INTRODUCTION

Primary insomnia (PI) is a disorder of sleeplessness including poor sleep quality or short sleep time. It afflicts 10%–15% of the adult people in the world (Cheung, Bartlett, Armour, & Saini, 2013; Taylor, Lichstein, & Durrence, 2003). Patients with PI commonly have impaired daytime function and many physiological dysfunctions (Buysse et al., 2007; Liu et al., 2014; Riedel & Lichstein, 2000). Electroencephalogram studies have found that PI patients showed elevated spectral power values in the beta and sigma frequency...
band (Spiegelhalder et al., 2012). Greater global cerebral glucose metabolism during sleep was also found using positron emission tomography in PI (Nofzinger et al., 2004). As a noninvasive technique, resting-state functional magnetic resonance imaging can detect spontaneous neural activity in the brain. Patients with insomnia exhibit decreased frontoparietal cortex activation during working memory task performance (Drummond et al., 2013; Li et al., 2014). However, the mechanism by which the PI influences the brain function of the specific brain regions has not been fully elucidated.

Iron contributes to many biological processes, including oxygen transport, protein expression regulation, and cell growth. Previously, it has been found that excessive brain iron deposition plays an important role in the arise and development of brain function disorders and cognitive impairment (Haller et al., 2010; Liu et al., 2015; Smith, Harris, Sayre, & Perry, 1997). Susceptibility-weighted imaging (SWI), which is combined by the phase images and the magnitude images, is sensitive to the paramagnetic effect of iron particles (Haacke, Mittal, Wu, Neelavalli, & Cheng, 2009; Mittal, Wu, Neelavalli, & Haacke, 2009). It has been proved reliable to measure tissue iron concentration, which is consistent with the autopsy examination results. We hypothesized that (a) there is brain iron deposition in PI patients in multiple brain regions and (b) individual cortical brain iron deposition in specific regions could correlate with the brain function alterations of PI patients.

### MATERIALS AND METHODS

#### 2.1 | Subjects

Thirty-five patients with PI were recruited from the sleep disorder clinic of our hospital (Table 1). The diagnosis criteria were made according to the International Classification of Sleep Disorders and Diagnostic and Statistical Manual of Mental Disorders, version 4 (DSM-IV). The Pittsburgh Sleep Quality Index (PSQI) was used to assess the sleep quality and disturbances. The Insomnia Severity Index (ISI) was used to evaluate subjective symptoms of insomnia. All patients underwent formal neuropsychological assessment using the following tests: Mini Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), Clinical Dementia Rating (CDR), Activities of Daily Living Scale (ADL), Figural Recognition Test, Verbal and Categorical Fluency Test, Auditory Verbal Learning Test, Boston Naming Test, Hamilton Anxiety Scale, and Hamilton Depression Scale. Exclusion criteria were as follows: (a) the patient who had an abnormal brain in conventional CT or MRI; (b) the patient who had serious organic disease or severe mental disease. Thirty-five age, gender, and education levels matched normal controls were recruited from the community. The exclusion criteria including: nervous system diseases such as brain trauma, tumor or hemorrhage, alcohol or drug abuse, systemic disease or other MRI contraindication. All subjects with severe depression (Hamilton Depression Rating Scale ≥18) or dementia (MMSE < 24) were also excluded. All of the participants were right-handed. Written informed consent was signed by every participant. This study was conducted in accordance with the Declaration of Helsinki. All the procedures were approved by the Medical Ethics Committee of our institution.

### 2.2 | Magnetic resonance imaging

All subjects were scanned on a 3.0 T whole body MRI scanner (Magnetom Trio, Siemens Healthcare, Erlangen, Germany). Susceptibility-weighted images were obtained with the following parameters: TR/TE: 56/42, flip angle: 20°, section thickness: 2 mm, field of view: 23 × 17 cm, matrix size: 348 × 320. For SWI, postprocessing was performed according to the previous literature (Haacke, Xu, Cheng, & Reichenbach, 2004). The conventional sequences include the transverse T1-weighted images (TR: 200 ms, TE: 2.78 ms, matrix: 384 × 384, flip angle: 70°, voxel size: 0.7 × 0.6 × 5 mm³) and fluid-attenuated inversion recovery (FLAIR) images and susceptibility-weighted images (TR:
9,000 ms, TE: 93 ms, TI: 2,500 ms, matrix: 256 × 256, flip angle: 130°, voxel size: 0.9 × 0.9 × 4 mm³).

