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Original Article

Analysis of the Relationship Between Sleep Quality and Brain Connections in Undergraduates: Study of Resting-State Functional Magnetic Resonance Imaging

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Purpose: Physiological statuses of college students and other age groups greatly differ. Past studies have mostly focused on sleep quality in the elderly to analyze their brain connections. In this study, AMG (amygdaloid), THL (thalamus), ACC (anterior cingulate cortex), and HPC (hippocampus) in 20-year-olds were examined to determine whether there is any difference in functional brain connections based on sleep quality.
Materials and Methods: Five patients (average age: 20.8 years) with insomnia and normal brain structures were assigned to the experimental group. For the control group, 10 healthy individuals were selected (average age: 20.3 years). Subjects were given instructions regarding resting-state fMRI acquisition.
Results: Experimental results showed significant differences in the AMG, THL, ACC, and HPC between the experimental and control groups ($p < 0.05$).
Discussion: There were key differences between healthy subjects and insomniac subjects in four regions of the brain: AMG, THL, ACC, and HPC. This preliminary investigation of young insomnia sufferers provides a basis for early detection and prevention, with the hope that insomnia can be adequately managed before the development of severe brain disease.

Keywords. insomnia, resting fMRI

Introduction

Increasing social pressures have resulted in increasing incidence of insomnia and sleep disorders. Insomnia refers to the inability to fall asleep or stay asleep, which results in sleep deprivation. Sleep disorders refer to abnormal sleep quantity and abnormal behaviors during sleep, which are also manifestations of the disorganization of normal sleep-wake rhythm. These may be caused by many different factors, which are commonly associated with physical disease. Insomnia can be classified as initial insomnia (difficulty in falling asleep), middle insomnia (difficulty in maintaining sleep), or terminal insomnia (early morning awakening) according to symptoms; transient insomnia (less than 1 week), short-term insomnia (1 week to 1 month), or chronic insomnia (more than 1 month)[1, 2, 3] according to duration; and physiological insomnia, psychological insomnia, pathologic insomnia, or drug-induced insomnia according to causative factors.

Sleep is intimately linked to human health. Studies have indicated that up to 30% of adults

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suffer from sleep disorder or sleep-related disease[4]. Insomnia typically causes great distress and mental burden, and some sufferers resort to drug abuse for relief, which can damage physical health. Experts have asserted that sleep is a physiological function that is extremely important for sustainment of life. Therefore, the purpose of this study was to investigate the differences in the brain structure of sleep disorder sufferers and normal sleepers, to provide a basis for early treatment. Many current studies on insomnia have been performed using magnetic resonance imaging (MRI) and analyses of BOLD signals during the resting state. At present, most studies have focused on subjects aged 35 and above, with little consideration for the fact that social progress has led to vast changes in the lifestyles of university students over the years. Therefore, there is great value in studying functional brain images of insomnia sufferers of university age. In this study, we aimed to determine if differences exist in functional connectivity in the anterior cingulate cortex (ACC), amygdala (AMG), thalamus (THL), and hippocampus (HPC) between insomnia sufferers and normal sleepers aged around 20.

Methods

Five patients with insomnia and normal brain structure were assigned to the experimental group. Among them, 4 were male and 1 was female, with a mean age of 20.8 years. For the control group, 10 healthy individuals were selected, of which 3 were male and 7 were female, with a mean age of 20.3 years. All participants were right-handed. They fulfilled the inclusion criterion of good sleep quality for 7 consecutive days. Individuals with a family history of disease, or with systemic organic disease, or previous use of anxiolytic, antidepressant, or sleep medication were excluded. Prior to the start of the experiment, Pittsburgh Sleep Quality Index (PSQI) was used to assess all subjects. Based on PSQI score, subjects were assigned to the experimental or control group. In addition, the Beck Depression Inventory (BDI) was used to exclude the possibility of depression.

The PSQI is a self-administered questionnaire that has been widely used to assess sleep quality over a 1-month time interval. It consists of two portions, namely 19 self-rated questions and 5 questions rated by the subject's bed partner or roommate. In this study, only the 19 self-rated questions were used to measure sleep disturbances along 7 dimensions: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction. The score can range from 0 to 3 for each component, with a maximum of 21 points. Higher total scores indicate worse sleep quality. Subjects with a PSQI score > 10 (indicating poor sleep quality) were assigned to the experimental group, while subjects with a PSQI score < 6 (indicating good sleep quality) were assigned to the control group.

