NEUROBIOLOGICAL BIOMARKERS FOR DEVELOPING NOVEL TREATMENTS OF SUBSTANCE AND NON-SUBSTANCE ADDICTION

EDITED BY: Yanhui Liao, Dara G. Ghahremani, Jianhua Chen, Kyoji Okita and Wenbin Guo

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Cortical Thickness in Alcohol Dependent Patients With Apathy

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Objectives: Many studies reported structural brain changes in patients with alcohol dependence (PADs). However, no studies identified structural correlates of apathy that might aggravate alcohol misuse. Here, we explored regional differences in cortical thickness in PADs relative to healthy controls (HCs), and examined the potential correlation of regional thickness with the severity of apathy.

Methods: Magnetic resonance imaging data were collected from 33 male PADs and 35 male age- and education-matched HCs. We used the FreeSurfer software to investigate group differences in cortical thickness across 148 regions. Apathy was evaluated using the Lille Apathy Rating Scale-Informant (LARS-I). Regression analyses examined the relationship between cortical thickness of regions of interest and apathy score in PADs.

Results: Compared to HCs, PADs showed significant decreases in the cortical thickness of occipito-temporal cortex (OTC), including the left middle occipital gyrus and occipital pole, right superior occipital gyri, and bilateral lingual gyrus; bilateral superior parietal cortex (SPC), including the right intraparietal sulcus; and bilateral inferior parietal cortex (IPC). Furthermore, the cortical thickness of all of the three regions was negatively correlated with the apathy total scores. The cortical thickness of the IPC was also negatively correlated with the action initiation subscore of the LARS-I.

Conclusions: The current results suggest the thickness of bilateral parietal and occipital temporal cortices as neural markers of apathy in PADs. These findings add to the literature by identifying the neural bases of a critical clinical feature of individuals with alcoholism.

Keywords: alcohol dependence, apathy, occipito-temporal cortex, superior parietal cortex, inferior parietal cortex

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INTRODUCTION

Alcohol dependence (AD) is a chronic illness characterized by poor treatment outcomes and high relapse rates (1). Individuals with AD frequently demonstrate motivational deficits, exacerbating personal and interpersonal health (2). Brain imaging was widely used to investigate the consequences of alcohol misuse, which included atrophy of the gray and white matters, sulcal widening, and ventricular enlargement. The structural changes resulted from excessive alcohol

consumption (3–7). These deficits involved multiple brain networks that support executive control, salience response, and emotion regulation, which may directly or indirectly contribute to cognitive and motivational dysfunction in patients with AD (8, 9).

The main clinical feature of apathy is lack of motivation (10) and reduced initiative, interest, and/or concern (11). It is also proposed that apathy be defined as dysfunction of volitional processes or a reduction in self-generated, voluntary, and purposeful behavior (i.e., goal-directed behavior) (12). Difficulties in goal-directed behavior, as a key feature of apathy, was significantly associated with symptoms of alcohol dependence (13). Almost all patients with severe alcohol dependence show diminished or lack of living practices except for drinking-related events, and executive dysfunction on alcohol abstinence (2, 14, 15). Importantly, patients undergoing treatment for AD often relapse despite better knowledge and their intention to remain abstinent, reflecting impairments in volition and goal-directed behavior (16). It is thus critical to investigate the neural correlates of apathy in AD.

In imaging studies of various brain disorders, apathy has been associated with structural and function deficits of multiple brain regions (17), particularly the dorsal anterior cingulate cortex (dACC) (18). Other studies implicated the fronto-subcortical circuits and lateral parietal cortex (19). Numerous studies have also shown structural and functional deficits of a wide range of cortical and subcortical circuits in individuals with AD (20). However, no studies to our knowledge have specifically examined the cerebral structural bases of apathy in AD.

The current study addressed this gap in research. On the basis of the literature that goal-directed behaviors require top-down attentional, cognitive and affective control as well as execution of complex motor movements (21), we hypothesized that the frontal and parietal cortical thickness would be diminished and may represent a neurobiological marker of apathy in AD.

METHODS

Participants and Consents

All patients with AD (PADs) were males, of Han Chinese descent, and recruited from Beijing Huilongguan Hospital between 2017 and 2018. Inclusion criteria were as follows: a) age between 18 and 65 years; b) diagnosis of AD in accordance with Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) criteria (22); c) right-handed; d) abstinent for 14 to 28 days after completion of medically assisted withdrawal, with no obvious withdrawal symptoms and a Clinical Institute Withdrawal Assessment for Alcohol (CIWA) (23) score of less than 3. Patients with other axis I mental disorders, including dependence on substances other than alcohol and nicotine, or severe medical conditions (e.g., unstable hypertension, poorly controlled diabetes, myocardial infarction, liver cirrhosis) were excluded. In addition, we recruited 35 male age- and education-matched healthy controls (HCs) from the general medical clinics of the hospital and local community. Prior to study inclusion, all participants provided written informed consent in accordance with the Declaration of Helsinki and a protocol approved by the ethics committee of Beijing Huilongguan Hospital.

Assessments

All PADs were evaluated with the Alcohol Use Disorder Identification Test (AUDIT) (24), a semi-structured questionnaire to investigate the severity of alcohol consumption (25). Lifetime Drinking History assessment to determine the number of daily drinks (one drink = 10 g of ethanol), age at first drink, and age at onset of alcohol dependence. The extent of apathy was assessed using the Lille Apathy Rating Scale-Informant (LARS-I) (11), which comprised 33 items across nine domains, each corresponding to a specific clinical dimension of apathy. Each sub-scale score was from −4 to +4, with higher positive scores indicating more severe symptoms. In brief, a negative or positive score indicates less or more apathy, and zero represents moderate apathy in everyday productivity and interests domains or non-applicable or non-classifiable reply in other domains. In this study, we focused on the apathy total score (T score) and action initiation (AI) subscore in data analyses.

MRI Data Acquisition and Analyses

All PADs (after detoxification) and HCs underwent MRI. MRIs were obtained by using a Siemens 3T MRI scanner. Head motion was minimized using foam pads. Structural MR images were acquired for the whole brain with a sagittal 3D-magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence, using the follow parameters: echo time (TE) = 2.98 ms, inversion time (TI) = 900 ms, repetition time (TR) = 2,300 ms, flip angle (FA) = 9°, field-of-view (FOV) = 240 mm×256 mm, matrix size = 256×240, thickness/gap = 1/0 mm.

Measures of cortical thickness (mm) of 148 cortical regions (bilateral; 74 x 2; Desikan-Killiany Atlas), total subcortical thickness, and total gray matter volume (TGV) were obtained using FreeSurfer (26) (http://surfer.nmr.mgh.harvard.edu). TGV was used as a covariate in all analyses to account for differences in head size. We followed the ENIGMA pipeline (http://enigma.ini.usc.edu/) for quality control of the images: All cortical regions with a thickness >1.5 or <1.5 times the interquartile range were identified and visually inspected by overlaying their segmentations on the participants' anatomical images. Only imaging data for which segmentation was judged to be accurate were subjected to statistical analyses.

Statistical Analysis

As all participants were male, continuous demographic variables were analyzed using independent samples t-tests and categorical data were analyzed with Chi-square (χ^2) tests to compare PADs and HCs. The level of statistical significance was set at $p < 0.05$ (two-tailed).

Cortical thickness values of the 148 regions were compared between PADs and HCs using univariate linear regression analysis, where the cortical thickness of each region was used as the dependent variable, and group (PADs/HCs), age, smoking status, education, and TGV were entered as independent

variables. The threshold for statistical significance was set at $p < 0.00033784$ (i.e., 0.05/148) to correct for multiple comparisons (Bonferroni correction).

We identified 12 cortical regions of interest (ROIs) that differed significantly between PADs and HCs in thickness, and these regions belong to the occipital temporal cortex (OTC) (lateral cortex including the left middle occipital gyrus, bilateral lingual gyrus, left occipital pole and right superior occipital gyrus), superior parietal cortex (SPC) (lateral cortex including the right intraparietal sulcus and bilateral superior parietal lobule), and inferior parietal cortex (IPC) (lateral cortex including the bilateral angular gyrus and supramarginal gyrus) (27). We performed bivariate correlation analyses to explore the relationship between cortical thickness of the OTC, SPC and IPC and apathy total score (T score), and action initiation subscore (AI score) in PADs. Statistical significance was tested at $p < 0.05/6$, two-sided, to correct for multiple comparisons. As many alcohol drinkers are heavy smokers, we performed two sets of regression, one without smoking status as a covariate, so that the results best reflect the typical populations of alcoholic participants, and the other with smoking status as a covariate, so the effects of smoking could be controlled for.

RESULTS

Demographic and Clinical Characteristics

There were no significant differences in age (t=1.257, $p = 0.213$) or years of education (t=−0.883, p=0.381) between PADs and HCs. More PADs were smokers than HCs ($\chi^2 = 11.87$, p=0.001). Smoking status was thus included as a covariate in the following data analyses. Compared to HCs, PADs showed higher LARS total or T score (t=5.85, $p < 0.001$) and action initiation or AI subscore (t=5.43, $p < 0.001$). The demographic and clinical characteristics of the two groups are presented in **Table 1**.

Cortical Thickness

The cortical thickness of the bilateral lingual gyrus in the OTC was significantly higher in PADs as compared with HCs; however, relative to HCs, PADs exhibited significant decreases in overall cortical thickness in the OTC (consisting of the left middle occipital gyrus and occipital pole, right superior occipital gyri, and bilateral lingual gyrus), SPC, as well as IPC, (all p's <0.00033784, **Table 2**) accounting for age, years of education, smoking status, and TGV. The mean cortical thickness and statistics for all 148 regions were shown in **Supplementary Table 1**.

For PADs, we focused on the OTC, SPC, and IPC and performed Pearson correlation analyses to evaluate the relationship between cortical thickness and apathy (T score and AI subscore). Evaluated at $p < 0.0083$ (i.e., 0.05/6, Bonferroni correction), the results revealed negative correlations between the thickness of the OTC, SPC as well as IPC and both apathy T score. The cortical thickness of the IPC was also negatively correlated with the AI subscore. These results are shown in **Figure 1**.

Because many more PADs are smokers, we performed covariance analyses with smoking status as a covariate. The results showed that apathy T score remained negatively correlated with the thickness of the OTC (r=−0.44, p=0.011), IPC (r=−0.47, p=0.007), and SPC (r=−0.58, $p < 0.001$); and the apathy AI subscore showed negative correlation with thickness of the IPC (r=−0.41, p=0.019) and SPC (r=−0.44, p=0.012) but not the OTC (r=−0.32, p=0.078). The findings suggest that after controlling for smoking status, the thickness of the parietal, but not that of occipital temporal cortex, was correlated with difficulties in action initiation in PADs.

DISCUSSION

We hypothesized that PADs would exhibit reduced thickness in the frontal and parietal cortex, and the extent of the reduction would be correlated with the severity of apathy in PADs. To this end, we determined differences in regional cortical thickness

TABLE 1 | Demographic and clinical characteristics in patients with alcohol dependence (PADs) and healthy controls (HCs) (mean ± SD).

	PAD (n = 33)	HCs (n = 35)	t/χ^2-value	p-value
Age, years	44.8 ± 9.8	41.6 ± 11.5	1.257	0.213
Education, years	11.8 ± 3.3	12.3 ± 2.4	−0.883	0.381
Smoker/non-smoker [a]	26/7	13/22	11.87	0.001
Action initiation (AI)	−1.21 ± 0.86	−2.19 ± 0.61	5.43	0.000
Total score of LARS	−5.85 ± 6.87	−14.78 ± 5.70	5.85	0.000
Age at onset of AD, years	32.5 ± 10.1	N/A	N/A	N/A
Age at first drink, years	19.9 ± 5.5	N/A	N/A	N/A
AUDIT total score	21.7 ± 5.2	N/A	N/A	N/A
Mean daily drinks [b]	19.1 ± 8.7	N/A	N/A	N/A

PADs, patients with alcohol dependence; HCs, healthy controls; LARS, Lille Apathy Rating Scale; AUDIT, Alcohol Use Disorders Identification Test; N/A, not applicable.
[a]Chi-square (χ^2) test: smoker = current or previous smoker; non-smoker: never smoker
[b]One drink contains 10 g of pure alcohol.

