Received: 8 June 2022 | Revised: 2 August 2022 | Accepted: 16 August 2022

DOI: 10.1002/hbm.26072

RESEARCH ARTICLE

WILEY

The role of ascending arousal network in patients with chronic insomnia disorder

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Funding information
Anhui Provincial Department of Science and Technology, Grant/Award Number: 202004j07020006; National Natural Science Foundation of China, Grant/Award Number: 82001803; Sichuan Provincial Science and Technology Department Project in China, Grant/Award Numbers: 2020YJ0176, 2020YJ0197, 2021YJ0162; Project of the Hefei Municipal Health Commission and Collaborative Innovation Center for Neuropsychiatric Disorders and Mental Health, Grant/Award Number: NDMHCI-19-01; Chengdu Science and Technology Department Project, Grant/Award Number: 2021-YF05-00247-SN

Abstract

The ascending arousal system plays a crucial role in individuals' consciousness. Recently, advanced functional magnetic resonance imaging (fMRI) has made it possible to investigate the ascending arousal network (AAN) in vivo. However, the role of AAN in the neuropathology of human insomnia remains unclear. Our study aimed to explore alterations in AAN and its connections with cortical networks in chronic insomnia disorder (CID). Resting-state fMRI data were acquired from 60 patients with CID and 60 good sleeper controls (GSCs). Changes in the brain's functional connectivity (FC) between the AAN and eight cortical networks were detected in patients with CID and GSCs. Multivariate pattern analysis (MVPA) was employed to differentiate CID patients from GSCs and predict clinical symptoms in patients with CID. Finally, these MVPA findings were further verified using an external data set (32 patients with CID and 33 GSCs). Compared to GSCs, patients with CID exhibited increased FC within the AAN, as well as increased FC between the AAN and default mode, cerebellar, sensorimotor, and dorsal attention networks. These AAN-related FC patterns and the MVPA classification model could be used to differentiate CID patients from GSCs with 88% accuracy in the first cohort and 77% accuracy in the validation cohort. Moreover, the MVPA prediction models could separately predict insomnia (data set 1, $R^2 = .34$; data set 2, $R^2 = .15$) and anxiety symptoms (data set 1, $R^2 = .35$; data set 2, $R^2 = .34$) in the two independent cohorts of patients. Our findings indicated that AAN contributed to the neurobiological mechanism of insomnia and highlighted that fMRI-based markers and machine learning techniques might facilitate the evaluation of insomnia and its comorbid mental symptoms.

KEYWORDS
anxiety, ascending arousal system, chronic insomnia disorder, functional connectivity, multivariate pattern analysis

1 | INTRODUCTION

Insomnia is a common sleep disorder characterized by difficulty in falling asleep, staying asleep, or waking up too early in the morning, with significant impairment of daytime functioning. Insomnia affects approximately 10–20% of the people, with about half of those suffering from a chronic course of the disease (Buysse, 2013), known as chronic insomnia disorder (CID). Recurrent insomnia is associated with a worse quality of life, a high rate of comorbidity with mental disorders, and an increased risk of depression and anxiety (Gong, Shi, et al., 2021; Gong, Yu, et al., 2021; Morin et al., 2015). Although insomnia is ranked as the second most common neuropsychiatric disorder (Wittchen et al., 2011), the understanding of the neurobiological mechanisms underlying CID is limited (Riemann et al., 2015; Van Someren, 2021).

Hyperarousal is commonly mentioned as the core subjective experience and persistent characteristic of patients with insomnia (Blanken et al., 2019; Pavlova et al., 2001). Recently, the brain hyperarousal process model has provided critical insights into the central pathogenesis of insomnia (Nofzinger et al., 2004; Riemann et al., 2010). In a positron emission tomography study, insomnia patients revealed higher brain metabolism across waking and sleep states, with the ascending reticular activating system failing to diminish metabolism during the transition from awake to asleep (Nofzinger et al., 2004). An electroencephalogram study also reported high levels of arousal in patients with insomnia during wakefulness and sleep (Colombo et al., 2016; Perlis et al., 2001). Using the resting-state functional connectivity (FC) approach, it has been observed that the insomnia-related hyperarousal state is particularly pronounced among the broadly cortical networks, including the default mode network (DMN), salience network (SN), sensorimotor network (SMN), dorsal attention network (DAN), and frontoparietal control network (FPN) (Y. Cheng et al., 2021; Fasiello et al., 2022; Schiel et al., 2020; Yu et al., 2018). Despite extensive studies on the functional brain networks of CID, limited neuroimaging studies have focused on the ascending arousal system in patients with insomnia.

The ascending arousal network (AAN) is defined as the subcortical neural network that supports human consciousness (Steriade, 1996). The AAN is located in the brainstem and connects the brainstem extensively to the hypothalamus, thalamus, basal forebrain, and cortex and activates cortical awareness networks (Edlow et al., 2012). The main neurotransmitters involved in AAN include noradrenergic neurons (locus coeruleus [LC]), serotonergic neurons (raphe nuclei), dopamine neurons (periaqueductal gray matter [PAG]), glutamatergic neurons (parabrachial complex [PBC]), and cholinergic neurons (pedunculopontine tegmental nucleus [PPN]), which are essential for sleep and circadian rhythms (Edlow et al., 2012; Saper et al., 2005). In recent years, using in vivo mapping of the human AAN, researchers have found that patients with acute and chronic disorders of consciousness (Snider et al., 2019; Snider et al., 2020) exhibit altered AAN connectivity. More recently, Guardia et al. investigated the effect of age on the AAN and found that AAN-cortical connectivity is significantly disrupted with age and that these connections could predict cognitive performance (Guardia et al., 2022). In our previous study, we found a disrupted LC (one nucleus of the AAN) FC network in patients with CID, and the alteration in FC connectivity was associated with symptoms of anxiety in patients (Gong, Shi, et al., 2021; Gong, Yu, et al., 2021). Although progress has been made in characterizing the brain hyperarousal network in insomnia, to the best of our knowledge, a complete understanding of the intranetwork and internetwork FC patterns between the AAN and cortical networks in human insomnia is still unavailable.