The Beck Depression Inventory (BDI) is a 21-question, multiple-choice, self-reported inventory. Questions in the BDI do not reflect any specific theories of depression but are based on actual descriptions of symptoms by depressed patients. A score of 0 to 3 is given to each component based on the severity of symptoms. A total score of ≤13 was the criterion for exclusion in this study.

A 1.5T MRI system (1.5T Signa Horizon LX/ EchoSpeed, General Electric) with an 8-channel radiofrequency coil was used. Subjects were instructed to relax, open their eyes and not fall asleep during resting-state fMRI acquisition. A T2*-weighted gradient echo-planer imaging sequence was used with the following parameters: repetition time (TR) = 2223 ms, echo time (TE) = 35 ms, flip angle = 75°, voxel size = 3.4 × 3.4 × 3.4 mm, matrix size = 64 x 64, field of view (FOV) = 220x220 mm, without intersection gap, and 150 continuous image volumes.

Processing of resting-state fMRI scans[5]: Imaging data were preprocessed using SPM8. Images of all subjects were sequentially subjected to calibration, normalization, and smoothing. The Resting-State fMRI Data Analysis Toolkit (REST V1.8 release 130615) in SPM8 was then used to configure seed voxels in AMG, THL, and ACC, with the following reference coordinates: AMG: [R(23,-5,-15)] [L(-23,-5,-15)]; THL: [R(11,-16,9)] [L(-

11,-16,9)]; and ACC: [R(11,17,32)] [L(-11,17,32)]. For the selected seed voxels, activation during resting state conditions is shown in the images.

Results

Experimental results showed strong interactions between the AMG and the contralateral brain region in the experimental group (Figure 1). Interactions in the THL were stronger in one hemisphere and weaker in the other in the experimental group. In contrast, interactions were balanced between the hemispheres in the control group (Figure 2). There were stronger interactions between the ACC and the contralateral ACC in the experimental group, with high functional connectivity ($p < 0.05$) (Figure 3). Higher functional connectivity ($p < 0.05$) was also observed between the HPC and surrounding brain regions in the experimental group (Figure 4). Thus, significant differences existed in the AMG, THL, ACC, and HPC between the experimental and control groups.

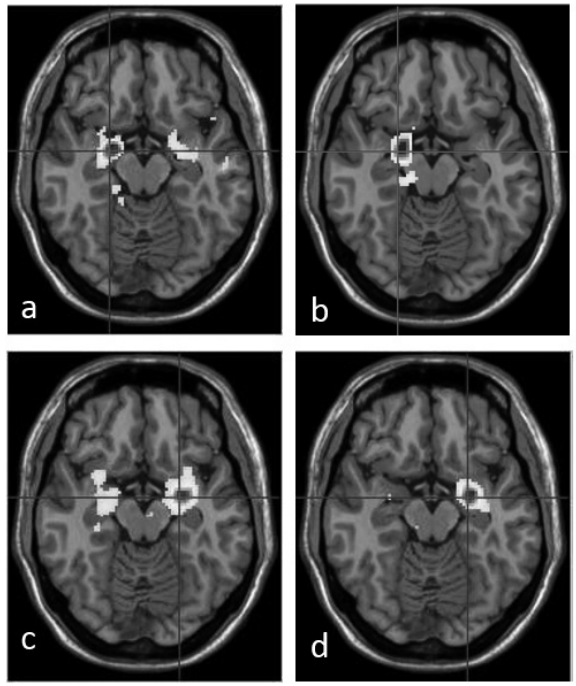

Figure. 1 Activation of associated brain regions of experimental group (a, c) and control group (b, d) in the right and left amygdala.