TABLE 2 | Brain regions with significant differences in cortical thickness between patients with alcohol dependence (PADs) and healthy controls (HCs).

Cortical thickness of Destrieux Atlas (mm)	PADs (n 33)	HCs (n = 35)	F-value	p-value
L-Middle occipital gyrus	2.165 ± 0.297	2.613 ± 0.123	29.545	9.768E–07
L-Lingual gyrus	2.118 ± 0.142	1.879 ± 0.131	44.499	7.960E–09
L-Angular gyrus	2.152 ± 0.329	2.705 ± 0.139	26.065	3.378E–06
L-Supramarginal gyrus	2.371 ± 0.271	2.741 ± 0.141	14.574	3.138E–04
L-Superior parietal lobule	1.931 ± 0.301	2.461 ± 0.117	32.666	3.351E–07
L-Occipital pole	1.672 ± 0.219	1.947 ± 0.145	20.949	2.321E–05
R-Superior occipital gyrus	1.881 ± 0.302	2.261 ± 0.145	17.749	8.313E–05
R-Lingual gyrus	2.137 ± 0.129	1.967 ± 0.156	16.656	1.303E–04
R-Angular gyrus	2.167 ± 0.324	2.704 ± 0.131	41.123	2.208E–08
R-Supramarginal gyrus	2.389 ± 0.299	2.741 ± 0.118	16.734	1.262E–04
R-Superior parietal lobule	1.929 ± 0.318	2.445 ± 0.118	38.473	5.044E–08
R-Intraparietal sulcus and transverse parietal sulci	1.892 ± 0.211	2.191 ± .089	20.290	3.004E–05

One-way ANOVA, Bonferroni corrected at p<0.00033784.

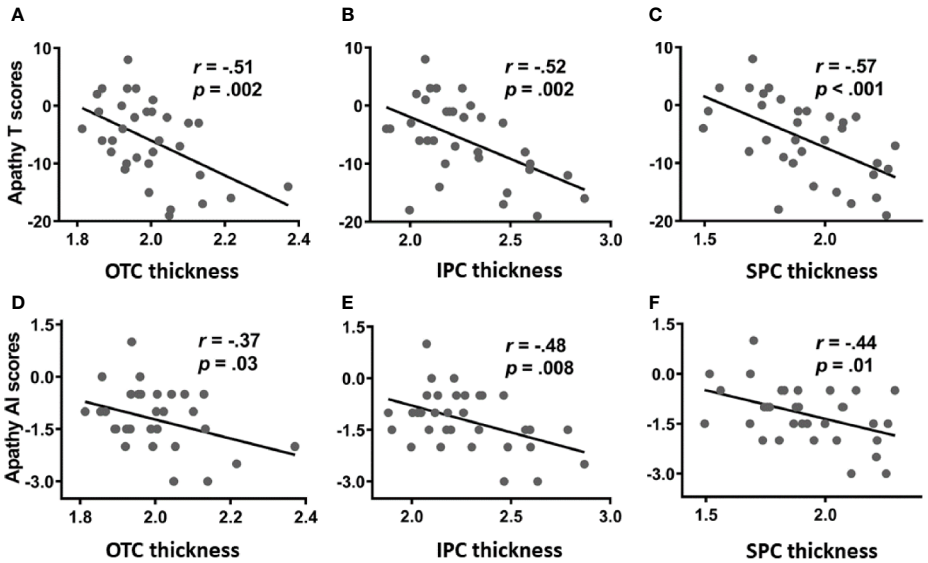

FIGURE 1 | Correlation of cortical thickness of the occipital temporal cortex (OTC), inferior parietal cortex (IPC), and superior parietal cortex (SPC) with apathy total score (T score; upper panel: **A**–**C**) and action initiation subscore (AI subscore; lower panel: **D–F**).

between PADs and HCs and investigated how these differences are related to apathy in PADs. The results suggest that AD was associated with reduced thickness in the bilateral occipital temporal cortex, the superior and inferior parietal cortices, and the extent of the reduction in cortical thickness was correlated with the severity of apathy. Although the cortical thickness of the bilateral lingual gyrus showed peculiar increases in PADs, this change, contrary to that shown in most of the previous studies (5, 28), did not affect the reduction of the whole OTC region in PADs compared with HCs. The mechanism of compensatory hypertrophy might be a potential explanation to this change (29) because a previous study found similar increases in alcohol abuse individuals, regardless of sex and brain regions (30). Greater reduction of parietal cortical thickness appears to be more specifically related to action initiation deficits in PADs. Together, these findings support structural changes of posterior cortical regions as a neural marker of apathy in alcoholism.

Structural imaging studies reported significant volume losses in the prefrontal cortex, insula, and hippocampus in AD (5, 31, 32); however, volumetric deficits of the posterior cortical regions have received less attention (30). For instance, parietal-occipital gray matter volumes were decreased in patients with AD and can be used to predict shorter time of any alcohol use and relapse to heavy drinking (33). A recent meta-analysis of 3,240 individuals with drug and alcohol dependence indicated that the most severe volumetric deficits are found in not only the frontal but also occipital, temporal, and parietal cortices in those with alcohol dependence (34). Consistently, we observed a significant reduction in cortical thickness in the occipital and parietal cortices. However, it is unclear why structural changes cannot be detected in the frontal cortex. One possibility is that the current work only focused on cortical thickness, a volumetric measure different from that as employed in the earlier work. This discrepancy clearly warrants further studies.

Many studies examined the neural mechanisms underlying cognitive and emotional deficits (35) but few focused on apathy in individuals with AD (36). In accordance with previous studies we observed more significant apathy symptoms, as reflected in the apathy total score in PADs than in HCs, suggesting motivational dysfunction in individuals with alcoholism (37). The cortical thickness of the occipital-temporal as well as superior and inferior parietal cortices was negatively correlated with the severity of apathy, consistent with earlier work associating apathy with structural changes in the parietal cortex, in addition to the prefrontal cortex (PFC) and basal ganglia (38). Research has indicated that the superior parietal lobule, including the intraparietal sulcus, play a key role in goal-directed behaviors and volitional processes (39). In addition, an [18F]-fluorodeoxyglucose (FDG) positron emission tomography (PET) study in a rat model of alcoholism revealed that alcohol significantly reduced whole-brain glucose metabolism, and that the effect was most pronounced in the parietal cortex (40). Together, the current along with these earlier studies support the importance of posterior cortical structures in mediating cognitive and emotional deficits (41), which may manifest as apathy in AD. Importantly, as apathy may impact the motivation for behavioral changes, the current findings are also consistent with our earlier work showing that volumetric deficits in the posterior-occipital region predicted relapse in AD patients undergoing treatment (42).

Limitations

A few limitations of present study included: 1) there were more smokers in PADs than in HCs. Although we have accounted for the potential effects of smoking in data analyses, one cannot completely rule out the effects of chronic smoking on the findings. On the other hand, compared to smoking, alcohol consumption appears to be an overriding factor leading to alterations in brain structures and functions (43). Further, alcohol dependent individuals are typically smokers; thus, one may argue that the current findings reflect that general alcohol dependent populations; 2) as a specialist hospital in Beijing for the PADs, we were exposed to patients with more serious clinical symptoms and social dysfunction and difficult to enroll those patients who have mild symptoms and no obvious apathy. This is the reason of correlation analysis without setting apathy-free patients as control in this study. 3) because there were much fewer women with alcohol dependence in China and no women were included in the present study, we were unable to examine the effects of gender on cortical thickness in relation to the impact of alcohol misuse. As sex differences in the etiological processes or consequences of alcohol misuse have been demonstrated in numerous studies (44, 45). We hope to study more women to examine sex differences, including the finding that neurotoxicity may be more severe among women with alcoholism. Lastly, the sample size of the present study is moderate and the findings would warrant replication.

CONCLUSIONS

The present study demonstrated significant differences in occipital, temporal, and parietal cortical thickness in individual with alcohol dependence as compared with healthy controls. These structural changes were related to the severity of apathy in alcohol dependent individuals. Future research with functional imaging may provide an opportunity to understand in more details how the posterior cortical regions participate in motivated behavior and how these processes may be compromised in alcohol dependent individuals with apathy.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/ **Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Beijing Huilongguan Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

YT designed the project, and took responsibility for the integrity of the data and the accuracy of the data analysis. KY, QY, and YN were responsible for recruiting the patients, performing the clinical rating, and collecting the samples. KY wrote the paper. Critical revision of the manuscript for important intellectual content was drafted by YT and C-SL. C-SL, XL, ST, ZW, JT, SC, FF, FY, and TL were invited in evolving the ideas, statistical analysis, and editing the manuscript. All authors have contributed to and have approved the final manuscript.

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SUPPLEMENTARY MATERIAL

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Smartphone Use and Sleep Quality in Chinese College Students: A Preliminary Study

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Background: Chinese college students are at high risk of sleep problems, and smartphone use is common among this population. However, the relationship between smartphone use characteristics and sleep problems in Chinese college students has been inadequately studied. In this preliminary study, we examined the association of poor sleep quality with smartphone use in a sample of Chinese college students from a health vocational college in Changsha, China.

Methods: A total of 439 college students completed a self-report questionnaire containing the Pittsburgh Sleep Quality Index (PSQI) and questions regarding demographic information, psychosocial factors, physical health, smartphone use characteristics, and mobile phone addiction (MPA).

Results: The results showed that the prevalence of poor sleep quality (PSQI > 7) in Chinese college students was 9.8%. In multiple logistic regression analysis, poor sleep quality was significantly associated with male gender (OR: 2.80, P: 0.022), not having good physical health (OR: 2.61, P: 0.020), headache (OR: 2.47, P: 0.014), more severe depressive symptoms (OR: 2.17, P: 0.049), > four years of smartphone use (OR: 3.38, P: 0.001), > five hours of daily smartphone use (OR: 2.19, P: 0.049), and more severe inability to control MPA craving (OR: 2.04, P: 0.040).

Conclusion: Our findings suggest that excessive smartphone use and MPA are associated with poor sleep quality in a sample of Chinese college students from a health vocational college. Because of the limited sample representativeness and cross-sectional design of this study, large-scale prospective representative studies are warranted to confirm these associations.

Keywords: poor sleep quality, smartphone use, mobile phone addiction, college students, association

INTRODUCTION

College students are at considerably high risk for sleep problems. Worldwide, the prevalence of insomnia in university students has been found to be 18.5%, much higher than the 7.4% rate in the general population (1). A cross-national survey reported an overall 10.4% prevalence of severe or extreme nocturnal sleeping problems in undergraduate university students during the previous month, with the lowest seen in Thailand (3.0%) and the highest in Indonesia (32.9%) (2). Chinese university students are also at increased risk of sleep problems; for example, 25.7% of university students have sleep problems, a rate two to three times that of the general Chinese population (7.4-15.0%) (1, 3, 4). Evidence from empirical studies has shown that sleep disturbance is a major risk factor for a variety of physical and mental morbidities and increased mortality, including obesity, cardiovascular diseases, depression, and cognitive impairment (5–10). For students, sleep disorder is also significantly associated with poor academic performance and elevated risk of suicidal behaviors (11, 12).

A diversity of factors have been reported to be associated with sleep problems in university students, such as smoking, academic failure, depression, anxiety, and stress (13–16). In recent years, because of the rapid development of communications and information technologies, smartphone use has been very popular in young people, particularly university students. While smartphone use has made our lives more convenient, it also has negative effects on the health of users. For example, in the international literature, sleep problems in university students and adolescents have been significantly associated with mobile phone addiction (MPA) and various measures of problematic smartphone use, including long duration of smartphone usage, late-night smartphone use, bedtime smartphone use, and excessive use of a smartphone (17–23). In addition, there is evidence that smartphone overuse impairs cognition *via* its negative influences on mood and sleep (24).

