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Evaluation of the age-related and gender-related differences in patients with primary insomnia by fractional amplitude of low-frequency fluctuation

A resting-state functional magnetic resonance imaging study

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Abstract

Insomnia patients with different gender and age usually had different sleep experience. Primary insomnia (PI) has been considered to be a disorder of hyper-arousal in the physiologic, emotional, or cognitive network. Although the hyper-arousal brain regions can be shown by comparing the brain activity of PI patients with normal people at rest, whether the brain activity of PI patients varied according to age and gender and whether age and gender could affect the distribution of hyper-arousal brain regions are still worthy of further exploration. Hence, a resting state functional magnetic resonance imaging study (No. NCT02448602) was designed to observe the brain activity of thirty PI patients and 15 healthy controls (HCs). The brain activity in resting state was measured by calculating the fractional amplitude of low-frequency fluctuations (fALFF), which reflected the idiopathic activity level of neurons. Multiple regression was performed to investigate the age and gender-related differences of brain activity in PI patients ($P < .001$, Family Wise Error (FWE) correct $P = .05$, cluster size >50) with age and gender as covariates. The hyper-arousal brain regions were measured by comparing the fALFF of PI patients and HCs. Multiple regression ($P < .001$, FWE correct $P = .05$, cluster size >50) was also performed for PI patients and HCs with group, age, and gender as covariates.

The results suggested that the gender-related difference of brain activity mainly existed in superior temporal gyrus, cerebellum posterior lobe, middle frontal gyrus, and the age-related difference mainly existed in cerebellum anterior lobe, superior temporal gyrus, brainstem, parahippocampa gyrus, anterior cingulate, cingulate gyrus. In addition, the altered fALFF regions between PI and HCs mainly existed in superior temporal gyrus, posterior cingulate, anterior cingulate, cingulate gyrus, middle frontal gyrus. Furthermore, the gender factor could not influence the distribution of the altered regions. While the age factor could affect the distribution of the altered regions.

Abbreviations: ACC = anterior cingulate cortex, AIS = Athens insomnia scale, BA = Brodmann area, BOLD = blood oxygenation level dependent, DPARSF = data processing assistant for resting-state fMRI, FA = flip angle, fALFF = fractional amplitude of low-frequency fluctuations, FOV = field of view, HCs = healthy controls, MNI = Montreal neurological institute, PI = primary insomnia, PSQI = Pittsburgh sleep quality index, Rs-fMRI = resting state functional magnetic resonance imaging, SAS = self-rating anxiety scale, SDS = self-rating depression scale, TE = echo time, TR = repetition time.

Keywords: amplitude of low-frequency fluctuations, functional magnetic resonance imaging, primary insomnia

1. Introduction

Primary insomnia (PI) is a globally prevalent and increasing incidence of sleep disorders characterized by difficulty in initiating sleep or maintaining sleep.[1,2] However, there were gender and age differences in the incidence of insomnia. Numerous researches showed that the prevalence of insomnia might be higher for women.[3–6] However, insomnia patients of different ages often have different insomnia experiences, younger

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The datasets generated and/or analyzed in the present study can be available from the corresponding author upon reasonable request.

The experiment was conducted in accordance with the ethical guidelines of the Declaration of Helsinki, and all methodologies were approved by the Ethics Committee of Changchun University of Chinese Medicine (Reference: CCZYFYLL2014-043). A written signed informed consent was provided by each participant.

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individuals often have difficulty falling asleep, while the elderly always have difficulty initiating sleep, maintaining sleep, and experiencing early morning awakenings.[4] Furthermore, there are gender and age differences in the response of insomnia patients to some interventions.[7] We are curious about the possible reasons for the difference in incidence and efficacy.

PI is generally considered to be a disorder of hyper-arousal in the physiologic, emotional, or cognitive network.[8,9] Evidence of hyper-arousal includes elevated whole-body metabolic rate during sleep and wakefulness, heightened body temperature, elevated cortisol and adrenocorticotropic hormone,[10] and increased high-frequency electroencephalographic activity during nonrapid eye movement sleep.[11] Previous studies have attempted to locate the specific brain areas with hyper-arousal in PI patients, and found that there was aberrant regional spontaneous brain activity in sleep disorders, and there were gender differences in these brain areas.[12,13] However, age-related differences in brain activity have not been fully studied. In addition, it is unclear whether and how gender and age-related differences affect the distribution of hyper-arousal brain regions. We believe that exploring the differences in the distribution of hyperarousal brain regions among PI patients of different genders and ages will be helpful to further study the gender and age differences in the efficacy of treatment.

