ORIGINAL RESEARCH

The Abnormal N-Acetylaspartate to Creatine Ratio of the Right Putamen is Linked to Wakefulness in Patients with Insomnia Disorder

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Purpose: Converging evidence implicates the putamen in sleep-wake regulation. However, its role remains unclear. We hypothesized that metabolic abnormalities in the putamen are linked to insomnia disorder, which has not been previously addressed, and investigated putaminal N-acetylaspartate (NAA), choline (Cho), and creatine (Cr) in patients with insomnia disorder compared to healthy controls.
Participants and Methods: In the present study, the concentrations of NAA, Cho, and Cr in the putamen of 23 patients with insomnia disorder and 18 healthy controls were determined using proton magnetic resonance spectroscopy. Sociodemographic, psychometric, and polysomnography data were obtained from all participants.
Results: We found that the mean NAA/Cr ratio of the right putamen was significantly greater in the insomnia group compared to the control group and also greater than the left putamen within the insomnia group. The NAA/Cr ratio of the right putamen distinguished insomnia disorder from normal sleep with 78.3% sensitivity and 61.1% specificity. Furthermore, this ratio positively correlated with both objective and subjective insomnia severity and sleep quality.
Conclusion: Our findings provide critical evidence for the dysfunctional putaminal metabolism of NAA/Cr in insomnia disorder, suggesting that the abnormal NAA/Cr ratio of the right putamen is linked to wakefulness in patients with insomnia disorder and may serve as a potential biomarker of insomnia disorder.
Keywords: insomnia disorder, wakefulness, putamen, proton magnetic resonance spectroscopy, NAA/Cr ratio, polysomnography

Introduction

Chronic insomnia is characterized as the dissatisfaction with the ability to initiate and maintain sleep and sleep duration and/or quality.[1,2] It is one of the most common medical complaints with a high prevalence of 4%-50% globally.[1–5] Affected individuals experience significant distress and impaired daytime functioning and are prone to developing psychiatric and cardiovascular disorders, resulting in increased healthcare burden.[1,2,6–8] Despite the high prevalence and socioeconomic burden, the neurobiology of insomnia, especially the brain mechanisms of insomnia, has remained elusive, hampering the development of effective treatments.[1,9,10]

The putamen, as a key component of the striatum and a critical component of the fronto-striatal circuit that governs reward and motivation, is well recognized for its role in motor control and movement disorders.[11–14] However, emerging studies have focused on the role of the putamen in sleep-wake regulation. Firstly, the putamen has been shown to regulate arousal by inhibitory GABAergic projections to pallidum and thalamus, promoting cortical activity and wakefulness.[15,16] Consistent with this, bilateral lesions of the putamen in rats reduced the time spent in wakefulness, and led to fragmentation of both sleep and wakefulness.[15–19] Secondly, morphometric studies in human studies show that insomnia is associated with altered anatomical measures of the putamen.[20–24] For instance, lower putaminal volume is associated with higher arousal indices measured by polysomnography in patients with chronic insomnia.[24] Similarly, smaller

volumes of the putamen and caudate have also been observed in psychophysiological and paradoxical insomnia patients, along with associations of both subjective and objective sleep parameters with the shape alterations of the putamen.[20] In contrast, in healthy older adults, greater bilateral putaminal volumes correlated with greater insomnia severity.[21] Thirdly, studies suggest a functional role of the putamen associated with sleep parameters.[20,21,24–27] For example, using resting-state functional magnetic resonance imaging (fMRI), the area under the curve of the nodal betweenness centrality of the right putamen was found to be positively associated with the three-item sleep subscale on the Hamilton Depression Scale-17.[25] In addition, using task-based fMRI with emotional stimuli, patients with chronic insomnia showed lower activation of the putamen, ventral striatum, right insula, orbitofrontal cortex and ventral tegmental area compared to healthy controls, which correlated with severity of insomnia.[26] Similarly, emotional reactivity to positive stimuli (humorous films) in patients with insomnia also demonstrated a potential link of insomnia to putaminal activity.[27] Fourthly, the putamen shows abnormalities in connectivity with multiple brain regions linked to insomnia.[25,28,29] By way of illustration, patients with insomnia disorder demonstrated decreased thalamic connectivity with the putamen across wakefulness and all three nonrapid eye movement sleep stages.[28] Similarly, increased resting-state functional connectivity of the putamen with the nucleus accumbens was found in patients with insomnia disorder.[29] Lastly, other relevant studies have also shed light on the link of putaminal function to sleep. Increased right putaminal activation was observed in subjects after total sleep deprivation, suggesting that arousal levels may be linked to putaminal activity.[30] Compared with the normal controls, increased phase shift values in the left putamen and left caudate were detected in patients with primary insomnia in an in vivo susceptibility-weighted imaging study.[31] Interestingly, as a manifestation of physiological arousal, restlessness has been linked to putaminal function, whereas motor regulation is linked to connectivity with the primary motor cortex and premotor areas.[13]

Noteworthily, clinical evidence indirectly reflects the sleep-wake regulatory role of the putamen as well. Sleep disorders are highly common in patients with Parkinson's disease characterized by loss of dopaminergic neurons in the dorsal striatum,[32] with an estimated prevalence of 60%-80%.[33] Using [^{11}C]DASB positron emission tomography, Parkinson's patients with sleep dysfunction showed decreased [^{11}C]DASB BP_{ND} values in the putamen, caudate, ventral striatum, thalamus, hypothalamus, and raphe nuclei, compared to those without sleep dysfunction.[34] Besides, in patients with Huntington's disease, where the putamen can also become severely impaired, multiple sleep disturbances have been observed including increased sleep latency, frequent nocturnal awakenings, more time spent awake, reduced sleep efficiency and slow wave sleep.[35,36]

Taken together, the above findings highlight the potential role of the putamen in the mechanisms of insomnia, although the findings are not always consistent with both increases and decreases in volume or connectivity, along with either positive or negative correlations with sleep parameters. Importantly, the role of metabolic function of the putamen in insomnia remains unclear.