In China, there have been a few studies examining the relationship between smartphone use and sleep problems in university students. Similar associations of sleep problems with MPA, long-term smartphone use, smartphone use before going to bed, and sleeping near the phone, have been reported (25–30). A prospective cohort study of Chinese college students has shown that long-time mobile phone use predicts sleep disturbances, and there is a bidirectional relationship between the duration of phone use and various sleep outcomes (25). Thus, results from both international and Chinese studies suggest that both some characteristics of smartphone use (i.e., duration of phone use, bedtime smartphone use, et al.) and MPA are involved in the etiology of the sleep problems of university students. However, it remains unknown which type of smartphone use variables (i.e., long duration of phone use versus MPA) is the strongest predictor of sleep problems, because these prior studies seldom examined the association of sleep problems with the two types of variables simultaneously. The relative contributions of the two groups of variables to sleep problems are potentially important, because these data may inform the level of intervention intensity required (i.e., health education vs. psychotherapy) if we want to address the sleep problems of university students *via* reducing problematic smartphone use.

Changsha is a major municipality in central-south China with a total population of 7,918,100, of which 672,000 are university students from its 51 universities. Due to societal concern about the misuse of smartphones by university students, our research team focuses on the prevention of MPA and its association with health problems in university campuses. To facilitate the design of a large-scale municipality-wide study on smartphone use and sleep health in university students of Changsha, China, a preliminary study was carried out to investigate the prevalence of poor sleep quality in a sample of Chinese college students, with a particular focus on its associated smartphone use factors. We hypothesized that long time phone use, high expense, and MPA would be negatively correlated with sleep quality in Chinese college students.

MATERIALS AND METHODS

Subjects

This cross-sectional study was conducted in May 2016. By using cluster sampling, all college students of the ten classes of the Department of Pharmacy, Changsha Health Vocational College, were invited to participate in this study. Students who were unwilling to join the study and had severe physical illnesses were excluded.

The study protocol was approved by the Institutional Review Board of Changsha Health Vocational College. Written consent was obtained from all participants, as well as their headteachers and parents, when necessary.

Procedures and Measures

We used a self-administered questionnaire to collect demographic information and data on psychosocial factors, physical health, smartphone use characteristics, and MPA. Before starting the study, the questionnaire was tested with a small sample of college students, and it was finalized after the pilot study.

Demographic data collected included gender, age, place of origin (rural vs. urban), and self-rated level of family income (good, fair, poor).

Psychosocial factors were depression and social support. In China, the University Personality Inventory (UPI) is the most widely used scale for assessing the mental health of college students (31). It consists of 60 items that assess a wide variety of psychopathological symptoms that an individual may have experienced in the previous year. Nevertheless, the administration time of UPI is a little long, and, therefore, it is not suitable for an epidemiological survey. Importantly, the factor structure of UPI in Chinese college students varies across studies (32–34), making it difficult to explain the test results of UPI. After expert consultation, we decided to use

12 items of the UPI to operationally construct a scale of depressive symptoms (SDS): poor appetite (item 1), feeling pessimistic (item 13), distracted (item 14), or restless (item 23), having suicidal thoughts (item 25), no interest in anything (item 26), a lack of judgment (item 29), good mood (item 35, reverse-scored), or lack of confidence (item 38), and feeling self-abased (item 44), physically exhausted (item 46), and hesitant (item 52). Because these items corresponded to the symptomatology of major depression according to DSM-IV criteria (35), SDS was defined in this manner. To avoid the overlap of the insomnia symptoms of depression and the outcome of our study, sleep quality, item 16 of the UPI (frequent insomnia) was not included in the SDS. All items were answered on a binary (yes/no, rated 1/0) scale. The total score of SDS ranged between 0 and 12, with a higher score denoting more severe depression. In our sample of college students, the internal consistency of SDS was satisfactory (Cronbach's α coefficient: 0.751).

We used two subscales of the Social Support Rating Scale (SSRS) to assess the level of social support: subjective social support and utilization of social support. Developed by Shuiyuan Xiao in 1986, the SSRS has good reliability and validity (36). A higher total score in each subscale represents a higher level of social support (37).

Two questions were used to evaluate physical health: "During the past year, did you often feel that you were in good physical health?" and "During the past year, did you often suffer from headaches?" Respondents who answered "yes" were categorized as having good physical health or headaches.

Smartphone use-related variables included length of smartphone use (years), smartphone charge per month (Yuan), duration of daily smartphone use and mobile internet use (hours), and MPA. MPA was assessed with the validated Chinese Mobile Phone Addiction Index (MPAI), which has 17 items and gauges four dimensions of MPA: inability to control cravings (ICC), feeling anxious and lost (FAL), withdrawal or escapism (WE), and productivity loss (PL) (38, 39). Each item of the MPAI is rated on a five-point Likert scale, from 1=never to 5=always. Higher scores indicate greater severity of MPA.

The primary outcome of the present study, sleep quality, was assessed with the validated Chinese Pittsburgh Sleep Quality Index (PSQI) (40, 41). The PSQI has 18 items,and its total score varies between zero and 21, with a higher score indicating poorer sleep quality. In China, a total score of eight or more is indicative of poor sleep quality (42).

Statistical Analysis

Respondents were categorized into subgroups according to demographic, psychosocial, physical health, and phone use characteristics. Prevalences of poor sleep quality in the whole sample and in different subgroups of college students were calculated. Chi-square (χ^2) test and Fisher's exact test were used to compare poor sleepers and normal sleepers between subgroups. Multicollinearity of independent variables was tested prior to the formal analysis. We found a very low degree of multicollinearity among independent variables because the Variance Inflation Factor values of all independent variables ranged from 1.02 to 1.16, substantially below the critical threshold of 10. Multiple logistic regression with a backward stepwise entry of all significant factors in univariate analysis was used to identify factors associated with poor sleep quality. Odds ratio (OR) and 95% confidence intervals (CIs) were generated for each variable. The statistical significance level was set at $p < 0.05$ (two-sided). SPSS software version 20.0 package was used for all analyses.

RESULTS

A total of 443 college students were invited to participate in the study, and all agreed. Four students were excluded due to an incomplete questionnaire, so the final sample consisted of 439 completers. The average age of the 439 college students was 18.8 years (standard deviation [SD]: 1.7, range: 15-24), and 86.8% were female. The mean length of smartphone use was 3.9 years (SD: 2.3). Detailed socio-demographic and smartphone use characteristics are displayed in **Table 1**.

The mean PSQI score was 4.5 (SD: 2.3). A total of 43 students (9.8%) had poor sleep quality (PSQI > 7). Results from Chi-square tests and Fisher's exact test (**Table 1**) show that poor sleepers were more likely to be male students (P=0.041), not to have good physical health (P=0.003), to suffer from headaches (P < 0.001), to have more severe depressive symptoms (as indicated by a score of two or more, P=0.006), to have been using a smartphone for more than four years (P=0.003), to pay a monthly phone charge of 50 Yuan or more (P=0.049), to use the phone for more than five hours per day (P=0.007), to use mobile internet for more than five hours per day (P < 0.001), and to have more severe MPA in terms of ICC (P=0.007), FAL (P=0.018), and PL (P=0.002).

Multivariable logistic regression analysis (**Table 2**) show that poor sleep quality in college students was significantly associated with male gender (OR: 2.80, P: 0.022), not having good physical health (OR: 2.61, P: 0.020), headaches (OR: 2.47, P: 0.014), more severe depressive symptoms (OR: 2.17, P: 0.049), > four years of smartphone use (OR: 3.38, P: 0.001), > five hours of daily smartphone use (OR: 2.19, P: 0.049), and more severe ICC (OR: 2.04, P: 0.040).

DISCUSSION

According to the statistics provided by the China Internet Network Information Center (CNNIC), by June 2019, the number of internet users in China was 854 million, and 847 million (99.1%) used mobile phones to access the internet. Of the total internet users, 41.5% were 10–29 years old, and 26% were students (the largest group) (43). Therefore, Chinese adolescents and young adults such as college students are the most vulnerable group to problematic smartphone use. To the best of our knowledge, our study is one of the very few to examine associations of poor sleep quality with smartphone use characteristics and MPA. We found that nearly one-tenth (9.8%)

TABLE 1 | Socio-demographic and smartphone use characteristics of college students and comparison between poor sleepers and normal sleepers by variable.

Variable		No.	Poor sleepers	Normal sleepers	χ^2	P
Gender	Male	58	10	48		
	Female	381	33	348	4.194	0.041
Age	≤19 years	274	22	252		
	≥20 years	165	21	144	5.573	0.109
Place of origin	Rural	246	27	219		
	Urban	193	16	177	0.883	0.347
Self-rated family economic status	Good	26	3	23		
	Fair	256	21	235		
	Poor	157	19	138	1.984#	0.364
Subjective social support*	≤9	227	25	202		
	>9	212	18	194	0.790	0.374
Utilization of social support*	≤8	288	32	256		
	>8	151	11	140	1.641	0.200
Depressive symptoms*	≤2	220	13	207		
	>2	219	30	189	7.537	0.006
Good physical health	Yes	381	31	350		
	No	58	12	46	8.978	0.003
Headaches	Yes	92	18	74		
	No	347	25	322	12.575	<0.001
Characteristics of phone use						
Years of smartphone use*	≤4	267	17	250		
	>4	172	26	146	9.063	0.003
Monthly smartphone charge (Yuan)*	≤50	236	17	219		
	>50	203	26	177	3.880	0.049
Hours of daily smartphone use*	≤5	249	16	233		
	>5	190	27	163	7.392	0.007
Hours of daily mobile internet use*	≤5	260	12	248		
	>5	179	31	148	19.362	< 0.001
Mobile phone addiction index						
Inability to control cravings*	≤14	239	15	224		
	>14	200	28	172	7.352	0.007
Feeling anxious and lost*	≤7	258	18	240		
	>7	181	25	156	5.625	0.018
Withdrawal or escapism*	≤7	259	25	234		
	>7	180	18	162	0.015	0.904
Productivity loss*	≤5	231	13	218		
	>5	208	30	178	9.583	0.002

**All continuous variables were dichotomized at the median value.*
#Fisher's exact test.

TABLE 2 | Multivariable logistic regression of factors associated with poor sleep quality.

Variable	Risk level	Reference level	P	OR(95%CI)
Gender	Male	Female	0.022	2.80(1.16,6.71)
Depressive symptoms*	>2	≤2	0.049	2.17(1.01,4.68)
Having good physical health	No	Yes	0.020	2.61(1.16,5.88)
Headaches	Yes	No	0.014	2.47(1.20,5.07)
Years of smartphone use*	>4	≤4	0.001	3.38(1.67,6.87)
Hours of daily smartphone use*	>5	≤5	0.049	2.19(1.04,4.63)
Inability to control cravings*	>14	≤14	0.040	2.04(1.01,4.14)

**All continuous variables were dichotomized at the median value.*

of the Chinese college students reported poor sleep quality and that poor sleep quality was significantly associated with male gender, not having good physical health, headaches, more severe depressive symptoms, >four years of smartphone use, >five hours of daily smartphone use, and more severe ICC of MPA.

The 9.8% prevalence of poor sleep quality revealed in our sample of college students is exactly the same as that of college students in Anhui, a central province in China (44) but lower than that of Chinese university students (25.7%) and the general population (15.0%) reported by two meta-analytic reviews (3, 4). The lower rate of poor sleep quality in this study might be related to the lower level of academic stress in college students of a health vocational college compared to college students of general universities. However, due to differences in sample characteristics (i.e., percentage of male

students) and assessments of sleep problem (i.e., PSQI vs. Athens Insomnia Scale) across existing studies, the relatively low prevalence of poor sleep quality does not necessarily mean a low risk of sleep problems in our sample of college students. Considering the many negative health effects associated with sleep problems and the very large number of Chinese college students, this 9.8% prevalence figure suggests that the sleep problems of college students still deserve preventive and clinical attention.