Resting state functional magnetic resonance imaging (rs-fMRI) could measure blood oxygenation level dependent (BOLD) changes in brain tissue in resting state.[14] Amplitude of low-frequency fluctuations (ALFF) can directly demonstrate BOLD signal and reflect idiopathic activity levels of neurons in the voxels according to their energy under the resting state.[15] The simple calculation and reliable characterization[16] of the ALFF measurement made it a useful tool to investigate the brain activity. Although ALFF was a useful tool in detecting the regional neural activity, physiological noise, such as the repetition times in MRI scan and so on, are not critically considered in the ALFF calculation. Therefore, a modified calculation called fractional amplitude of low-frequency fluctuation (fALFF), which means the ratio of the power spectrum of low frequency (0.01–0.08 Hz) to that of the entire frequency range, has been proven to suppress nonspecific noise components and improve the effectiveness in exploring local BOLD signals.[17] Therefore, 15 normal people as healthy controls (HCs) and 30 PI patients were recruited for rs-fMRI study (No. NCT02448602, 14/04/2015). Our study investigated gender-related and age-related differences in brain activity in PI patients. Furthermore, the distribution of hyper-arousal regions was studied by comparing the fALFF between PI and HCs, and the gender-related and age-related differences of hyper-arousal regions were further analyzed. By exploring the influence of age and gender on the brain activity and the distribution of hyper-arousal regions in PI patients, we hoped that our trial could contribute to the further understanding of the hyper-arousal theory of PI.

2. Method

2.1. Participants

From September 2017 to September 2018, 15 healthy subjects without insomnia and 30 PI patients from the outpatient clinic in the Neurological Department of China-Japan Union Hospital of Jilin University and the Neurological Department of Changchun University of Chinese Medicine were recruited in this study. A written signed informed consent was provided by each participant. The experiment was conducted in accordance with the ethical guidelines of the Declaration of Helsinki, and all methodologies were approved by the Ethics Committee of Changchun University of Chinese Medicine (Reference: CCZYFYLL2014-043). All PI patients should satisfy the following criteria.

Inclusion criteria:

(1) patients range from 18 to 65 years old;
(2) patients with sleep onset latency or wake after sleep onset of >30 minutes at least 3 nights per week, with symptoms lasting for ≥3 months;
(3) patients with a Pittsburgh sleep quality index (PSQI) score of >7 and Athens insomnia scale (AIS) score of ≥6.

Exclusion criteria:

(1) patients with uncontrolled medical or psychiatric conditions;
(2) patients with self-rating anxiety scale (SAS) or self-rating depression scale (SDS) of ≥50;
(3) patients diagnosed with comorbid sleep disorders, such as obstructive sleep apnea;
(4) patients with alcohol and/or other drug abuse or dependence;
(5) patients who received hypnotic or sedating medications in the recent 1 month.

The clinical data, including age, gender, PSQI, AIS, SAS, and SDS of PI patients and HCs were analyzed with SPSS 18.0 statistical software and expressed as mean ± standard deviation.

2.2. Rs-fMRI data acquisition

HCs and PI patients all received a rs-fMRI assessment at 8:00 AM to 10:00 AM in awake state. The fMRI scan was completed on a 3.0 T whole-body MRI scanner (MAGNETOM-skyra-SIEMENTS). The MRI sequences included the following:

(1) T1-weighted MRI: data were acquired using a magnetization-prepared rapid gradient-echo sequence with 192 continuous sagittal slices that covered the whole brain, with TR/TE at 700 ms/11 ms, FOV at 256 × 256 mm, and a voxel size of 1 × 1;
(2) rs-fMRI: data were acquired using an echo planar imaging sequence sensitive to BOLD contrast with 36 slices that covered the whole brain, with TR/TE/FA at 2020 ms/30 ms/90°, and FOV at 106 × 106 mm, and a voxel size of 2.4 × 2.4. The rs-fMRI scan duration lasted 200 TR.

2.3. Rs-fMRI data processing

Rs-fMRI data were pre-processed with the data processing assistant for resting-state fMRI (DPARSF, http://rfmri.org/DPARSF)[18] package and analyzed with statistical parametric mapping toolbox 8 (Welcome Department of Imaging Neuroscience, Institute of Neurology, London; http://www.fil.ion.ucl.ac.uk/spm). Digital imaging and communications in medicine data were converted into Neuroimaging Informatics Technology Initiative data. The first 10 images of each functional time series were discarded, all slices of the remaining images were processed by slice-timing adjustment, and realigned to the middle volume. Then, the time series of images was motion-corrected. The data set in which the translation or rotation parameters exceeded 1.5 mm or 1.5° of the rotation were discarded. Then, the realigned functional images were spatially normalized to the Montreal neurological institute (MNI) space using the normalization parameters estimated by the T1 structural image

unified segmentation, and re-sampled to a resolution of $3 \times 3 \times 3$ mm^3 voxels. Then the normalized data were spatially smoothed using a 6 mm full-width half-maximum Gaussian kernel. Linear detrending and nuisance linear regression (including the white matter, the cerebrospinal fluid, and head motion parameters) were performed, and a temporal bandpass filter (0.01–0.08 Hz) was applied to reduce the effects of head motion and nonneuronal BOLD fluctuations.[19] ALFF and fALFF were calculated with DPARSF package for each subject.[17]