Proton magnetic resonance spectroscopy (^{1}H-MRS) offers a noninvasive method to measure brain metabolism and neurochemistry.[37] As measured using ^{1}H-MRS, N-acetylaspartate (NAA, in which a decrease can reflect neuronal degeneration),[37–39] choline (Cho, which reflects non-specific cell membrane breakdown),[37,40] and creatine (Cr, whose content is relatively stable and not easily affected by various pathological conditions and often used as a reference to measure the content of other metabolites)[37,41] are common neurobiochemical metabolites used to assess disease status.[37] Therefore, in order to further understand the role of the putamen in sleep-wake regulation, especially in insomnia disorder, we investigated putaminal metabolites in patients with insomnia disorder and healthy normal sleepers by utilizing 2D multi-voxel ^{1}H-MRS. Specifically, we hypothesized that (1) NAA/Cr and/or Cho/Cr ratios of the bilateral or unilateral putamen in patients with insomnia disorder would differ from those in healthy normal sleepers, and the direction of this difference would reflect the pathophysiological significance of NAA, Cho, and Cr within the putamen in insomnia disorder; (2) there are close correlations between putaminal metabolites and sleep parameters; and (3) altered putaminal metabolites may be a potential biomarker of insomnia.

Materials and Methods
Study Procedure and Participants
In the present study, right-handed participants were recruited through advertisements from community population and from outpatient units of the Department of Psychiatry, The First Affiliated Hospital of Jinan University, Guangzhou,

China. As shown in Figure 1, patients with insomnia disorder (insomnia group) were selected from 195 individuals with insomnia complaints. Age-sex-education-nationality matched healthy normal sleepers (control group) were selected from 50 individuals without insomnia complaints and medical history who were willing to participate in the present study. All participants were evaluated with an unstructured clinical interview for history of medical and sleep disorders, and a structured interview for lifetime history of psychiatric disorders using the Mini-International Neuropsychiatric Interview (M.I.N.I.),[42] Chinese version.[43] Insomnia disorder was diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria by a senior psychiatrist, based on participants' primary complaints of sleep disturbance despite adequate opportunity for sleep, distresses or impairments of functioning caused by the sleep disturbance, and duration of the sleep difficulty. Meanwhile, it was required that the insomnia was not attributable to any coexisting mental disorder and medical condition, or the physiological effects of a substance.

The inclusion criteria of our study included (1) age ≥ 18 years and ≤ 60 years; (2) capability to complete psychometric assessments and polysomnography (PSG); (3) unmedicated condition (to avoid the potential effects of medication, we recruited only unmedicated participants); (4) no previous or current non-pharmacological treatment for insomnia disorder, such as cognitive-behavioral therapy, was received; (5) no shift work and no staying up late in last 3 months; (6) informed consent; and (7) a current diagnosis of DSM-5 primary insomnia disorder for the insomnia subjects; no
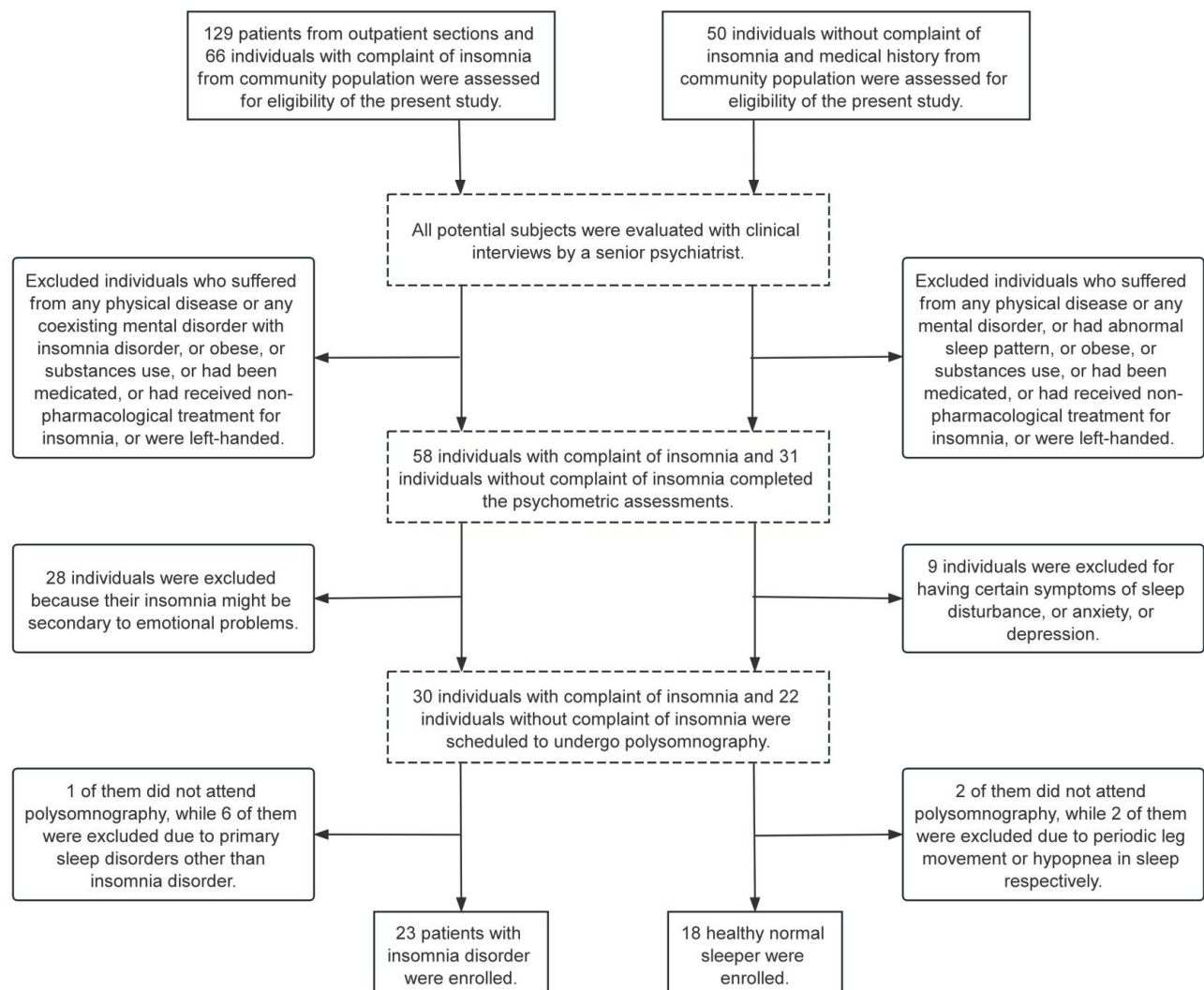