Overall, the significant associations between poor sleep quality and poor physical health (as indicated by not having good physical health and headache) and depression are consistent with prior studies (45–47). These could be explained by the complex reciprocal relationships between sleep disorders and pain, chronic conditions, and depression (46, 48–50). For example, poor physical and mental health would cause insomnia, and insomnia would exacerbate physical and mental health problems.

Numerous studies have confirmed the female predisposition to sleep problems in various populations, including college students, young adults, and adults (51–55), but we found a significantly elevated risk of poor sleep quality in male compared to female students. This unexpected phenomenon might be related to some unknown male-specific stressors, because, in most Chinese health vocational colleges, male students are the minority group, usually accounting for less than 5% of the total students. Nevertheless, since the sample size of male students is small (n=58), further investigation is warranted to validate this finding.

Our findings on the significant relationships between poor sleep quality and excessive smartphone use (as indicated by more years of smartphone use and more hours of daily smartphone use) and more MPA-associated ICC are generally in line with existing international and Chinese studies, despite the different measures of smartphone overuse and MPA used (17–23, 25–30). The difference between this study and other studies is that excessive smartphone use and MPA simultaneously contributed to the poor sleep quality of college students. Although the long-term effect of smartphone use on health remains unclear, the identified association between more years of smartphone use and poor sleep quality in our study might suggest a cumulative adverse effect of smartphone use on sleep.

Several mechanisms have been proposed to explain the negative effect of smartphone use on sleep. First, long-time phone use directly reduces the user's time spent on sleep, in particular bedtime smartphone use, resulting in insufficient sleep. Second, some phone users prefer to browse mobile websites before bed. Inappropriate content on these websites may make the users feel tension and excitement, leading to difficulties in initiating sleep. Third, excessive smartphone use may affect sleep *via* some physiological and psychological pathways (27, 56). For example, exposure to the light of smartphone screens and radiation from the phone during bedtime could influence the onset time and secretion of melatonin, which in turn causes sleep-wake rhythm disorders (25, 57). Fourth, morphological evidence from an fMRI study has confirmed alterations in gray matter volume and white matter integrity in college students with MPA (58); we speculate that MPA would affect sleep *via* several neurobiological pathways, which need to be further understood.

As a preliminary study, some limitations are unavoidable. First, this is a cross-sectional study, so the significant associations of poor sleep with poor physical health, depression, unhealthy smartphone use, and MPA found are not, strictly speaking, causal relationships. The influences of these factors on sleep need to be examined by prospective longitudinal studies. Second, our sample of college students was recruited from one health vocational college only, so the sample representativeness is very limited. Caution is needed when generalizing our findings to students of other colleges and universities. Third, although the internal consistency of our measure of depression was satisfactory and its items were believed to mirror depressive symptoms, empirical data on its validity were still unavailable in this study. Studies using common depression scales are warranted to examine the sleep–smartphone use associations. Fourth, our assessment of perceived physical health was based on only a single question, which may be unable to capture all dimensions of physical well-being. Fifth, there are no clinical diagnoses of mobile phone addiction, and the results from MPA assessment can only indicate the severity of phone addiction. Sixth, there are some other limitations, such as inadequate controlling of other variables associated with poor sleep, such as anxiety, and no assessment of the contents browsed on the phone before falling asleep, which could influence sleep quality.

CONCLUSIONS

In summary, in this sample of Chinese college students from a health vocational college in Changsha, China, one out of every ten have poor sleep quality, and poor sleep quality is significantly associated with poor physical health, depression, excessive smartphone use, and MPA. Given the common use of smartphones in Chinese colleges and the association of this use with sleep problems, administrators and health workers at Chinese colleges should pay more attention in terms of research and interventions to problematic smartphone use among students. Further investigation is justified into whether reducing smartphone use and MPA can improve the sleep quality of Chinese college students. Nevertheless, because of the limited sample representativeness and cross-sectional design of this study, large-scale prospective representative studies are warranted to confirm these associations.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of Changsha Health Vocational College. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to this study. QH and HC conceptualized and designed the research. XZ, JQ, and SL prepared the assessment tools. TS, ZL, and XC performed the experiments. YC and YL undertook the statistical analysis. QH and SH wrote the first draft of the manuscript and contributed to the final manuscript. All authors critically reviewed content and approved the final version for publication.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Relationship Between Buddhist Belief and Suicide Risk in Chinese Persons Undergoing Methadone Maintenance Therapy for Heroin Dependence

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Background: In western countries, there is a negative association between religious belief and suicide risk, while in China this association is positive. Nevertheless, few data are available on the association between one specific type of religion and suicide risk, which might be different from the overall positive religion-suicide association in China. This study examined the association between Buddhist belief and suicide risk in Chinese persons receiving methadone maintenance therapy (MMT) for heroin dependence.

Methods: In total, 61 Buddhist believers and 425 age, gender, and clinic frequency-matched non-religious believers were selected from a sample of patients with heroin dependence treated in three MMT clinics in Wuhan, China. The suicidality module of the Chinese version of the Mini-international Neuropsychiatric Interview 5.0 was used to assess current suicide risk. Patients' demographic and clinical characteristics were also collected. Multiple ordinary logistic regression was used to analyze the association between Buddhist belief and current suicide risk, controlling for the confounding effects of demographic and clinical factors.

Results: In Chinese patients receiving MMT for heroin dependence, Buddhist believers had significantly higher levels of current suicide risk than non-religious believers (low: 45.9% vs. 24.7%, medium: 4.9% vs. 3.5%, high: 19.7% vs. 12.5%, $P < 0.001$). After adjusting for demographic and clinical covariates (including depressive symptoms), Buddhist belief was still significantly associated with an increase in the level of current suicide risk (OR: 2.98, $P < 0.001$).

Conclusion: Buddhist belief is significantly associated with elevated current suicide risk in Chinese patients receiving MMT for heroin independence. In Chinese MMT clinics, patients with Buddhist belief may have a high current suicide risk and a timely psychiatric assessment and crisis intervention (when necessary) should be provided to these patients.

Keywords: Buddhist belief, suicide risk, heroin dependence, methadone maintenance treatment, association, China

INTRODUCTION

Religious beliefs can affect a person's mental health help-seeking behaviors and perceptions of mental well-being, meaning of life, pain, and psychiatric symptomatology (1–4). Despite some controversies over the positive effect of religion on mental health, in western countries, most studies have shown that religious involvement is associated with better mental health, including lower risk of depression, substance abuse, and death due to suicide (5). In general, religion can provide material, moral, emotional, and social supports to people who regularly participate in religious organizations and activities, which in turn buffer the negative effect of stressful life events, thereby maintaining/promoting mental health and reducing suicide risk (6). It is also speculated that religious beliefs could lower an individual's suicide risk by increasing the sense of coherence, because suicide can be viewed as a manifestation of the lack of social cohesion in Durkheim's theory of suicide (7, 8). Accordingly, researchers have developed some religious psychotherapies or integrated religious beliefs into psychotherapies to address patients' mental health and suicidal problems, and some empirical studies have provided promising results on the effectiveness of such treatments (9–12).

However, most evidence on the beneficial effects of religion is derived from studies conducted in Christian countries that are economically developed and generally believed to be religious (5, 13). As suggested by findings from two systematic reviews, although religion does protect against attempted and completed suicide, this effect varies depending on the cultural and religious context because both religion and suicidal behaviors are multidimensional and complex (14, 15). Due to cultural differences, it might be difficult to generalize western findings to people of less religious and non-Christian countries such as China. For example, in Chinese general hospitals, outpatients with religious beliefs are at significantly greater risk for depressive disorders than those without religious beliefs, and religious beliefs are significantly associated with higher levels of suicide intent among suicide attempters (16, 17), and, in the Chinese general population, religious beliefs are positively associated with an elevated risk of mental disorders and attempted and completed suicide (18–20). So it seems that religion is harmful for the mental health of people in China. Some Chinese researchers have ascribed this phenomenon to the weaker sense of cultural identity and belonging of religious believers, because they are a minority group in this atheist-dominated country, China, accounting for only 10% of the total population (21, 22). Nevertheless, we must be cautious to generalize the above findings in China, because an overall significant association does do not mean that all types of religion are associated with elevated risk of mental health problems and suicide and, in fact, there has been evidence showing that the risk of psychological distress and suicidal behaviors differs across believers of various religions (23). China has a variety of religions, including Buddhism, Taoism, Christianity, and Islam (22), it is therefore necessary to further examine the association between one specific religious belief and risk of mental health problems and suicidal behaviors, which may help deepen our understanding on the mechanisms underlying the religion-mental health association. Unfortunately, due to the small numbers of religious believers in previous studies, few of them did stratified analyses according to religions.

In China, heroin use remains a public health concern (24), and in 2016, there were 162,000 Chinese individuals receiving methadone maintenance therapy (MMT) for heroin dependence (25). These patients with heroin dependence, although they are undergoing MMT, are still at higher risk of completed suicide and other non-fatal suicidal behaviors (26–29). Some studies have reported a variety of factors associated with suicidal behaviors in this patient population, including female gender, a low level of education, inadequate social support, and depression (26–28). However, as far as we know, the association of religious affiliation with suicide risk has not been examined in Chinese patients undergoing MMT for heroin dependence.

The present study investigated the association between Buddhist belief and suicide risk among a sample of Chinese MMT patients. Buddhism was the religion of interest in this study, because it is the most common type of religion in China and suicide is generally contrary to Buddhism ethics (22, 30).

MATERIALS AND METHODS

Participants

This study was a secondary data analysis of a large-scale survey, which was conducted to investigate the mental health, self-destructive behaviors, sexual life satisfaction, and quality of life among Chinese patients of three municipality-owned MMT clinics in Wuhan, China, between June 2009 and July 2010 (31–33). By using consecutive sampling method, all patients who were 20 years old or older, met DSM-IV criteria for heroin dependence, and were undergoing MMT in the three clinics were invited to participate in the study. We excluded patients with severe physical illnesses and psychotic symptoms, as well as those with diagnoses of alcohol dependence and brain organic mental disorders.

In total, 743 patients were receiving MMT at the three selected clinics. Among them, 652 met the inclusion criteria after assessment for eligibility and all participated in the study. Because 16 patients withdrew the survey and 33 patients did not complete the survey, our study finally collected complete questionnaires from 603 MMT patients: 86 with and 517 without religious belief. Proportions of men (31.7% v. 28.6%, $\chi 2 = 0.202$, $P = 0.653$) and mean ages (38.1 ± 7.0 v. 36.5 ± 6.8, $t = 1.542$, $P = 0.124$) did not differ significantly between the 603 completers and 49 non-completers.

Among the 86 believers, 61 were Buddhist believers. The current study was designed as a comparative study, comparing the suicide risk between Buddhist and those without religious belief (non-religious believers). Therefore, a comparison group of 425 non-religious believers was purposively selected from the 517 non-religious believers, which was age, gender, and clinic frequency-matched to the group of 61 Buddhist believers.

The study was reported according to STROBE (the Strengthening the Reporting of Observational Studies in Epidemiology Statement) guideline (34). Because this was a secondary data analysis, the association between Buddhist belief and suicide risk was not of primary interest of the primary study, we did not pre-calculate sample size required for the current analysis. Nevertheless, results of the post-hoc analysis suggested that the sample size of our study was large enough for the current analysis, because we obtained a statistical power of 0.996 given that proportions of persons at risk for suicide were 0.705 in Buddhist believers and 0.407 in non-religious believers, sample sizes were 61 for Buddhist believers and 425 for non-religious believers, and a statistical significance level was targeted at 0.05.