2.4. Rs-fMRI data analysis

First, when studying the age and gender-related difference of brain activity in PI patients, the fALFF value of PI patients was analyzed by multiple regression analysis with age and gender as covariables ($P < .001$, FWE correct $P = .05$, cluster size >50). The fALFF value of HCs was also analyzed by multiple regression analysis with age and gender as covariables ($P < .001$, FWE correct $P = .05$, cluster size >50).

Then, when studying the difference of fALFF regions between PI patients and HCs, 15 PI patients were randomly selected to be compared with the HCs. Multiple regression ($P < .001$, FWE correct $P = .05$, cluster size >50) was performed for PI patients and HCs with group, age and gender as covariates. Based on altered fALFF regions, the interaction between group and age, and the interaction between group and gender as covariates, multiple regression analysis was performed to investigate the age and gender-related difference. The above analysis was repeated twice. A conjunction analysis was performed for the results of 2 times. Overlap areas with cluster size over 20 would be listed in results.

3. Results

3.1. Participant demography

Fifteen HCs (7 males, 8 females) range from 26 to 59 years old (45.53 ± 12.68) and thirty PI patients (12 males, 18 females) range from 22 to 59 years old (49.67 ± 9.58) were recruited in our study. There was no significant difference in age ($P = .228$) and gender ($P = .678$) between the PI patients and HCs. The PSQI, AIS, SAS, SDS of PI patients had significant differences with HCs ($P < .01$) (Table 1).

Table 1

Demographic characteristic in PI patients and HCs.

Parameter	PI	HCs	P
Gender			.678
Male	12	7	
Female	18	8	
Age (yr)	49.67 ± 9.58	45.53 ± 12.68	.228
PSQI	15.23 ± 1.70	3.53 ± 0.92	<.001
AIS	13.47 ± 2.29	2.73 ± 1.16	<.001
SAS	36.97 ± 2.06	18.53 ± 4.14	<.001
SDS	37.70 ± 2.42	17.53 ± 4.58	<.001

Independent 2-sample *T*-test was performed for PI patients and HCs.
AIS = Athens insomnia scale, HCs = healthy controls, PI = primary insomnia, PSQI = Pittsburgh sleep quality index, SAS = self-rating anxiety scale, SDS = self-rating depression scale.

3.2. Rs-fMRI result

The age-related difference of fALFF value in PI patients mainly existed in bilateral cerebellum posterior lobe, right cerebelluma anterior lobe, right superior temporal gyrus, bilateral brainstem, left parahippocampa gyrus, bilateral anterior cingulate, right cingulate gyrus, and the older PI patients had the lower fALFF value ($P < .001$, FWE correct $P = .05$, cluster size >50) (Table 2, Fig. 1). The gender-related difference of fALFF value in PI patients mainly existed in right superior temporal gyrus, right cerebellum posterior lobe, left middle frontal gyrus. The fALFF value in right superior temporal gyrus of male PI patients were higher than female PI patients ($P < .001$, FWE correct $P = .05$, cluster size >50), while the fALFF value in right cerebellum posterior lobe and left middle frontal gyrus of male PI patients were lower than female PI patients ($P < .001$, FWE correct $P = .05$, cluster size >50) (Table 2, Fig. 2).

The age-related difference of fALFF value in HCs were not been found when $P < .001$, which were mainly existed in right cerebellum posterior lobe and left precuneus when $P < .02$, and the older HCs had the lower fALFF value (FWE correct $P = .05$, cluster size >20) (Table 2). The gender-related difference of fALFF value in HCs were not been found when $P < .001$, which were mainly existed in right superior temporal gyrus and right middle temporal gyrus when $P < .01$ (FWE correct $P = .05$, cluster size >20) (Table 2). And the fALFF value in right superior temporal gyrus and right middle temporal gyrus of male HCs were higher than female HCs.