Figure 1 Recruitment flow chart of the subjects.
Note: Diagnosis of insomnia disorder was made according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition criteria by a senior psychiatrist.

sleep complaints for the control subjects. The exclusion criteria were as follows: (1) any other current psychiatric disorder (including substance abuse and dependence); (2) any current sleep disorder other than insomnia disorder; (3) known acute or chronic medical conditions; (4) a trans-meridian flight during the last three months; (5) use of medications or substances known to affect sleep or mood, or abnormal coffee and/or alcohol consumption; (6) known dementia or other cognitive impairment; and (7) pregnant and lactating women. Individuals who had abnormal sleep patterns were also excluded from the control group. In addition, participants with either an apnea-hypopnea index or a periodic limb movements of sleep index greater than 5 were excluded from the present study. For control subjects, a sleep efficiency greater than 90%, combined with a subjective report of sleep similar to usual, was required.

All participants provided written informed consent before their participation in the study. This study was conducted in accordance with the Declaration of Helsinki and as per the protocol approved by the Human Research and Ethics Committee of The First Affiliated Hospital of Jinan University, Guangzhou, China (approval number: KY-2023-110).

Clinical Assessment Instruments
Sociodemographics
The weight and height of all subjects were measured and used to calculate body mass index (BMI). Other basic information, including age, sex, education level, occupation, smoking status, and alcohol intake, was measured using binary or ordinal questions.

Psychometric Assessments
Assessments including the Chinese versions of the Pittsburgh Sleep Quality Index (PSQI),[44,45] Insomnia Severity Index (ISI),[46,47] State-Trait Anxiety Inventory (STAI),[44,48] and Beck Depression Inventory, 2nd edition (BDI-II)[44,49] were performed on all subjects.

PSGs
All subjects underwent one night of in-laboratory PSG to eliminate first-night effects and to preliminarily screen them for potential sleep disorders, and one additional night for the assessment of sleep architectures, utilizing a dynamic 64-lead PSG device (Compumedics, Australia) with a continuous recording time of at least 8 hours.[46] Subjects were instructed not to take stimulating substances and psychoactive substances, including nicotine, alcohol, and coffee and to abstain from high intensity exercise for 24 hours before the PSG measurements. Surface electrodes were used to record electroencephalograms, electrooculograms, electromyograms (EMG), and electrocardiograms. Apnea and hypopnea were monitored via oximetry, respiratory efforts (abdominal and thoracic efforts), nasal airflow, and nasal pressure. Periodic limb movements were monitored via EMG of the anterior tibialis. The sleep parameters of all the subjects were reviewed and confirmed by a skilled PSG technician.

Neuroimaging Data Acquisition and Analysis
The subjects were taken to the Medical Imaging Center for MRS acquisition on the second night before PSG recording. All subjects underwent ^{1}H-MRS scans between 19:00–20:00 (ie, approximately 3 h before their usual bedtime).

Data Acquisition
MRI/MRS data were acquired on a 3.0T MR scanner (Discovery MR 750, GE Healthcare, Milwaukee, WI, USA) with an eight-channel phased array head coil.[50] Subjects were scanned in a supine, head-first position with symmetrically placed cushions on both sides of the head to decrease motion. Ear plugs were used to reduce the noise. Subjects were instructed to relax with their eyes closed but remain awake for the duration of the scanning session.

Routine axial T1-weighted fluid attenuation inversion recovery (T1 Flair) [repetition time (TR) = 1750 ms, echo-time (TE) = 24 ms, numbers of excitation (NEX) = 1, field of view (FOV) = 24×24 cm, matrix = 320 × 256, slice thickness = 5 mm, gap = 1.5 mm, acquisition time = 1 min and 22 sec] and fast spin echo T2 -weighted images (T2WI) [TR = 8400 ms, TE = 145 ms, NEX = 1, FOV = 24×24 cm, matrix = 256 × 256, slice thickness = 5 mm, gap = 1.5 mm, acquisition

time = 2 min and 15 sec] were obtained for anatomical localization and excluding any anatomic abnormalities including any brain tumors, vascular disease, and other cerebral illnesses.

