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Cerebral Blood Flow Changes and Their Spatial Correlations With GABAa and Dopamine-D1 Receptor Explaining Individual Differences in Chronic Insomnia and the Therapeutic Effects of Acupuncture

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ABSTRACT

This study integrated neuroimaging and neurochemistry data to explore brain mechanisms in chronic insomnia disorder (CID) and the neuromodulatory effects of acupuncture. We analyzed a cross-sectional arterial spin labeling (ASL) dataset ($N = 197$) of CID patients and healthy controls to identify cerebral blood flow (CBF) changes. Additionally, a longitudinal ASL dataset ($N = 44$) examined CBF changes in CID patients after a 4-week acupuncture treatment or on a waitlist. We then assessed the impact of 19 neurotransmitter receptors/transporters on these CBF alterations. In cross-sectional comparisons, CID patients exhibited increased CBF in cortical areas and decreased CBF in subcortical regions, correlating with insomnia severity. In longitudinal comparisons, acupuncture treatment enhanced subcortical CBF and alleviated insomnia symptoms, changes not observed in the waitlist group. The left putamen was identified as an overlapping subcortical region involved in both CID-related changes and post-treatment alterations. Moreover, the CBF patterns induced by acupuncture negatively correlated with the abnormal patterns in CID patients, and both were significantly associated with GABAa and dopamine-D1 receptor densities. The observed decrease in CBF in the left putamen could potentially serve as a neural biomarker for CID, while acupuncture may alleviate insomnia symptoms by increasing CBF in this region, potentially through the modulation of GABAa and D1 receptor expressions.

1 | Introduction

Chronic insomnia disorder (CID) is the second-most common psychiatric condition and is linked to multi-scale brain changes (Yu, Hu, et al. 2024; Zhang and Wing 2006). Neuroimaging studies have uncovered macroscopic functional changes in various brain regions in individuals with CID (van Someren 2021). Simultaneously, at the microscopic level, substantial alterations

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within the neurotransmitter system (Lancel et al. 2021; Yu, Peng, et al. 2024) are evident in those suffering from insomnia. Consequently, the therapeutic approach for CID should not only consider these macroscopic brain changes but also delve into the underlying microscopic neurochemical mechanisms driving these alterations.

Acupuncture, as a multifaceted therapeutic approach, exerts its efficacy in treating CID through various levels and factors, including modulating brain function as well as neurotransmitter expression. For instance, acupuncture can modulate functional connectivity (FC) between the locus coeruleus and supramarginal gyrus to improve insomnia symptoms (Chen et al. 2023). Simultaneously, acupuncture can modulate the activity of the neurotransmitter receptor to treat insomnia (Xi et al. 2023). However, to date, few studies have simultaneously integrated neuroimaging and neurotransmitter expression to fully understand CID's pathogenesis and the mechanisms behind acupuncture's benefits.

Whole-brain neuroimaging techniques, especially blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI), have significantly advanced our understanding of CID (Shen et al. 2024; Yu et al. 2023). Additionally, arterial spin labeling (ASL), a specialized fMRI technique renowned for its ability to quantify regional cerebral blood flow (CBF). The greater sensitivity of ASL to low-frequency signals compared to BOLD fMRI (Watts et al. 2013) makes it an invaluable tool to explore the underlying macroscopic brain mechanisms of insomnia. However, there have been few studies (Huang et al. 2022; Luo, Li, et al. 2023; Park et al. 2019; Xu et al. 2023; Zhou et al. 2019) to date that combine ASL with CBF analysis to investigate both the individual differences of CID and the effectiveness of acupuncture as a treatment.

While neuroimaging techniques like ASL have been valuable in understanding the neurovascular coupling of CID, they fall short in identifying the underlying neurochemical substrates. To bridge this gap, the integration of positron emission tomography (PET) with fMRI offers a more comprehensive view. PET, using selective radiotracers, can probe the neurochemical signatures involved in the functional networks observed through fMRI (Sander et al. 2020). For instance, a recent study (Kim et al. 2023) using simultaneous PET-fMRI was able to correlate amyloid β burden in the brain with altered functional connectivity in the salience network, providing molecular insights into sleep-related aberrant macro-scope connectivity networks.

However, the use of hybrid PET/fMRI is limited by its high costs, hardware demands, and invasiveness. Additionally, PET scans are typically selective for one neurotransmitter at a time and are restricted by radiation exposure and tracer availability. To circumvent these limitations, researchers have turned to constructing a comprehensive atlas of neurotransmitter receptor densities across the brain (Hansen et al. 2022). This approach assumes that the magnitude of neurotransmitter-related activity correlates linearly with receptor distribution and availability across brain regions (Sander et al. 2020), providing a more flexible and less invasive method to map macroscopic brain alterations onto the distributions of target receptors. This innovative strategy has been effectively employed in various neuroimaging studies (Hansen et al. 2022; Luo, Dong, et al. 2023; Luppi et al. 2023; Vamvakas et al. 2022) to enhance our understanding of macroscopic functional alterations at the molecular level.

In this current work, our approach involves analyzing a cross-sectional neuroimaging dataset ($N = 197$) encompassing both CID patients and healthy controls (HCs) to identify specific CBF changes in CID. Additionally, a multi-linear regression model (LRM) was utilized to interpret CID-related CBF changes from normalized cortical distribution maps of 19 neurotransmitter receptors/transporters (Hansen et al. 2022) in the Neuromaps database (Markello et al. 2022). Simultaneously, ASL data from a randomized neuroimaging dataset ($N = 44$) examining patients undergoing 4-week acupuncture therapy for CID was utilized to explore the corresponding mechanisms underlying the effectiveness of acupuncture (Figure 1). This study represents an attempt to integrate neuroimaging and neurochemistry data to explore the brain mechanisms associated with CID and the neuromodulatory effects of acupuncture. We hypothesized that: (1) CID patients would exhibit abnormal CBF patterns in both cortical and subcortical regions compared to HCs; (2) acupuncture treatment could reverse these abnormal CBF patterns and alleviate insomnia symptoms; and (3) the abnormal CBF patterns in CID patients and the CBF changes induced by acupuncture would significantly correlate with specific neurotransmitter expressions.

2 | Methods

2.1 | Dataset 1: Cross-Sectional Study of CID Patients and Matched HCs

In this dataset, our objective was to identify CID-related CBF changes compared to HCs and to interpret these alterations from a neurochemical perspective.

2.1.1 | Participant Recruitment and Assessment

A total of 108 patients diagnosed with CID were enrolled in the outpatient clinics of the Affiliated Hospital of Chengdu University of Traditional Chinese Medicine (CDUTCM) and Chengdu Second People's Hospital from 2017 to 2022. Additionally, 96 HCs were recruited through social media advertisements from surrounding communities. The inclusion and exclusion criteria, aligned with our previous research (Shen et al. 2024; Yu et al. 2023), are detailed in the Supporting Information. We collected the PSQI and Insomnia Severity Index (ISI) questionnaires from each participant and the Self-Rating Depression Scale (SDS) and Self-Rating Anxiety Scale (SAS) from patients with CID. Ethical approval for this study was granted by the Ethics Committee of the Affiliated Hospital of CDUTCM, and each participant provided written informed consent before participating in the study.

2.1.2 | MRI Acquisition

In our study, the MRI scans for all participants were performed in the afternoon, on average about 7 h after waking up in the