Discussion

The experimental results of this study demonstrated key differences between healthy subjects and

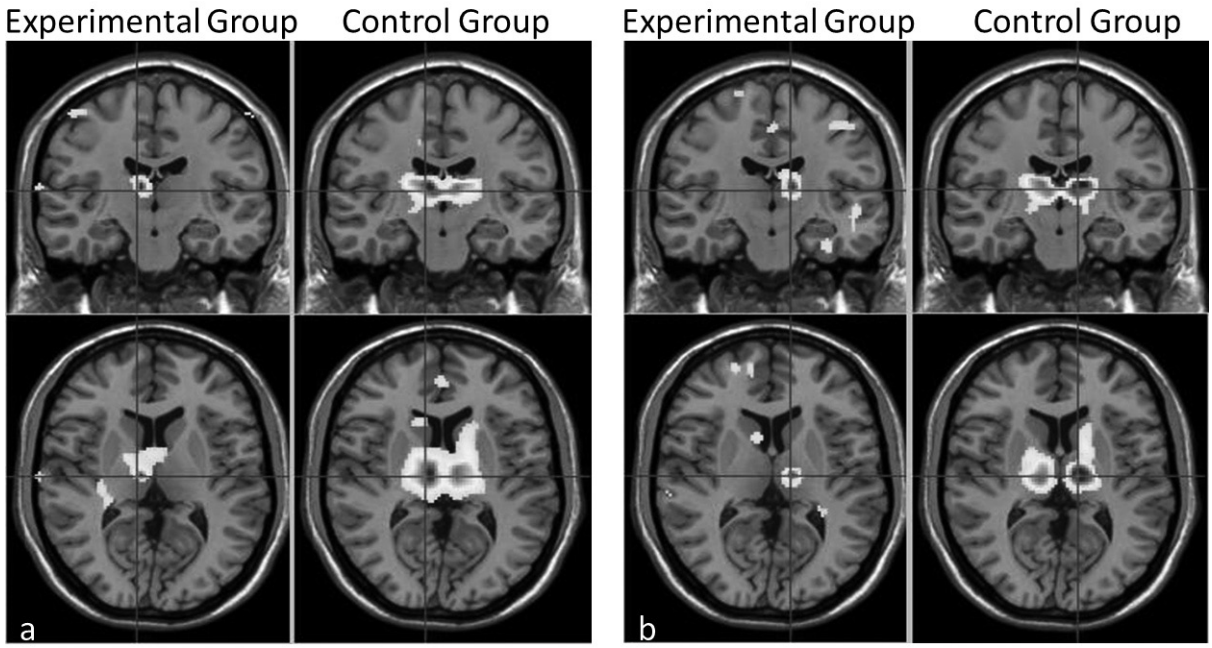

Figure. 2 Activation of associated brain regions in experimental group and control group in the right (a) and left thalamus (b).

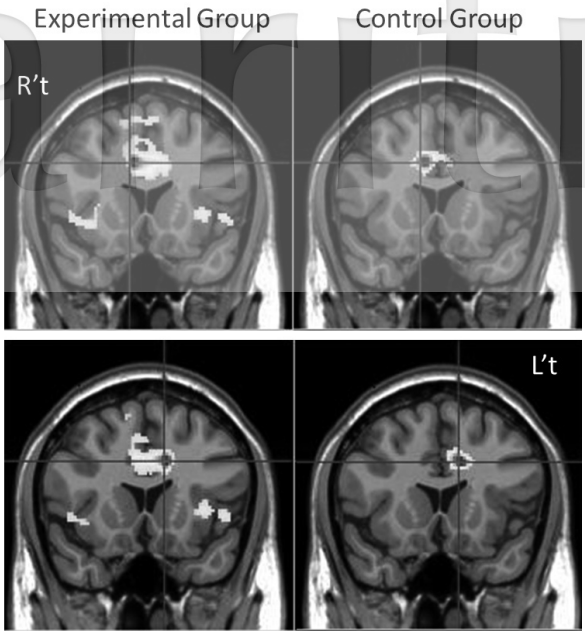

Figure. 3 Activation of associated brain regions of experimental group and control group in the right and left anterior cingulate cortex.

insomniac subjects in four regions of the brain: the AMG, THL, ACC, and HPC. The functions and effects of these four regions have been proven in previous studies. Compared with subjects without insomnia, there was higher functional connectivity in the AMG, ACC, and HPC and lower connectivity in the THL of insomniac subjects. Our experimental results showed strong interactions between the AMG and the contralateral brain region in the experimental group. The AMG[6,7], being the control center for emotions and behavior, is located within the limbic system and receives various types of sensory information from the sensory cortex and lateral nuclei. Activation within this region represents interactions in the limbic system and strong activity in the contralateral region in the experimental group indicates that the brains of insomnia sufferers are more prone to disturbances during the resting state. We also found that interactions in the THL[8] were stronger in one hemisphere and weaker in the other in the experimental group, while interactions were balanced between the hemispheres in the control group. The THL serves as a central relay hub for afferent and efferent information. Afferent (sensory) information passes through the THL before reaching the cerebral cortex and, similarly, efferent (motor) information sent from the brain passes through the THL before transmission to