The spectra were acquired using a 2D multi-voxel technique. The volume of interest (VOI) was placed in a uniform manner by the same investigator, a trained radiologist who was blinded to the diagnosis of each participant. Figure2 shows the locations of the bilateral putamen, which were determined based on anatomical localization acquired by axial T2WI (TR = 3500 ms, TE = 102 ms, NEX = 2, FOV = 24×24 cm, spatial matrix = 256 × 256, slice thickness = 5 mm without a gap, acquisition time = 1 min and 45s), VOI, and the voxels. The MRS acquisition parameters were as follows: TR = 1000ms, TE = 144ms, FOV = 24×24 cm, NEX = 2, matrix = 18 × 18, slice thickness = 10 mm, and nominal voxel size = 13×13 × 10 mm^3.

Automatic pre-scanning was performed before each spectroscopic scan to achieve an optimal full-width half-maximum of 10 Hz. General quality standard spectra with a line width greater than 10 Hz or water suppression less than 98% efficiency were excluded. Additional saturation bands were placed outside the VOI to minimize lipid contamination from the scalp. The total acquisition time for each participant in ^{1}H-MRS sequence was 5 min 28s.
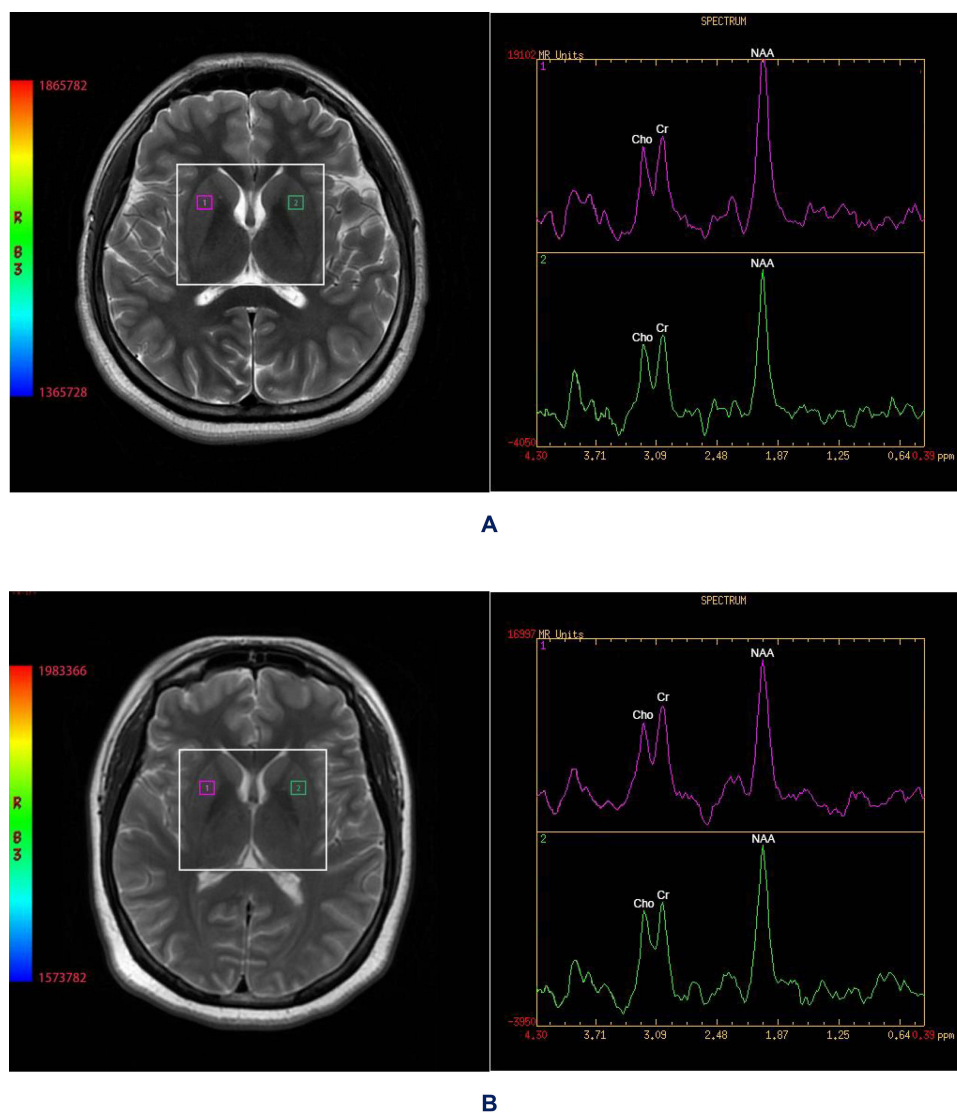

Figure 2 Examples of volume of interests (VOIs) and chemical shifts for the two groups.
Note: As shown in Figure2, the images on the left show the locations of VOIs placed in the bilateral putamen for insomnia subject (**A**) and control subject (**B**). While the images on the right show the chemical shifts of metabolites including N-acetylaspartate (NAA), choline (Cho), and creatine (Cr) corresponding to the locations on the left images. The NAA, Cr, and Cho resonances spiked at 2.02 ppm, 3.02 ppm and 3.23 ppm, respectively.