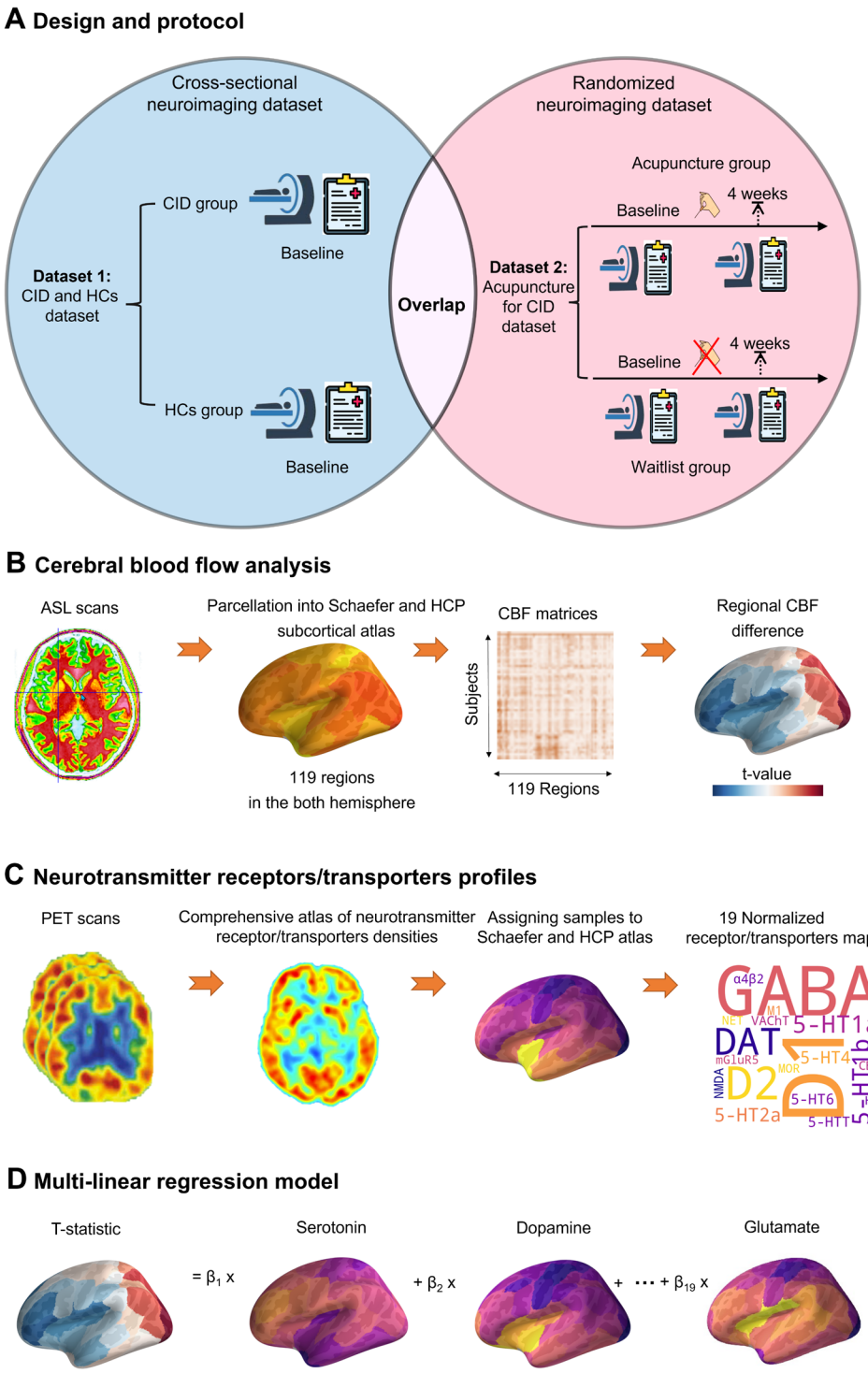

FIGURE 1 | Study overview. (A) Design and protocol. Our approach involves analyzing a cross-sectional neuroimaging dataset and a randomized neuroimaging dataset to explore the correlation between ASL-derived CBF changes and neurotransmitter receptor profiles for CID. (B) CBF analysis profiles. ASL scans from two different datasets were processed to calculate CBF and mapped into 119 regions for each participant. Then, through both between-group and within-group comparisons across different datasets, regional CBF differences were explored. (C) Neurotransmitter receptors/transporters profiles. Positron emission tomography studies with radioligands for 15 receptors and 4 transporters were normalized and mapped, resulting in a map of 119 regions with 19 neurotransmitters. (D) A multi-linear regression model. A multi-linear regression model was used to identify neuroimaging-neurochemistry associations in different datasets. The relative contribution of each receptor/transporter was also calculated within each model. Intercept and error terms are not displayed. Abbreviations: ASL, arterial spin-labeled; CBF, cerebral blood flow; CID, chronic insomnia disorder; HCs, healthy controls; PET, positron emission tomography.

morning (Yang et al. 2023). MRI scans were conducted on a 3.0-Tesla MR system (Discovery MR750w, General Electric, Milwaukee, WI, USA) equipped with a 32-channel head coil. To mitigate scanner noise, earplugs were used, and to minimize head motion, tight yet comfortable foam padding was employed. High-resolution 3D T1-weighted (T1w) structural images were acquired with the following parameters: repetition time (TR) = 7.06 ms; echo time (TE) = 3.04 ms; inversion time (TI) = 450 ms; flip angle (FA) = 12°; field of view (FOV) = 256 mm × 256 mm; matrix size = 256 × 256; slice thickness = 1 mm, no gap; 192 sagittal slices. Resting-state perfusion imaging was performed using a pseudo-continuous ASL sequence with a 3D fast spin-echo acquisition and background suppression (TR = 4860 ms, TE = 10.86 ms; voxel resolution = 3.75 × 3.75 × 4 mm; post-label delay = 2025 ms; label duration = 1450 ms; spiral in readout of eight arms with 512 sample points; FA = 111°; FOV = 240 mm × 240 mm; reconstruction matrix = 128 × 128; slice thickness = 4 mm, no gap; number of excitations = 3). A total of 36 pairs of label and control volumes were acquired.

2.2 | Dataset 2: Longitudinal Study of CID Patients Undergoing Acupuncture Therapy or Waitlist Control

This dataset aimed to validate whether the regional CBF changes and specific neurotransmitter expression patterns related to CID, identified in Dataset 1, could be modifiable by acupuncture therapy. The acupuncture protocol received ethical approval from the Ethics Committee of the Affiliated Hospital of CDUTCM (2021KL-125) and was registered with the Chinese Clinical Trial Registry (ChiCTR2200058878). Participants in the cross-sectional (Dataset 1) and longitudinal (Dataset 2) cohorts were recruited independently, with no individuals included in both datasets.

2.2.1 | Sample Size Calculation

The effect size is based on post-treatment PSQI scores and their standard deviations on a prior study (Fu et al. 2017). It reported post-treatment PSQI scores of 8.62 ± 2.93 for the acupuncture group and 14.76 ± 3.35 for the control group. The required sample size is calculated using the formula: $N = \left[\frac{(z_\alpha + Z_\beta)\sigma}{\delta - \Delta}\right]^2$. Where $Z_\alpha = 1.96$ ($\alpha = 0.05$), $Z_\beta = 1.28$ (power = 90%), σ is the pooled standard deviation, $\delta = 6.14$ the intergroup PSQI difference, and $\Delta = 3.8$ is the clinically meaningful difference (Cheuk et al. 2012). The calculation yields 19 participants per group. Accounting for a 20% dropout rate, 24 participants are required per group. With a 1:1 allocation ratio, a total of 48 participants is needed.

2.2.2 | Participant Recruitment and Assessment

Adhering to the inclusion and exclusion criteria from Dataset 1, this longitudinal study enrolled 48 eligible patients diagnosed with CID. Participants were randomly assigned in a 1:1 ratio to either the acupuncture treatment group or the waitlist control group using a computer-generated randomization sequence. Throughout the study, outcome assessors, data collectors, and statisticians remained blinded to the group assignments. Both groups were assessed using the PSQI, ISI, SAS, and SDS scores at baseline and at the 4-week visit.

2.2.3 | Acupuncture Intervention

Acupuncture treatments were administered by two licensed acupuncturists with more than 3 years of experience. The acupuncture group received 20 sessions over 4 weeks (five sessions weekly), each lasting 30 min. We used a combination of acupuncture points commonly employed in the treatment of insomnia, including Baihui (GV20), Sishencong, Anmian, Shenmen (HT7), Neiguan (PC6), and Sanyinjiao (SP6), as depicted in Figure 2. These points were selected based on traditional clinical theory, practices, and existing evidence, which suggest that their combined action has a synergistic effect in improving insomnia symptoms. Specifically, Baihui (GV20) is known for its ability to calm the mind and harmonize Qi, enhancing cerebral circulation and promoting relaxation (Li, Xu, et al. 2023). Sishencong is used to calm the spirit and improve cognitive function, helping to promote restful sleep (Gao et al. 2013). Anmian is a well-established point for insomnia, known for its sedative effects and ability to regulate the autonomic nervous system, thus reducing stress and anxiety (Huo et al. 2013). Shenmen (HT7) calms the heart and mind, reducing anxiety and promoting deeper sleep (Song et al. 2022). Neiguan (PC6) is often selected for its calming effects on the mind and its ability to regulate the autonomic nervous system, especially in cases where insomnia is linked

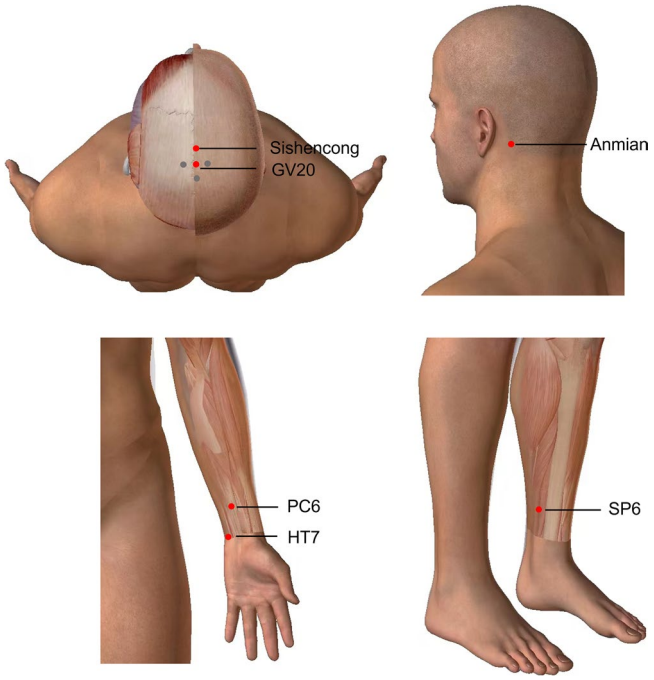

FIGURE 2 | Location of acupoints for acupuncture treatment: GV20, Sishencong, Anmian, HT7, PC6, and SP6. Acupoints where stimulation was provided via needles were marked as red points.

to anxiety (Gao et al. 2013). Finally, Sanyinjiao (SP6) regulates internal organs, harmonizes the blood, and promotes relaxation, which is particularly beneficial in cases of insomnia associated with hormonal imbalances (Xu et al. 2022). Treatments involved the use of disposable stainless-steel needles (0.30 × 25 mm/0.30 × 40 mm), with stimulation achieved through lifting, thrusting, twirling, and rotating the needle to elicit the "Deqi" sensation—characterized by feelings of pain, heaviness, numbness, swelling, or radiation indicating effective treatment. Patients in the waitlist group did not receive acupuncture but were informed that they would be provided with 20 sessions of acupuncture treatment without charge after the completion of the full observational period.

2.2.4 | MRI Acquisition

The time, location, and parameters for MRI acquisition in the acupuncture therapy dataset were consistent with those utilized in Dataset 1.