The primary purpose of this study was to explore the patterns of altered FC within the AAN and between the AAN and eight cortical networks in patients with CID, using resting-state functional magnetic resonance imaging (fMRI) data. We hypothesized that FC within the AAN and FC between the AAN-cortical networks would be altered in patients with CID. The second purpose was to examine the accuracy and reliability of a classification model based on AAN-cortical network features and machine learning methods (i.e., multivariate pattern analysis [MVPA]) to distinguish between patients and healthy individuals using two independent data sets. Furthermore, given the common comorbidity of depressive and anxiety symptoms in CID and our previous work confirming the association between the LC network and anxiety symptoms (Gong, Shi, et al., 2021; Gong, Yu, et al., 2021), the third purpose was to develop a predictive model of mental symptoms in patients with CID. We hypothesized that AAN-related connectivity would be important in distinguishing insomniacs from good sleepers as well as in predicting the severity of insomnia and anxiety symptoms in patients with CID.

2 | METHODS

2.1 | Participants

A total of 60 patients with CID and 60 demographically matched good sleeper controls (GSCs) were enrolled in this study. All participants were recruited from the outpatients of Chengdu Second People's Hospital and underwent a series of neuropsychological tests and MRI scans. The study was approved by the Institutional Review Board Ethics Committee of Chengdu Second People's Hospital (ethics approval number: 2020021), and written informed consent was obtained from each subject. Seven participants (three patients with CID and four GSC) were excluded because of excessive head motion artifacts (above 2 mm or 2°, as detailed in the preprocessing of fMRI data). The final analysis included 57 patients with CID and 56 GSCs.

We also included an independent data set of 32 patients with CID and 33 GSCs for classification and regression model validation. Individuals in the independent data set were recruited from outpatients, and all provided written informed consent at the Third Affiliated Hospital of Anhui Medical University (ethics approval number: 2019-010-1). Five participants (two patients with CID and three GSCs) were excluded because of excessive head motion artifacts. The

final analysis included 30 patients with CID and 30 GSCs in the independent data set.

The inclusion criteria for patients with CID were as follows: (1) meeting the diagnostic criteria for CID as outlined in the International Classification of Sleep Disorders, third version (Sateia, 2014); (2) a Pittsburgh Sleep Quality Index (PSQI) score higher than 7 (X. Liu et al., 1996; Mollayeva et al., 2016); (3) not taking any hypnotic medication 2 weeks prior to the neuropsychological test and MRI scan; and (4) between 18 and 65 years of age. The inclusion criteria for GSCs were similar to those for CID, but without sleep complaints and with a PSQI score below 7. The exclusion criteria for all participants included: (1) history of other neuropsychiatric disorders and serious chronic diseases (e.g., diabetes, heart disease, and cancer); (2) other sleep disorders, such as sleep-related breathing disorders (sleep apnea syndrome), central disorders of hypersomnolence, circadian rhythm sleep–wake disorders, sleep-related movement disorders, parasomnia, and hypersomnia; (3) a history of substance addiction (e.g., drugs, nicotine, alcohol); (4) contraindications to MRI; and (5) brain lesions or white matter hyperintensities detected by routine T2-weighted MRI scans.

2.2 | Clinical evaluation

The PSQI scale was used to assess subjective sleep quality and the severity of insomnia (Backhaus et al., 2002). Zung's Self-Rating Depression Scale (SDS) and Zung's Self-Rating Anxiety Scale (SAS) were used to evaluate depression and anxiety (Zung, 1971; Zung et al., 1965). All neuropsychological tests were performed before the imaging scan.

2.3 | Imaging data

The imaging of data set 1 was performed at Chengdu Second People's Hospital using a GE 3.0-Tesla scanner (GE Healthcare Discovery Pioneer, General Electric, Milwaukee, WI). Imaging of data set 2 was performed at the Third Affiliated Hospital of Anhui Medical University using a Siemens 3.0-Tesla scanner (Siemens, Erlangen, Germany). The same MRI scan parameters were used for both data sets. Structural images were acquired using a high-resolution spoiled gradient-recalled echo sequence with the following parameters: repetition time/echo time (TR/TE), 7.06/3.04 ms; flip angle (FA), 12°; acquisition matrix, 256 × 256; field-of-view, 240 × 240 mm; thickness, 1.0 mm; gap, 0 mm; number of slices, 192; and number of excitations, 1.0. Functional images were obtained using an 8-min gradient-recalled echo-planar imaging pulse sequence with the following parameters: TR/TE, 2000/30 ms; FA, 90°; acquisition matrix, 64 × 64; thickness, 3.5 mm; number of slices, 33; and number of time points, 240. All participants were instructed to relax and keep their eyes closed during the scan, and stabilizers were used to immobilize their head. After the scan, each participant was asked if they were awake, and all participants claimed to be awake during the study.

2.4 | Imaging preprocessing

The two data sets underwent the same imaging preprocessing. The imaging data were preprocessed using SPM12 (http://www.fil.ion.ucl.ac.uk/spm) and the DPABI 6.0 (Data Processing & Analysis of Brain Imaging; http://rfmri.org/dpabi) implemented in MATLAB 9.0 (The MathWorks, Inc., Natick, MA) (Yan et al., 2016). Preprocessing included the following steps: removal of the first five initial volumes, slice time correction, reorientation, realignment, co-registration with T1-weighted structural images, normalization to standard stereotactic Montreal Neurological Institute space (resampled to $3 \times 3 \times 3\ mm^3$ voxels), detrending, filtering (0.01–0.08 Hz), regression out of white matter/cerebrospinal fluid/whole brain signals, 24 head motion-related covariates, 24 head motion-related covariates, and smoothing (full-width at half-maximum, 6 mm). Participants with head motions exceeding 2 mm or 2° were excluded from the imaging analysis. There was no significant difference in the mean framewise displacement between the groups in the two data sets.

2.5 | Region of interest definition and network construction

The structure of the AAN was acquired from the Ascending Arousal Network Atlas (Martinos Center for Biomedical Imaging, Charlestown, MA; https://www.nmr.mgh.harvard.edu/resources/aan-atlas) (Edlow et al., 2012). Nine regions of interest (ROIs) were defined in the AAN atlas (Figure 1): the dorsal raphe nucleus, LC, mesencephalic reticular formation (MRF), median raphe nucleus (MR), PAG, PBC, pontine nucleus oralis (PO), PPN, and ventral tegmental area. The 32 nodes of the eight cortical networks were obtained from the FSL Harvard-Oxford atlas available in the Conn Toolbox v.21 (https://web.conn-toolbox.org/) (see Table S1 for details). The eight cortical functional networks included DMN, four nodes; SN, seven nodes; DAN, four nodes; FPN, four nodes; SMN, three nodes; visual network (VN), four nodes; language network (LN), four nodes; and cerebellar network (CBN), two nodes. For each participant, Pearson's correlation analyses were conducted to obtain the correlation coefficients between the preprocessed fMRI time series of each ROI and all other ROIs (Wang et al., 2018). In addition, Fisher's Z-transformation was applied to improve the correlation coefficients so that they approached a normal distribution [$Z = 0.5\ln(1 + CC)/(1 - CC)$] (F. Liu et al., 2017). Finally, a 41 × 41 FC matrix (intra- and inter-ANN and eight cortical networks) was obtained for each participant and used for subsequent analyses (Xu et al., 2020).

