PSQI score	12.7 ± 4.6	14.7 ± 3.7	9.3 ± 3.9	4.6 ± 3.5	$t = 5.23$, $p < 0.001^a$	$t = -10.50$, $p < 0.001^a$
CGI-S score	20.8 ± 4.0	4.4 ± 1.0	3.9 ± 1.3	7.8 ± 3.9	$t = 1.68$, $p = 0.100^a$	$t = 20.12$, $p < 0.001^a$

Values are presented as number only or mean ± standard deviation.

BDI, Beck Depression Inventory; BHS, Beck Hopelessness Scale; CGI-S, Clinical Global Impression Severity Scale; HC, healthy control; HDRS, Hamilton Depression Rating Scale; ISI, Insomnia Severity Index; MDD, major depressive disorder; MDD_w/INS, MDD with significant insomnia; MDD_wo/INS, MDD without significant insomnia; MRI, magnetic resonance imaging; N/A, not available; PSQI, Pittsburgh Sleep Quality Index.

^aStudent's t test; ^bChi-square test.

Table 2. Difference of seed-to-voxel connectivity between MDD_wo/INS and MDD_w/INS groups

Contrast	Seed	Cluster			Peak			Peak MNI coordinates			AAL3 Label
		p (FDR)	p (unc.)	K	T	Z	p (unc.)	X	Y	Z	
MDD_wo/INS > MDD_w/INS	Septal	0.001	< 0.001	311	4.61	4.19	< 0.001	20	−80	−28	Cerebellum_Crus1_R
MDD_wo/INS < MDD_w/INS	Habe_L	0.003	< 0.001	218	4.91	4.42	< 0.001	40	−18	18	Rolandic_Oper_R
		< 0.001	< 0.001	343	4.32	3.97	< 0.001	16	−72	24	Cuneus_R
	Habe_R	< 0.001	< 0.001	393	5.14	4.59	< 0.001	14	−20	18	Thal_PuL_R

One-way ANCOVA covariate control was used to compare differences in RSFC between MDD_wo/INS and MDD_w/INS groups. The potential confounders (i.e., age, sex, education years, and Hamilton Depression Rating Scale score) were adjusted in this analysis.

AAL, Automated Anatomical Labelling; Cerebellum_Crus1_R, Crus I of the right cerebellar hemisphere; Cuneus_R, right cuneus; FDR, false discovery rate corrected; Habe_L, left habenula; Habe_R, right habenula; MDD, major depressive disorder; MDD_w/INS, MDD with significant insomnia; MDD_wo/INS, MDD without significant insomnia; MNI, Montreal Neurological Institute; Rolandic_Oper_R, right rolandic operculum; Septal, septal nuclei; Thal_PuL_R, lateral pulvinar of right thalamus; unc., uncorrected.

Table 3. Correlation between RSFC and sleep questionnaire score

Contrast	Connectivity pairs	ISI		PSQI	
		Pearson's <i>r</i>	<i>p</i> value	Pearson's <i>r</i>	<i>p</i> value
MDD_wo/INS > MDD_w/INS	Septal - Cerebellum_Crus1_R	−0.308	0.007*	−0.228	0.040*
MDD_wo/INS < MDD_w/INS	Habe_L - Rolandic_Oper_R	0.306	0.007*	0.096	0.393
	Habe_L - Cuneus_R	0.315	0.006*	0.166	0.136
	Habe_R - Thal_PuL_R	0.340	0.003*	0.199	0.074

Partial correlation analysis controlling for age, sex, education duration, and Hamilton Depression Rating Scale score.

Cerebellum_Crus1_R, Crus I of the right cerebellar hemisphere; Cuneus_R, Right cuneus; Habe_L, left habenula; Habe_R, right habenula; ISI, Insomnia Severity Index; MDD, major depressive disorder; MDD_w/INS, MDD with significant insomnia; MDD_wo/INS, MDD without significant insomnia; Pearson's *r*, Pearson correlation coefficient; PSQI, Pittsburgh Sleep Quality Index; Rolandic_Oper_R, right rolandic operculum; RSFC, resting state functional connectivity; Septal, septal nuclei; Thal_PuL_R, lateral pulvinar of right thalamus.

* $p < 0.05$.