2.3 | MRI Data Preprocessing

For both Datasets 1 and 2, the preprocessing of ASL and T1w data and the calculation of regional CBF values were conducted using identical methodologies. Initially, DICOM data were converted into the brain imaging data structure (BIDS) format using the ExploreASL toolbox (Mutsaerts et al. 2020), version v1.10.0. The ASL MRI images formatted in BIDS were then preprocessed with ASLPrep 0.6.0 (Adebimpe et al. 2022). For anatomical data preprocessing, each subject's T1w image underwent intensity non-uniformity correction, skull-stripping, brain tissue segmentation, and spatial normalization. ASL data preprocessing involved generating a smoothed reference volume from M0 images, which was later used for CBF calculation. Co-registration between ASL and T1w images was performed using FSL's flirt (Jenkinson and Smith 2001), with a boundary-based registration cost function (Greve and Fischl 2009). CBF was quantified using a standard model (Alsop et al. 2015; Detre et al. 1992), extracting parcellated CBF estimates for 100 cortical regions as defined in the Schaefer atlas (Schaefer et al. 2018) and 19 subcortical regions, including the bilateral cerebellum, thalamus, caudate, putamen, pallidum, hippocampus, amygdala, accumbens, diencephalon ventral, and brain stem as delineated in the HCP subcortical atlas (Glasser et al. 2013). As part of quality assurance (Adebimpe et al. 2022) in both Datasets 1 and 2, we excluded participants whose mean frame-wise displacement exceeded 1 mm or whose CBF gray matter and white matter ratio was below 1.

2.4 | Acquire Neurotransmitter Distribution

To investigate the impact of neurotransmitter distributions on CBF changes in both datasets, we utilized the Neuromaps database (Markello et al. 2022) to acquire mean cortical distribution maps for 19 specific neurotransmitter receptors/transporters (Hansen et al. 2022). These include receptor/transporter for dopamine (D1, D2, DAT), serotonin (5-HT1a, 5-HT1b, 5-HT2a, 5-HT4, 5-HT6, 5-HTT), acetylcholine (α4β2, M1, VAChT), GABA, glutamate (NMDA, mGluR5), norepinephrine (NET), histamine (H3), cannabinoids (CB1), and opioids (MOR), which were parcellated into the Schaefer 100 atlas (Schaefer et al. 2018) and the HCP subcortical atlas (Glasser et al. 2013).

2.5 | Statistical Analysis

Statistical analyses were performed using R 4.1.0. For demographic and clinical data, between-group differences in age and clinical scores were assessed using two-sample *t*-tests, Welch's two-sample *t*-test, or Mann–Whitney *U* tests, based on the normality of the data and the homogeneity of variances. Gender differences were evaluated using the chi-squared test or Fisher's exact test, depending on the expected frequencies in the contingency table. Within-group differences were tested with paired *t*-tests for normally distributed data and paired Wilcoxon tests for non-normally distributed data. For correlation analyses, Pearson correlation or Spearman rank correlation was used based on data normality. Effect sizes (Cohen's *d*) were calculated for all group comparisons to evaluate the magnitude of CBF alterations.

In Dataset 1, to assess between-group differences in CBF, we used LRMs, adjusting for age, sex, and education as covariates. To account for multiple comparisons across 119 regions, we applied a false discovery rate (FDR) correction with a significance threshold of $p_{FDR} < 0.025$ (two-tailed). Within the CID group, correlation analysis explored associations between regional CBF values that survived the LRM and various clinical scales, using a significance threshold of $p_{FDR} < 0.05$. Additionally, the relationship between mean regional CBF in HCs and case–control CBF differences (*t*-values) was also evaluated using correlation analysis with spin tests (see Section 2.6, Null Models for details).

In Dataset 2, within-group effects were evaluated by comparing CBF values at baseline and after 4 weeks using paired *t*-tests or paired Wilcoxon tests, depending on the data distribution. In the acupuncture group, correlation analysis was performed to investigate the relationship between changes in regional CBF (calculated as the difference between post-treatment and baseline values) and variations in clinical scores (ΔPSQI, ΔISI, ΔSAS, ΔSDS). Additionally, spatial correlations between (1) acupuncture- and waitlist-induced CBF changes, (2) acupuncture-induced changes and CID-related alterations, and (3) waitlist-induced changes and CID-related alterations were assessed using correlation analysis with spin tests (see Section 2.6, Null Models for details).

To assess the impact of 19 neurotransmitter distributions on CBF differences between CID patients and HCs, and on the treatment-induced CBF alterations, we applied a multi-LRM. The robustness and significance of this model were validated against a spatial permutation-preserving null model. Furthermore, we conducted a dominance analysis (Azen and Budescu 2003; Yu, Peng, et al. 2024) to determine the relative contribution of each receptor/transporter to the model's total fit (adjusted R^2). This involved fitting the regression model to all possible combinations of input variables ($2^n - 1$ submodels for n variables). Total dominance is the average relative increase in

R^2 when adding a variable across all submodels. The sum of all variables' dominance equals the model's total adjusted R^2, partitioning the effect size across predictors. Dominance was normalized by the model's total fit for comparability.

2.6 | Null Models

We used spatial autocorrelation-preserving permutation tests (Markello and Misic 2021; Váša et al. 2018), also known as "spin" tests, to assess the statistical significance of associations across brain regions. The threshold for significance in these spatial permutation tests was set at $p_{spin} < 0.05$. Further details can be found in the Supporting Information.

3 | Results

3.1 | Cerebral Blood Flow Changes in CID Patients Are Aligned With Brain Neurochemical Systems

3.1.1 | Participant Characteristics in Dataset 1

After image quality control, 3 CID patients and 4 HCs were excluded, resulting in a final analysis of 105 CID patients and 92 HCs in Dataset 1. The demographic and clinical characteristics of the participants are summarized in Table 1. No significant group differences were found in age, sex, or education levels. These variables were regarded as covariates in the LRM to improve the accuracy of the case–control comparisons.

3.1.2 | Case–Control Differences in Regional CBF Values

Regional CBF residuals behaved as an approximately normal distribution (Figure 3A) across cortical and subcortical areas of both hemispheres. Global CBF, calculated by averaging regional CBF across 119 areas for each participant, did not differ significantly between CID patients and HCs (Figure S1). CBF values were mapped onto 119 brain areas for both groups (Figure 3B). Case–control differences were assessed using linear LRM, with a positive t-value indicating higher CBF in CID patients compared to controls and a negative t-value indicating lower CBF in CID patients. A significant positive association was discerned between the mean CBF value in HCs and the magnitude of the regional case–control t value (Figure 3C; Pearson's $r = 0.22$, $p_{spin} < 0.014$). This correlation implies that the areas with greater blood flow in the HCs may be more susceptible to alterations in CID. We identified 16 regions with statistically significant case–control differences at the threshold of $p_{FDR} < 0.025$ (Figure 3D). CBF increased in CID patients, mainly located in the visual cortex and some regions of the dorsal attention networks, and decreased mainly in the subcortical areas, such as the left putamen, bilateral pallidum, and bilateral diencephalon ventral (Figure 3D, Table S1).

3.1.3 | Correlation Between Case–Control CBF Changes and Clinical Scales

Negative correlations were found between the CBF values of the left putamen, left pallidum, and bilateral diencephalon ventral, and the PSQI scores, reaching a significant level at $p_{FDR} < 0.05$ (Figure 3E, Figure S2). No significant correlations were identified between the CBF values of regions where CBF was significantly increased or decreased and the other scales.

3.1.4 | Neurotransmitter Receptor/Transporter Profiles Are Associated With CBF Changes in CID Patients

Using a multi-LRM, we assessed the impact of neurotransmitter receptor/transporter densities on CID-related CBF alterations (Figure 4A). Compared to a spin-permuted null model with 10,000 iterations, we observed robust correlations between the densities of these receptors/transports and the CBF alterations observed in CID (total adjusted $R^2 = 0.68$, $p_{spin} = 0.019$, Figure 4B). Through dominance analysis, we identified M1, MOR, GABAa, VAChT, H3, 5HTT, 5HT1a, D1, and CB1 spatial distributions as major contributors (each dominance > 5%) to the CID-related CBF changes (Figure 4B).

TABLE 1 | Demographic and clinical characteristics in CID and HCs dataset.

Demographics	CID ($n = 105$)	HCs ($n = 92$)	Test statistic	p
Gender, female (%)	67 (63.81)	63 (68.48)	0.29*	0.59
Age, years	34.44 (10.801)	36.32 (10.14)	4172.5▲	0.10
Education, years	15.69 (2.28)	15.33 (2.10)	5403.00▲	0.13
PSQI	13.09 (2.97)	2.88 (1.77)	9660.00▲	< 0.001
ISI	17.03 (4.55)	3.20 (3.68)	9471.5▲	< 0.001
SDS	51.66 (12.69)	/	/	/
SAS	53.50 (12.71)	/	/	/
CBF_{global} (mL/100 g/min)	46.65 (6.39)	46.77 (5.53)	0.04Δ	0.97

Note: *Represents the chi-squared value from the chi-squared Test, ▲Represents the U-value from the Mann–Whitney U test, and Δrepresents the t-value from the two-sample t test.
Abbreviations: ISI, Insomnia Severity Index; PSQI, Pittsburgh Sleep Quality Index; SAS, Self-rating Anxiety Scale; SDS, Self-Rating Depression Scale.