We randomly selected the 15 PI patients' fALFF data (10 females, 5 males; age 48.40 ± 10.81 years old) to analyze the difference between PI patients and HCs. The altered fALFF regions between PI patients and HCs were mainly in left cerebellum posterior lobe, right superior temporal gyrus, right extra-nuclear, left posterior cingulate, left anterior cingulate, bilateral medial frontal gyrus, left middle frontal gyrus, left superior frontal gyrus ($P < .001$, FWE correct $P = .05$, cluster size >50) (Table 3). Based on the altered fALFF regions between PI patients and HCs, only the left middle frontal gyrus (cluster size: 61; MNI: −33 24 39; *t*-value: −5.1834) was found could be influenced by age factor ($P < .01$, FWE correct $P = .05$, cluster size >50). No region was found could be influenced by gender factor ($P < .01$, FWE correct $P = .05$, cluster size >50).

We repeated the above steps, randomly selected the 15 PI patients' fALFF data (9 females, 6 males; age 49.73 ± 9.46 years old) to analyze the difference between PI patients and HCs. The altered fALFF regions between PI patients and HCs were mainly in right superior temporal gyrus, left inferior frontal gyrus, right extra-nuclear, left anterior cingulate, left cingulate, left posterior cingulate, right postcentral gyrus, bilateral middle frontal gyrus ($P < .001$, FWE correct $P = .05$, cluster size >50) (Table 4). Based on the altered fALFF regions between PI patients and HCs, the left middle frontal gyrus (cluster size: 62; MNI: −51 42 −6; *t*-value: −4.5373) and left anterior cingulate (cluster size: 70; MNI: 0 36 12; *t*-value: −4.8757) were found could be influenced by age factor ($P < .01$, FWE correct $P = .05$, cluster size >50). No region was found could be influenced by gender factor ($P < .01$, FWE correct $P = .05$, cluster size >50).

The overlap areas of the first and second comparison of PI patients and HCs were mainly in right superior temporal gyrus, left posterior cingulate, left anterior cingulate, left cingulate gyrus, left middle frontal gyrus (cluster size >20) (Table 5, Fig. 3)

4. Discussion

Sleep has a critical role in promoting health. Research over the past decades have documented that sleep disturbance has a

Table 2

The age-related and gender-related differences of fALFF value in PI patients and HCs.

Group		Brain regions	BA	Side	Cluster size	MNI			*t*-value
						X	*Y*	*Z*	
PI	Age-related	Cerebellum posterior lobe		R	220	36	−72	−45	−9.11
		Cerebellum posterior lobe		L/R	173	0	−69	−12	−8.04
		Cerebellum posterior lobe		L	65	−48	−72	−48	−7.05
		Cerebellum anterior lobe		R	65	24	−48	−27	−6.36
		Superior temporal gyrus	22	R	69	54	0	3	−7.75
		Brainstem		L/R	167	−9	−12	−21	−9.13
		Parahippocampa gyrus							
		Brainstem		R/L	54	−6	−15	−3	−7.75
		Anterior cingulate		R/L	65	0	36	−3	−7.90
		Cingulate gyrus	31	R	52	15	−21	39	−9.37
	Gender-related	Superior temporal gyrus	39	R	68	54	−63	18	6.90
		Cerebellum posterior lobe		R	116	42	−66	−42	−7.66
		Middle frontal gyrus	9	L	61	−30	24	36	−9.16
HCs	Age-related*	Cerebellum posterior lobe		R	23	9	−90	−36	−4.11
		Precuneus		L	20	−21	−48	39	−5.97
	Gender-related†	Superior temporal gyrus		R	25	48	15	−27	4.91
		Middle temporal gyrus	37	R	20	42	−66	3	4.63

Anatomical locations, approximate Brodmann areas (BA), and Montreal Neurological Institute (MNI) coordinates, correspond to the *t*-values of representative peaks within each cluster were reported. L: left. R: right. Negative *t*-value in age-related study suggested older patients (or HCs) had lower fALFF values in this area, negative *t*-value in gender-related study suggested male patients (or HCs) had lower fALFF values in this area. All regions shown in PI group reached a voxel-level significance threshold of $P < .001$, FWE correct $P = .05$, cluster size >50.

fALFF = fractional amplitude of low-frequency fluctuations, HCs = healthy controls, PI = primary insomnia.

*Regions shown in HCs group reached a voxel-level significance threshold of $P < .02$, FWE correct $P = .05$, cluster size >20.

†Regions shown in HCs group reached a voxel-level significance threshold of $P < .01$, FWE correct $P = .05$, cluster size >20.