2.6 | Statistical analysis

2.6.1 | Clinical data analysis

For demographic and clinical information, two-sample *t* tests and chi-square tests were employed to compare the differences between the

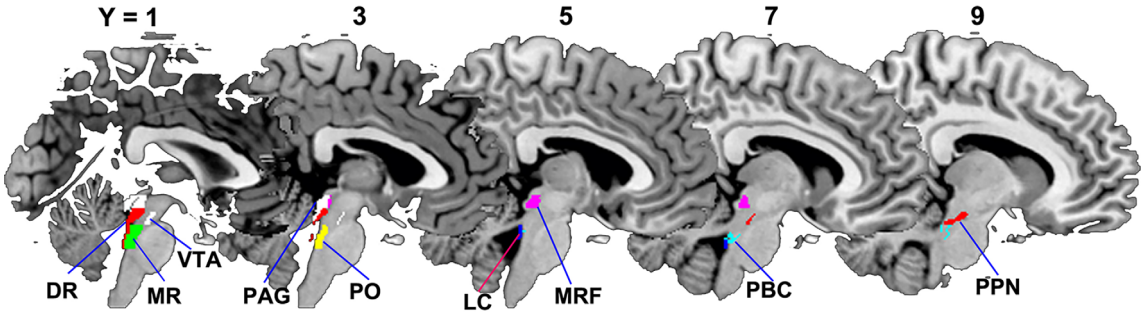

FIGURE 1 The nodes of Harvard ascending arousal network. DR, dorsal raphe nucleus; LC, locus coeruleus; MR, median raphe nucleus; MRF, mesencephalic reticular formation; PAG, periaqueductal gray; PBC, parabrachial complex; PO, pontine nucleus oralis; PPN, pedunculopontine tegmental nucleus; VTA, ventral tegmental area

CID and GSC groups (SPSS 24.0, SPSS Inc.). The significance threshold was set at *p*-value <.05.

2.6.2 | Functional brain network analysis

For the AAN and cortical networks, two-sample *t* tests were utilized to determine group differences in the FC matrix between CID patients and GSCs, after controlling for the effects of age, gender, and years of education. The significance level was set at *p*-value <.05, and the false discovery rate method was used for multiple comparison correction.

2.6.3 | Multivariate pattern analysis

The toolbox of MVPA of Neuroimaging Data (http://funi.tmu.edu.cn/index.php?c=article&a=type&tid=444) was employed for machine learning analysis with fMRI data, based on MATLAB 9.0 platform (Peng et al., 2020). In rs-fMRI research, support vector machine (SVM) is one of the most widely used machine learning algorithms and is well-generalized (James et al., 2013; Meier et al., 2012). Hence, we chose the SVM algorithm to construct a classification model (support vector classification [SVC]) and regression model (support vector regression [SVR]) in this study (Chang & Lin, 2011).

The SVC was employed for the classification of patients with CID from GSCs, and the FC matrix for each participant was used as the input feature. A 10-fold cross-validation was performed to avoid overfitting the training set. The C-SVC with linear kernel and default SVC parameters (penalty coefficient $c = 1$, gamma $g = 0.1$, degree $d = 3$, coefficient $r = 0$, nu $n = 0.5$, and epsilon in the loss function $p = 0.1$) were set for the classification model. SVC results were reported in terms of mean accuracy, specificity, sensitivity, and area under the receiver operating characteristic curve (AUC).

For the prediction of clinical symptoms in the CID group, SVR was employed, the FC matrix for each participant was used as input features, and the clinical scores (i.e., PSQI, SAS, and SDS) were set as labels. Leave-one-out cross-validation was used for the prediction model to ensure separation between the training and testing samples. The e-SVR with a linear kernel was used for regression analyses with default SVR parameters. The squared prediction-outcome correlation (R^2) and mean absolute error (MAE) were calculated to assess the predictive power of SVR (Lindquist et al., 2017; Wager et al., 2013).

2.6.4 | Permutation testing and weight calculation

The statistical significance of the classification and regression models was tested by using a permutation test (1000 times). In addition, the corresponding mean weight of each FC was computed for each SVC and SVR model.

2.7 | Validation analysis in an independent data set

We also tested the external validity of the SVC and SVR models using the following steps. First, we tested whether the FC classifier features in data set 1 could distinguish CID patients from GSCs in an independent data set. The SVC model trained in the first data set was applied to an independent sample of patients with CID and GSCs, without model fitting. Second, we tested whether the FCs predictor feature in data set 1 could predict clinical symptoms (PSQI, SAS, and SDS scores) in patients with CID in the independent data set. The SVR model trained with the first data set was applied to data set 2 without model fitting.

3 | RESULTS

3.1 | Demographic and clinical features

Table 1 shows that there were no significant group differences between patients with CID and GSCs in terms of sex, age, and years of education ($p > .05$). The mean disease duration in the CID group was 57.80 months. The CID group demonstrated worse sleep quality, greater anxiety, and higher depression scores than the GSC group ($p < .001$).

TABLE 1 Demographic, clinical characteristics, and brain volume for two groups

Characteristic	Data set 1				Data set 2			
	CID ($n = 57$)	GSC ($n = 56$)	T/χ^2 value	p-Value	CID ($n = 30$)	GSC ($n = 30$)	T/χ^2 value	T/χ^2 value
Age	34.49 ± 11.46	34.43 ± 8.81	0.03	.97	39.86 ± 11.39	39.10 ± 9.76	0.28	0.78
Gender (female/male)	37/20	36/20	0.01	.94[a]	19/11	18/12	0.07	0.79[a]
Year of education	15.73 ± 3.07	15.27 ± 3.41	0.77	.44	12.67 ± 3.99	11.13 ± 4.34	1.42	0.16
Duration (months)	57.80 ± 59.72	-	-	-	52.40 ± 54.02	-	-	-
PSQI	12.96 ± 2.63	3.58 ± 1.81	22.04	<.001	13.73 ± 1.92	3.56 ± 2.14	19.31	<0.001
SDS	44.05 ± 10.97	30.80 ± 7.81	7.39	<.001	44.00 ± 5.07	27.50 ± 12.07	6.90	<0.001
SAS	42.19 ± 9.68	27.19 ± 5.87	10.07	<.001	43.10 ± 8.56	24.75 ± 7.16	9.00	<0.001

Abbreviations: CID, chronic insomnia disorder; GSC, good sleep control; PSQI, Pittsburgh Sleep Quality Index; SAS, Zung's Self-Rating Anxiety Scale; SDS, Zung's self-Rating Depression Scale.
[a]The p value was obtained by chi-square test; other p values were obtained by a two-way t test.