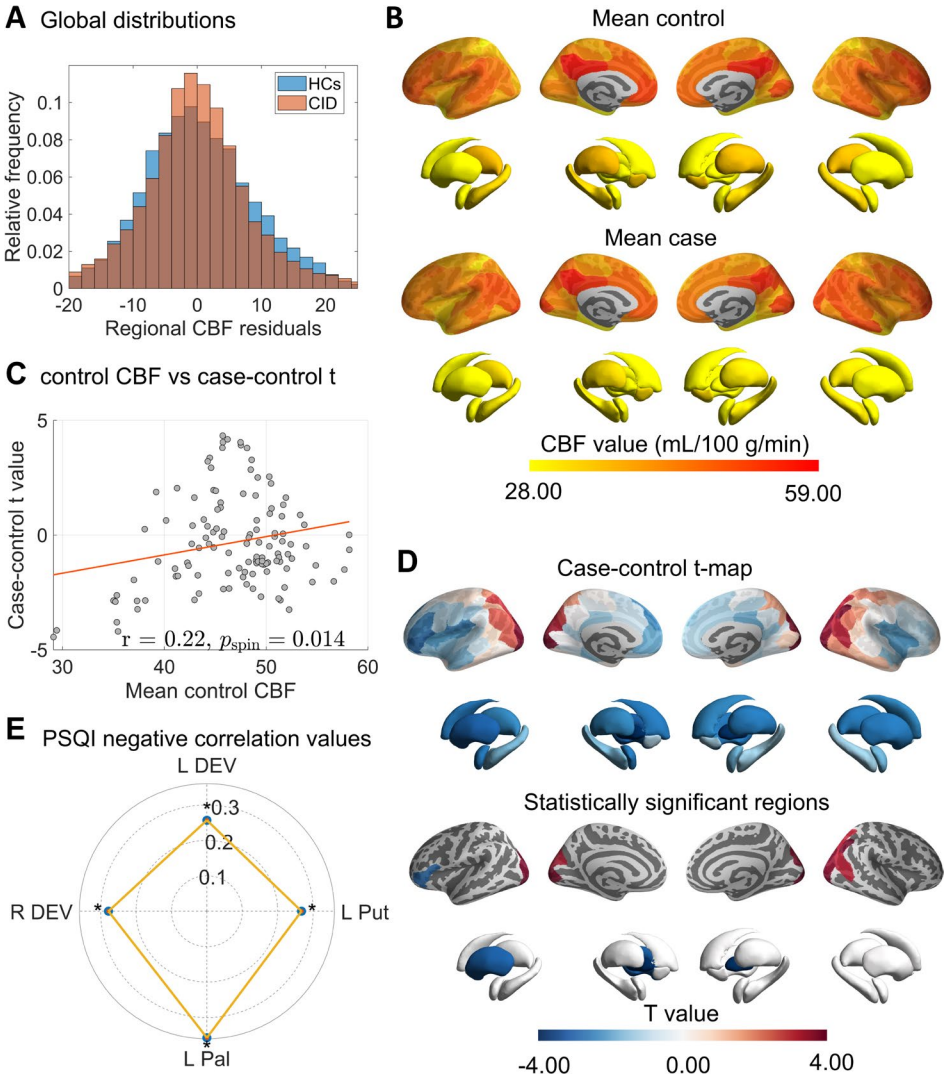

FIGURE 3 | Case–control differences in regional cerebral blood flow. (A) Global distribution of regional CBF residuals, that is, the averaged CBF value across 119 regions for each participant, after regressing age, sex, and education for patients with CID (red) and HCs (blue). (B) Mean regional CBF pattern of control subjects and patients with CID. The frontal, temporal, and occipital lobes exhibited high CBF values, whereas the parietal lobe and subcortical areas showed low CBF values. (C) Scatter plot of mean control regional CBF (x axis) versus case–control t value (y axis). Control CBF (from B) is positively correlated with case–control CBF differences (from D; Pearson's $r = 0.22$, $p_{spin} < 0.014$). (D) Case–control comparison (t-map) of regional CBF. In the bottom row, 16 regions in cortical and subcortical areas were presented for a threshold of $p < 0.025$, FDR corrected. (E) The radar map shows a negative correlation between the CBF values of the left putamen ($r = -0.36$, $p_{FDR} = 0.002$), the left pallidum ($r = -0.27$, $p_{FDR} = 0.007$), the left diencephalon ventral ($r = -0.26$, $p_{FDR} = 0.009$), the right diencephalon ventral ($r = -0.28$, $p_{FDR} = 0.005$), and the PSQI scores. Abbreviations: CBF, cerebral blood flow; CID, chronic insomnia disorder; DEV, diencephalon ventral; FDR, false discovery rate; HCs, healthy controls; L, left; Pal, pallidum; Put, putamen; R, right.

3.2 | Acupuncture Therapy for Insomnia: Its Effect on CBF and Underlying Neurochemical Mechanisms

3.2.1 | Participant Characteristics in Dataset 2

One patient from the acupuncture group and three from the waitlist group were excluded due to scheduling conflicts. No additional patients were excluded due to image quality. The final sample consisted of 23 patients in the acupuncture group and 21 in the waitlist group. The detailed demographic and clinical characteristics of these patients are summarized in Table 2.

3.2.2 | Effects of Acupuncture Treatment on Clinical Symptoms

In the acupuncture group, the post-treatment scores for PSQI, ISI, and SAS were all significantly reduced ($p < 0.05$), while the SDS score did not exhibit a significant change ($p > 0.05$, Table S2). Conversely, in the waitlist group, there were no significant changes in any of the clinical indicators before and after the trial. Comparatively, the reductions in PSQI, ISI, and SAS scores in the acupuncture group were significantly greater than those in the waitlist group ($p < 0.05$), while no significant difference was noted in SDS scores ($p > 0.05$, Table 2).

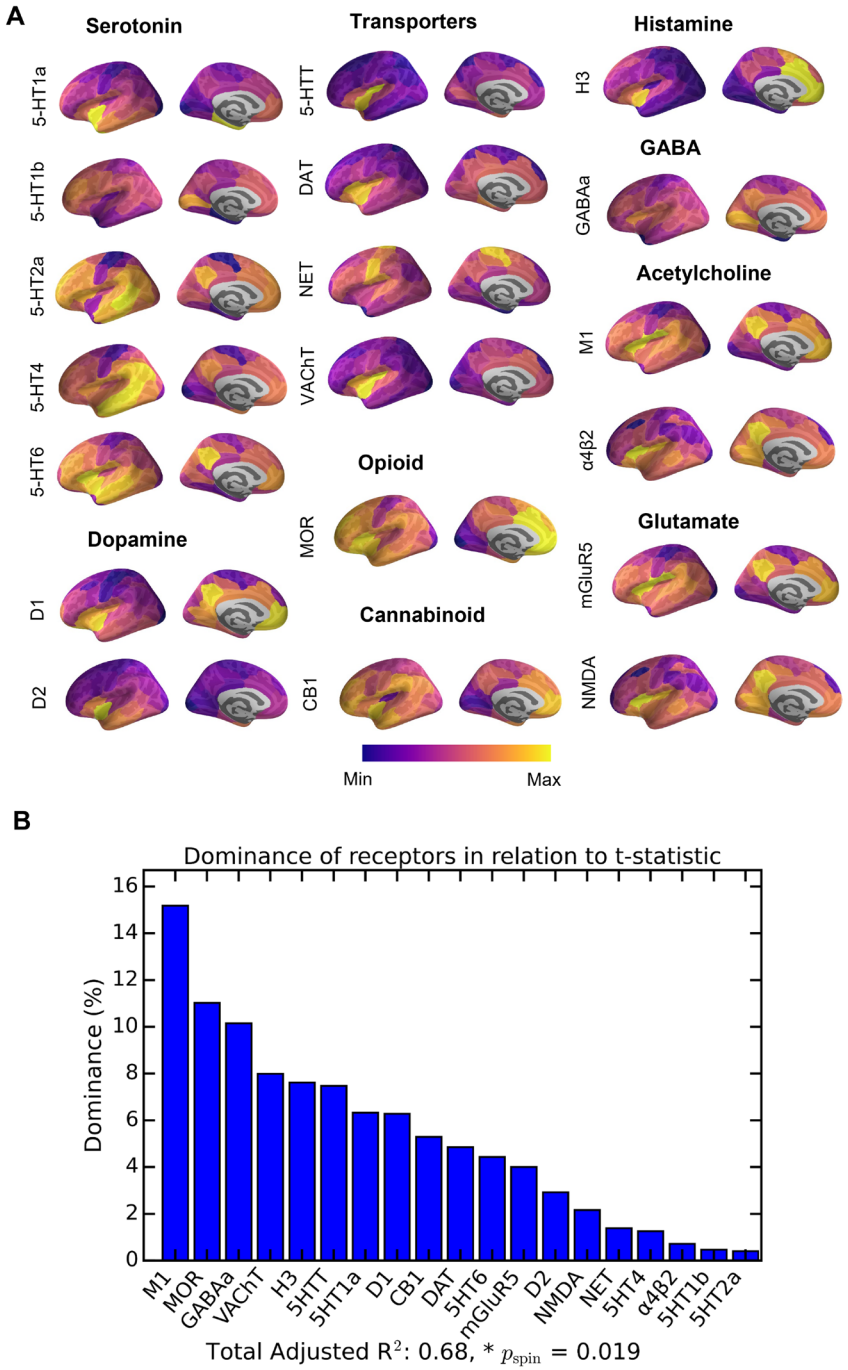

FIGURE 4 | Neurotransmitter distribution association with CBF changes. (A) Neurotransmitter systems are mapped with positron emission tomography with radioligands for 15 receptors and 4 transporters, resulting in a map of 100 cortical regions with 19 neurotransmitters. Data for this plot can be found in supplementary Dataset 1. (B) We fit a multi-linear regression model that predicts CID-related CBF differences from 19 receptors/transporters distributions. Receptor distributions closely correspond to CID-related CBF changes (total adjusted $R^2 = 0.68$, $p_{spin} = 0.019$). Through dominance analysis, the percent dominance of each input variable is defined as the variable's dominance normalized by the total adjusted R^2 of the model. We identified M1, MOR, GABAa, VAChT, H3, 5HTT, 5HT1a, D1, and CB1 spatial distributions as major contributors (each dominance > 5%) to the CID-related CBF changes. Abbreviations: CBF, cerebral blood flow; CID, chronic insomnia disorder.