Spectral Fitting

All spectra were fitted using the automated fitting software Functool (version 9.4.05a, GE Advantage Workstation 4.5), provided by the manufacturer. The metabolite basis sets in vivo proton spectral quantitation were simulated using software developed on site.

Statistical Analyses

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) 26.0 software. Descriptive statistics were presented as percentages for discrete variables and as means (standard deviations) for continuous variables. The Shapiro–Wilk test was used to assess whether the data were normally distributed. Chi-square test and independent-samples t-test or paired-samples t-test were used to compare the differences in demographic factors, scores of psychometric assessments, PSG parameters, and metabolites measured by ^{1}H-MRS between the insomnia and control groups, where appropriate. Multivariate Analysis of Covariance (MANCOVA) was used to study the differences in bilateral putaminal metabolites between the two groups, with adjustment for sex, age, BMI, education, occupation, and total scores of SAI, TAI, and BDI-II. A partial correlation analysis was conducted to analyze the associations between putaminal metabolites and sleep parameters after controlling for sex, age, BMI, education, and occupation. Receiver operating characteristic (ROC) curve analysis was performed to explore the role of putaminal metabolites in distinguishing insomnia disorder from normal sleep. A P value less than 0.05 was considered statistically significant.

Results

Sociodemographics and Clinical Characteristics

As shown in Figure 1 and Table 1, we enrolled 23 patients with insomnia disorder (65.2% female; mean [S.D.] age, 31.3 [8.1] years; mean [S.D.] BMI, 20.7 [2.1] kg/m^2; mean [S.D.] education, 15.4 [3.4] years; 91.3% employee/student) from 195 individuals with insomnia complaints. Similarly, we enrolled 18 age-sex-education-nationality matched healthy

Table 1 Demographic, Clinical and Sleep Characteristics of the Two Groups

	Insomnia Group (n = 23)	Control Group (n = 18)	P
Sex (Female, %)	15 (65.2)	10 (55.6)	0.529
Age (years)	31.3 ± 8.1	28.2 ± 6.9	0.203
BMI (kg/m^2)	20.7 ± 2.1	21.1 ± 2.7	0.623
Education (years)	15.4 ± 3.4	14.9 ± 2.3	0.558
Occupation (Employee/Student, %)	21 (91.3)	17 (94.4)	0.513
PSQI total score	12.0 ± 2.9	3.6 ± 1.7	**< 0.001**
ISI total score	17.0 ± 4.6	3.1 ± 3.8	**< 0.001**
SAI total score	45.3 ± 11.2	32.5 ± 10.0	**< 0.001**
TAI total score	47.1 ± 12.2	33.6 ± 8.4	**< 0.001**
BDI-II total score	11.6 ± 6.0	3.7 ± 3.8	**< 0.001**
Sleep onset latency (min)	35.1 ± 14.1	12.3 ± 9.2	**< 0.001**
Sleep efficiency (%)	77.3 ± 11.3	87.6 ± 5.2	**0.001**
Total sleep time (min)	388.2 ± 51.8	421.7 ± 60.6	0.063
Awake time after sleep onset (min)	82.5 ± 65.3	36.8 ± 20.1	**0.004**
Arousal index (/h)	18.5 ± 7.5	9.5 ± 3.9	**< 0.001**
NREM sleep stage 1 (%)	10.7 ± 4.0	7.0 ± 4.1	**0.006**
NREM sleep stage 2 (%)	53.1 ± 7.2	53.5 ± 5.1	0.846
NREM sleep stage 3 (%)	16.4 ± 6.2	21.2 ± 6.1	**0.019**
REM sleep (%)	19.7 ± 3.4	18.3 ± 4.5	0.236

Notes: Data are presented as sample size (percentage) for categorical variables, and mean ± standard deviation for continuous variables. *P*-values are given for the comparison of each characteristic between the insomnia group and the control group. *P*-values ≤ 0.05 are indicated in bold.
Abbreviations: BMI, body mass index; PSQI, Pittsburgh Sleep Quality Index; ISI, Insomnia Severity Index; SAI, State Anxiety Inventory; TAI, Trait Anxiety Inventory; BDI-II, Beck Depression Inventory (2nd edition); NREM, non-rapid eye movement; REM, rapid eye movement.

normal sleepers (55.6% female; mean [S.D.] age, 28.2 [6.9] years; mean [S.D.] BMI, 21.1 [2.7] kg/m^2; mean [S.D.] education, 14.9 [2.3] years; 94.4% employee/student) who served as the control group from 50 individuals without insomnia complaints and medical history.

There were no significant differences in demographics profile between the insomnia group and the control group with regard to sex (χ^2 = 0.40, P = 0.529), age (t = 1.29, P = 0.203), BMI (t = −0.50, P = 0.623), education (t = 0.59, P = 0.558) and occupation (χ^2 = 0.43, P = 0.513) (Table 1).

Compared to the control group, the insomnia group displayed higher PSQI total score (12.0 ± 2.9 vs 3.6 ± 1.7, t = 11.89, P < 0.001), ISI total score (17.0 ± 4.6 vs 3.1 ± 3.8, t = 10.39, P < 0.001), SAI total score (45.3 ± 11.2 vs 32.5 ± 10.0, t = 3.81, P < 0.001), TAI total score (47.1 ± 12.2 vs 33.6 ± 8.4, t = 4.01, P < 0.001), and BDI-II total score (11.6 ± 6.0 vs 3.7 ± 3.8, t = 5.13, P < 0.001) (Table 1).