3.2 | Functional brain network results

The average FC matrix for each group is illustrated in Figure 2a,b. In the GSC and CID groups, the FC patterns within the AAN and cortical networks were positive, whereas most of the connections between the nodes of the cortical functional network and AAN were negative.

Regarding group differences in FC matrices, the CID group exhibited both increased and decreased FC compared to the GSC group (Figure 2c,d). Notably, the FCs of AAN-SMN and AAN-DAN were negative in the GSC group (Figure 2a). Thus, a decrease in the FCs of AAN-SMN and AAN-DAN imply that FCs in patients with CID were increased compared to GSCs. Detailed FC alteration results are presented in Table S2. Overall, compared to the GSC group, the enhanced positive FCs in the CID group were located within the AAN, between the AAN and DMN and between the AAN and CBN, while the elevated negative FCs in the CID group were situated between the AAN and SMN and between the AAN and DAN.

3.3 | SVC classification results

The SVC classification results are illustrated in Figure 3a. The classification model showed a total accuracy of 88.33%, a specificity of 86.67%, and a sensitivity of 90.00%. The area under the curve of the classification model was 0.93. The permutation test showed that the classification model was significantly higher than the chance-level classification accuracy ($p < .001$). The top 10 FCs that contributed to SVC classification included FCs within the AAN and LN, AAN and DMN, AAN and DAN, AAN and SMN, SN and VN, and DMN and VN (Table 2). It should be noted that all the top 10 FCs in the classification model were dysfunctional in the CID group.

3.4 | External validity of the SVC classification model

The SVC classification model obtained from data set 1 was used to discriminate patients with CID from GSCs in an independent cohort. The SVC model yielded an accuracy of 77.34%, a specificity of 78.94%, a sensitivity of 75.44%, and an AUC of 0.84 ($p = .001$), indicating good generalizability in the independent data set 2 (Figure 3b).

3.5 | SVR prediction results

The SVR prediction results showed that FC patterns could predict the severity of clinical symptoms in patients with CID. Specifically, FC patterns could predict sleep quality ($R^2 = .34$; $p = 1.96 \times 10^{-6}$; MAE = 1.62; permutation $p = .005$; Figure 4a), anxiety symptoms ($R^2 = .35$; $p = 1.06 \times 10^{-6}$; MAE, 6.41; permutation $p = .003$; Figure 4b), and depressive symptoms ($R^2 = .38$; $p = 3.32 \times 10^{7}$; MAE, 6.99; permutation $p = .013$; Figure 4c). The top 10 FCs contributing to the SVR prediction model for PSQI scores included FCs within the AAN and DMN as well as FCs between the FPN and VN, DMN and LN, SN and VN, DAN and LN, and DAN and CBN. The top 10 FCs contributing to the SVR prediction model for SAS scores included FCs within the CBN and VN as well as FCs between the AAN and FPN, FPN and DMN, FPN and SN, FPN and LN, DAN and VN, SN and VN, and DMN and CBN. The top 10 FCs that contributed to the SVR prediction model for SDS scores included the FCs within the AAN, FPN, and SN as well as the FCs between the DAN and VN, and between SN and VN. The top 10 FCs that contributed to the clinical symptom prediction models are presented in Table 2.

3.6 | External validity of the SVR prediction model

The SVR predictive model obtained from data set 1 was used to predict the clinical symptoms of patients with CID in an independent insomnia cohort. The SVR prediction model could predict insomnia symptoms ($R^2 = .15$; $p = .04$; MAE = 2.02) and anxiety symptoms ($R^2 = .34$; $p = 1.07 \times 10^{6}$, MAE = 5.43) in independent data set 2. The SVR prediction model could not predict depressive symptoms in patients with CID ($R^2 = .09$; $p = .07$).