Written informed consent was obtained from all participants. The institutional review board of Wuhan Mental Health Center approved the study protocol before the formal survey.

Instruments

The study used a standardized self-administered questionnaire to collect data. Trained investigators were arranged to read out the questions for patients who had difficulties in completing their questionnaires.

Demographic variables in the questionnaire included age, gender, education, marital status, and employment status.

Clinical factors included the main route of past heroin use (smoking vs. injecting), duration of past heroin use, duration of MMT, dosage of methadone (md/d), and pain. The pain intensity was assessed with the five-point Verbal Rating Scale ("Overall, how intense is your pain now?") (35). The five response options were "1=none", "2=mild", "3=moderate", "4=severe", and "5=very severe". In concordance with previous studies, patients who selected 3–5 were regarded as having clinically significant pain (31, 36). This single-item measure of pain has been widely in a variety of clinical and non-clinical settings with satisfactory validity for assessing pain intensity (35, 37).

Depressive symptoms were assessed with the Chinese version of Zung's Self-rating Depression Scale (SDS) (38). SDS has 20 items with each item being rated on a 4-point Likert scale from "1=a little of the time" to "4=most of the time". The total raw score of SDS ranges from 20 to 80, with higher scores corresponding to more severe depressive symptoms. The Chinese SDS has been proved to be valid and reliable in assessing the severity of depressive symptoms for Chinese population, and a total SDS score of 40 and above indicates clinically significant depressive symptoms (39).

In this study, two standardized questions were used to determine the presence of Buddhist belief. The first question, asked of all patients, was ''Do you have religious beliefs?''. Patients answering "yes" were further asked a second question: "what is your religion?'' Respondents who answered 'Buddhism'' were classified as having Buddhist belief.

The suicidality module of the validated Chinese version of the Mini-international Neuropsychiatric Interview 5.0 was used to assess current suicide risk (40). This module includes five specific questions regarding death wish (if present, the score is 1), self-harm (if present, the score is 2), suicidal ideation (if present, the score is 6), suicide plan (if present, the score is 10), and suicide attempt (if present, the score is 10) within the past month and one question regarding lifetime suicide attempt (if present, the score is 10). Based on the total score of this module, current suicide risk of a respondent is categorized into four mutually exclusive levels: no (0 score), low (1–5 score), medium (6–9 score), and high (≥10 score).

Statistical Analysis

SPSS software version 17.0 package (SPSS Inc., Chicago, IL, USA) was used for all analyses. Demographic and clinical variables, the presence of depression, and suicide risk between Buddhist and non-religious believers were described and compared by t-test or Chi-square test, as appropriate. The association between Buddhist belief and suicide risk was examined with multiple ordinary logistic regression that entered the level of current suicide risk as the outcome variable, Buddhist belief as the predictor, and demographic and clinical variables and depression all together as covariates. This analytic approach was used to adjust for the potential confounding effects of demographic and clinical variables and depression. Because these demographic and clinical variables and depression have been reported to be factors associated with or risk factors of suicidal behaviors among general population and patients with heroin dependence (29, 41–46), they were included as covariates in the above adjustment analysis. Odds ratios (ORs) and their 95% confidence intervals (95%CIs) were used to quantify associations between factors and the increase in suicide risk. The statistical significance level was set at $P < 0.05$ (two-sided).

RESULTS

As shown in **Table 1**, although Buddhist and non-religious believers were comparable with respect to most demographic and clinical variables, Buddhist believers were significantly more likely to have an educational level of junior high school and above, pain, and depressive symptoms ($P \leq 0.004$).

Levels of current suicide risk were significantly higher in Buddhist than non-religious believers (low: 45.9% vs. 24.7%, medium: 4.9% vs. 3.5%, high: 19.7% vs. 12.5%, $P < 0.001$) (**Table 1**). After adjusting for demographic and clinical covariates and depression, Buddhist belief was still significantly associated with an increase in the level of current suicide risk (OR: 2.98, 95%CI: 1.66, 5.34, $P < 0.001$) (**Table 2**).

DISCUSSION

To the best of our knowledge, this is the first empirical study in China examining the association between Buddhist belief and suicide risk in patients with heroin dependence. Results from between-group comparison showed a significantly higher level of current suicide risk in Buddhist than non-religious believers. The elevated suicide risk in Buddhist believers was further

TABLE 1 | Characteristics and current suicide risk of Buddhist and non-religious believers among Chinese persons receiving methadone maintenance therapy (MMT) for heroin dependence.

Characteristics	Buddhist believers (n=61)	Non-religious believers (n=425)	Statistics	P
Clinic*				
Diyi	38 (62.3%)	280 (65.9%)		
Hanyangweimin	14 (23.0%)	74 (17.4%)		
Gutian	9 (14.8%)	71 (16.7%)	$\chi2 = 1.132$	0.568
Gender: male*	39 (63.9%)	298 (70.1%)	$\chi2 = 0.959$	0.327
Age (years)#	40.1 (7.2)	39.1 (6.0)	t=1.118	0.264
Education: junior high school and above*	34 (55.7%)	156 (36.7%)	$\chi2 = 8.115$	0.004
Marital status: non-married*$	30 (49.2%)	211 (49.6%)	$\chi2 = 0.005$	0.946
Unemployment*	27 (46.6%)	189 (46.4%)	$\chi2 = 0.001$	0.987
Main route of heroin administration: smoking*	13 (21.3%)	68 (16.1%)	$\chi2 = 1.032$	0.310
Duration of heroin use (years)#	10.8 (3.7)	10.2 (4.2)	t = 1.073	0.284
Duration of MMT (months)#	24.7 (10.6)	25.4 (10.5)	t = 0.452	0.652
Dosage of methadone (mg/d)#	68.3 (27.6)	68.8 (27.6)	t = 0.149	0.882
Pain*	49 (80.3%)	216 (50.8%)	$\chi2 = 18.728$	<0.001
Depressive symptoms*	36 (62.1%)	145 (35.5%)	$\chi2 = 15.046$	<0.001
Current suicide risk*				
No	18 (29.5%)	252 (59.3%)		
Low	28 (45.9%)	105 (24.7%)		
Medium	3 (4.9%)	15 (3.5%)		
High	12 (19.7%)	53 (12.5%)	$\chi2 = 19.622$	<0.001

**Categorical variables are expressed as counts (percentages).*
#Continuous variables are expressed as means (standard deviation).
$"Non-married" includes never-married, separated, cohabitating, divorced, and widowed.
MMT, methadone maintenance therapy.

TABLE 2 | Multiple ordinal logistic regression on the association between Buddhist belief and current suicide risk in Chinese persons receiving methadone maintenance therapy for heroin dependence, controlling for demographic and clinical factors and depressive symptoms.

Characteristics	OR(95%CI)	P
Buddhist belief (vs. non-religious)	2.98 (1.66, 5.34)	<0.001
Clinic		
Hanyangweimin (vs. *Diyi*)	0.82 (0.55, 1.19)	0.485
Gutian (vs. *Diyi*)	1.28 (0.32, 2.23)	0.698
Gender: female (vs. male)	1.64 (1.04, 2.60)	0.034
Age (years)	1.02 (0.98, 1.06)	0.318
Education: primary school and illiterate (vs. junior high school and above)	3.71 (1.94, 7.07)	<0.001
Marital status: non-married (vs. married)*	1.34 (0.87, 2.08)	0.184
Unemployment (vs. employment)	2.28 (1.50, 3.45)	<0.001
Main route of heroin administration: injecting (vs. smoking)	4.47 (2.28, 8.79)	<0.001
Duration of heroin use (years)	1.07 (1.01, 1.12)	0.014
Duration of methadone maintenance therapy (months)	0.99 (0.97, 1.01)	0.415
Dosage of methadone (mg/d)	1.01 (0.99, 1.02)	0.111
Pain	1.89 (1.20, 2.96)	0.006
Depressive symptoms	3.59 (2.18, 5.86)	<0.001

**"Married" includes married and remarried; "non-married" includes never-married, separated, cohabitating, divorced, and widowed.*
CI, confidence interval; OR, odds ratio; MMT, methadone maintenance therapy.

strengthened by the significant association between Buddhist belief and an increase in the level of current suicide risk after adjusting for potential confounders, that is, the significantly higher current suicide risk in Buddhist than non-religious believers is independent of their demographic and clinical factors (including depressive symptoms). Notably, the strength of the association of suicide risk with Buddhism (OR: 2.98) is close to that with depression (OR: 3.59) and higher than that with pain (OR: 1.89) (**Table 2**). Because the latter two are well-known major risk factors for suicidal behaviors (41, 47–49), Buddhist belief appears to be the other major factor associated with elevated suicide risk in Chinese patients receiving MMT for heroin dependence.

The first code of ethics from the five precepts within Buddhism doctrine is the abstinence from killing living beings, including intentionally ending one's own life by one's own hand, which is strictly prohibited by Buddhism (30, 50). In the Buddhism teaching, suicidal behaviors are regarded as an "unskilled action", which would cause "*dukkha*" (unsatisfactoriness or suffering) to persons after the self-destructive behaviors (51). Although human life is also full of "*dukkha*" in Buddhism, suicide is useless in escaping from suffering and difficulties, because Buddhism views life and death are a great cycle, and death does not mean the end of life but a beginning of a new cycle. In this case, death by suicide would result in the premature cessation of a valuable human rebirth and trigger another cycle of life with more "*dukkha*" (51, 52). As confirmed by an qualitative study, Buddhism lowers an individual's risk of suicidal ideation *via* its influence on reasons to not attempting suicidal behaviors (53). Due to these reasons, Buddhist belief has been associated with lower risk of suicidal ideation in community-dwelling residents in Vietnam, and with lower risk of suicide attempt in university students of 12 countries (23, 54). However, our study found a positive association between Buddhist belief and elevated suicide risk, which is opposite to the Buddhist perspectives on suicide and findings from the above-mentioned studies.

In China, although people can enjoy the freedom of religious belief, believing in religion is not a common phenomenon in this world's most atheistic country. We therefore consider that the elevated suicide risk in Buddhist believers of MMT patients may result from their social identity pressures. Moreover, unlike religious believers in western countries, the religiosity of religious believers and followers in China is relatively low, including Buddhist believers (55). Many Buddhist believers seldom attend the religious activities unless they are mentally distressed and suffer from suicidal problems, so Buddhist belief may not work in preventing or reducing suicide due to the lack of continuous religious group support. In fact, a very common phenomenon in the Chinese general population is that people often turn to religion when they are emotionally distressed or have suicidal tendency and need to seek help from the religion (55, 56). Because of their own mental health and suicidal problems and their religious help-seeking behaviors, Buddhism would "passively" have a positive association with suicide risk. We believe that this explanation is more likely because the majority of patients with heroin dependence, including those receiving MMT, have a high unmet need for their physical and

psychosocial problems (31–33). In addition, the unexpectedly higher prevalence of Buddhist belief in our sample of patients with heroin dependence (10.1%, 61/603) than the Chinese general population (6.8%) (22) seems to support this speculation, because this is not in line with the Buddhist precept of abstaining from intoxicating drinks and drugs. The most possible answer is that some patients turn to Buddhism for seeking help and supports for their physical and psychosocial problems caused by heroin dependence, or even confessing for their past misconducts.