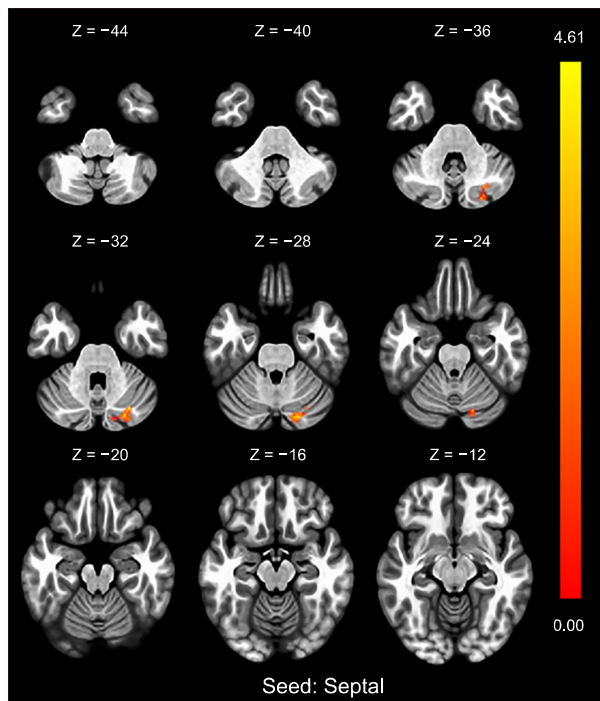


Fig. 1. Functional connectivity regions between MDD_wo/INS > MDD_w/INS contrast at the septal nucleus seed to voxel analysis. Target regions of the septal nucleus in MDD_wo/INS > MDD_w/INS contrast are presented.

MDD, major depressive disorder; MDD_w/INS, MDD with significant insomnia; MDD_wo/INS, MDD without significant insomnia; Septal, septal nuclei.

ence in RSFC of the septal nucleus with the Crus I of the right cerebellar hemisphere (Cerebellum_Crus1_R, p -FDR = 0.001) appeared in MDD_wo/INS > MDD_w/INS contrast (Table 2, Fig. 1). On the other hand, the significantly increased RSFC of left habenula (Habe_L) and right habenula (Habe_R) appeared in MDD_wo/INS < MDD_w/INS contrast (Table 2, Fig. 2). The right Rolandic oper-

culum (Rolandic_Oper_R, p -FDR = 0.003) and the right cuneus (Cuneus_R, p -FDR < 0.001) had significant RSFC with Habe_L. Furthermore, the lateral pulvinar of the right thalamus (Thal_PuL_R, p -FDR < 0.001) had significant RSFC with Habe_R (Table 2, Fig. 2).

All connectivity pairs with significant differences in RSFC between MDD_wo/INS and MDD_w/INS had a significant correlation with clinical sleep questionnaires. The RSFC between Septal and Cerebellum_Crus1_R in MDD_wo/INS > MDD_w/INS had a negative correlation with ISI ($p = 0.007$) and PSQI ($p = 0.04$) (Table 3, Fig. 3). Conversely, the RSFC of MDD_wo/INS < MDD_w/INS (Habe_L and Rolandic_Oper_R, Habe_L and Cuneus_R, Habe_R and Thal_PuL_R) had a positive correlation with ISI total score ($p = 0.007$, $p = 0.006$, and $p = 0.003$, respectively) (Table 3, Fig. 4), but there was no significant correlation with PSQI total score. The significant regions showing differences in RSFC between the MDD_w/INS and MDD_wo/INS groups were also observed in the seed-to-voxel RSFC analysis of the whole MDD group (Supplementary Tables 1-3, Supplementary Figs. 1-3; available online).

The comparison of RSFC difference between MDD (MDD_wo/INS + MDD_w/INS groups) and HC showed increased RSFC of Habe_L and Habe_R in HC > MDD contrast (Supplementary Table 4, Supplementary Fig. 4; available online). Habe_L had significant RSFC with lobule 8 of the right cerebellar hemisphere (Cerebellum_8_R, p -FDR = 0.049) and lobule 6 of the left cerebellar hemisphere (Cerebellum_6_L, p -FDR = 0.022). Furthermore, Habe_R had significant RSFC with left precuneus cortex (Precuneus_L, p -FDR < 0.001) and right angular gyrus (Angular_R, p -FDR = 0.005). On the contrary, the RSFC of