Images were analyzed by two independent radiologists who have 10 and 11 years of experience in MRI with SPIN software (Signal Processing in NMR, Version 1751, MRI Institute for Biomedical Research, Detroit, MI; http://www.wayne.edu/download.htm). SWI original images were first processed through a 32 × 32 high-pass filter. Then, the high-pass filtered images were weighted by the coil sensitivity factor and combined to a single complex image. Finally, the corrected phase images and normalized phase mask were created and multiplied with the magnitude image to produce the final SWI and phase images. Bilateral anterior cingulate cortex, posterior cingulate cortex, hippocampus, caudate nucleus, globus pallidus, putamen, thalamus, red nucleus, substantia nigra, parietal cortex, and frontal white matter were selected as region of interest (ROI) (Figure 1). The area of the ROI was 100 pixels in a circular shape. The Siemens Phase Unit was measured from each ROI and converted into radians using according to the previous literature (Haacke et al., 2007).

2.3 | Statistical analysis

SPSS software (version 18.0; SPSS, Chicago, IL) was used for statistical analysis. Comparisons of phase shift values between the PI patients and the normal controls were performed using two-sample t tests. Partial correlations between phase shifts values and the scores of MMSE, MoCA, ADL, PSQI, and ISI tests were analyzed for the PI group. Age, gender, and education levels were imported as covariates and the significance was set at \( p < 0.05 \) corrected with Bonferroni correction. Intraobserver variability was analyzed by using intraclass correlation coefficients.

3 | RESULTS

The PI patients and normal control volunteers had no significant difference in age, sex, and education years (\( p > 0.05 \)).

The phase shift values in the putamen, caudate nucleus, globus pallidus, substantia nigra, red nucleus, thalamus, frontal white matter, and hippocampus of our normal controls were -0.0106, 0.0199, 0.0431, 0.0137, 0.0398, 0.0339, −0.0046, 0.0024, respectively. A close positive correlation (\( R = 0.771, p < 0.05 \)) between the phase shift values of our normal controls and the regional real iron concentrations reported by the literatures (13.32, 9.28, 21.30, 18.64, 19.48, 4.76, 4.24, and 3.13 mg per 100 g of wet weight) was found (Figure 2).

Compared with normal controls, the PI patients have significantly lower MMSE, MoCA scores, and higher PSQI, HAMA, HAMD scores. The PI patients showed significant increased phase shift values in the left caudate nucleus, left putamen, left hippocampus, and bilateral thalamus (\( p < 0.05 \); Table 2). Close correlation was found between the phase shift value of the left hippocampus and MMSE scores (\( R = -0.447, p < 0.05 \); Figure 3).

The intraclass correlation coefficients were >0.90 for all the ROIs.

4 | DISCUSSION

Insomnia is a widespread problem in the world. According to the literature, about 30% of adults experience occasional sleep difficulties, and 6%-13% meet the insomnia diagnostic criteria (Morin, LeBlanc, Daley, Gregoire, & Merette, 2006; Morin et al., 2011; Ohayon, 2002). In this study, the insomnia patients had poorer MMSE and
MoCA scores compared with the normal controls. Cognitive impairments were frequent in people with insomnia. These findings are in agreement with the prior literature. Previously, many studies have compared the neuropsychological performance of individuals with insomnia and normal sleepers. Reliable cognitive differences between the insomnia patients and normal sleepers have been identified in many cognitive domains including working memory, episodic memory, and problem solving (Fortier-Brochu, Beaulieu-Bonneau, Ivers, & Morin, 2012).