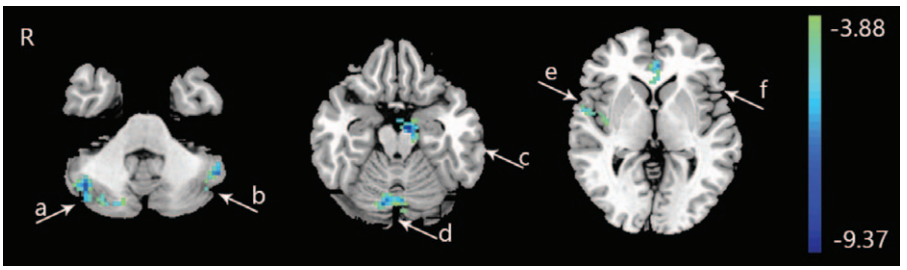

Figure 1. The maps of age-related fALFF differences in PI patients' brain regions. R: right brain. a: right cerebellum posterior lobe, b: left cerebellum posterior lobe, c: left brainstem, d: cerebellum posterior lobe, e: right superior temporal gyrus, f: anterior cingulate. All regions shown reached a voxel-level significance threshold of $P < .001$, FWE correct $P = .05$, cluster size >50. Negative *t*-value suggested elder PI patients had lower fALFF values in this area than younger PI patients. fALFF = fractional amplitude of low-frequency fluctuations, PI = primary insomnia.

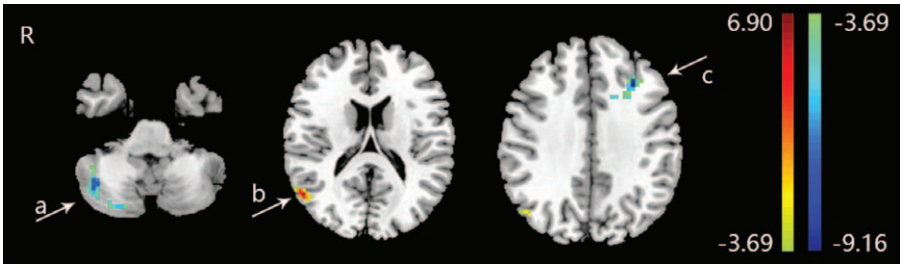

Figure 2. The maps of gender-related fALFF differences in PI patients' brain regions. R: right brain. a: right cerebellum posterior lobe, b: right superior temporal gyrus, c: left middle frontal gyrus. All regions shown reached a voxel-level significance threshold of $P < .001$, FWE correct $P = .05$, cluster size >50. Negative *t*-value suggested male PI patients had lower fALFF values in this area than female PI patients. fALFF = fractional amplitude of low-frequency fluctuations, PI = primary insomnia.

Table 3

The altered fALFF regions between PI patients (n = 15) and HCs (n = 15).

Brain regions	BA	Side	Cluster size	MNI			t-value
				X	Y	Z	
Cerebellum posterior lobe	37	L	74	−39	−75	−24	−6.45
Superior temporal gyrus	13	R	87	48	6	0	−5.68
Posterior cingulate		L	50	−9	−60	12	−6.34
Extra-nuclear		R	171	24	3	21	−6.82
Medial frontal gyrus	9	R	71	18	30	30	−5.25
Anterior cingulate	32	L	531	−27	15	36	−6.97
Medial frontal gyrus							
Middle frontal gyrus							
Superior frontal gyrus	6	L	246	−24	12	54	−8.84

Anatomical locations, approximate Brodmann areas (BA), and Montreal Neurological Institute (MNI) coordinates, correspond to the *t*-values of representative peaks within each cluster were reported. L: left. R: right. Negative *t*-value suggested PI patients had lower fALFF values in this area than HCs. All regions shown reached a voxel-level significance threshold of $P < .001$, FWE correct $P = .05$, cluster size >50.

fALFF = fractional amplitude of low-frequency fluctuations, HCs = healthy controls, PI = primary insomnia.

Table 4

The altered fALFF regions between PI patients (n = 15) and HCs (n = 15).

Brain regions	BA	Side	Cluster size	MNI			t-value
				X	Y	Z	
Superior temporal gyrus	13	R	100	48	0	3	−5.09
Inferior frontal gyrus	13	L	125	−42	27	9	−4.88
Extra-nuclear		R	54	27	−42	18	−5.01
Anterior cingulate	32	L	86	−3	36	21	−4.90
Cingulate gyrus	31	L	529	−6	−36	30	−5.77
Posterior cingulate							
Postcentral gyrus	40	R	80	54	−24	33	−5.20
Middle frontal gyrus	9	L	66	−39	39	33	−5.23
Middle frontal gyrus		R	108	39	9	51	−5.30
Middle frontal gyrus	6	L	114	−27	−3	63	−5.24

Anatomical locations, approximate Brodmann areas (BA), and Montreal Neurological Institute (MNI) coordinates, correspond to the *t*-values of representative peaks within each cluster were reported. L: left. R: right. Negative *t*-value suggested PI patients had lower fALFF values in this area than HCs. All regions shown reached a voxel-level significance threshold of $P < .001$, FWE correct $P = .05$, cluster size >50.

fALFF = fractional amplitude of low-frequency fluctuations, HCs = healthy controls, PI = primary insomnia.