The major limitation of this study is that we did not collect data on reasons for believing in Buddhism and whether Buddhist belief preceded suicidal behaviors. Due to these, we cannot ascertain the causality between Buddhist belief and elevated suicide risk. The second limitation is no assessment of frequency of attending Buddhist activities, which would provide more useful information on the relationship between Buddhist belief and suicide risk. Third, some variables associated with increased suicide risk such as inadequate social support and negative life events were not collected in this study. Studies collecting these data are warranted to clarify the influence of these variables on the association between Buddhist belief and suicide risk in patients receiving MMT. Despite these limitations, the increased suicide risk in Buddhist believers of patients with heroin dependence is an interesting and clinically relevant phenomenon in Chinese MMT settings. Health care providers in Chinese MMT clinics may consider Buddhist belief as a marker or clue of a high current suicide risk of the patients and provide a timely psychiatric assessment and crisis intervention (if necessary) to those at risk of suicide.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Institutional Review Board of Wuhan Mental Health Center. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

J-XH and B-LZ were responsible for the design of the study and interpretation of data, and Y-MX for the manuscript draft, statistical analysis, the data collection, and critical revision of the manuscript. All authors reviewed the data and analysis, revised the manuscript, had full access to all of the data in the study, can take responsibility for the integrity of the data and the accuracy of the data analysis, and had authority over approval of final manuscript version and the decision to submit for publication.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Electrophysiological Evidence of Event-Related Potential Changes Induced by 12 h Abstinence in Young Smokers Based on the Flanker Study

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The cognitive control processes may be disrupted by abstinence in smokers, which may be helpful in the development and maintenance of addictive behavior. The purpose of this study was to measure the performance of cognitive task after 12 h of smoking abstinence by using event-related potentials (ERPs), including the error-related negativity (ERN) and the error positivity (Pe). In Eriksen flanker task, electroencephalography (EEG) signals of 24 smokers were recorded in two conditions: satiety and 12 h abstinence. In the behavioral data, both conditions exhibited more errors and more time on the incongruent trials than congruence. Meantime, the Minnesota Nicotine Withdrawal Scale (MNWS) score was increased during abstinence. Smokers showed reduced ERN and Pe after 12 h of abstinence, compared with satiety condition. The results indicate that the diminished error processing in young smokers after 12 h of abstinence. It may be related to increased withdrawal symptoms. In conclusion, the disrupted neurophysiological indexes in the general behavior monitoring system may be caused by abstinence. The results of this study may provide us with new ideas about the effects of short-term abstinence on brain cognitive neuroscience and be helpful for the solution of relapse.

Keywords: error processing, adolescent smokers, abstinence, event-related potential (ERP), performance monitoring

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INTRODUCTION

According to statistics from the Global Youth Tobacco Survey (GYTS) in 2014, the rate of attempted smoking among adolescents in China was 19.9% (30.1% for boys and 8.7% for girls, 82.3% of them tried smoking for the first time before the age of 13), the current smoking rate (the smoking behavior in the past month) is 6.9% (11.2% for boys and 2.2% for girls) (1). In addition, at least 20% of young smokers with high nicotine dependence will develop into ordinary smokers (2). Previous studies have shown that the brain structure and nervous system development of adolescent smokers may be affected by nicotine (3–7). But most attempts to quit ended in relapse, even though they realized the adverse consequences of smoking and expressed their strong desire to quit (8).

Abstinence from smoking disrupts cognitive performance that may help maintain smoking behavior (9, 10). Previous studies reported that a general performance monitoring system involving a range of cognitive functions after acute abstinence from smoking may be a neurophysiological indicator (11). Besides, our group research showed that the interaction of neurological mechanisms can be significantly changed, which affects the balance between cognitive control and reward (8, 12–15). Studies have shown that most smokers are prone to relapse within 24 h of withdrawal (16) and have a series of withdrawal symptoms (17).

The core function of cognitive control is to monitor and regulate our behavior. In this study, we investigated the error monitoring, as an aspect of cognitive control, which plays an important role in behavioral regulation and cognitive control (18–20). Using two event-related potential (ERP) components associated with error processing: the error-related negativity (ERN or Ne) and the error positivity (Pe), which appears after an error and seems to be sensitive (21). Researches particularly relevant to this study have been shown the reduction of ERN and Pe, indicating that initial error processing and motivational significance of smokers are affected after errors. Few ERP studies have investigated performance monitoring in substance dependence (22–25). Meantime, there are few studies to investigate the error monitoring of young smokers under the abstinence condition. Nor can we identify any studies investigating the link between error processing and smoking abstinence.

Previous neurophysiological studies conducted that lower ERN and Pe amplitudes during abstinence in adults (11), suggested that the neurophysiological indicators of performance monitoring are affected in the process of acute abstinence. Based on these results, we expect to observe the decrease of ERN or Pe during abstinence condition by using two ERP components related to error monitoring: ERN and Pe. In conclusion, the main purpose of this study is to test the prediction of whether the general performance monitoring system would be disrupted after overnight abstinence from smoking.

MATERIALS AND METHODS

Ethics Statement

The study was approved by the Medical Ethical Committee of the First Affiliated Hospital of Baotou Medical College, Inner Mongolia University of Science and Technology, and was in agreement with the Declaration of Helsinki. All procedures were carried out with the adequate understanding and written informed consent of the subjects and their legal guardian after understanding the purpose of the study.

Participants

Participants were 24 young male smokers recruited from Inner Mongolia University of Science and Technology by using Internet and print media advertisements in the present study. All participants are right-handed. Mean age of the smokers was 21.04 ± 1.27 (mean ± SD). The criteria for inclusion of all young smokers are as follows: 1) correspond with DSM-V criteria for current nicotine dependence; 2) consumed 10 or more cigarettes per day in the last 6 months; 3) ≥3 points on the Fagerstrom Test for Nicotine Dependence (FTND) (26); 4) no period of smoking abstinence longer than 3 months; 5) expired air carbon monoxide (CO)>6 ppm (ppm) (by Smokelyzer, Bedfont Scientific, Kent, UK); 6) there are currently no physical, neurological, or psychiatric or extracranial lesions assessed by alcohol or substance abuse. **Table 1** presents the demographic and smoking characteristics of the sample. Several standards and widely used self-measures were completed prior to or after the lab session. In this study, 10 items of smoking impulse questionnaire (QSU) were used to measure the craving level of subjects (27). Current nicotine withdrawal was assessed before and after the 15 min testing session using a revised Minnesota Nicotine Withdrawal Scale [MNWS; (28)]. Breath carbon monoxide (CO) was measured before the testing session.

Procedure

Participants completed the demographic questionnaire before the experiment began. We designed two identical EEG sessions in 1–3 weeks (1) smoking as usual (satiety) and (2) ≥ 12-h abstinence (abstinence). The time interval between withdrawal and EEG data collection is ~12 h. During satisfied condition, smokers can smoke their first cigarette of the day (of their preferred brand) within 30 min before the experiment. All participants were asked to ensure adequate sleep before the experiment. The specific CO measurement standard is to measure the CO level concentration of the participants within 10 min before the start of the experiment. In particular, under the state of abstinence, the participants were required to tell the time of the last cigarette. Abstinence was verified by a carbon monoxide level of less than 6 ppm (29). Expired air carbon monoxide (CO) readings were obtained using the Smokerlyzer system (Bedfont Scientific Ltd., Rochester, UK) in two sessions before the beginning of the experiment. Meanwhile, participants completed the MNWS and Brief-QSU scales. Participants were placed in a comfortable environment free from outside interference. Participants then sat in comfortable chairs and electrodes were attached. And participants are told that errors are inevitable, but they need to complete the task as quickly and correctly as possible and pay attention during the task. Then the participants began to complete the Flanker task. The study used

TABLE 1 | Demographic and smoking characteristics of young smokers.

Clinical details	Young smokers (n = 24)
Age	21.04 ± 1.27
Age range (years)	19-24
Education (years)	14.13 ± 0.61
Age of smoking initiation	15.33 ± 2.78
Smoking duration (years)	4.21 ± 2.21
Pack-years	2.95 ± 2.73
Cigarettes per day (CPD)	14.25 ± 4.59
FTND total score	4.75 ± 1.48

Values are expressed as means ± standard deviations.
FTND, Fagerström Test for Nicotine Dependence.
Pack-years: smoking years × daily consumption/20.

E-Prime 2.0 software to present stimuli and collect behavioral data. Participants were remunerated in the study.

Task Paradigm

Task paradigm the Eriksen flanker task (30) takes the most typical subtitle stimulus paradigm as an example. The experimenter provides participants with a series of five-letter strings. Four different strings (SSSSS SSHSS, HHHHH HHSHH) appear in the center of the computer screen. If the middle letter is H, the participant presses a button with the right index finger. If the middle letter is S, the participant presses a button with the left index finger. When the participant responds correctly, a "+" will be feedback in the center of the screen; when the participant responds incorrectly, a red rectangle will be received; when the participant does not respond, a "!" will be feedback in the center of the screen. The entire task consists of five groups, each group will present 80 strings, and the number of occurrences of the congruent group and the incongruent group is the same, 40 times (n = 40). Since the string in the center of the screen is presented for a very short time, participants are required to respond quickly to the stimulus (**Figure 1**).

Electroencephalography Recording and Data Analysis

All EEG data were collected in a quiet, dimly lit, and good sound-attenuated EEG laboratory. Recording EEG data with BrainAmp MR plus amplifier (Brain Products GmbH. Munich. Germany) from 64 scalp sites (positioned following the 10–20 International System) with Ag/AgCl electrodes mounted in a cap (EasyCap, GmbH). Stimuli were presented on a computer using E-Prime 2.0 software and about 45 cm away from the participants. Two active Ag/AgCl electrodes were placed in the outer canthus of right eye and above the left eye to record the vertical electrooculogram (EOG). All signals were digitalized with a sample rate of 1,000 Hz with a frequency band from 0.15 to 35 Hz [infinite impulse response (IIR) filter 24/dB/octave roll of], and electrode impedances were reduced to less than 10 kΩ. BrainVision Analyzer 2 (Brain Products GmbH. Munich. Germany) software is used to analyze all offline data. TP9 and TP10 were employed as re-referenced and the FCz electrode was a reference electrode. The EEG signals were segmented in 1,000 ms epochs (200 ms before and 800 ms after response) and artifact rejection was done for all epochs. The rejection criteria for all epochs were: 1) maximal allowed voltage skip (gradient) is 50 μV for each ample point; 2) minimum allowed activity 0.5 μV in a 100 ms interval and maximal allowed value difference 200 μV in a 200 ms interval. The independent component analysis (ICA) was applied to remove eye movements and eye blinks. The mean 200 ms pre-response period was served as baseline. After baseline correction, average ERP waves were calculated for artifact-free trials at each scalp site in the two response (correct and incorrect). The ERN and Pe amplitudes were defined as the mean value at the signal subject level (ERN, 50–150 ms; Pe, 200–400 ms) (22, 31, 32) after onset of the response. For both the ERN and Pe, we studied the midline electrodes Fz, Cz, and Pz.

Statistics

All data are analyzed and processed by SPSS 20.0 software (SPSS Statistics, IBM, Armonk, NY). Two-sample paired t-test was employed to compare demographic data [pack-years, FTND, cigarettes per day (CPD)], questionnaires score, behavior performance (the number of errors, reaction time), and ERP data (amplitude and latency of the ERN and Pe) differences between the satiety and abstinence condition. Eventually, calculate the Pearson correlation coefficients between them.

RESULTS

Questionnaires and Breath Carbon Monoxide-Concentration Analysis

During abstinence, carbon monoxide in the expired air was significantly lower (t = −9.326, p <.000). On the MNWS assessment of withdrawal symptoms, the score was increased during abstinence (t = 2.172, p =.040) (**Figure 2**).