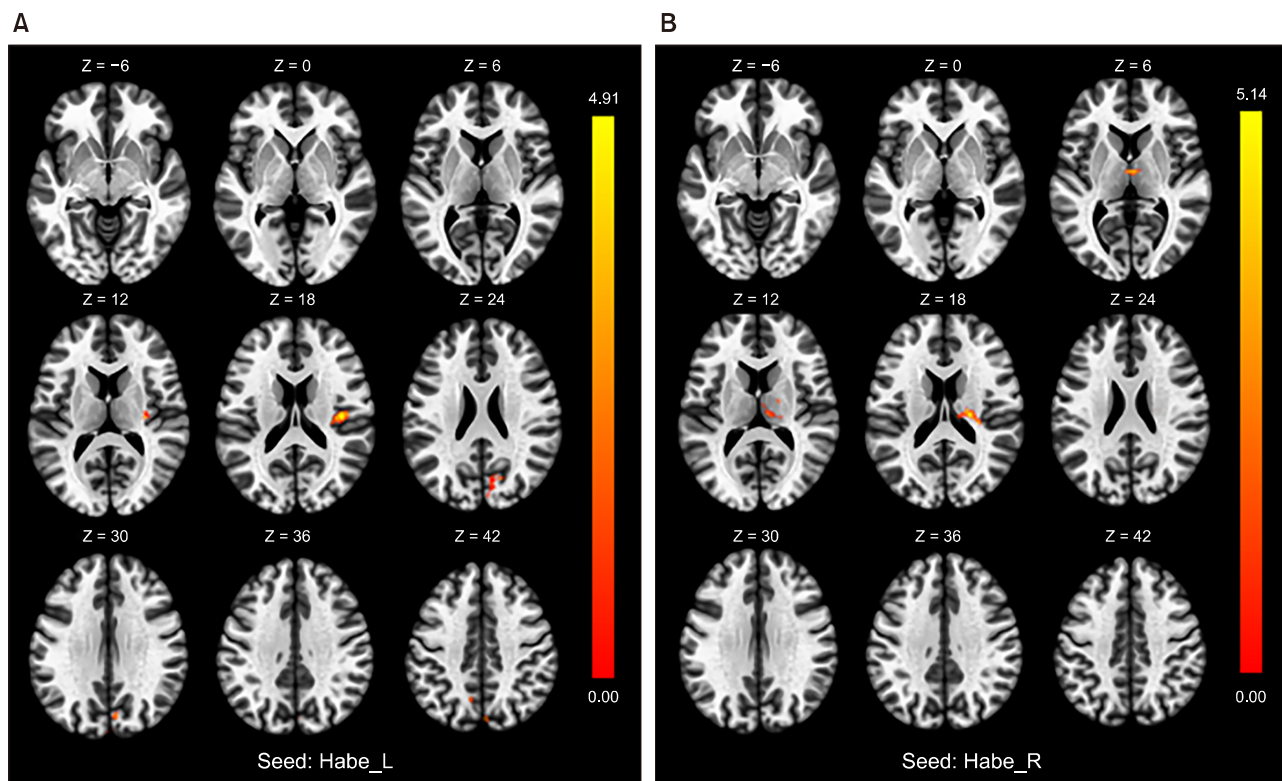


Fig. 2. Functional connectivity regions between MDD_wo/INS < MDD_w/INS contrast at the seed-to-voxel analysis. Target regions of (A) Habe_L and (B) Habe_R in MDD_wo/INS < MDD_w/INS contrast are presented, respectively. Habe_L, left habenula; Habe_R, right habenula; MDD, major depressive disorder; MDD_w/INS, MDD with significant insomnia; MDD_wo/INS, MDD without significant insomnia.

Habe_R with right middle cingulate and paracingulate gyri (Cingulate_Mid_R), right precentral gyrus (Precentral_R) and left postcentral gyrus (Postcentral_L) appeared in HC < MDD contrast (Supplementary Table 4, Supplementary Fig. 5; available online).

DISCUSSION

We found that MDD with insomnia showed increased RSFC in Habe_L - Rolandic_Oper_R, Habe_L-Cuneus_R, Habe_R-Thal_Pul_R and decreased RSFC in Septal - Cerebellum_Crus1_R compared to MDD without insomnia. All regions with significant differences were significantly correlated with insomnia severity.