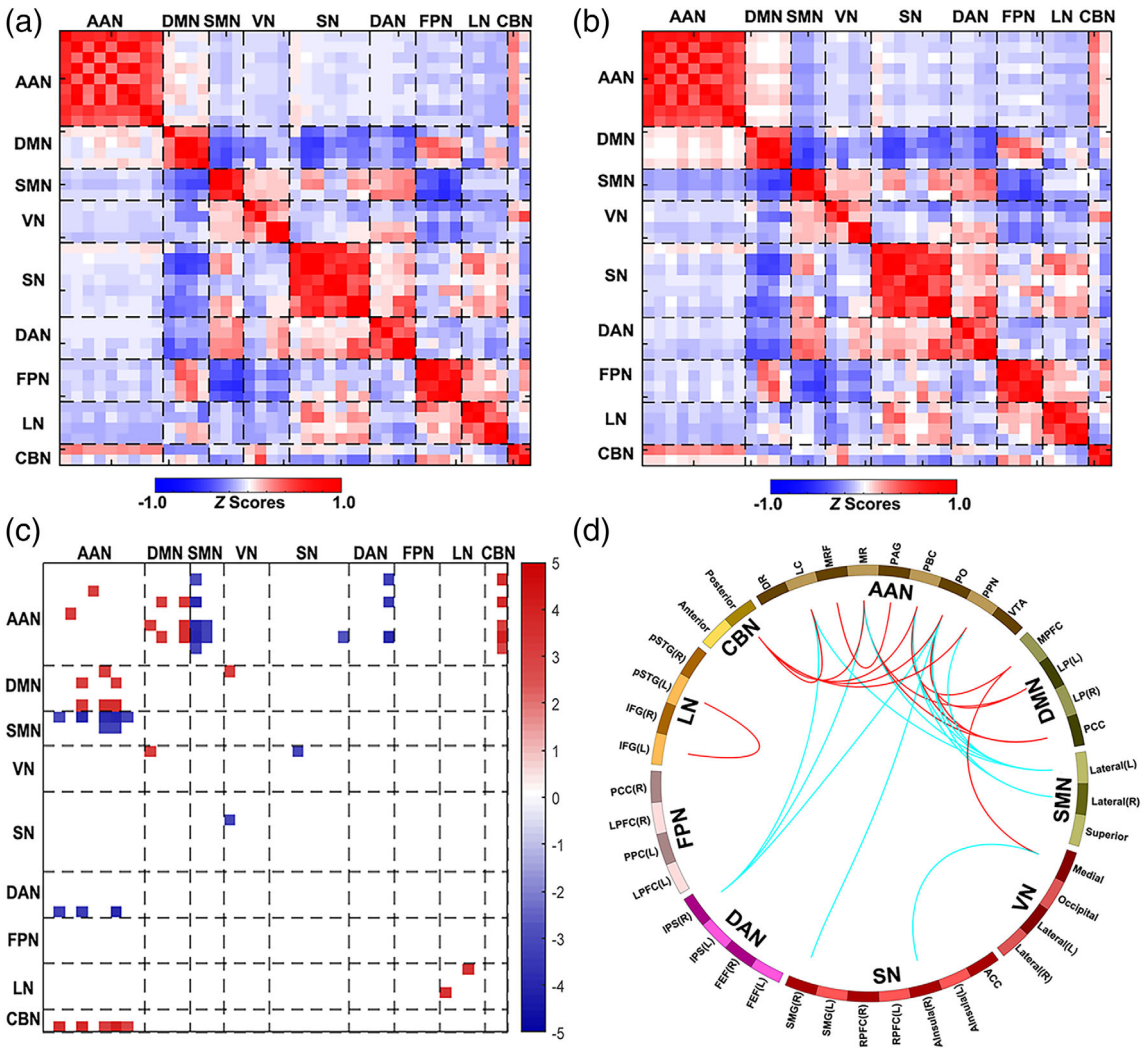

FIGURE 2 The AAN and cortical networks FC patterns in each group and group differences between CID and GSC. (a) The average FC pattern of GSC group; (b) the average FC pattern of CID group; and (c,d) the group difference of ANN and cortical networks between CID and GSC. The result was illustrated with matrix (c) and Circos (d). AAN, ascending arousal network; CBN, cerebellar network; CID, chronic insomnia disorder; DAN, dorsal attention network; DMN, default mode network; FC, functional connectivity; FPN, frontoparietal control network; GSC, good sleep control; LN, language network; SMN, sensorimotor network; SN, salience network; VN, visual network

4 | DISCUSSION

The current study demonstrated abnormal FC patterns across the AAN and eight resting-state cortical networks in patients with CID and explored potential AAN-based biomarkers for discriminating insomniacs from controls and predicting clinical symptoms in patients with CID. First, we found that the AAN plays a critical role in information transfer between the brainstem and cerebral cortex in patients with CID, with elevated positive FCs found in AAN-DMN and AAN-CBN and elevated negative FCs found in AAN-SMN and AAN-DAN. Second, the cross-validation accuracy of the AAN-based FCs pattern in discriminating patients with CID from GSCs was 88%, and there was an independent validation accuracy of 77%. Third, the AAN-based FCs pattern can predict clinical symptoms in patients with CID and, in particular, the predictive models for insomnia and anxiety symptoms can also be externally validated in the independent data set. Cumulatively, we believe that AAN may serve as an important network for the brain mechanisms underlying CID and its comorbidities with mental symptoms.

4.1 | Disruption of AAN-cortical network coupling patterns in patients with CID

In recent years, advances in in vivo neuroimaging have allowed us to investigate the function of the ascending arousal system in human brainstem nuclei (Beissner et al., 2014; Bianciardi et al., 2015; Singh et al., 2022). The present study found that the ascending arousal nuclei were positively connected to each other within the AAN. These intranetwork results are consistent with those of recent studies on the FC of ascending arousal nuclei using resting-state fMRI data (Beissner et al., 2014; Guardia et al., 2022; Singh et al., 2022). In