Susceptibility-weighted imaging is a new imaging technique that can measure the tissues iron concentration in vivo. Until now, the golden standard of brain iron concentrations is the autopsy

| ROI     | PI (n = 35)        | NC (n = 35)        | t value | p-Value |
|---------|--------------------|--------------------|---------|---------|
| Left PC | 0.0011 ± 0.0111    | −0.0016 ± 0.0096   | 0.903   | 0.370   |
| Right PC| −0.0086 ± 0.0192   | −0.0119 ± 0.0109   | 1.751   | 0.085   |
| Left CN | 0.0563 ± 0.0104    | 0.0387 ± 0.0174    | 5.013   | 0.000*  |
| Right CN| 0.0500 ± 0.0266    | 0.0409 ± 0.0185    | 1.576   | 0.120   |
| Left PU | 0.0450 ± 0.0156    | 0.0195 ± 0.0148    | 6.712   | 0.000*  |
| Right PU| 0.0079 ± 0.0087    | 0.0073 ± 0.0254    | 0.131   | 0.896   |
| Left GP | 0.0459 ± 0.0340    | 0.0357 ± 0.0196    | 1.452   | 0.151   |
| Right GP| 0.0313 ± 0.0280    | 0.0323 ± 0.0188    | 0.162   | 0.872   |
| Left TH | 0.0235 ± 0.0272    | −0.0101 ± 0.0244   | 5.201   | 0.000*  |
| Right TH| 0.0143 ± 0.0139    | 0.0009 ± 0.0141    | 3.867   | 0.000*  |
| Left FWM| 0.0063 ± 0.0181    | −0.0011 ± 0.0129   | 1.390   | 0.140   |
| Right FWM| 0.0083 ± 0.0153   | 0.0059 ± 0.0223    | 0.517   | 0.607   |
| Left HP | −0.0151 ± 0.0191   | −0.0220 ± 0.0225   | 2.473   | 0.016*  |
| Right HP| 0.0026 ± 0.0087    | 0.0008 ± 0.0104    | 1.330   | 0.188   |
| Left RN | 0.0293 ± 0.0229    | 0.0247 ± 0.0249    | 0.903   | 0.370   |
| Right RN| 0.0785 ± 0.0280    | 0.0616 ± 0.0415    | 0.041   | 0.055   |
| Left SN | 0.0198 ± 0.0255    | 0.0067 ± 0.0320    | 1.832   | 0.072   |
| Right SN| 0.0341 ± 0.0391    | 0.0332 ± 0.0298    | 0.096   | 0.924   |
| ACC     | 0.0279 ± 0.0322    | 0.0221 ± 0.0126    | 0.917   | 0.363   |
| PCC     | 0.0179 ± 0.0174    | 0.0130 ± 0.0283    | 0.851   | 0.398   |

ACC, anterior cingulate cortex; CN, caudate nucleus; FWM, frontal white matter; GP, globus pallidus; HP, hippocampus; PC, parietal cortex; PCC, posterior cingulated; PU, putamen; RN, red nucleus; SN, substantia nigra; TH, thalamus.

*denotes p < 0.05.
examinations of 81 normal brains performed by Hallgren and Sourander (1958). In this study, a close correlation was found between regional phase shifts values of our healthy controls and the golden standard in the putamen, caudate nucleus, globus pallidus, substantia nigra, red nucleus, thalamus, frontal white matter, and hippocampus. These results indicated that our SWI method is effective and reliable.

In this study, compared with normal controls, the PI patients showed significant increased phase shift values in the left caudate nucleus, left putamen, left hippocampus, and bilateral thalamus. Close correlation was found between the phase shift value of the left hippocampus and MMSE scores. These results suggest that iron deposition in the left hippocampus plays an important role in the pathophysiological mechanism of cognitive impairment. The hippocampus is responsible for memory function including memory encoding and memory retrieval. Patients with PI commonly showed memory function deficits (Backhaus et al., 2006; Fuldá & Schulz, 2001). Negative correlations of disturbed sleep and memory tasks performance have been reported (Fortier-Brochu & Morin, 2014; Ooesterman, van Someren, Vogels, Van Harten, & Scherder, 2009).