There have been several previous fMRI studies between HC and insomnia patients without other mental disorders, albeit with limited numbers of subjects. Findings such as abnormal amygdala connectivity, differences in RSFC between parietal and prefrontal cortices, increased insular connectivity, and abnormal thalamocortical functional

connectivity in insomnia have been reported [6,25-27]. However, few studies have compared RSFC of fMRI with and without insomnia in depression. Ye *et al.* [28] found that participants with MDD with high insomnia had increased amygdala-based RSFC with bilateral superior temporal gyrus and decreased RSFC with left supplementary motor area and bilateral postcentral gyrus compared to MDD with low insomnia. Deng *et al.* [29] also reported that MDD with high insomnia patients had a lower dynamic amplitude of low-frequency fluctuation values in the right middle and superior temporal gyrus than MDD with low insomnia patients. Gong *et al.* [30] found that global functional connectivity in the left inferior temporal gyrus and posterior parahippocampal/hippocampal gyrus was associated with insomnia. In contrast, the global functional connectivity in the left anterior parahippocampal/hippocampal gyrus was correlated with non-insomnia symptoms in the MDD group.

Habenula is a structure regulating the activities of monoaminergic neurons in the brain and is involved in sleep,

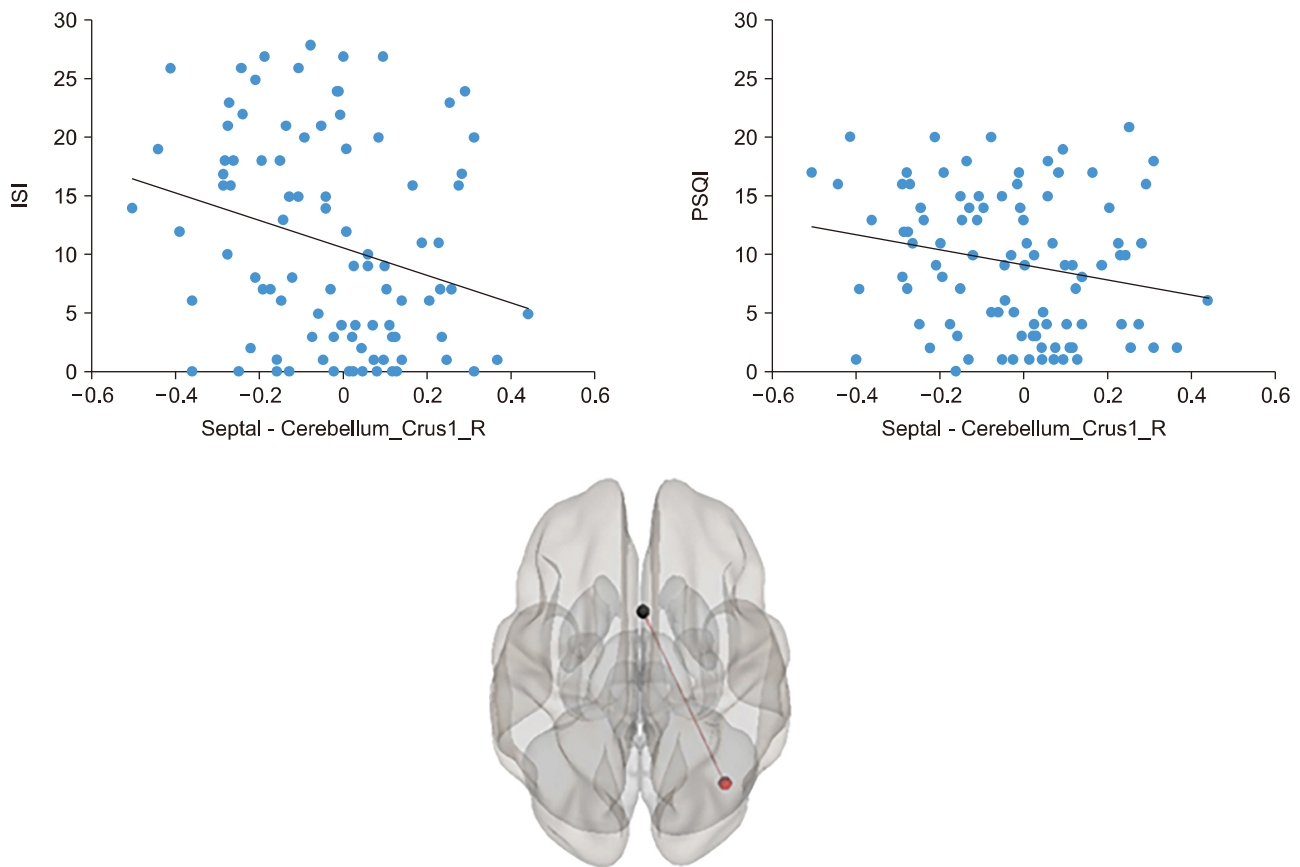


Fig. 3. Scatterplot of negative correlation between RSFC and sleep questionnaire score in MDD_wo/INS > MDD_w/INS. Cerebellum_Crus1_R, Crus I of the right cerebellar hemisphere; ISI, Insomnia Severity Index; MDD, major depressive disorder; MDD_w/INS, MDD with significant insomnia; MDD_wo/INS, MDD without significant insomnia; PSQI, Pittsburgh Sleep Quality Index; RSFC, resting state functional connectivity; Septal, septal nuclei.