3.2.3 | Changes in Regional Cerebral Blood Flow From Baseline to 4-Week Post-Treatment

CBF values were averaged and mapped into 119 areas for both the acupuncture group and the waitlist group at baseline and at 4-week after randomization (Figure 5A, Figure 5B). After 4 weeks of treatment, the acupuncture group exhibited increases in regional CBF in the left putamen, bilateral cerebellums, and brain stem ($p < 0.05$, Figure 5C, Table S3). In contrast, the waitlist group showed decreases in regional CBF within the control network, salience network, and default mode network after 4 weeks' wait ($p < 0.05$, Figure 5D, Table S4). However, none of these changes

TABLE 2 | Comparison of the treatment effects between two groups in the acupuncture treatment for CID dataset.

Variable	Acupuncture ($n=23$)	Wait ($n=21$)	Test statistic	p
Female (%)	19 (82.61)	13 (61.90)	0.35*	0.18
Age (years)	36.83 (9.87)	33.33 (9.94)	302.50▲	0.15
PSQI				
Baseline	13.30 (2.46)	12.48 (2.64)	1.07	0.28
4-weeks	7.35 (2.53)**	11.81 (2.06)	/	/
Change	−5.96 (3.04)	−0.67 (1.91)	−6.98$^{\Delta}$	0.00
ISI				
Baseline	17.26 (3.86)	15.57 (3.40)	1.53	0.13
4-weeks	9.22 (4.36)**	15.43 (4.01)	/	/
Change	−8.04 (5.12)	−0.14 (2.73)	31.50▲	0.00
SAS				
Baseline	40.04 (7.67)	36.29 (7.71)	302.00▲	0.16
4-weeks	34.00 (5.60)**	37.44 (10.02)	/	/
Change	−6.04 (7.06)	1.16 (5.89)	−3.65	0.00
SDS				
Baseline	41.39 (6.99)	38.38 (8.15)	1.32	0.19
4-weeks	39.22 (5.95)	38.86 (8.70)	/	/
Change	−2.17 (7.43)	0.48 (4.76)	−1.42$^{\Delta}$	0.16

Note: *Represents the statistical value from the Fisher's exact test, and ▲ represents the U-value from the Mann–Whitney U test, and $^{\Delta}$ represents the t-value from the Welch Two Sample t-test. The remaining statistical values represent the t value from the two-sample t test. **Represents significant improvement within the acupuncture group after 4 weeks of treatment.
Abbreviations: ISI, Insomnia Severity Index; PSQI, Pittsburgh Sleep Quality Index; SAS, Self-rating Anxiety Scale; SDS, Self-Rating Depression Scale.

in either group survived FDR correction for multiple comparisons ($n=119$ comparisons). Additionally, spatial patterns of CBF changes induced by acupuncture did not significantly correlate with those in the waitlist group (Pearson's $r=-0.06$, $p_{spin}=0.552$). Interestingly, a negative correlation was found between acupuncture-induced CBF changes in Dataset 2 and CID-related CBF alterations in Dataset 1 (Pearson's $r=-0.39$, $p_{spin}=0.034$, Figure 6A). Pearson correlation analysis between acupuncture-induced regional CBF changes (ΔCBF) and improvements in clinical scores (ΔPSQI, ΔISI, ΔSAS, ΔSDS) revealed no statistically significant associations (all $p>0.05$, Table S5).

3.2.4 | Acupuncture-Induced CBF Changes Associated With Neurotransmitter Receptor Profiles

There were significant correlations between neurotransmitter receptor/transporter densities and changes in CBF induced by acupuncture (total adjusted $R^2=0.37$, $p_{spin}=0.011$, Figure 6B). The spatial distributions of GABAa, α4β2, D1, 5HT4, mGluR5, and 5HT1b were identified as major contributors to these CBF changes (each dominance >5%, Figure 6B). Notably, the left putamen (Figure 6C) emerged as a shared region, with reduced CBF in CID patients compared to HCs (Dataset 1) and significant CBF increases following acupuncture treatment (Dataset 2). GABAa and D1 receptors (Figure 6D) were identified as major contributors in both contexts, underscoring their potential roles in CID neurobiology and acupuncture modulation.

4 | Discussion

To our knowledge, this is the first study combining ASL and neurotransmitter receptor density maps to elucidate the brain's mechanisms underlying CID and the modulation effects of treatment. In the cross-sectional dataset, CID patients exhibited increased CBF in several cortical areas and decreased CBF in subcortical regions (e.g., left putamen), correlating with insomnia severity. And GABAa and D1 were identified as major contributors to the abnormal patterns in CID patients. In the longitudinal dataset, acupuncture enhanced subcortical CBF (e.g., left putamen) and alleviated insomnia symptoms, which were absent in the waitlist group. The CBF patterns induced by acupuncture were negatively correlated with the abnormal patterns in CID patients and were also significantly associated with GABAa and dopamine-D1 receptor expressions. These findings provide a multiscale model of the brain, suggesting that the left putamen may be a diagnostic and therapeutic neuro biomarker for CID and that acupuncture may modulate this region's CBF through key neurotransmitter receptor expression, thereby ameliorating insomnia symptoms. This

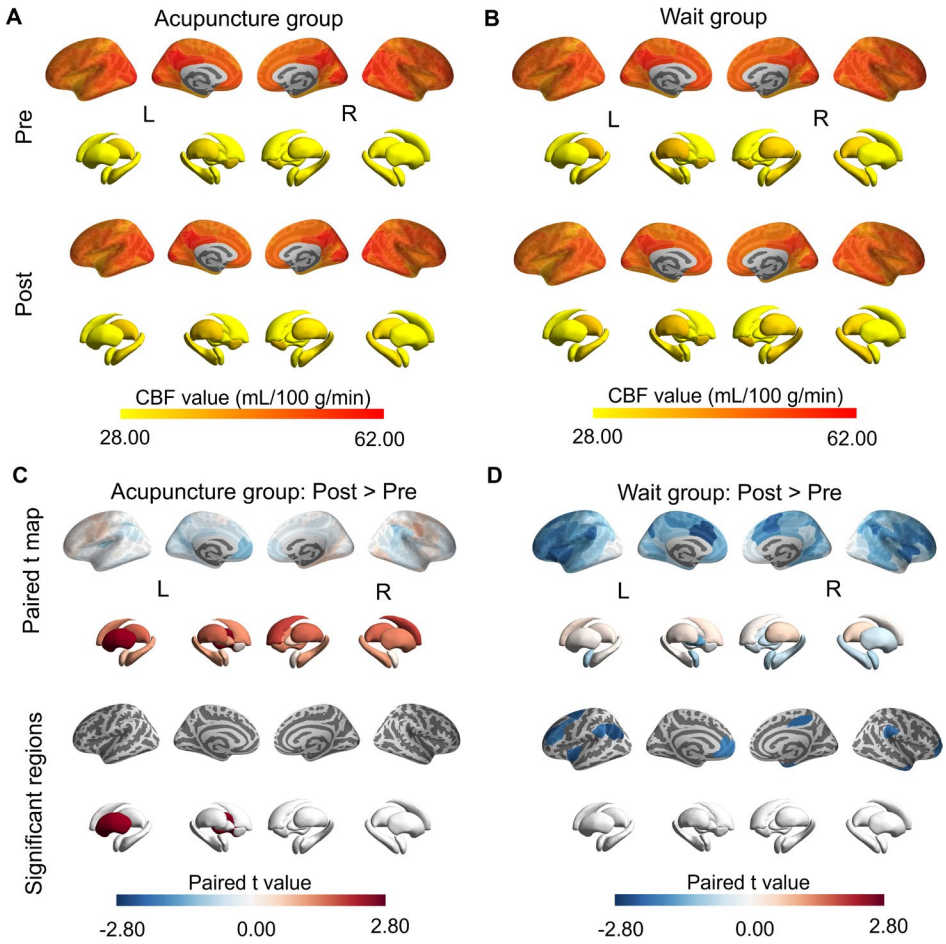

FIGURE 5 | Regional cerebral blood flow at baseline and post-administration. Regional distribution of CBF for each cortical (upper row) and subcortical (lower row) region in the acupuncture group (A) and wait group (B). The upper row of each panel shows the CBF value at baseline; the lower row shows the CBF value at 4-week administration. The regional distribution of changes in CBF in cortical and subcortical regions after 4 weeks of acupuncture treatment (C) and 4 weeks of administration without treatment (D) compared to baseline. The upper row of each panel shows an unthresholded paired *t*-value; the lower row shows all significant regions at $p < 0.05$, uncorrected. No region survived FDR correction for the total of 119 regions tested within each group. Abbreviations: L, left; R, right.

comprehensive approach deepens our understanding of the neural and molecular mechanisms of CID and supports the development of targeted treatments.

ASL, a non-invasive perfusion MRI technique, was utilized for quantitative CBF measurement in this study. ASL measures arterial blood delivery to brain tissues with neural function and metabolism (Detre et al. 2012) and allows for rapid, quantitative data collection, making it ideal for both cross-sectional and longitudinal studies (Watts et al. 2013). Recent studies (Huang et al. 2022; Luo, Li, et al. 2023; Park et al. 2019; Xu et al. 2023; Zhou et al. 2019) have shown varied CBF patterns associated with insomnia. For instance, increased cortical CBF in the right hemisphere's lobes has been observed in insomnia patients (Luo, Li, et al. 2023). Different subtypes and symptoms of insomnia have been linked to specific CBF alterations, such as decreased subcortical CBF in insomnia comorbid with depression (Xu et al. 2023) and increased CBF in the inferior occipital cortex in shift workers with insomnia (Park et al. 2019). In our study, CID patients showed increased CBF in cortical areas, notably within the visual and dorsal attention networks, and decreased CBF in subcortical regions, including the left putamen, bilateral pallidum, and bilateral diencephalon ventral. These previous studies (Huang et al. 2022; Luo, Li, et al. 2023; Park et al. 2019; Xu et al. 2023; Zhou et al. 2019) primarily focused on voxel-level analysis; our study corroborates these findings and extends the analysis to the regional level. Regional-level analysis enhances interpretability and statistical robustness by reducing the multiple comparisons burden inherent to voxel-wise approaches while aligning with anatomically and functionally meaningful brain subdivisions (Glasser et al. 2013; Schaefer et al. 2018). This approach has been widely adopted in neuroimaging studies to improve statistical power and facilitate cross-study comparisons (Hansen et al. 2022; Luppi et al. 2023). Additionally, we found a significant negative correlation between insomnia severity and decreased CBF in these subcortical areas, underscoring the importance of reduced CBF in subcortical regions in the pathogenesis of insomnia. However, the exploration of treatment effects on CBF in insomnia remains relatively uncharted, and the neurobiological markers identified in CID require further validation in longitudinal studies.