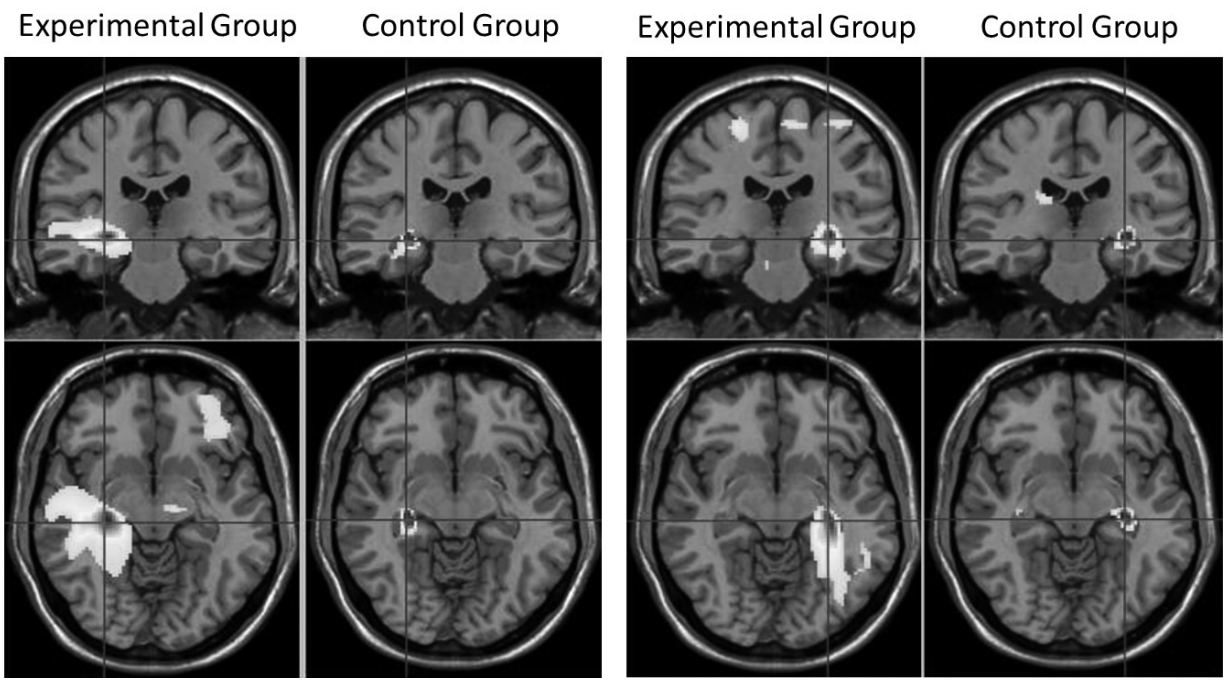

Figure. 4 Activation of associated brain regions of experimental group and control group in the right (a) and left hippocampus (b).

effector organs. The left and right halves of the THL function independently and are connected by a flattened band of tissue known as the massa intermedia, which is responsible for information exchange between the two halves. As the THL is a control center for physical coordination, the balance between the left and right halves is extremely important. An imbalance in activated connections between the two halves in the experimental group demonstrated that the excessive activated responses of the surrounding brain regions in insomnia sufferers result in unapparent activation of the THL. Thus, insomniacs experience more chaotic emotions during resting state.

In addition, interactions between the ACC and the contralateral region were stronger in the experimental group, with higher functional connectivity with the surrounding brain regions, while interactions between the ACC and the contralateral region were not found in the control group. The cingulate cortex is the largest structure of the limbic system and is involved in emotions, learning, and memory. It functions as an integration center for emotions and feelings and the areas that it controls are similar to those of the cerebral cortex. The ACC[9,10,11,12,13] is responsible for motor control, while the posterior cingulate cortex (PCC) is responsible for sensory information processing. Our findings indicated stronger interactions in brain regions in the experimental group, signifying that the ACC, an integration center for emotions and feelings, sends a continuous stream of information and exchanges information with adjacent brain regions during the resting state. This provides clear evidence of the chaotic emotions experienced by insomniac subjects and that they are less relaxed during MRI examination. Therefore, their muscles remain tense. As ACC interacts with the surrounding brain regions of insomniac subjects at rest, these subjects experience chaotic emotions.