Behavioral Data

Table 2 shows the number of errors and reaction time both two conditions made on the flanker task. The total number of errors was similar between the smoking satiety condition and the 12-h abstinence smoking condition (t = −.182, p =.858). However, there are more errors on the incongruent trials than on the congruent trials (t = −7.560, p <.000) in both conditions. Responses were markedly faster on congruent trials (t =

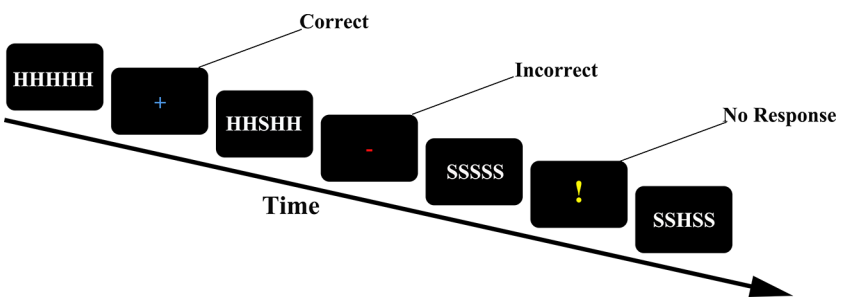

FIGURE 1 | Trial structure of the flanker task. Feedback was informative ("+"for correct; red for incorrect; "!" for missing or not responding in time).

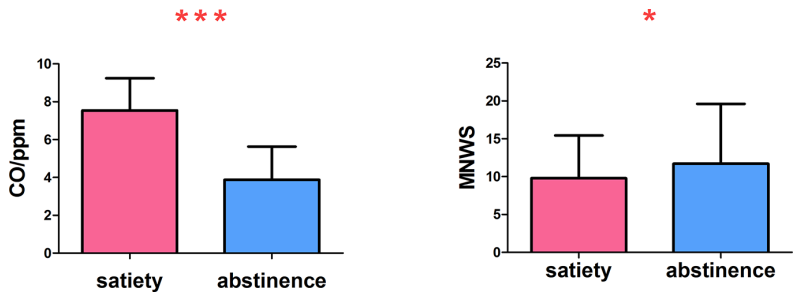

FIGURE 2 | The carbon monoxide in expired air was significantly reduced and the withdrawal symptoms increased *p < 0.05, ***p < 0.001. * and *** Represents that there were significant differences in CO level and withdrawal symptoms between satiety and abstinence in smokers.

TABLE 2 | Means and standard deviations for task performance variables.

	Satiety (n=24)	12-h abstinence (n=24)	p-Value
Number of errors of all response	40.17 ± 23.31	40.96 ± 27.78	0.858
Number of errors on congruent trails	12.25 ± 11.78	12.46 ± 11.72	0.851
Number of errors on incongruent trails	22.04 ± 18.35	20.58 ± 15.63	0.527
Correct	425.87 ± 46.42	426.67 ± 39.31	0.869
Incorrect	367.87 ± 60.55	371.34 ± 52.53	0.734
Congruent	416.13 ± 46.77	419.64 ± 40.03	0.486
Incongruent	434.66 ± 45.95	433.48 ± 39.28	0.809

Values are expressed as means ± standard deviations.

−9.842, p <.000). Moreover, it spent more time to respond on the correct trials as compared to incorrect trials (t = 10.993, p <.000) (**Figure 3**).

Electroencephalography Data

Error-Related Negativity

Figure 4 shows the ERN and Pe amplitude of both conditions on the incorrect trials at the midline electrodes Fz, Cz, and Pz. After 12 h of abstinence, the ERN was significantly decreased at Fz (t = −2.268, p =.033), but no differ at Cz (t = −1.668, p =.109) and Pz (t = −.976, p =.339) on the incorrect trials.

Error Positivity

Remarkably, the amplitude of Pe in 12-h smoking abstinence condition was reduced compared with smoking satiety conditions in young smokers (Fz: t = −3.084, p =.005, Cz: t = −2.974, p =.007, Pz: t = −2.562, p =.016).

Correlations Between Smoking Variables, Behavioral Data, and Event-Related Potentials

There was no significant correlation between any of the smoking clinical data, behavioral data, and ERPs.

DISCUSSION

The smoking rate of adolescents in China is increasing continuously, and the age of smoking tends to be younger, which may promote nicotine dependence in later life. In the present study, the error-related negativity (ERN) and error positivity (Pe) were used as indicators to test the effect of overnight smoking abstinence on performance monitoring. Compared with smoking as usual, Pe amplitude decreased significantly during abstinence condition, which was consistent with our prediction. More specifically, this study showed the ERN amplitude was significantly decreased after 12 h of abstinence. Our findings may reveal electrophysiological evidence for the reduction error processing in the flanker task during abstinence condition. In addition, the level of self-reported withdrawal symptoms was higher after 12 h abstinence, which was not associated with the reduction in the ERN and Pe brain potentials. On the behavioral level, more errors were shown in the two conditions of incongruent trials, and more reaction time was spent in the incorrect trials. Participants abstinence for at least 12 h and the carbon monoxide in expired air was significantly reduced.

The ERN appears immediately after committing errors that peaks at approximately 50–150 ms after error commission. Although researchers lack of consensus on the specific functional significance of ERN, some believe that ERN may reflect the early, automatic, and unconscious processing of errors (33). To test the effect of abstinence on one aspect of cognitive performance, ERN may be a useful indicator (34). In the present study, we found that the ERN amplitude was significantly decreased at Fz after making an error. Several studies have shown that there are neural systems in the medial frontal cortex (MFC) for monitoring errors, including the rostral cingulate motor area, the supplementary eye field, and the dorsal anterior cingulate cortex (dACC) (35). A study documenting both EEG and functional MRI (fMRI) more strongly shows that

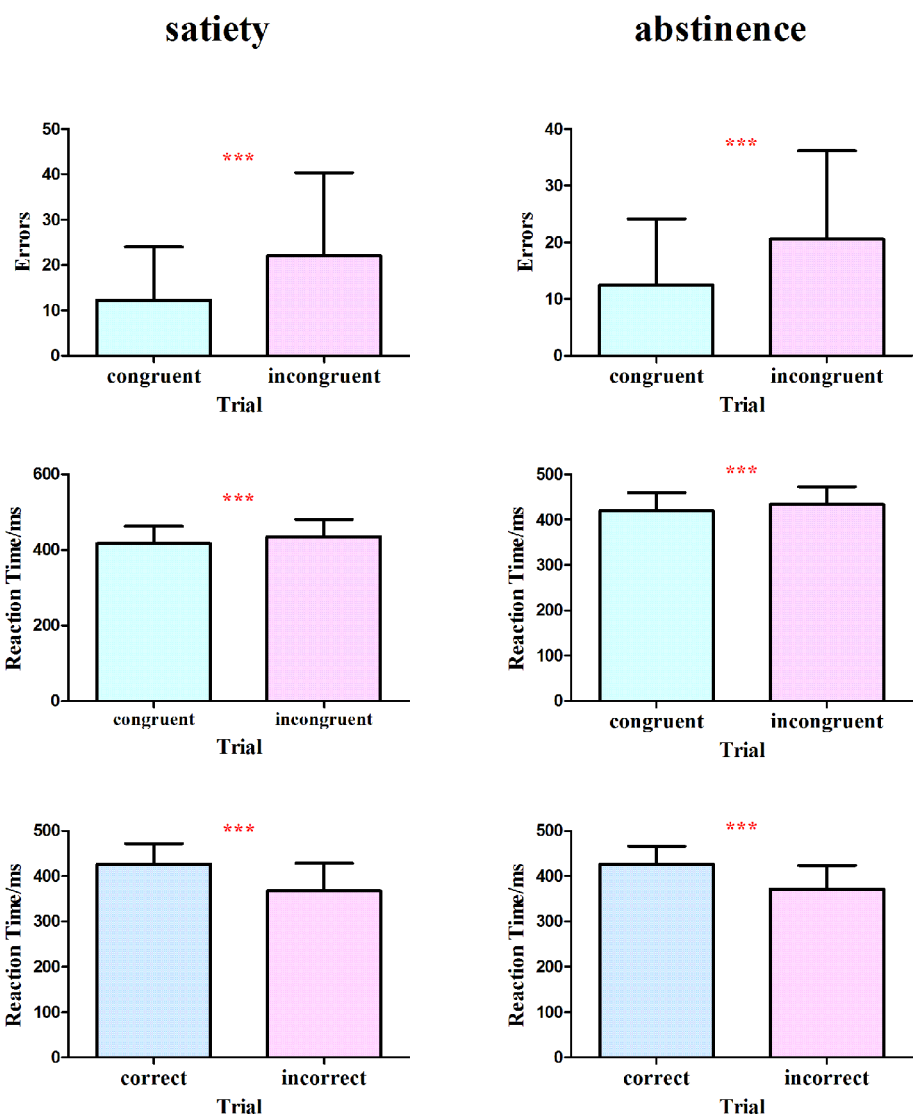

FIGURE 3 | No significant difference was found in the total number of errors between the smoking satiety condition and the 12-h abstinence smoking condition ($t = -.182$, $p = .858$). More errors were found on the incongruent trials than on the congruent ($t = -7.560$, $p < .000$) and responses were markedly faster on congruent trials ($t = -9.842$, $p < .000$) in both two conditions. Moreover, it took more time to respond on the correct trials as compared to incorrect trials ($t = 10.993$, $p < .000$). ***$p < 0.001$. *** Represents that there are significant differences in the number of errors and reaction time between different trial conditions.

there is a strong link between ERN and the activation of error-related ACC (36). Previous fMRI studies have shown that ERN and Pe are produced by the anterior cingulate cortex, and plays an important role in motivation and regulation problems. Therefore, the decreased of ERN at Fz electrode in the state of abstinence reflects the decrease in error processing. Consistent with this interpretation, Luijten *et al.* found young smokers with modestly deprived healthy had a reduced ERN compared to a non-smoker control group. Several other studies were also in accordance with our findings, such as cocaine (24) and juvenile violent offenders (37), suggesting that a deficit in performance monitoring. All of the above reveals that reduced ERN is associated with error processing. In a previous study on smoking, Franken et al. (22) showed that the amplitude of ERN did not decrease, indicating that the initial error processing seems not to be disrupted. However, Mayra L. Padilla et al. (38) showed that larger ERN amplitudes improved performance monitoring enabling abstinent alcoholics to overcome response conflicts. The differences found in these studies may be due to differences in dependent substances (nicotine *vs.* cocaine, resp.) or abstinence states (satiety *vs.* abstinent) (22).

Immediately after ERN, a positive ERP component (error positivity, Pe) appeared, which peaks at approximately 200–400 ms after the error (39). The Pe is considered to represent a conscious, more elaborate post-processing of an error (39).

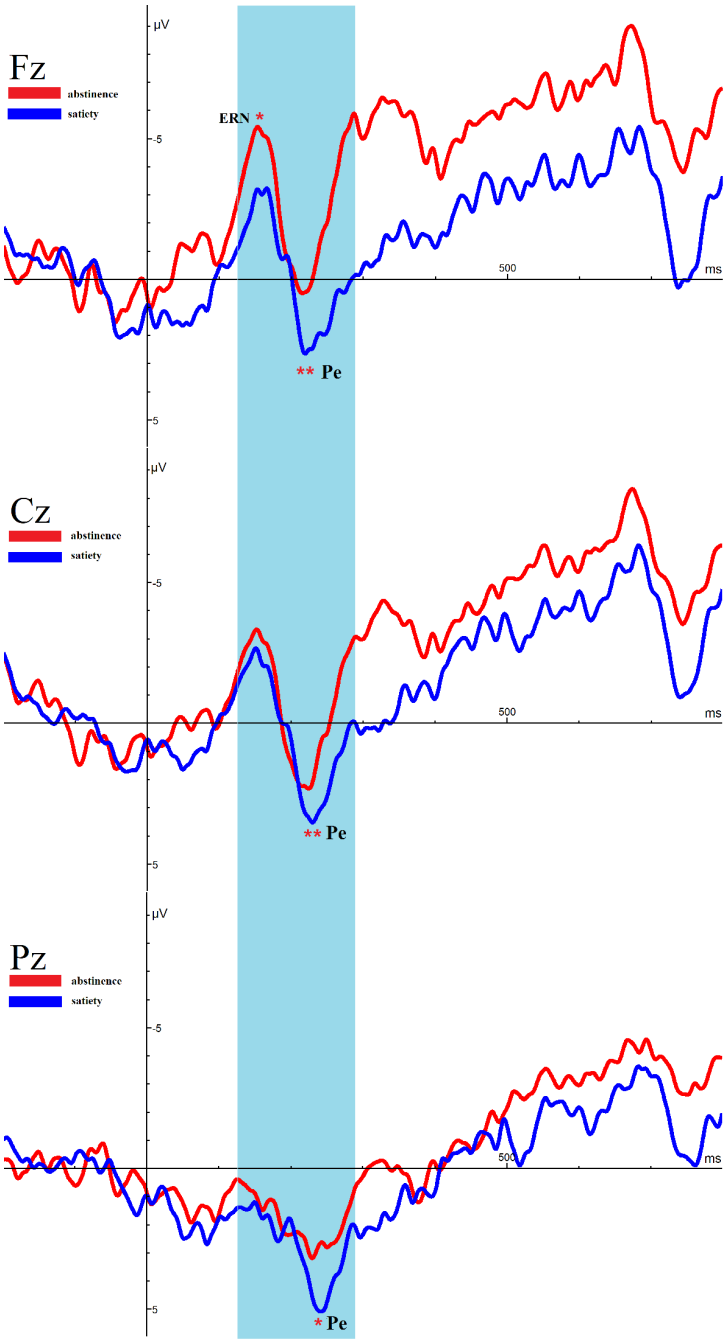

FIGURE 4 | Average event-related potential (ERP) activity during error trials of the Eriksen Flanker Task for satiety and abstinence. *p < 0.05, **p < 0.01. * and ** Represents an area where there are significant differences in ERP between satiety and abstinence in young smokers.