Acupuncture has been recognized as a promising nonpharmacological intervention for CID (Peng et al. 2024; Wang

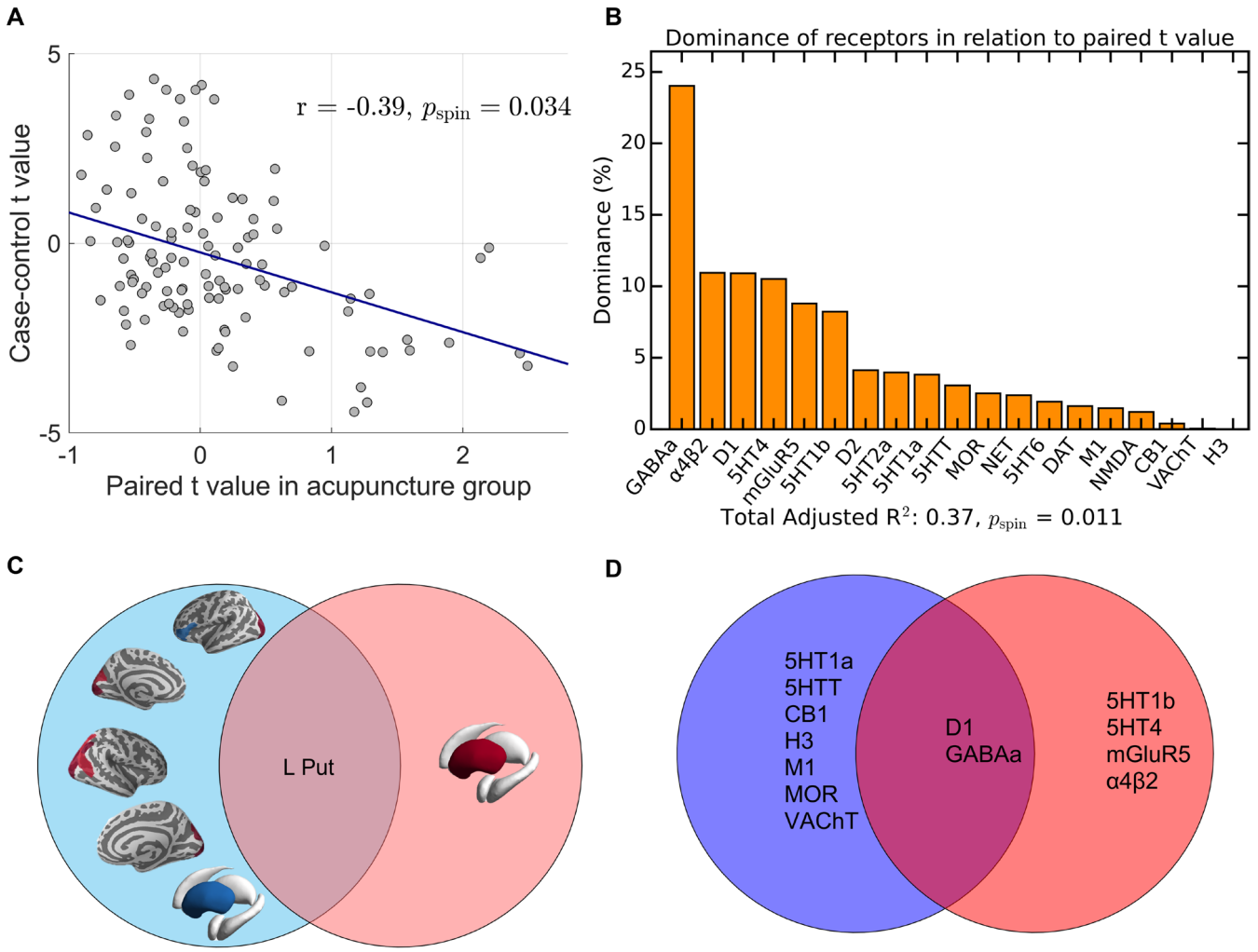

FIGURE 6 | (A) Scatter plot of the paired *t* value (*x* axis) in the acupuncture group versus the case–control *t* value in the CID and HCs dataset (*y* axis). Acupuncture-induced CBF changes (paired *t* value) are negatively correlated with CID-related CBF differences (case–control *t*) (Pearson's $r = -0.39$, $p_{spin} < 0.034$). This negative correlation indicates that an increase in cerebral blood flow induced by acupuncture (reflected by higher paired *t*-values) is associated with a reduction in the difference in cerebral blood flow between patients with CID and HCs (indicated by lower case–control *t*-values). (B) We fit a multi-linear regression model that predicts acupuncture-induced CBF changes from 19 receptors/transporters distributions. Receptor distributions significantly correspond to acupuncture-induced CBF changes (total adjusted $R^2 = 0.37$, $p_{spin} = 0.011$). Through dominance analysis, the percent dominance of each input variable is defined as the variable's dominance normalized by the total adjusted R^2 of the model. We identified GABAa, α4β2, D1, 5HT4, mGluR5, and 5HT1b spatial distributions as major contributors (each dominance > 5%) to the acupuncture-induced CBF changes. (C) A Venn diagram shows overlapping and distinct areas between baseline CID-related changes versus HCs and post-acupuncture alterations. Notably, CID patients had reduced left putamen CBF compared to HCs, which significantly increased after acupuncture treatment. (D) A Venn diagram illustrates neurotransmitter receptors/transporters with over 5% dominance in both CID-related and post-acupuncture CBF changes. Notably, D1 and GABAa receptors correlate with CBF alterations in both contexts. Abbreviations: CBF, cerebral blood flow; CID, chronic insomnia disorder. HCs, healthy controls; L Put, left putamen.

et al. 2023), offering significant benefits by minimizing the confounding influences of medication on brain function (Luppi et al. 2023). Our study highlights three key aspects of acupuncture's effectiveness in treating CID. First, patients receiving acupuncture showed considerable symptom relief, with notable improvements in PSQI, ISI, and SAS scores compared to the waitlist group. This indicates acupuncture's effectiveness in alleviating insomnia and anxiety symptoms. Second, the spatial patterns of CBF changes induced by acupuncture did not correlate significantly with those in the waitlist group, suggesting distinct underlying processes. The waitlist group showed a decrease in cortical CBF in the control network, salience network, and default mode network, likely reflecting the natural progression of insomnia (van Someren 2021). In contrast, acupuncture appeared to halt or reverse these changes. Third, we identified a negative correlation between acupuncture-induced CBF changes and case–control-related alterations. Regions showing elevated or reduced CBF in CID patients experienced a decrease or increase in CBF following 4-week acupuncture treatment, respectively. This negative correlation pattern suggests that acupuncture effectively reverses the abnormal CBF patterns characteristic of CID, aligning brain function more closely with that of healthy individuals. Despite these findings indicating the potential of modulating CBF in acupuncture's

effectiveness in treating CID, the specific key brain regions involved remain unidentified.

A highlight of this study is the identification of the left putamen as a shared region that is involved in both CID-related changes and post-acupuncture treatment alterations. Specifically, CID patients had reduced left putamen CBF compared to HCs, which significantly increased after acupuncture treatment (Figure 6C). The putamen, traditionally associated with motor and cognitive functions (Anderson et al. 2019), is now increasingly acknowledged for its role in insomnia. Genome-wide association studies have revealed a significant correlation between the putamen's medium spiny neurons and insomnia (Ketchesin et al. 2023). Structural MRI study (Falgàs et al. 2021) has shown a correlation between increased putamen volume and insomnia severity, while PET study (Stokholm et al. 2017) indicates that neurochemical and neuroinflammatory changes within the putamen are associated with sleep disorders. Additionally, previous studies suggest that acupuncture effectively modulates the function of the putamen. For instance, a PET study (Schlünzen et al. 2007) has shown changes in the putamen's CBF during acupuncture in healthy individuals, while an fMRI study (Hui et al. 2000) has demonstrated that acupuncture modulates putamen activity to exert its complex multisystem effects. These findings suggest that the putamen is a key region in the insomnia mechanism, and acupuncture may modulate its function to alleviate insomnia symptoms. It should be noted that we also observed significant CBF changes in regions like the pallidum (Luo, Ge, et al. 2023) and brain stem (Mu and Huang 2019), both crucial for sleep regulation, in separate cross-sectional and longitudinal comparisons. However, our primary focus centered on the left putamen, which not only exhibited significant CBF changes in CID compared to HCs but also showed responsiveness to acupuncture interventions. Despite these findings indicating the potential of targeting specific brain regions for effective treatment, the underlying neurochemical mechanisms driving these CBF changes remain unclear.