wakefulness, and mood [31], and electrophysiological studies in animal experiments have found that the habenular structure is involved in the regulation of REM sleep [11,32]. Moreover, hyperactivation or dysfunction of the habenular neurons in the habenular circuit may cause behavioral and sleep disturbance in depression [11]. Abnormal functional connectivity in the habenula is reportedly associated with subjective hyperarousal state in chronic insomnia [33].

In this study, the RSFC between the left habenula and right Rolandic operculum was larger in the MDD with insomnia group. The previous study showed a significant difference in RSFC between the right Hb - Rolandic operculum between the past anorexia nervosa group and psychiatric patient controls, although multiple comparison correction was not used [34]. The Rolandic operculum is implicated in sleep processes, showing altered connectivity and potential involvement in sleep-wake tran-

sitions, as observed in conditions like insomnia [35]. The Rolandic operculum exhibited increased brain degree centrality before intervention in a real-time fMRI neuro-feedback training study [36], and brain imaging taken before auricular electroacupuncture also revealed increased functional connectivity in the right Rolandic operculum [37], suggesting that it may be a target for intervention to treat insomnia.

The right cuneus is a region that showed decreased nodal centrality in patients with chronic insomnia compared to controls in a study that identified brain functional connectome patterns [38]. The observed alteration of cuneus connectivity in individuals with insomnia aligns with prior research [39], implying that disruptions in visual processing may contribute to the neurobiological mechanisms linking depression and sleep disturbances. The lateral pulvinar thalamus showed volume decline and hypometabolism in a PET/MRI imaging study of fatal familial