Recently it has been found that insomnia patients show decreased hippocampal volume and atrophy in the cornu ammonis and dentate gyrus which associated with impaired cognitive functions (Joo, Kim, Suh, & Hong. 2014). In this study, we also found iron deposition in thalamus, caudate nucleus, and putamen of the PI patients. The thalamus plays a major role in arousal, awareness level, and activity regulation. It can control the sleep and wakefulness states (Steriade & Llinás, 1988). The caudate nucleus and putamen are parts of the basal ganglia. They are responsible for voluntary movement control and took part in many cognitive functions including memory, emotion, and learning (Bastos-Leite et al., 2007; Kantarci et al., 2010).

The precise mechanisms of the abnormal iron deposition in these brain regions of PI are not understood.

Using SWI technology, brain iron deposition has been reported in several other diseases previously. The iron concentrations of the substantia nigra in PD patients have been proved significantly in several other diseases previously. The iron concentrations of the brain regions of PI are not understood. The precise mechanisms of the abnormal iron deposition in these brain regions of PI are not understood.

ACKNOWLEDGMENT

This work was supported by the National Natural Science Foundation of China (81471194) and the Science and Health Joint Research Project of Chongqing (2018DZXM005).

ORCID

Chen Liu http://orcid.org/0000-0001-5149-2496
Chuanming Li http://orcid.org/0000-0003-3428-7831

REFERENCES

Backhaus, J., Junghans, K., Born, J., Hohaus, K., Faasch, F., & Hohagen, F. (2006). Impaired declarative memory consolidation during sleep in patients with primary insomnia: Influence of sleep architecture and nocturnal cortisol release. Biological Psychiatry, 60, 1324–1330. https://doi.org/10.1016/j.biopsych.2006.03.051
Bastos-Leite, A. J., van der Flier, W. M., van Straaten, E. C., Staekenborg, S. S., Scheltens, P., & Barkhof, F. (2007). The contribution of medial temporal lobe atrophy and vascular pathology to cognitive impairment in vascular dementia. Stroke, 38, 3182–3185. https://doi.org/10.1161/STROKEAHA.107.490102
Buysse, D. J., Thompson, W., Scott, J., Franzen, P. L., Germain, A., Hall, M., ... Kupfer, D. J. (2007). Daytime symptoms in primary insomnia: A prospective analysis using ecological momentary assessment. Sleep Medicine, 8, 198–208. https://doi.org/10.1016/j.sleep.2006.10.006
Chawla, S., Kister, I., Wuerfel, J., Brisset, J.C., Liu, S., Sinnecker, T., ... Ge, Y. (2016). Iron and Non-iron-related characteristics of multiple sclerosis and neuromyelitis optica lesions at 7T MRI. American Journal of Neuroradiology, 37, 1223–1230 https://doi.org/10.3174/ajnr.A4729
Cheung, J. M., Bartlett, D. J., Armour, C. L., & Saini, B. (2013). The insomnia patient perspective, a narrative review. Behavioral Sleep Medicine, 11, 369–389. https://doi.org/10.1080/15402002.2012.694382
Drummond, S. P., Walker, M., Almklov, E., Campos, M., Anderson, D. E., & Straus, L. D. (2013). Neural correlates of working memory performance in primary insomnia. Sleep, 36, 1307–1316.
Fortier-Brochu, E., Beauchesne-Bonneau, S., Ivers, H., & Morin, C. M. (2012). Insomnia and daytime cognitive performance: A meta-analysis. Sleep Medicine Reviews, 16, 83–94. https://doi.org/10.1016/j.smrv.2011.03.008
Fortier-Brochu, E., & Morin, C. M. (2014). Cognitive impairment in individuals with insomnia: Clinical significance and correlates. Sleep, 37, 1787–1798. https://doi.org/10.5665/sleep.4172
Fuldá, S., & Schulz, H. (2001). Cognitive dysfunction in sleep disorders. Sleep Medicine Reviews, 5, 423–445. https://doi.org/10.1053/smrv.2001.0157
Haacke, E.M., Ayaz, M., Khan, A., Manova, E.S., Krishnamurthy, B., Gollapalli, L., ... Kirsch, W. (2007). Establishing a baseline phase behavior in magnetic resonance imaging to determine normal vs. abnormal iron content in the brain. JMRI 26, 256–264. https://doi.org/10.1002/jmri.1522-2586