Table 5

The overlap regions of altered fALFF regions between PI patients (n = 15) and HCs (n = 15).

Brain regions	Side	Cluster size	MNI		
			X	Y	Z
Superior temporal gyrus	R	37	48	6	−3
Posterior cingulate	L	85	−12	−47	18
Anterior cingulate	L	38	−3	43	8
Cingulate gyrus	L	85	−5	−38	36
Middle frontal gyrus	L	52	−39	−9	42

Anatomical locations and Montreal Neurological Institute (MNI) coordinates, correspond to the overlap regions were reported. L: left. R: right. All regions shown reached a voxel-level significance threshold of $P < .001$, FWE correct $P = .05$, cluster size >20.

fALFF = fractional amplitude of low-frequency fluctuations, HCs = healthy controls, PI = primary insomnia.

powerful influence on the occurrence and progression of several major medical illnesses, including cardiovascular disease and cancer, and the incidence of depression.[2] Previous studies on the mechanism of PI suggest that insomnia is caused by hyper-arousal in physiological, emotional, or cognitive networks.[8,9] Furthermore, some studies have shown that the brain activity in PI patients may vary depending on gender.[12] However, few studies have explored the effect of age on brain activity. Therefore, a resting state fMRI study was designed to detect brain activity of 30 PI patients and 15 HCs. The fALFF, measures the relative contribution of low frequency fluctuations within a specific frequency band to the whole detectable frequency range, was used to reveal the strength of inter-regional cooperation and potentially identifying brain areas with abnormal local functioning.[17]

In the study, the age-related differences in the brain activity of PI patients were mainly in bilateral cerebellum posterior lobe, right cerebellum anterior lobe, right superior temporal gyrus, bilateral brainstem, left parahippocampa gyrus, bilateral anterior cingulate, right cingulate gyrus, and the older PI patients had lower activity in these regions. While the older healthy people might have lower activity in right cerebellum posterior lobe. Therefore, the low activity in left cerebellum posterior lobe, right cerebellum anterior lobe, right superior temporal gyrus, bilateral brainstem, left parahippocampa gyrus, bilateral anterior cingulate, right cingulate gyrus might be the main changes of brain activity in elderly PI patients. Brainstem as a central regulating node for arousal, are critically involved in the regulation of rapid eye movement (REM) sleep and wake,[20] which also interact with forebrain as a circuits to produce and regulate sleep-wake rhythms.[21] The cerebellum posterior lobe is associated with cognitive, linguistic, and emotional functions in

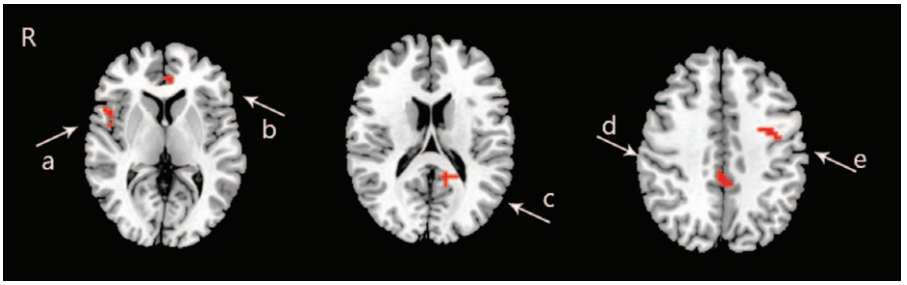

Figure 3. The maps (overlap regions) of the difference between PI patients and HCs. R: right brain. a: right superior temporal gyrus, b: left anterior cingulate, c: left posterior cingulate, d: left cingulate gyrus, e: left middle frontal gyrus. All regions shown reached a voxel-level significance threshold of $P < .001$, FWE correct $P = .05$, cluster size >20. fALFF = fractional amplitude of low-frequency fluctuations, PI = primary insomnia.