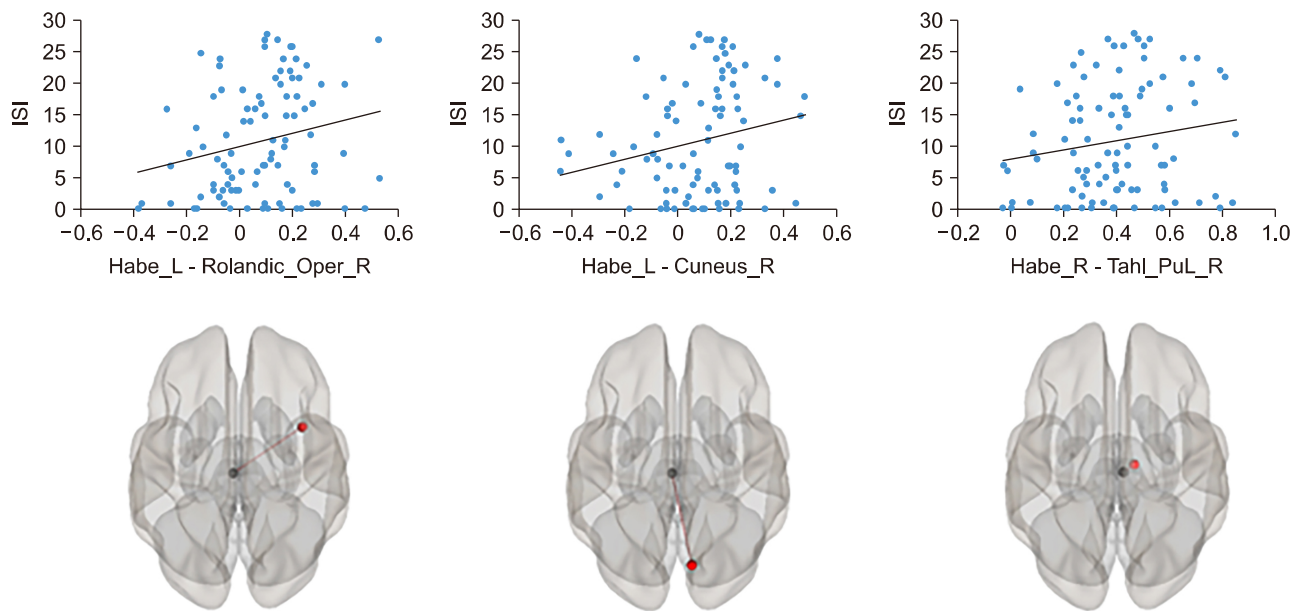


Fig. 4. Scatterplot of positive correlation between RSFC and sleep questionnaire score in MDD_wo/INS < MDD_w/INS. Cuneus_R, right cuneus; Haba_L, left habenula; Haba_R, right habenula; ISI, Insomnia Severity Index; MDD, major depressive disorder; MDD_w/INS, MDD with significant insomnia; MDD_wo/INS, MDD without significant insomnia; Rolandic_Oper_R, right Rolandic operculum; RSFC, resting state functional connectivity; Thal_PuL_R, lateral pulvinar of right thalamus.

insomnia [40]. The thalamic pulvinar, known for its role in attention and arousal, may contribute to the manifestation of insomnia in depressive disorders [41,42]. The interplay between the habenula and thalamic pulvinar connectivity may modulate emotional and regulatory processes within the thalamus, potentially influencing sleep-wake cycles and contributing to the distinctive sleep disturbances observed in MDD [43,44]. Also, Crus I of the cerebellum showed decreased connectivity with the right thalamus in a previous brain imaging study of insomnia patients [45]. The decreased RSFC between septal nuclei and the right cerebellar Crus I (Cerebellum_Crus1_R) in MDD with insomnia of our study highlights a potential disruption in the interaction between mood regulatory structures and cerebellar regions implicated in cognitive and emotional processing [46,47].

The significant difference in RSFC between MDD with and without insomnia and these regions in MDD is supported because the RSFC of all regional pairs with significant differences between the two groups in this study were also significantly correlated with insomnia severity (i.e., ISI score). In addition to this, these findings not only implicate the altered brain connectivity of insomnia in MDD but also highlight the correlation between the de-

gree of RSFC and insomnia severity in MDD. However, only RSFC of Septal - Cerebellum_Crus1_R showed a significant correlation with PSQI, which indicates sleep quality, and this difference might be because PSQI is a clinical measurement of sleep quality and does not directly reflect insomnia severity.

Notably, our findings highlight a lateralization pattern of habenular connectivity in patients with MDD and insomnia, where the left habenula was more strongly connected to regions implicated in sensory integration (i.e., the Rolandic operculum and cuneus). In contrast, the right habenula was linked to the pulvinar thalamus. This lateralized pattern is consistent with emerging evidence suggesting a functional asymmetry of the habenula, with the left side more involved in affective processing and the right side in arousal regulation [30,33,48]. The distinct involvement of the right habenula-pulvinar circuit in MDD with insomnia may reflect a hyperarousal component characteristic of insomnia, whereas the enhanced connectivity between the left habenula and sensory-related cortices may be related to altered internal salience processing and hypervigilance. Further research is warranted to explore these hemispheric differences in the context of depression and sleep regulation.

Limitations

Although this study provides valuable insights into the neurobiological differences associated with insomnia in MDD, several limitations should be acknowledged. First, the cross-sectional design limits causal inference and precludes assessment of longitudinal changes in RSFC patterns over time. In addition, the relatively small sample size may limit the generalizability of the findings to broader populations of individuals with MDD and insomnia. Furthermore, the inclusion of objective measures of sleep, such as polysomnography, may provide deeper insights into the relationship between RSFC patterns and sleep disturbances in MDD.

Conclusion

The present study provides valuable insights into the distinct RSFC patterns associated with insomnia in MDD, emphasizing the interconnectedness of mood and sleep at the neural level. Previous studies have examined the RSFC in fMRI with and without insomnia, but few studies have examined the RSFC in MDD with insomnia, so this study is particularly meaningful because it was conducted with and without insomnia within MDD. In addition, the significance of this study is that it is the fMRI study to seed the habenula and septal nuclei, which play an important role in insomnia, sleep, and mood. In the future, the features of brain function according to depression subtype and clinical presentation need to be further studied, and follow-up studies with larger, more diverse samples and longitudinal designs are needed to confirm and extend the findings of this study and to elucidate the dynamic interplay between depression and insomnia at the neural level. Further research is warranted to elucidate the underlying mechanisms of MDD with insomnia and ultimately contribute to the development of targeted treatment interventions for individuals experiencing the complex interplay of depression and insomnia.

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Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Author Contributions

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