Another highlight of this study is the significant correlation between CBF changes observed in CID patients and the densities of various neurotransmitter receptors/transporters. Notably, GABAa (Steiger 2007) and D1 (McCullough et al. 2021) were identified as major contributors to these CBF changes in our study, consistently validated through both cross-sectional and longitudinal comparisons (Figure 6D). Pharmacological treatments for CID often target the dopaminergic and GABAergic systems (López-Muciño et al. 2022; Oishi et al. 2023). GABAa and D1 receptors, crucial for nervous system development (Vosberg et al. 2020; Wang and Kriegstein 2009), play key roles in regulating CBF by influencing neuronal activity (Tekin and Cummings 2002). D1 receptors modulate CBF through effects on neuronal excitability and synaptic activity, often enhancing perfusion in areas linked to reward and motivation (Li, Nakano, et al. 2023). Conversely, GABAa receptors, being inhibitory, tend to reduce neuronal firing and synaptic activity, thus potentially decreasing CBF (Laufs et al. 2011). This calming effect of GABAergic activity can reduce overall brain metabolism and CBF. The interplay of these excitatory and inhibitory systems is vital for maintaining cerebral blood flow dynamics (Katz et al. 2023; Yu, Hu, et al. 2024), essential for brain function and effective insomnia treatment. Previous studies suggest that acupuncture may ameliorate sleep disturbances by modulating neurotransmitters. For instance, acupuncture has been shown to increase GABA levels (Zhang et al. 2023) and decrease D1 receptor activity (Xi et al. 2023) to improve sleep quality. Although other neurotransmitter receptors/transporters, such as 5-HT1a (Singh et al. 2023), CB1 (Silvani et al. 2014), and M1 (Nissen et al. 2006), are implicated in sleep regulation, their contribution to CID-related CBF changes and their modulation by acupuncture have not been as extensively validated. These receptors may individually influence CID-related CBF alterations but appear less involved in modulating responses to acupuncture treatment. Further experiments are warranted to clarify the specific roles of these neurotransmitters in the context of CID and the action of acupuncture.

Some limitations existed in our study. First, the modest sample size in the acupuncture treatment for the CID dataset may have limited the ability to detect subtle effects in multiple comparisons. Future research should aim for a larger sample size, enhancing the robustness of findings related to CBF changes in CID patients. Second, the independence assumption in our multiple LRM and two-brain map correlation analysis was challenged by spatial autocorrelation. We addressed this using null models (Markello et al. 2021; Váša et al. 2018) that preserve spatial autocorrelation to correct the significant p-values. Third, participants did not undergo brain vascular exams (such as Magnetic Resonance Angiography), and we acknowledge that vascular abnormalities could potentially influence cerebral blood flow measurements. To address this concern, we carefully screened participants for any known cardiovascular or cerebrovascular conditions during the recruitment process. Finally, while we identified correlations between neurotransmitter receptors/transporters and CBF changes in CID, the causal relationships remain to be definitively established. Future research using PET/fMRI could provide a more in-depth examination of the dopaminergic and GABAergic systems, thereby validating and expanding our preliminary findings.

In summary, our study delineates the neural and molecular basis of the onset and therapeutic regulatory role of CID. We identified significant CBF changes in the left putamen and other regions, correlating with insomnia severity and acupuncture responsiveness. Neurotransmitter receptors, especially GABAa and D1, were key contributors to these brain changes, highlighting their roles in CID pathophysiology and modulation effects in acupuncture. While promising, further research with larger samples and advanced imaging techniques is needed to establish causality and validate these findings. This work underscores the importance of integrating macroscopic neuroimaging with microscopic neurochemical expression in understanding CID and developing targeted therapies.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The original MRI data that support the findings of this study are available on request from the corresponding author. The supporting data for the figures, tables, and customized code are available at https://github.com/xiaoyi-142/CBF_Neurotransmitter.

References

Adebimpe, A., M. Bertolero, S. Dolui, et al. 2022. "ASLPrep: A Platform for Processing of Arterial Spin Labeled MRI and Quantification of Regional Brain Perfusion." *Nature Methods* 19: 683.

Alsop, D. C., J. A. Detre, X. Golay, et al. 2015. "Recommended Implementation of Arterial Spin-Labeled Perfusion MRI for Clinical Applications: A Consensus of the ISMRM Perfusion Study Group and the European Consortium for ASL in Dementia." *Magnetic Resonance in Medicine* 73: 102–116.

Anderson, A. J., P. Ren, T. M. Baran, Z. Zhang, and F. Lin. 2019. "Insula and Putamen Centered Functional Connectivity Networks Reflect Healthy Agers' Subjective Experience of Cognitive Fatigue in Multiple Tasks." *Cortex* 119: 428–440.

Azen, R., and D. V. Budescu. 2003. "The Dominance Analysis Approach for Comparing Predictors in Multiple Regression." *Psychological Methods* 8: 129–148.

Chen, Z., T. Jiang, X. Yin, B. Li, Z. Tan, and J. Guo. 2023. "The Increased Functional Connectivity Between the Locus Coeruleus and Supramarginal Gyrus in Insomnia Disorder With Acupuncture Modulation." *Frontiers in Neuroscience* 17: 1131916. https://doi.org/10.3389/fnins.2023.1131916.

Cheuk, D. K., W.-F. Yeung, K. Chung, and V. Wong. 2012. "Acupuncture for Insomnia." *Cochrane Database of Systematic Reviews* 2012: CD005472.

Detre, J. A., J. S. Leigh, D. S. Williams, and A. P. Koretsky. 1992. "Perfusion Imaging." *Magnetic Resonance in Medicine* 23: 37–45.

Detre, J. A., H. Rao, D. J. J. Wang, Y. F. Chen, and Z. Wang. 2012. "Applications of Arterial Spin Labeled MRI in the Brain." *Journal of Magnetic Resonance Imaging* 35: 1026–1037.

Falgàs, N., I. Illán-Gala, I. E. Allen, et al. 2021. "Specific Cortical and Subcortical Grey Matter Regions Are Associated With Insomnia Severity." *PLoS One* 16: e0252076.

Fu, C., N. Zhao, Z. Liu, et al. 2017. "Acupuncture Improves Peri-Menopausal Insomnia: A Randomized Controlled Trial." *Sleep* 40: zsx153. https://doi.org/10.1093/sleep/zsx153/4259343.

Gao, X., C. Xu, P. Wang, et al. 2013. "Curative Effect of Acupuncture and Moxibustion on Insomnia: A Randomized Clinical Trial." *Journal of Traditional Chinese Medicine* 33: 428–432.

Glasser, M. F., S. N. Sotiropoulos, J. A. Wilson, et al. 2013. "The Minimal Preprocessing Pipelines for the Human Connectome Project." *NeuroImage* 80: 105–124.

Greve, D. N., and B. Fischl. 2009. "Accurate and Robust Brain Image Alignment Using Boundary-Based Registration." *NeuroImage* 48: 63–72.

Hansen, J. Y., G. Shafiei, R. D. Markello, et al. 2022. "Mapping Neurotransmitter Systems to the Structural and Functional Organization of the Human Neocortex." *Nature Neuroscience* 25: 1569–1581.

Huang, G., Y. Fang, W. Zhang, et al. 2022. "Altered Thalamic Functional Connectivity and Cerebral Blood Flow in Insomnia Disorder: A Resting-State Functional Magnetic Resonance Imaging Study." *Clinical Imaging* 88: 17–23.

Hui, K. K., J. Liu, N. Makris, et al. 2000. "Acupuncture Modulates the Limbic System and Subcortical Gray Structures of the Human Brain: Evidence From fMRI Studies in Normal Subjects." *Human Brain Mapping* 9: 13–25.

Huo, Z.-J., J. Guo, and D. Li. 2013. "Effects of Acupuncture With Meridian Acupoints and Three Anmian Acupoints on Insomnia and Related Depression and Anxiety State." *Chinese Journal of Integrative Medicine* 19: 187–191.

Jenkinson, M., and S. Smith. 2001. "A Global Optimisation Method for Robust Affine Registration of Brain Images." *Medical Image Analysis* 5: 143–156.

Katz, B. M., L. R. Walton, K. M. Houston, D. H. Cerri, and Y.-Y. I. Shih. 2023. "Putative Neurochemical and Cell Type Contributions to Hemodynamic Activity in the Rodent Caudate Putamen." *Journal of Cerebral Blood Flow and Metabolism* 43: 481–498.

Ketchesin, K. D., W. Zong, M. A. Hildebrand, et al. 2023. "Diurnal Alterations in Gene Expression Across Striatal Subregions in Psychosis." *Biological Psychiatry* 93: 137–148.

Kim, H., X. Zhu, Y. Zhao, et al. 2023. "Resting-State Functional Connectivity Changes in Older Adults With Sleep Disturbance and the Role of Amyloid Burden." *Molecular Psychiatry* 28, no. 10: 4399–4406. https://doi.org/10.1038/s41380-023-02214-9.

Lancel, M., G. J. Boersma, and J. Kamphuis. 2021. "Insomnia Disorder and Its Reciprocal Relation With Psychopathology." *Current Opinion in Psychology* 41: 34–39.

Laufs, H., M. P. Richardson, A. Salek-Haddadi, et al. 2011. "Converging PET and fMRI Evidence for a Common Area Involved in Human Focal Epilepsies." *Neurology* 77: 904–910.

Li, W.-R., T. Nakano, K. Mizutani, et al. 2023. "Neural Mechanisms Underlying Uninstructed Orofacial Movements During Reward-Based Learning Behaviors." *Current Biology: CB* 33: 3436–3451.

Li, Z.-H., Y.-J. Xu, and Y.-M. Wu. 2023. "Acupuncture at "Umbilical Four-Acupoints" for Chronic Insomnia and Its Comorbid Symptoms." *Zhongguo Zhen Jiu* 43: 629–633.

López-Muciño, L. A., F. García-García, J. Cueto-Escobedo, M. Acosta-Hernández, A. Venebra-Muñoz, and J. C. Rodríguez-Alba. 2022. "Sleep Loss and Addiction." *Neuroscience and Biobehavioral Reviews* 141: 104832.

Luo, X.-W., Q.-X. Li, L.-S. Shen, et al. 2023. "Quantitative Association of Cerebral Blood Flow, Relaxation Times and Proton Density in Young and Middle-Aged Primary Insomnia Patients: A Prospective Study Using Three-Dimensional Arterial Spin Labeling and Synthetic Magnetic Resonance Imaging." *Frontiers in Neuroscience* 17: 1099911.