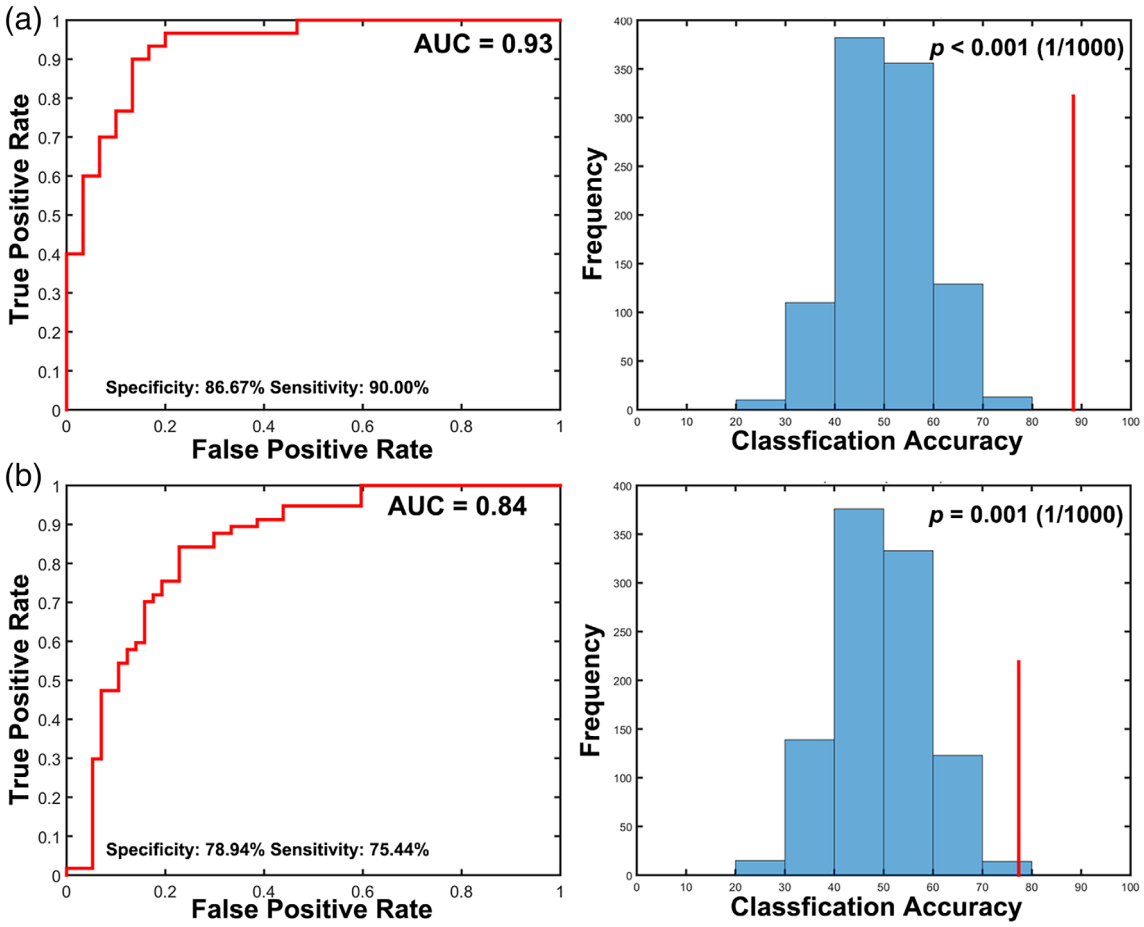

FIGURE 3 The classification results of SVC analysis based on functional connectivity between AAN and cortical networks. (a) The SVC classification performance in data set 1. (b) The classification performance in data set 2 based on the model obtained from data set 1. Left, the classification performance. Right, the permutation tests results. AAN, ascending arousal network; SVC, support vector classification

addition, internetwork results showed increased positive FCs of AAN-DMN, AAN-CBN, and MRF-PAG and increased negative FCs of AAN-SMN and AAN-DAN in patients with CID compared to GSCs. Considered together, these findings indicate that AAN-based intranetwork and internetwork functional coupling was altered in patients with CID, that is, there was an increased information transfer between wake-promoting brain regions (ascending arousal systems) and cortical regions.

LC, MR, PBC, PO, and PPN were the major nodes that connected the AAN to other resting-state networks. The LC in AAN produces most of the brain's noradrenaline (NE), which is thought to be a crucial neurotransmitter for brain wakefulness and arousal (Berridge et al., 2012; Moruzzi & Magoun, 1949). Our previous fMRI study found increased FCs in the somatosensory association cortex (supramarginal gyrus) and visual cortex (occipital cortex) of the LC in patients with CID, and disruption of LC connectivity has also been linked to the duration of insomnia (Gong, Shi, et al., 2021; Gong, Yu, et al., 2021). In this study, we observed increased connectivity of the LC to the SMN, DAN, and CBN in CID patients, suggesting that insufficient silencing of LC-NE activity may lead to disruption of overnight adaptive processes and restless sleep (Swift et al., 2018).

MR is the core nucleus for GABA, hypocretin, and serotonin transmission, and MR neurons are thought to be involved in the regulation of fear response and sleep–wake activity (Hsiao et al., 2019; Varga et al., 2002). This study found that patients with CID exhibited increased intrinsic FC of the DMN, SMN, DAN, and CBN with MR, indicating an abnormal modulation of serotonin and GABA transmission from the ascending arousal system to the cortical cortex. The PBC receives visceral afferent information from the brainstem and outputs this information to the hypothalamus, amygdala, and cortical cortex (Herbert et al., 1990). Recent research has linked PBC to facilitating arousal in the cerebral cortex (Fuller et al., 2011) and to the pathological mechanism of obstructive sleep apnea (Kaur et al., 2013). In addition, PBC, PO, and PPN are considered to be involved in cortical activation via the glutamatergic pathway (Pedersen et al., 2017). The increased intrinsic FCs of the PBC, PO, and PPN with the DMN, SMN, and CBN in the present study may indicate an abnormal mechanism of glutamatergic pathway-promoted cortical arousal in CID.

Overall, the current study demonstrated increased communication between the major nodes in the AAN and multiple cortical networks in patients with CID. These findings confirm and extend previous work (Fernandez-Mendoza et al., 2016; Nofzinger

TABLE 2 The top 10 FCs contribute to the SVC classification model and SVR prediction model

SVC classification		PSQI prediction		SAS prediction		SDS prediction	
FC	**Weight**	**FC**	**Weight**	**FC**	**Weight**	**FC**	**Weight**
DMN.MPFC-VN. Medial	1.74	AAN.DR-AAN.PPN	2.52	FPN.PPC(R)-SN.SMG(L)	−4.20	FPN.PPC(R)-AAN. PAG	3.97
SN.AInsula(R)-VN. Medial	−1.27	FPN.LPFC(R)-VN. Lateral(R)	1.78	VN.Lateral(L)-VN. Occipital	−3.12	FPN.PPC(R)-AAN.DR	3.75
LN.pSTG(L)-LN.IFG(L)	1.13	DMN.LP(L)-DMN. MPFC	−1.52	DAN.IPS(L)-VN.Lateral(L)	2.68	DAN.IPS(L)-VN. Lateral(L)	3.73
AAN.MRF-AAN.PAG	1.07	LN.IFG(L)-DMN.PCC	−1.41	CBN.Posterior-DMN.LP (R)	−2.60	FPN.LPFC(L)-AAN.DR	2.59
DAN.IPS(R)-AAN.LC	−0.81	SN.RPFC(R)-VN. Occipital	−1.34	CBN.Posterior-CBN. Anterior	2.47	SN.SMG(L)-VN.Lateral (L)	2.54
DAN.IPS(R)-AAN.PO	−0.75	LN.IFG(L)-DAN.IPS(L)	1.30	LN.IFG(R)-FPN.LPFC(R)	2.41	FPN.PPC(R)-AAN. PBC	2.48
DMN.MPFC-AAN. PBC	0.72	CBN.Anterior-DAN.IPS (L)	−1.25	FPN.PPC(R)-AAN.VTA	2.11	DAN.IPS(R)-VN. Lateral(L)	2.44
SMN.Lateral(L)-AAN. PPN	−0.71	SN.AInsula(R)-VN. Medial	1.15	FPN.PPC(R)-AAN.MR	2.05	SN.RPFC(L)-AAN.PAG	2.34
SMN.Lateral(L)-AAN. PO	−0.64	LN.IFG(L)-DAN.IPS(R)	1.06	FPN.LPFC(L)-DMN.PCC	−1.95	SN.RPFC(L)-AAN.DR	2.32
SMN.Lateral(L)-AAN. MR	−0.60	DAN.IPS(L)-VN. Occipital	−0.93	SN.SMG(L)-VN.Lateral(L)	1.61	SN.RPFC(L)-AAN. MRF	2.23