The functional connectivity of the HPC with surrounding brain regions in the experimental group was stronger than that in the control group. The HPC[14] is a component of the brain's limbic system and is located beneath the cerebral cortex. It is involved in short-term memory, long-term memory, and spatial orientation, and is mainly responsible for the formation and storage of long-term memories. A higher connectivity in the experimental group represents higher activity in the limbic system, which may be due to poorer control of emotions or incessant thoughts in the insomniac subjects.

Based on the assessment of the default mode network in patients with primary insomnia on resting-state fMRI by Li et al[15]., 7 males and 13 females with a mean age of 38.5 ± 8.57 years and symptoms of insomnia were selected for the experimental group, while 7 males and 13 females with a mean age of 35.40 ± 10.41 years without symptoms of insomnia were selected for the control group. On resting-state fMRI scans, brain regions with decreased functional connectivity with the PCC in the experimental and control groups included the left occipital lobe, dentate gyrus, lingual gyrus, parahippocampal gyrus and fusiform gyrus, right superior temporal gyrus, temporal pole, middle temporal gyrus, and middle occiptal gyrus. Brain regions with increased functional connectivity with the PCC in the experimental group were the bilateral superior frontal lobes and middle cingulated gyrus. The results of this study showed increased functional connectivity in the HPC of insomniac subjects. There are several differences between our study and Li's study: (1) Our study focused on the ACC, while Li's study focused on the PCC. (2) The subjects were of different age groups, with the age range of subjects in Li's study higher than that in our study. Li's study was conducted on subjects aged 30-40 years, i.e., working adults whose insomnia may be primarily caused by social factors such as economic or work-related stress. For younger insomniac subjects, insomnia may be caused by an overabundance of energy. Observations of physical structure and physiological actions revealed pronounced muscle tension in our experimental subjects, which indicated that they were not able to relax during the experimental process. As the subjects in our study were aged around 20, insomnia was less likely to be caused by emotional factors (i.e., different possible factors for insomnia between studies). (3) In Li's study, PCC was set as a seed region to test the connectivity

of different brain regions, i.e., they used a single seed for exploration of other regions. In the present study, several seed voxels were configured for comparisons with other brain regions. Therefore, the observation points and statistical methods also differed.

In a 2004 study on insomnia using functional brain imaging, conducted by Nofzinger et al.[16], 4 females and 3 males with a mean age of 34.2 ± 8.9 years and symptoms of insomnia were selected as the experimental group, while 13 females and 7 females with a mean age of 32.6 ± 8.4 years and without symptoms of insomnia were selected as the control group. Images revealed signals of lower intensity (inhibitory) in the left frontal lobe, left upper region, superior temporal cortex occipital cortex, THL, hypothalamus, and brainstem in insomniac subjects compared with healthy subjects. Although their experimental results are similar to those of our study, their focus was on functional images rather than on resting-state images. Their conclusion was that insomniacs have lower levels of activation in the left frontal lobe, left upper region, superior temporal cortex and occipital cortex, THL, hypothalamus, and brainstem. Lower levels of activation indicate decreased functional connectivity, signifying lack of inhibitory effect and more chaotic emotions in insomnia sufferers.

In a 2011 study on sleep deprivation by De Havas et al.[17], 11 males and 15 females with a mean age of 22.5 ± 2 years underwent fMRI examination twice: once after a normal night of sleep and once after 24 hours of sleep deprivation. Images obtained during resting state and activity indicated reduced functional connectivity at most node locations in sleep-deprived subjects. The age range of subjects in that study was closer to that of our study. However, the subjects were healthy normal sleepers without primary insomnia who were merely subjected to sleep deprivation for the purpose of the study. Their results demonstrated the activation status of functional connectivity in the brain under conditions of sleep deprivation. In particular, significant reductions in connectivity in the left lateral temporal cortex (LLTC) and left temporoparietal junction (LTPJ), compared with other brain regions, were observed after sleep deprivation. When their results were compared with the findings of our study, brain activation outcomes differed. Therefore, the increases and decreases in connectivity observed in the AMG, THL, ACC, and HPC in our study are not associated with sleep deprivation, and the possibility of short-term sleep deprivation resulting in the observed changes in functional connectivity can be excluded.