Table 1 also shows the sleep parameters of all subjects measured on the second night of the laboratory PSG. The insomnia group had a significantly lower sleep efficiency (77.3 ± 11.3 vs 87.6 ± 5.2%, t = −3.85, P = 0.001) and a lower stage 3 sleep percentage (16.4 ± 6.2 vs 21.2 ± 6.1%, t = −2.44, P = 0.019) than the control group. The insomnia group also displayed longer sleep latency (35.1 ± 14.1 vs 12.3 ± 9.2 min, t = 5.68, P < 0.001), greater awake time after sleep onset (82.5 ± 65.3 vs 36.8 ± 20.1 min, t = 3.17, P = 0.004), greater arousal index (18.5 ± 7.5 vs 9.5 ± 3.9 per hour, t = 4.96, P < 0.001), and greater stage 1 sleep percentage (10.7 ± 4.0 vs 7.0 ± 4.1%, t = 2.89, P = 0.006) than the control group.

MRS Characteristics

None of the subjects displayed any anatomical abnormalities on routine MRI.

The NAA resonance spikes at 2.02 ppm, while Cr and Cho resonance spikes at 3.02 ppm and 3.23 ppm in subjects, respectively (Figure 2). Notably, altered resonance was noted in the right putamen of the subjects with insomnia disorder.

We compared the differences in bilateral putaminal metabolites between the two groups and within the groups. As shown in Table 2, the results of Independent-samples t test showed that the NAA/Cr ratio of the right putamen in the insomnia group was significantly greater than that in the control group (1.69 ± 0.16 vs 1.52 ± 0.15, t = 3.46, P = 0.001). Furthermore, as shown in Table 3, the results of the paired-samples t-test showed that the NAA/Cr ratio of the right putamen was significantly greater than that of the left putamen in the insomnia group (1.69 ± 0.16 vs 1.45 ± 0.17, t = 5.17, P < 0.001), but not in the control group (1.52 ± 0.15 vs 1.44 ± 0.19, t = 1.31, P = 0.207).

Additionally, MANCOVA was conducted to control for factors including sex, age, BMI, education, occupation, SAI total score, TAI total score, and BDI-II total score, which showed that there was only a significant group difference with regard to the NAA/Cr ratio in the right putamen (1.71 ± 0.04 vs 1.50 ± 0.05 (adjusted mean ± standard error), F = 8.633, P = 0.006). The TAI score tended to influence the NAA/Cr ratio in the right putamen (F = 3.986, P = 0.055).

To further evaluate the significance of the right putaminal NAA/Cr ratio difference between the two groups, ROC curve analysis was used to assess the ability of this difference to distinguish insomnia disorder from normal sleep. As shown in Figure 3, the results revealed that the NAA/Cr ratio of the right putamen could distinguish insomnia disorder from normal sleep with 78.3% sensitivity and 61.1% specificity when the cut-off value was 1.57 in all subjects (area under the curve (AUC) = 0.79, P = 0.002). ROC curve analysis was further used to determine if its efficacy varied by sex. In males, the NAA/Cr ratio of the right putamen could distinguish insomnia disorder from normal sleep with 100% sensitivity and 62.5% specificity when the cut-off

Table 2 Comparison of Metabolites in Bilateral Putamen Between the Two Groups

Metabolite	Left/Right Putamen	Insomnia Group (n=23)	Control Group (n=18)	P
NAA/Cr	Left putamen	1.45 ± 0.17	1.44 ± 0.19	0.975
	Right putamen	1.69 ± 0.16	1.52 ± 0.15	**0.001**
Cho/Cr	Left putamen	0.76 ± 0.17	0.74 ± 0.17	0.647
	Right putamen	0.70 ± 0.13	0.66 ± 0.13	0.364

Notes: Data are presented as mean ± standard deviation for continuous variables. *P*-values are given for the comparisons of NAA/Cr and Cho/Cr between the insomnia group and the control group. *P*-values ≤ 0.05 are indicated in bold.
Abbreviations: NAA, N-acetylaspartate; Cho, choline; Cr, creatine.

Table 3 Comparison of Metabolites in Bilateral Putamen Within Each Group

Metabolite	Group	Left Putamen	Right Putamen	P
NAA/Cr	Insomnia group (n=23)	1.45 ± 0.17	1.69 ± 0.16	**< 0.001**
	Control group (n=18)	1.44 ± 0.19	1.52 ± 0.15	0.207
Cho/Cr	Insomnia group (n=23)	0.76 ± 0.17	0.70 ± 0.13	0.155
	Control group (n=18)	0.74 ± 0.17	0.66 ± 0.13	0.178

Notes: Data are presented as mean ± standard deviation for continuous variables. *P*-values are given for the comparisons of NAA/Cr and Cho/Cr within the insomnia group and within the control group. *P*-values ≤ 0.05 are indicated in bold.
Abbreviations: NAA, N-acetylaspartate; Cho, choline; Cr, creatine.

value was 1.54, or with 62.5% sensitivity and 75% specificity when cut off value was 1.62 (AUC = 0.813, P = 0.036). In females, 80% sensitivity and 60% specificity when the cut-off value was 1.57, or with 66.7% sensitivity and 70% specificity when the cut-off value was 1.64 (AUC = 0.780, P = 0.020).