Abbreviations: ACC, anterior cingulate cortex; CBN, cerebellar network; DAN, dorsal attention network; DMN, default mode network; DR, dorsal raphe nucleus; FEF, frontal eye field; FPN, frontoparietal control network; IFG, inferior frontal gyrus; IPS, intraparietal sulcus; LC, locus coeruleus; LN, language network; LP, lateral parietal; LPFC, lateral prefrontal cortex; MPFC, medial prefrontal cortex; MR, median raphe nucleus; MRF, mesencephalic reticular formation; PAG, periaqueductal gray; PBC, parabrachial complex; PCC, precuneus cortex; PO, pontine nucleus oralis; PPC, posterior parietal cortex; PPN, pedunculopontine tegmental nucleus; pSTG, posterior superior temporal gyrus; RPFC, rostral prefrontal cortex; SMG, supramarginal gyrus; SMN, sensorimotor network; SN, salience network; VN, visual network; VTA, ventral tegmental area.

et al., 2004), demonstrating the importance of AAN in the hyperarousal model of insomnia in humans. Specifically, the brainstem arousal system contains multiple arousal-promoting neurotransmitters (e.g., monoaminergic-, cholinergic-, and glutamate-related nuclei). Thus, increased AAN-cortical network signaling in insomnia patients may lead to increased cortical excitability, which ultimately affects sleep initiation and maintenance.

4.2 | AAN-cortical network connectivity to identify patients with CID from good sleepers

In recent years, the SVM algorithm combined with resting-state fMRI data have been widely applied to identify brain signatures in several neuropsychiatric diseases (Bleich-Cohen et al., 2014; Gong et al., 2022; Khazaee et al., 2016; Zeng et al., 2012) and for treatment response prediction (Pang et al., 2022). In our study, a classification model based on AAN and intrinsic cortical network features successfully identified patients with CID from the control group. Notably, seven of the top 10 classifying features contained AAN, indicating that FC associated with AAN contributed substantially to classifying patients with CID and GSCs. This SVC model implies that AAN-based connectivity may serve as a brain marker for CID diagnosis to distinguish insomniac brains from healthy ones. These results further support the significance of AAN in the neuropathological mechanisms of insomnia.

Compared to previous studies using machine learning to classify insomnia from healthy brains (Dai et al., 2020; C. Li et al., 2019), our model exhibited higher accuracy, from 81.5% (data-driven approach) to 88.0%. This improvement in accuracy may be attributed to our feature selection method, which extracts an underlying set of meaningful features, that is, AAN-related FC. More importantly, the classifier discriminated insomnia in an independent cohort of participants, with an accuracy of 77%. Thus, the above cross-validation and independent validation results confirm the generalizability and reliability of the AAN-related connectivity classifier for insomnia diagnosis.

The DSM-5 defines insomnia as the subjective experience of difficulty in initiating sleep, difficulty in maintaining sleep, and waking up too early in the morning. In clinical practice, insomnia is diagnosed mostly by self-reported symptoms that can be confused with the symptoms of other disorders (Benjamins et al., 2017). For instance, individuals with delayed sleep phase syndrome are commonly mislabeled as having insomnia (Murray et al., 2017). In addition to subjective questionnaire assessment, polysomnography can serve as the "gold standard" tool for the diagnosis of most sleep disorders, but the complexity of its construction limits its clinical applicability. Hence,