In conclusion, in this study we found the PI patients have significant cognitive impairment and increased iron deposition in several brain regions. The iron concentration of the left hippocampus is a biomarker of cognitive impairment and may play an important role in the pathophysiological mechanism. This study had several limitations. Firstly, there is a relatively small sample size in this study. Secondly, as in previous literature, we considered that increasing susceptibility reflects increasing iron concentration. However, sometime several other metals such as manganese and copper may have the potential to cause susceptibility (Schenck & Zimmerman, 2004).
Haacke, E. M., Mittal, S., Wu, Z., Neelavalli, J., & Cheng, Y. C. N. (2009). Susceptibility-weighted imaging: Technical aspects and clinical applications, part 1. *American Journal of Neuroradiology*, 30, 19–30. https://doi.org/10.3174/ajnr.A1400

Haacke, E. M., Yang, R., Cheng, Y. C., & Reichenbach, J. R. (2004). Susceptibility weighted imaging (SWI). *Magnetic Resonance in Medicine*, 52, 612–618. https://doi.org/10.1002/mrm.1522-2594

Haller, S., Bartsch, A., Nguyen, D., Rodriguez, C., Emch, J., Gold, G., ... Giannakopoulos, P. (2010). Cerebral microhemorrhage and iron deposition in mild cognitive impairment: Susceptibility-weighted MR imaging assessment. *Radiology*, 257, 764–773. https://doi.org/10.1148/radiol.10100612

Hallgren, B., & Sauerland, P. (1958). The effect of age on the non-haem iron in the human brain. *Journal of Neurochemistry*, 3, 41–51. https://doi.org/10.1016/0022-3042(58)95198-5

Joo, E. Y., Kim, H., Suh, S., & Hong, S. B. (2014). Hippocampal substructural vulnerability to sleep disturbance and cognitive impairment in patients with chronic primary insomnia: Magnetic resonance imaging morphometry. *Sleep*, 37, 1189–1198.

Kantarci, K., Avula, R., Senjem, M. L., Samikoglu, A. R., Zhang, Z., Weigand, S. D., ... Jack, C. R. (2010). Dementia with Lewy bodies and Alzheimer disease: Neurodegenerative patterns characterized by DTI. *Neurology*, 74, 1814–1821. https://doi.org/10.1212/WNL.0b013e3181ee7bcf

Li, Y., Wang, E., Zhang, H., Dou, S., Liu, L., Tong, L., ... Zhang, Q. (2014). Functional connectivity changes between parietal and prefrontal cortices in primary insomnia patients: Evidence from resting-state fMRI. *European Journal of Medical Research*, 19, 32. https://doi.org/10.1186/s12978-014-0045-5

Liu, C., Li, C., Yang, J., Gui, L., Zhao, L., Evans, A. C., ... Wang, J. (2015). Characterizing brain iron deposition in subcortical ischemic vascular dementia using susceptibility-weighted imaging: An in vivo MR study. *Behavioral Brain Research*, 288, 33–38. https://doi.org/10.1016/j.bbr.2015.04.003

Liu, H., Wang, D., Li, Y., Li, Z., Zhang, Y., Lei, F., ... Tang, X. (2014). Examination of daytime sleepiness and cognitive performance testing in patients with primary insomnia. *PLoS ONE*, 9(6), e100965. https://doi.org/10.1371/journal.pone.0100965

McCrea, R. P., Harder, S. L., Martin, M., Buist, R., & Nichol, H. (2008). A comparison of rapid-scanning X-ray fluorescence mapping and magnetic resonance imaging to localize brain iron distribution. *European Journal of Radiology*, 68, S109–S113. https://doi.org/10.1016/j.ejrad.2008.04.048

Mittal, S., Wu, Z., Neelavalli, J., & Haacke, E. M. (2009). Susceptibility-weighted imaging: Technical aspects and clinical applications, part 2. *American Journal of Neuroradiology*, 30, 232–252. https://doi.org/10.3174/ajnr.A1461

Morin, C. M., LeBlanc, M., Belanger, L., Ivers, H., Merette, C., & Savard, J. (2011). Prevalence of insomnia and its treatment in Canada. *Canadian Journal of Psychiatry*, 56, 540–548. https://doi.org/10.1177/070674371105600905