The Cho/Cr ratio of bilateral putamen did not vary significantly between the two groups (0.76 ± 0.17 vs 0.74 ± 0.17, t = 0.46, P = 0.647, and 0.70 ± 0.13 vs 0.66 ± 0.13, t = 0.92, P = 0.364) (Table 2) or within the insomnia group (0.76 ± 0.17 vs 0.70 ± 0.13, t = −1.47, P = 0.155) (Table 3). The ROC curve analysis also showed no significant differences with regard to the Cho/Cr ratio of the bilateral putamen between the two groups (AUC = 0.604, P = 0.259) or within the male (AUC = 0.406, P = 0.529) and female (AUC = 0.720, P = 0.067) subjects.

Associations Between Putaminal Metabolites and Sleep Parameters of the Subjects

After controlling for sex, age, BMI, education, occupation, SAI total score, TAI total score, and BDI-II total score, the results of the partial correlation analyses showed that the NAA/Cr ratio of the right putamen was significantly correlated with the arousal index measured by PSG (r = 0.452, P = 0.008), the PSQI total score (r = 0.405, P = 0.020), and the ISI total score (r = 0.439, P = 0.011).

Discussion

In the present study, we investigated the neurometabolites in the bilateral putaminal regions of patients with insomnia disorder and healthy normal sleepers. We found differences in the NAA/Cr ratio between the insomnia patients and the
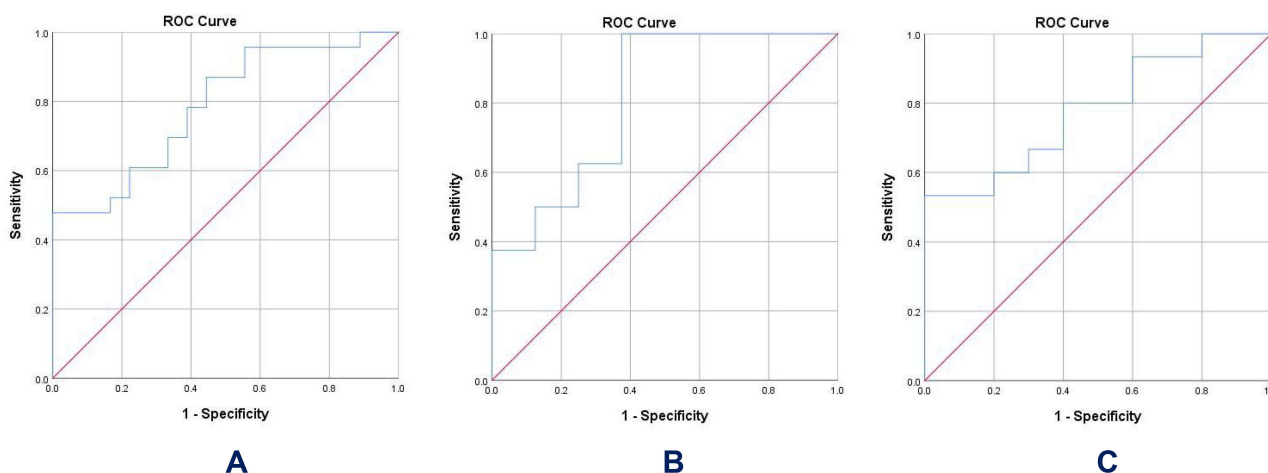

Figure 3 Results of receiver operating characteristic (ROC) curve analyses.
Note: ROC curve analyses were used to establish the efficacy and the cut off value of the right putaminal NAA/Cr ratio in distinguishing insomnia disorder from normal sleep. The right putaminal NAA/Cr ratio could distinguish insomnia disorder from normal sleep with 78.3% sensitivity and 61.1% specificity when cut off value was 1.57 in all subjects (area under the curve (AUC) = 0.785, P = 0.002) (**A**); with 100% sensitivity and 62.5% specificity when cut off value was 1.54 (or with 62.5% sensitivity and 75% specificity when cut off value was 1.62) in male subjects (AUC = 0.813, P = 0.036) (**B**); with 80% sensitivity and 60% specificity when cut off value was 1.57 (or with 66.7% sensitivity and 70% specificity when cut off value was 1.64) in female subjects (AUC = 0.780, P = 0.020) (**C**), respectively.

healthy controls, with a hemispherical lateralization effect in the right putamen. In particular, the NAA/Cr ratio of the right putamen positively correlated with objective and subjective insomnia severity. Additionally, robust results in discriminating insomnia disorder from normal sleep using MRS data were demonstrated.

To the best of our knowledge, the present study is the first to demonstrate putaminal NAA/Cr metabolic abnormalities in patients with insomnia disorder. Important confounders such as sex, age, and BMI were controlled between the two groups, and all subjects were unmedicated and carefully screened to be free of medical illnesses and other psychiatric disorders or secondary causes of insomnia, in order to better understand the role of putaminal metabolites including NAA and Cho in insomnia.

Consistent with our hypothesis, ^{1}H-MRS data of the present study showed a greater NAA/Cr ratio in the right putamen of patients with insomnia disorder than in healthy normal sleepers. Specifically, the NAA/Cr ratio of the right putamen was significantly and positively correlated with the arousal index measured by PSG, PSQI total score, and ISI total score, indicating that more severe insomnia was associated with more disrupted right putaminal NAA/Cr metabolism. This is consistent with previous studies that demonstrated close associations between putaminal characteristics and insomnia.[20,21,24–27] Importantly, the identified neurobiochemical marker demonstrated that insomnia without any underlying psychiatric comorbidity may be characterized by the altered NAA/Cr ratio of the putamen, which together with previous studies further confirmed the sleep-wake regulatory role of the putamen. Additionally, the identified neurobiochemical marker was independent of sex, age, BMI, educational level, occupation status, and level of anxiety and depression symptoms, which were controlled for in group comparisons. These novel findings also provide evidence that disrupted neurobiochemical metabolites of the frontostriatal circuits are associated with insomnia disorder, suggesting that these networks contribute to the neural circuitry that underlies insomnia disorder.[51,52]