Luo, Y., D. Dong, H. Huang, et al. 2023. "Associating Multimodal Neuroimaging Abnormalities With the Transcriptome and Neurotransmitter Signatures in Schizophrenia." *Schizophrenia Bulletin* 49: 1554–1567.

Luo, Y.-J., J. Ge, Z.-K. Chen, et al. 2023. "Ventral Pallidal Glutamatergic Neurons Regulate Wakefulness and Emotion Through Separated Projections." *iScience* 26: 107385.

Luppi, A. I., J. Y. Hansen, R. Adapa, et al. 2023. "In Vivo Mapping of Pharmacologically Induced Functional Reorganization Onto the Human Brain's Neurotransmitter Landscape." *Science Advances* 9: eadf8332.

Markello, R. D., A. Arnatkeviciute, J.-B. Poline, B. D. Fulcher, A. Fornito, and B. Misic. 2021. "Standardizing Workflows in Imaging Transcriptomics With the Abagen Toolbox." *eLife* 10: e72129.

Markello, R. D., J. Y. Hansen, Z.-Q. Liu, et al. 2022. "Neuromaps: Structural and Functional Interpretation of Brain Maps." *Nature Methods* 19: 1472–1479.

Markello, R. D., and B. Misic. 2021. "Comparing Spatial Null Models for Brain Maps." *NeuroImage* 236: 118052.

McCullough, K. M., G. Missig, M. A. Robble, et al. 2021. "Nucleus Accumbens Medium Spiny Neuron Subtypes Differentially Regulate Stress-Associated Alterations in Sleep Architecture." *Biological Psychiatry* 89: 1138–1149.

Mu, P., and Y. H. Huang. 2019. "Cholinergic System in Sleep Regulation of Emotion and Motivation." *Pharmacological Research* 143: 113–118.

Mutsaerts, H. J. M. M., J. Petr, P. Groot, et al. 2020. "ExploreASL: An Image Processing Pipeline for Multi-Center ASL Perfusion MRI Studies." *NeuroImage* 219: 117031.

Nissen, C., E. A. Nofzinger, B. Feige, et al. 2006. "Differential Effects of the Muscarinic M1 Receptor Agonist RS-86 and the Acetylcholine-Esterase Inhibitor Donepezil on REM Sleep Regulation in Healthy Volunteers." *Neuropsychopharmacology* 31: 1294–1300.

Oishi, Y., Y. C. Saito, and T. Sakurai. 2023. "GABAergic Modulation of Sleep-Wake States." *Pharmacology & Therapeutics* 249: 108505.

Park, Y. K., J. H. Kim, S. J. Choi, S. T. Kim, and E. Y. Joo. 2019. "Altered Regional Cerebral Blood Flow Associated With Mood and Sleep in Shift Workers: Cerebral Perfusion Magnetic Resonance Imaging Study." *Journal of Clinical Neurology* 15: 438–447.

Peng, W., H. Xu, C. Zhang, Y. Hu, and S. Yu. 2024. "The Altered Hypothalamic Network Functional Connectivity in Chronic Insomnia Disorder and Regulation Effect of Acupuncture: A Randomized Controlled Neuroimaging Study." *BMC Complementary Medicine and Therapies* 24: 396.

Sander, C. Y., H. D. Hansen, and H.-Y. Wey. 2020. "Advances in Simultaneous PET/MR for Imaging Neuroreceptor Function." *Journal of Cerebral Blood Flow & Metabolism* 40: 1148–1166.

Schaefer, A., R. Kong, E. M. Gordon, et al. 2018. "Local-Global Parcellation of the Human Cerebral Cortex From Intrinsic Functional Connectivity MRI." *Cerebral Cortex* 28: 3095–3114.

Schlünzen, L., M. S. Vafaee, and G. E. Cold. 2007. "Acupuncture of LI-4 in Anesthetized Healthy Humans Decreases Cerebral Blood Flow in the Putamen Measured With Positron Emission Tomography." *Anesthesia and Analgesia* 104: 308–311.

Shen, Z., X. Yang, T. She, et al. 2024. "Deficits in Brain Default Mode Network Connectivity Mediate the Relationship Between Poor Sleep Quality and Anxiety Severity." *Sleep* 47: zsad296.

Silvani, A., C. Berteotti, S. Bastianini, et al. 2014. "Multiple Sleep Alterations in Mice Lacking Cannabinoid Type 1 Receptors." *PLoS One* 9: e89432.

Singh, D., P. Singh, P. Srivastava, D. Kakkar, M. Pathak, and A. K. Tiwari. 2023. "Development and Challenges in the Discovery of 5-HT1A and 5-HT7 Receptor Ligands." *Bioorganic Chemistry* 131: 106254.

Song, X.-J., Y.-H. Zhu, P. Wu, L. Du, and Z.-W. Li. 2022. "Acupoint Compatibility Effect and Mechanism of Shenmen (HT7) and Sanyinjiao (SP6) in Improving Daytime Fatigue and Sleepiness of Insomnia." *Zhen Ci Yan Jiu* 47: 630–635.

Steiger, A. 2007. "Neurochemical Regulation of Sleep." *Journal of Psychiatric Research* 41: 537–552.

Stokholm, M. G., A. Iranzo, K. Østergaard, et al. 2017. "Assessment of Neuroinflammation in Patients With Idiopathic Rapid-Eye-Movement Sleep Behaviour Disorder: A Case-Control Study." *Lancet Neurology* 16: 789–796.

Tekin, S., and J. L. Cummings. 2002. "Frontal-Subcortical Neuronal Circuits and Clinical Neuropsychiatry: An Update." *Journal of Psychosomatic Research* 53: 647–654.

Vamvakas, A., T. Lawn, M. Veronese, S. C. R. Williams, I. Tsougos, and M. A. Howard. 2022. "Neurotransmitter Receptor Densities Are Associated With Changes in Regional Cerebral Blood Flow During Clinical Ongoing Pain." *Human Brain Mapping* 43: 5235–5249.

van Someren, E. J. W. 2021. "Brain Mechanisms of Insomnia: New Perspectives on Causes and Consequences." *Physiological Reviews* 101: 995–1046.

Váša, F., J. Seidlitz, R. Romero-Garcia, et al. 2018. "Adolescent Tuning of Association Cortex in Human Structural Brain Networks." *Cerebral Cortex* 28: 281–294.

Vosberg, D. E., M. Leyton, and C. Flores. 2020. "The Netrin-1/DCC Guidance System: Dopamine Pathway Maturation and Psychiatric Disorders Emerging in Adolescence." *Molecular Psychiatry* 25: 297–307.

Wang, D. D., and A. R. Kriegstein. 2009. "Defining the Role of GABA in Cortical Development." *Journal of Physiology* 587: 1873–1879.

Wang, S., Y. Lan, Z. Liu, S. Xu, and X. Wu. 2023. "Effects of Different Interventions on Insomnia in Adults: Systematic Review and Network Meta-Analysis." *Journal of Psychiatric Research* 165: 140–149.

Watts, J. M., C. T. Whitlow, and J. A. Maldjian. 2013. "Clinical Applications of Arterial Spin Labeling." *NMR in Biomedicine* 26: 892–900.

Xi, H., W. Wu, S. Qin, X. Wang, and C. Liu. 2023. "Effects of Electroacupuncture on the Ventral Tegmental Area- Nucleus Accumbens Dopamine Pathway in Rats With Chronic Sleep Deprivation." *Acupuncture in Medicine* 41: 336–344.

Xu, M., Q. Wang, B. Li, et al. 2023. "Cerebellum and Hippocampus Abnormalities in Patients With Insomnia Comorbid Depression: A Study on Cerebral Blood Perfusion and Functional Connectivity." *Frontiers in Neuroscience* 17: 1202514.

Xu, X.-Y., J.-H. Ma, J.-X. Ou, et al. 2022. "Effect of Fang's Scalp Acupuncture on Perceived Stress and Sleep Structure in Insomnia Patients: A Randomized Controlled Trial." *Zhongguo Zhen Jiu* 42: 371–376.

Yang, L., X. Xiao, L. Yu, et al. 2023. "Neural Mechanisms of Working Memory Dysfunction in Patients With Chronic Insomnia Disorder." *Sleep Medicine* 112: 151–158.

Yu, L., D. Hu, Y. Luo, et al. 2024. "Transcriptional Signatures of Cortical Structural Changes in Chronic Insomnia Disorder." *Psychophysiology* 61: e14671.

Yu, L., W. Peng, W. Lin, et al. 2024. "Electroencephalography Connectome Changes in Chronic Insomnia Disorder Are Correlated With Neurochemical Signatures." *Sleep* 47: zsae080.

Yu, S., Z. Shen, H. Xu, et al. 2023. "Top-Down and Bottom-Up Alterations of Connectivity Patterns of the Suprachiasmatic Nucleus in Chronic Insomnia Disorder." *European Archives of Psychiatry and Clinical Neuroscience* 274, no. 2: 245–254. https://doi.org/10.1007/s00406-022-01534-1.

Zhang, B., and Y.-K. Wing. 2006. "Sex Differences in Insomnia: A Meta-Analysis." *Sleep* 29: 85–93.

Zhang, F., X. Zhang, Q. Peng, and L. Tang. 2023. "Electroacupuncture of the Cymba Concha Alleviates p-Chlorophenylalanine-Induced Insomnia in Mice." *Acupuncture in Medicine* 41: 345–353.

Zhou, F., M. Huang, L. Gu, et al. 2019. "Regional Cerebral Hypoperfusion After Acute Sleep Deprivation: A STROBE-Compliant Study of Arterial Spin Labeling fMRI." *Medicine (Baltimore)* 98: e14008.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.