Morin, C. M., LeBlanc, M., Daley, M., Gregoire, J. P., & Merette, C. (2006). Epidemiology of insomnia: Prevalence, self-help treatments, consultations, and determinants of help-seeking behaviors. *Sleep Medicine*, 7, 123–130. https://doi.org/10.1016/j.sleep.2005.08.008

Nakada, T., Matsuzawa, H., Igarashi, H., Fujii, Y., & Kwee, I. L. (2008). In vivo visualization of senile-particle-like pathology in Alzheimer's disease patients by MR microscopy on a 7T system. *Journal of Neuroimaging*, 18, 125–129. https://doi.org/10.1111/j.1552-6569.2007.00179.x

Nofzinger, E. A., Buysse, D. J., Germain, A., Price, J. C., Miewald, J. M., & Kupfer, D. J. (2004). Functional neuroimaging evidence for hyperarousal in insomnia. *American Journal of Psychiatry*, 161, 2126–2128. https://doi.org/10.1176/appi.ajp.161.11.2126

Ohayon, M. M. (2002). Epidemiology of insomnia: What we know and what we still need to learn. *Sleep Medicine Reviews*, 6, 97–111. https://doi.org/10.1053/smrv.2002.0186

Oosterman, J. M., van Someren, E. J., Vogels, R. L., Van Harten, B., & Scherder, E. J. (2009). Fragmentation of the rest-activity rhythm correlates with age-related cognitive deficits. *Journal of Sleep Research*, 18, 129–135. https://doi.org/10.1111/j.1365-2869.2008.00704.x

Riedel, B. W., & Lichstein, K. L. (2000). Insomnia and daytime functioning. *Sleep Medicine Reviews*, 4, 277–298. https://doi.org/10.1053/smrv.1999.0074

Schenck, J. F., & Zimmerman, E. A. (2004). High field magnetic resonance imaging of brain iron: Birth of a biomarker? *NMR in Biomedicine*, 17, 433–445. https://doi.org/10.1002/(ISSN)1099-1492

Schwietzer, A. D., Liu, T., Gupta, A., Zheng, K., Seedial, S., Shitlabinas, A., ... Tsouris, A. J. (2015). Quantitative susceptibility mapping of the motor cortex in amyotrophic lateral sclerosis and primary lateral sclerosis. *American Journal of Roentgenology*, 204(5), 1086–1092. https://doi.org/10.2214/AJR.14.13459

Smith, M. A., Harris, P. L., Sayre, L. M., & Perry, G. (1997). Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. *Proceedings of the National Academy of Sciences of the United States of America*, 94, 9866–9868. https://doi.org/10.1073/pnas.94.18.9866

Spiegelhalder, K., Regen, W., Feige, B., Holz, J., Piosczyk, H., Baglioni, C., ... Nissen, C. (2012). Increased EEG sigma and beta power during NREM sleep in primary insomnia. *Biological Psychology*, 91(3), 329–333. https://doi.org/10.1016/j.biopsycho.2012.08.009

Steriade, M., & Linas, R. R. (1988). The functional states of the thalamus and the associated neuronal interplay. *Physiological Reviews*, 68(3), 649–742. https://doi.org/10.1152/physrev.1988.68.3.649

Taylor, D. J., Lichstein, K. L., & Durren, H. H. (2003). Insomnia as a health risk factor. *Behavioral Sleep Medicine*, 1, 227–247. https://doi.org/10.1016/S1540-2101(03)00104_5

Zhang, J., Zhang, Y., Wang, J., Cai, P., Luo, C., Qian, Z., ... Feng, H. (2010). Characterizing iron deposition in Parkinson's disease using susceptibility-weighted imaging: An in vivo MR study. *Brain Research*, 1330, 124–130. https://doi.org/10.1016/j.brainres.2010.03.036

How to cite this article: Chen L, Wei X, Liu C, Li C, Zhou Z. Brain iron deposition in primary insomnia—An in vivo susceptibility-weighted imaging study. *Brain Behav*. 2019;9:e01138. https://doi.org/10.1002/brb3.1138