However, it is worth noting that previous studies have revealed inconsistent putamen volume changes in individuals with insomnia, as well as variable results for the link between the characteristics of the putamen and insomnia severity. Notably, an increased NAA/Cr ratio in the right putamen of patients with insomnia disorder was observed in the present study when compared with healthy normal sleepers. As NAA/Cr is generally considered to be a marker of neuronal viability and integrity and is often used to assess neuronal density,[37–39] our findings indicate dysfunction (enhanced) of the right putamen in patients with insomnia disorder. Thus, our results support the effect of up-regulated putaminal functional status on insomnia severity, in line with its suggested role of promoting and maintaining wakefulness.[15–19] Consistent with our findings, Emamian et al reported that poor subjective and objective sleep quality is linked with greater volumes of the lateral putamen, and speculated that this might be a compensatory mechanism to dysfunction of the limbic circuits.[20]

Based on our findings and those of other related studies, we inferred that an altered right putaminal NAA/Cr ratio is strongly linked to wakefulness (arousal) in insomnia disorder. It has been suggested that the putamen regulates arousal by inhibitory GABAergic projections to pallidum and thalamus, promoting cortical activity and wakefulness.[15] Along the same lines, bilateral putamen lesions produced both a reduction in the amount of wakefulness and alterations in sleep-wake architecture.[15–19] Furthermore, dopamine D1 receptor-positive neurons within the dorsal striatum, where the putamen is located, has been implicated in promoting wakefulness.[53] Together these findings are consistent with our observations of greater right putaminal NAA/Cr ratio with greater severity of insomnia. Rats with core lesions in the nucleus accumbens exhibited an increase in wakefulness and a reduction in nonrapid eye movement sleep-bout duration.[15–19] This suggests that the putamen and nucleus accumbens core may play opposing roles in sleep-wake regulation. Thus, in this line, our findings support the crucial function of the putamen in promoting and maintaining wakefulness, which is consistent with the hyperarousal model of insomnia.[54]

The putamen is highly interconnected with frontal cortical areas, which constitute important components of the frontostriatal circuits implicated in sleep-wake regulation.[25] Altered patterns of connectivity between the putamen and frontal cortical regions in insomnia patients have been observed in fMRI studies.[28,55–57] Therefore, our findings provide further evidence for impairments in the frontostriatal circuits correlated with insomnia disorder.

Hemispheric asymmetry of the NAA/Cr ratio in the right putamen is particularly intriguing. Brain hemispheric asymmetry in structure and function has been demonstrated across a range of processes,[58] such as the neural processing of language, faces, or emotions.[59,60] Bilateral age-related shrinkage of the putamen also shows hemispherical

lateralization with a significant rightward asymmetry in the putamen and the caudate.[61] Patients with psychiatric disorders often demonstrate atypical hemispheric asymmetry.[58] This phenomenon was further confirmed by our finding of the NAA/Cr metabolism in the right putamen of patients with insomnia disorder. However, the exact mechanism linking this hemispheric asymmetry and insomnia disorder requires further investigation.

Notably, the results of the ROC curve analysis in this study revealed the cut-off value of the NAA/Cr ratio in the right putamen between insomnia and normal sleep. Taken together with other results, we suggest that the NAA/Cr ratio of the right putamen may be a potential candidate biomarker of insomnia disorder, warranting future comprehensive neurochemical profiling of the putamen. In addition, our reliance on the single-mode neuroimaging technique limited our ability to simultaneously verify potential morphological and other functional differences between the two groups.

This study has several limitations that merit further discussion. First, the cross-sectional design of the study makes it unclear whether the putaminal neurochemical profile in this study is inherent to insomnia disorder or generalized to the effects of chronic sleep disturbance. Future longitudinal studies are warranted to investigate whether treatment normalizes the NAA/Cr ratio of the right putamen in patients with insomnia disorder. Second, the anatomical specificity of metabolites in all subdivided functional units of the putamen is challenging to determine using ^{1}H-MRS. Future neuroimaging investigations, including a combination of multi-mode imaging methods, are warranted, and may yield more comprehensive and precise results. Moreover, we did not assess other neurotransmitters and metabolites such as gamma-aminobutyric acid, which has a notable role in the etiology of insomnia.[62] Third, our results were only generalizable to young and middle-aged adults with primary insomnia disorder. Evaluation of a wider age range and those with comorbid insomnia would be more instructive. Last, our data are preliminary, in that they are from a small cohort of patients with insomnia disorder, but lend further support to the growing evidence of putaminal regulation on sleep and wakefulness. Further studies with larger cohorts are warranted.

Conclusion

In summary, the present study provides the first evidence of hemispherically lateralized abnormalities of NAA/Cr metabolism in the right putamen of patients with insomnia disorder, which positively correlated with objective and subjective insomnia severity. Our findings address the potential role of the right putaminal NAA/Cr metabolic abnormality in the underlying mechanism of insomnia disorder, suggesting that besides its crucial role in motor and cognitive behaviors, the putamen may also exert its regulatory influence on the sleep-wake cycle (ie, wakefulness (arousal) promotion and maintenance), which might aid in providing more evidence for elucidating the etiology and progression of insomnia disorder.

Acknowledgments

Disclosure

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