addition to initiation and planning of coordinating movement.[12] Cingulate gyrus, an integral part of the limbic system, is involved with emotion formation and processing, learning and memory.[22] Anterior cingulate cortex (ACC) is a region known to integrate attention and emotion, which is not only responsible for implementing attentional control during tasks that require response inhibition, selective attention, target selection, or novel responses,[23,24] but also has been repeatedly shown to be active during the regulation of emotional responses.[25] Furthermore, ACC also participates in the sleep process, as the backbone of the brain network, which could be activated during REM sleep.[26,27] Some studies have shown that the activity of ACC was different between PI and healthy people.[28] Parahippocampa gyrus and cingulate gyrus are critical memory-related structures, which are related to the emotions and cognitive functions, such as memory, learning, and visuospatial tasks, and plays an active role in the generation of arousal and insomnia. These results suggested that the emotional and cognitive function were downregulated in elder PI patients. Another study also suggested that insomnia disorder in older adults is associated with worse cognitive function than adults with insomnia symptoms, which was consistent with our results.[29]

The gender-related differences in the brain activity of PI patients were mainly in right superior temporal gyrus, right cerebellum posterior lobe, left middle frontal gyrus. The activity of right superior temporal gyrus in male PI patients were higher than that of female PI patients, and similar results were found between male HCs and female HCs. Therefore, the lower activity of right cerebellum posterior lobe, left middle frontal gyrus might be the main changes of brain activity in male PI patients. Because the posterior lobe of cerebellum is related to cognitive and emotional function,[12] we believed that the clinical difference between male and female patients is also caused by the different activity in areas related to cognitive and emotional function.

In addition to the brain activity of PI patients, we were concerned about the difference of brain activity between PI patients and HCs, which was also meaningful for the mechanism and clinical treatment of PI. The altered fALFF regions between PI patients and HCs when regressing out the influence of age and gender were found in right superior temporal gyrus, left posterior cingulate, left anterior cingulate, left cingulate gyrus, and left middle frontal gyrus which might be influenced by age factor. ACC was not only participant the emotional processing, but also associated with sleep progress. Research have shown that the activity of ACC and posterior cingulate were different between PI and healthy people.[13] Increased activation were found in emotion related regions such as the ACC and superior temporal gyrus during the sleep.[30]

Insomnia is usually associated with the emotional disorders, the excitable increase in emotion is an important factor in the etiology of insomnia.[9] The emotional network is a necessary factor for the emergence and maintenance of consciousness in a developing brain, which is maintained through the sleeping process. Sleep disturbances could lead to emotional and cognitive dysfunctions[31] and versa vice.[32] Therefore, we considered that the altered brain activity of brain regions related to emotional regulation might mediate the occurrence of PI.

Above all, the age-related and gender-related difference of brain activity in PI patients were found to be associated with emotional and cognitive function, which could be the possible cause of different insomnia incidence, sleep experience and efficacy of intervention in PI patients with different gender and age. In addition, the gender factor should be considered in further research when investigating the difference in brain activity between PI patients and healthy people.

5. Limitation

The conclusion of our research is based on the current subjects, and further research should expand the number of subjects to verify this conclusion. In addition, our PI subjects were screened out from the insomnia patients in the hospital. The subjects generally experienced the problem of long-term insomnia, so the degree of insomnia was relatively consistent (the scores of AIS and PSQI were concentrated) and there was no statistical difference among the female and male groups. Therefore, the correlation between AIS/PSQI scores and results was not studied in this experiment. We suggest that the correlation between AIS/PSQI score and results should be further considered when the degree of insomnia of the included subjects is inconsistent. The exploration of the effect of insomnia degree on the brain activity and the distribution of hyper-arousal brain regions may help to perfect the theory of hyper-arousal of insomnia.

Author contributions

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References

[1] Kessler RC, Berglund PA, Coulouvrat C, et al. Insomnia and the performance of US workers: results from the America insomnia Survey. Sleep 2011;34:1161–71.
[2] Irwin MR. Why sleep is important for health: a psychoneuroimmunology perspective. Annu Rev Psychol 2015;66:143–72.
[3] Tang J, Liao Y, Kelly BC, et al. Gender and regional differences in sleep quality and insomnia: a general population-based study in Hunan Province of China. Sci Rep 2017;7:43690.
[4] Roth T. Insomnia: definition, prevalence, etiology, and consequences. J Clin Sleep Med 2007;3(5 Suppl):S7–10.
[5] Green MJ, Espie CA, Benzeval M. Social class and gender patterning of insomnia symptoms and psychiatric distress: a 20-year prospective cohort study. BMC Psychiatry 2014;14:152.
[6] Bjorvatn B, Meland E, Flo E, et al. High prevalence of insomnia and hypnotic use in patients visiting their general practitioner. Fam Pract 2017;34:20–4.
[7] Kredlow MA, Capozzoli MC, Hearon BA, et al. The effects of physical activity on sleep: a meta-analytic review. J Behav Med 2015;38:427–49.
[8] Levenson JC, Kay DB, Buysse DJ. The pathophysiology of insomnia. Chest 2015;147:1179–92.
[9] Riemann D, Spiegelhalder K, Feige B, et al. The hyperarousal model of insomnia: a review of the concept and its evidence. Sleep Med Rev 2010;14:19–31.
[10] Chapman JL, Comas M, Hoyos CM, et al. Is metabolic rate increased in insomnia disorder? A systematic review. Front Endocrinol (Lausanne) 2018;9:374.
[11] Wu YM, Pietrone R, Cashmere JD, et al. EEG power during waking and NREM sleep in primary insomnia. J Clin Sleep Med 2013;9:1031–7.
[12] Dai XJ, Gong HH, Wang YX, et al. Gender differences in brain regional homogeneity of healthy subjects after normal sleep and after sleep deprivation: a resting-state fMRI study. Sleep Med 2012;13:720–7.