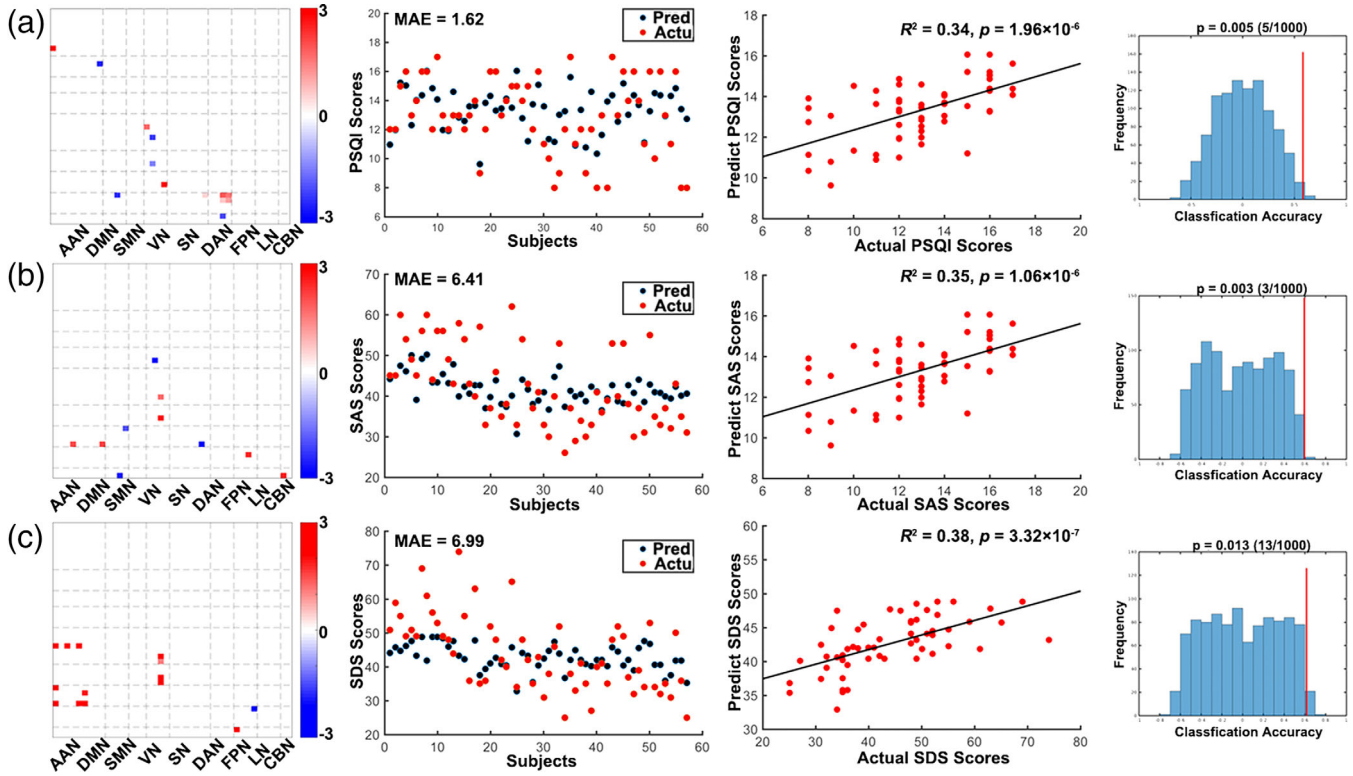

FIGURE 4 The SVR-based clinical prediction results. (a) Insomnia symptom prediction; (b) anxiety symptom prediction; and (c) depression symptom prediction; left, the weight of each regions in SVR model; middle left, the actual clinical scores and predicted clinical scores for each patient; middle right, the FC patterns predict the clinical performance in patients with CID; and right, the permutation tests of the prediction model. AAN, ascending arousal network; CBN, cerebellar network; CID, chronic insomnia disorder; DAN, dorsal attention network; DMN, default mode network; FPN, frontoparietal control network; LN, language network; MAE, mean absolute error; PSQI, Pittsburgh Sleep Quality Index; SAS, Zung's self-rating anxiety scale; SDS, Zung's self-rating depression scale; SMN, sensorimotor network; SN, salience network; SVR, support vector regression; VN, visual network

the objective fMRI-based biomarkers identified by us show potential as complements to the clinical diagnosis of CID. Therefore, the results were consistent with the hypothesis that the functional brain network contains valuable information reflecting the condition of patients with CID and could be regarded as a biomarker to distinguish them from GSC using the MVPA method. However, further studies are necessary to assess the clinical scenarios and populations in which these markers are suitable.

4.3 | AAN-cortical network connectivity may attribute to predict clinical symptoms in patients with CID

We also explored potential brain network features to predict clinical symptoms. SVR models based on resting-state fMRI data have been widely used to predict individual brain maturity (Dosenbach et al., 2010), physical pain (Wager et al., 2013), and clinical responses (Qin et al., 2015). Recently, Zhou et al. (2020) developed a prediction model for sleep quality based on dynamic functional network connectivity features in a healthy population using fMRI data from the Human Connectome Project. A previous study found that CBN FC strength is associated with anxiety and postpartum depression (B. Cheng et al., 2022). Correlation analyses from our previous study also confirmed that brain connectivity between the LC and dorsal anterior cingulate cortex was associated with SAS scores in patients with CID (Gong, Shi, et al., 2021; Gong, Yu, et al., 2021).

In the present study, we found that in two independent data sets, the SVR model based on the AAN and cortical networks predicted insomnia and anxiety symptoms but not depressive symptoms in CID. The FCs between the AAN, VN, DMN, and FPN contributed more to the prediction model. Thus, our study suggests that neural markers based on AAN-related connectivity exhibit potential as early treatment biomarkers for predicting treatment response, particularly for insomnia and anxiety symptom improvement. Indeed, many fields, from oncology and cardiology to internal medicine, have developed biomarkers that indicate specific pathophysiological mechanisms. In recent years, the combination of machine-learning-based MVPA approaches and neuroimaging data features has yielded some encouraging diagnostic and predictive models for individual-level disease diagnosis and efficacy prediction (H. Li et al., 2022; Pang et al., 2022; Zhang et al., 2022). However, it is still in the early stages of brain biomarker development; future applications should extend our predictive model to multiple time-visits longitudinal experimental designs, such

as predicting the effect of pharmacological and non-pharmacological therapies on insomnia by collecting data from two time points observed before and after treatment.

4.4 | Limitations

Certain limitations should be considered when interpreting our findings. First, because cross-sectional fMRI studies limit the inference of causality, future longitudinal research should investigate whether disrupted AAN connectivity is a consequence or cause of sleep disturbances, especially in the different stages of insomnia. Second, the existence of insomnia subtypes such as difficulty in initiating sleep, difficulty in maintaining sleep, and early morning awakening has been supported by several studies (Benjamins et al., 2017). Hence, it would be interesting to study the specific and common neural mechanisms underlying the different subtypes of CID and provide individual neuroimaging markers for the identification of CID subtypes. Third, we did not assess arousal status before and after the fMRI scan, and future studies should consider whether an individual's arousal state affects classification performance. Fourth, sleep quality was not monitored using subjective (i.e., sleep log) and objective (i.e., actigraphy and polysomnography) tools. Future studies that assess sleep quality are warranted to extend our findings. Fifth, we used a supervised learning algorithm (SVM) for classification and regression, and future research may use unsupervised learning algorithms (i.e., hidden Markov models) to verify the role of AAN in insomnia. Finally, the resting-state fMRI data in this study were acquired using a 3.0 Tesla MRI scanner. Hence, our results should be validated in additional work using 7.0 Tesla fMRI data to ensure the reproducibility of the small seed region connectivity map (Singh et al., 2022).

5 | CONCLUSION

To the best of our knowledge, our observations provide the first in vivo evidence of strong connections between AAN and cortical networks across the DMN, CBN, SMN, and DAN in patients with CID. Furthermore, combining the machine learning approach of MVPA and AAN-cortical network features, a classification model that could discriminate CID patients from good sleepers and a prediction model that could predict insomnia and anxiety symptoms in patients with CID were identified in two independent data sets. Collectively, the findings of our study highlight the involvement of the AAN in the pathophysiology of human insomnia and the comorbidities of mental symptoms.

ACKNOWLEDGMENT

The authors would like to thank Editage (www.editage.cn) for English language editing.

FUNDING INFORMATION

This study was supported by the Sichuan Provincial Science and Technology Department Project in China (2020YJ0197, 2020YJ0176, and 2021YJ0162); the National Natural Science Foundation of China (82001803); Chengdu Science and Technology Department Project (2021-YF05-00247-SN); the Project of the Hefei Municipal Health Commission and Collaborative Innovation Center for Neuropsychiatric Disorders and Mental Health (NDMHCI-19-01); and the Department of Science and Technology of Anhui Province (202004j07020006).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding authors.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Gong, L., He, K., Cheng, F., Deng, Z., Cheng, K., Zhang, X., Zhou, W., Ou, J., Wang, J., Zhang, B., Ding, X., Xu, R., & Xi, C. (2023). The role of ascending arousal network in patients with chronic insomnia disorder. *Human Brain Mapping*, *44*(2), 484–495. https://doi.org/10.1002/hbm.26072