In a study comparing the HPC volumes of insomniac patients and normal sleepers by Winkelman et al[18]., 10 males with a mean age of 39.3 ± 8.7 years and insomnia were selected as the experimental group. MRI was performed twice to obtain mean data (n = 20). Nine males with a mean age of 38.8 ± 5.3 years and without symptoms of insomnia were selected as the control group (n = 15). The results of that study showed that HPC volume is reduced in insomniac subjects relative to normal sleepers. In our study, strong brain functional connectivity in the HPC was clearly seen on obtained images. However, changes in HPC volume were not observed. In contrast, in Winkelman's study, the number of HPC cells and HPC volume were reduced in male insomniac subjects who were close to 40 years of age and had experienced insomnia for at least 6 months. This is attributable to the lack of rest of overused HPC cells, which results in HPC cell loss and, subsequently, HPC atrophy in the long term. In our study, excitation of HPC tissue was observed in a younger insomniac population. Therefore, overly active HPC tissue may shrink due to long-term lack of rest and, subsequently, cause adverse reactions.

In another study on chronic insomniac sufferers by Winkelman[19], insomniac subjects were classified as treated or untreated. A total of 41 insomniac subjects and 35 healthy subjects were included in that study. The results indicated an increased rostral ACC volume in insomniac subjects compared with normal subjects. As the ACC is responsible for motor control, the long-term inability to relax muscles results in pronounced activity in the ACC, which is consistent with our experimental results.

In a study on insomnia in older adults by Bastien et al[20]., subjects were divided into three groups with a mean age of 62.5 ± 5.8 years across all groups: insomnia sufferers with chronic drug

use, drug-free insomnia sufferers, and good sleepers without symptoms of insomnia. The results of this study indicated that, irrespective of drug usage, insomnia sufferers had poorer concentration and lack of concentration factors compared with healthy subjects. However, more negative effects were observed in insomnia sufferers who used medication than in those who were drug-free. This shows that insomniacs are relatively unable to concentrate, resulting in more meaningless brain connections. The increased number of excess connections in the limbic system with other brain regions observed in this study serves as further proof of this theory. In parts of the limbic system investigated in our study, i.e., the cingulate gyrus, HPC and THL, increased activity was observed in most regions, indicating relatively chaotic emotions in insomniac subjects.

In view of the results reported in the literature on insomnia, insomnia sufferers aged 35 years and above have a higher level of activity in the cerebral cortex during resting state. The results of our study showed significant differences in the AMG, THL, ACC, and HPC of young insomniac and control subjects. Compared with the control group, higher functional connectivity in AMG, ACC, and HPC, which are responsible for reception and activation, and lower connectivity in the THL, which is responsible for coordination and inhibition, were observed in the experimental group. Thus, we can deduce that sleep difficulty in insomnia sufferers is due to a higher level of activity in some brain regions.

Our experimental results provide evidence of higher functional connectivity in the ACC and HPC. The results of previous studies indicate that insomnia sufferers and sleep-deprived subjects share common attributes of increased brain activation and increased brain functional connectivity. However, such increased activity usually occurs in meaningless connections, which results in chaotic emotions. Certain discrepancies exist between the experimental results of this study and previous studies due to differences in study methods and age ranges of subjects. From a physiological standpoint, the results of the present study are clinically significant.

Insomnia can result in many types of brain disease. In a study on the correlation between insomnia and ischemic stroke by Chen[21], data from 508 ischemic stroke patients revealed that insomnia sufferers have a higher risk of stroke. In addition to keeping a lookout for signs of depression[22], insomnia sufferers must be vigilant for signs of prefrontal infarction or diabetes mellitus. Most brain diseases are a consequence of long-term effects and disease onset may occur after several decades. However, symptoms of inducing factors usually manifest at a younger age. Therefore, early prevention or treatment of insomnia is extremely important. The preliminary investigation of young insomnia sufferers in this study provides a basis for early detection and prevention, with the hope that insomnia can be adequately managed before the development of severe brain disease.

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