[13] Dai XJ, Nie X, Liu X, et al. Gender differences in regional brain activity in patients with chronic primary insomnia: evidence from a resting-state fMRI study. J Clin Sleep Med 2016;12:363–74.
[14] Chen JE, Glover GH. Functional magnetic resonance imaging methods. Neuropsychol Rev 2015;25:289–313.
[15] Fransson P. Spontaneous low-frequency BOLD signal fluctuations: an fMRI investigation of the resting-state default mode of brain function hypothesis. Hum Brain Mapp 2005;26:15–29.
[16] Zuo XN, Di-Martino A, Kelly C, et al. The oscillating brain: complex and reliable. Neuroimage 2010;49:1432–45.
[17] Zou QH, Zhu CZ, Yang Y, et al. An improved approach to detection of amplitude of low-frequency fluctuation (ALFF) for resting-state fMRI: fractional ALFF. J Neurosci Methods 2008;172:137–41.
[18] Yan CG, Zang YF. DPARSF. A MATLAB toolbox for "pipeline" data analysis of resting-state fMRI. Front Syst Neurosci 2010;4:13.
[19] He Y, Wang L, Zang Y, et al. Regional coherence changes in the early stages of Alzheimer's disease: a combined structural and resting-state functional MRI study. Neuroimage 2007;35:488–500.
[20] Coulon P, Budde T, Pape HC. The sleep relay-the role of the thalamus in central and decentral sleep regulation. Pflugers Arch 2012;463:53–71.
[21] Blumberg MS, Gall AJ, Todd WD. The development of sleep-wake rhythms and the search for elemental circuits in the infant brain. Behav Neurosci 2014;128:250–63.
[22] Kozlovskiy SA, Vartanov AV, Nikonova EY, et al. The cingulate cortex and human memory processes. Psychol Russia: State of the Art 2012;5: 231–43.
[23] Brown JW, Braver TS. Risk prediction and aversion by anterior cingulate cortex. Cogn Affect Behav Neurosci 2007;7:266–77.
[24] Ma N, Dinges DF, Basner M, et al. How acute total sleep loss affects the attending brain: a meta-analysis of neuroimaging studies. Sleep 2015;38:233–40.
[25] Eippert F, Veit R, Weiskopf N, et al. Regulation of emotional responses elicited by threat related stimuli. Hum Brain Mapp 2007;28:409–23.
[26] Perogamvros L, Schwartz S. Sleep and emotional functions. Curr Top Behav Neurosci 2015;25:411–31.
[27] Murphy M, Riedner BA, Huber R, et al. Source modeling sleep slow waves. Proc Natl Acad Sci USA 2009;06:1608–13.
[28] Yang W, Chen Q, Liu P, et al. Abnormal brain activation during directed forgetting of negative memory in depressed patients. J Affect Disord 2016;190:880–8.
[29] Cross NE, Carrier J, Postuma RB, et al. Association between insomnia disorder and cognitive function in middle-aged and older adults: a cross-sectional analysis of the Canadian Longitudinal Study on Aging. Sleep 2019;42:zsz114.
[30] Schabus M, Dang-Vu TT, Albouy G, et al. Hemodynamic cerebral correlates of sleep spindles during human nonrapid eye movement sleep. Proc Natl Acad Sci USA 2007;104:13164–9.
[31] Medic G, Wille M, Hemels ME. Short- and long-term health consequences of sleep disruption. Nat Sci Sleep 2017;9:151–61.
[32] Baglioni C, Nanovska S, Regen W, et al. Sleep and mental disorders: a meta-analysis of polysomnographic research. Psychol Bull 2016;142: 969–90.