Although the role of cognition in nicotine dependence has aroused great interest (40), and in the cognitive neuroscience of the Pe (37, 41–43), it seems to be the first study to show that Pe is sensitive to acute abstinence from smoking. Previous ERP studies have shown that the Pe possibly reflects the error awareness (44), motivational significance (45), or emotional assessment of an error (46). Of particular relevance to the present study, some studies found smokers are affected by the initial motivational significance attributed to an error during smoking cue exposure (47). As expected, we found that the Pe amplitude of smokers decreased after 12 h abstinence. The current findings converge with a previous study that the

Franken et al. (22) found that a decrease in Pe amplitude suggests that smoking is associated with a reduction in error processing. Meantime, they found that the Pe amplitude was reduced in cocaine dependent subjects as compared to healthy controls, suggesting that cocaine addiction was related to error processing (23). In addition, Luijten M et al. (47) found the same results and suggests that this may contribute to the development and maintenance of addictive behaviors. Some other studies also have confirmed that the change of Pe reflects the abnormality of performance monitoring (32, 48). These findings again demonstrate a reduced error processing after 12 h of abstinence, and may contribute to the maintenance of addictive behavior. Unexpectedly, we did not find significant relationship between behavioral data and the Pe. This may be due to the short withdrawal time. Furthermore, we observed the withdrawal (MNWS) was increased during abstinence. Smokers commonly report that difficulty concentrating during smoking abstinence (28), and standard assessments of withdrawal include cognitive items (e.g., Minnesota Nicotine Withdrawal Scale). The current results seem to be associated with smoking abstinence. Previous studies found that abstinence led to reduced heart rate, feelings of depression, irritability, poor task performance, stress, poor concentration, restlessness, and smoking impulses (49). This may be provided the idea for explaining the reduction of performance monitoring under abstinence condition.

In sum, results of the present study showed the diminished error processing in young smokers after 12 h of abstinence at the physiological level. At the same time, reduced error processing might be the result of abstinence. It reveals that abstinence may disrupt the neurophysiological indicators of performance monitoring. These results supplement the extant literature. Importantly, this may be the first study to assess and demonstrate the effect of neurophysiological indicators of performance monitoring in young smokers after short-time abstinence. The results of this study are helpful to understand the cognitive and motivational factors of relapse behavior after abstinence from smoking.

Limitations

First, the current study is the relatively small size of the samples, and the participants were all men in this study. Second, at present, smokers are young smokers with a low degree of smoking dependence, and they are relatively in the early stage. The effect of abstinence may be more significant in more serious smokers. In the future, more subjects should be added in the research, while smokers with severe nicotine dependence are better.

DATA AVAILABILITY STATEMENT

The datasets generated for this study will not be made publicly available because there are multiple collaborating units, and research and experiments have not been completed, the data cannot be made public for other researchers to share. Requests to access the datasets should be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Medical Ethical Committee of the First Affiliated Hospital of Baotou Medical College, Inner Mongolia University of Science and Technology. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DY, KY, and YL conceived and designed the experiments. XL, DX, YCh, TX, ST, and MZ performed the experiments. YCu, FD, XL, and YCh analyzed the data. YCu and FD wrote the manuscript. TX, YR, DY, and KY provided the critical revision of the manuscript. All authors critically reviewed the content and approved the final version for publication.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Clarification of the Cut-off Score for Nine-Item Internet Gaming Disorder Scale–Short Form (IGDS9-SF) in a Chinese Context

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Background: The nine-item Internet Gaming Disorder Scale–Short-Form (IGDS9-SF) is a self-reported screening measure based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) criteria. It has been used to assesses symptoms and prevalence of Internet Gaming Disorder (IGD) in general population. Despite its widespread use, there is confusion arising from the recommended cutoff score for a positive diagnosis. This study aimed to identify the appropriate cutoff score for IGDS9-SF in a Chinese context.

Methods: The present study included a sample from clinical settings (n = 131) and another from universities (n = 3742). IGDS9-SF measurement and structured clinical interviews based on DSM-5 criteria for IGD were conducted in the sample from clinical settings. The cutoff score was determined using the receiver operating characteristics (ROC) curve. The validity of this cutoff score was further assessed in a sample from universities.

Results: Mathematical models suggest that the score of 32 is the optimal cutoff point (Youden's index, 96.2%; diagnostic accuracy, 96.1%; sensitivity, 98.0%; specificity, 91.9%; NPV, 91.9%; and NPY, 100%). The prevalence of IGD is 2.9% in this study.

Conclusion: This study suggested that the optimal cutoff score of IGDS9-SF is 32 for the positive diagnosis of IGD in a Chinese context.

Keywords: Internet gaming disorder, Internet Gaming Disorder Scale–Short-Form, cutoff score, clinical settings, universities

INTRODUCTION

Internet gaming disorder (IGD) has been described as a preoccupation with online/offline gaming characterized by impaired control over gaming behaviors that take precedence over other life interests and daily activities despite increasing negative consequences to the individual's psychosocial functioning for a period of at least 12 months (1). According to the Diagnostic and

Statistical Manual of Mental Disorders-5 (DSM-5), the clinical diagnosis of IGD requires the endorsement of at least five (or more) of the following nine criteria: 1) preoccupation with online/offline gaming; 2) experience of unpleasant symptoms when gaming is taken away; 3) the need to spend increasing amounts of time engaged in games; 4) unsuccessful attempts to control participation in games; 5) loss of interest in previous hobbies and entertainment as a result of, and with the exception of, games; 6) continued excessive use of games despite knowledge of psychosocial problems; 7) deceiving family members, therapists, or others regarding the amount of gaming; 8) use of games to escape or relieve negative moods; and 9) jeopardizing or losing a significant relationship, job, or education or career opportunity because of participation in games (1). In addition to its negative influences of their social functions, individuals with IGD also displayed the alternation of brain function (2, 3). Early diagnosis of IGD may increase the chances for successful treatment.

Diagnosing IGD in accordance with the DSM-5 requires a clinical interview, but this process is both expensive and time-consuming. As a result, many researchers have developed multiple psychometric questionnaires to screen IGD for clinical and nonclinical settings. The nine-item Internet Gaming Disorder Scale–Short-Form (IGDS9-SF) developed by Pontes and Griffiths (4) is a short psychometric tool based on the nine core criteria defining IGD as suggested by the DSM-5. The IGDS9-SF assesses symptoms and prevalence of IGD by examining both online and/or offline gaming activities occurring over a 12-month period. The scale has been widely used, particularly in the research context. The scale produces final scores between 9 and 45. However, questions have been raised about the appropriateness of cutoff scores, especially when it has been used in different language context.

Internet use and video-game playing are increasing available and accessible for both youth and adult populations in China. A minority of users may experience Internet or Internet gaming addiction. The rate of Internet addiction was higher among Chinese than their counterparts in other countries, like the United States (5). So far, there is, however, no research on the cutoff score of IGDS9-SF in a Chinese context, as well as the prevalence of IGD in adolescents and adults in China. This study aims to examine the optimal cutoff score for the IGDS9-SF in a Chinese context.

METHODS

Participants and Procedure

The study included samples from clinical settings (n = 131) and from universities (n = 3,742). Clinical samples of were collected from the outpatient clinics of three hospitals (the Department of Psychology of Tsinghua University Hospital, Beijing, China; the Department of Psychology of Yingtan people's Hospital, Jiangxi, China; and the Department of Psychology of Jiangxi Mental Hospital of Nanchang University, Jiangxi, China) between November 12, 2019, and January 7, 2020. The sample from outpatient clinics was composed of 131 participants who were 15- to 35-year-old (M = 18.70, SD = 3.29; male = 74.8%) with problematic gaming behaviors as their chief complaint. After the psychometric assessment of IGD, a blind structured interview was conducted individually by two certified psychiatrists (LQ and TL) to validate and determine the cutoff score of IGDS9-SF.

The sample size assessment for samples from clinical settings are mainly based on the primary outcome of a sample of 555 Brazilian gamers using IGDS9-SF (6). A subsample of 18.9% (n = 104) participants were selected to conduct a clinical evaluation by the certified psychiatrists. Among them, 31.7% was classified as positive diagnosis (4.8% participants diagnosed with IGD and 26.9% at risk) and 68.3% as negative diagnosis (not endorsing at least five out of the nine IGD criteria). Thus, this study aimed to recruit a sample no less than 104 participants. We finally recruited a sample of 131 participants from the outpatient clinics.

To confirm the validity of the IGDS9-SF cutoff score proposed in this study, data collection was also conducted in the classroom setting at three public universities in Beijing, China, during the same period. Students were selected based on availability (convenience sampling). The sample from universities was composed of 3724 participants who were 17- to 35-year-old (M = 20.31, SD = 2.87; male = 44%).

Ethics

Informed consent was obtained from all participants, while parents' permission was also obtained for those less than 18 years of age. The procedures were carried out in accordance with the Declaration of Helsinki. The ethical approval for this study was also obtained from the Medical Ethics Committee of Tsinghua University.

Measures

In addition to the collection of demographic information (i.e., gender, age) and relevant characteristics of IGD (i.e., weekly online gameplay time), the survey involved completion of the following scales.

Chinese Version of the IGDS9-SF

The Chinese version of the IGDS-9SF Test was self-administered by the participants to assess their IGD symptom severity (7, 8). The test has a 5-point Likert response scale (ranging from 1 = never to 5 = very often). The final score ranging from 9 to 45, with higher scores being indicative of a higher degree of disordered gaming. In this study, the IGDS9-SF presented with a high internal consistency (Cronbach α was 0.91).

DSM-5 Diagnostic Criteria of IGD

The diagnostic features of the IGD in DSM-5 comprise nine criteria reflecting its key aspects, including: preoccupation, withdrawal, tolerance, loss of control, give up other activities, continue despite problems, deception, escape, and negative consequences. A structured interview schedule was developed to examine the DSM-5 criteria of IGD based on two recent international recommendations (9, 10). The diagnosis for IGD in

the structured interview was based on the cutoff value of 5 suggested in the DSM-5.

Statistical Analysis

The diagnostic ability of the IGDS9-SF for IGD was evaluated through a ROC analysis using SPSS 25.0 for Windows. The area under the ROC curve (AUC) is a measure of the diagnostic efficacy of IGDS9-SF. The sensitivity, specificity, and Youden's index of IGDS9-SF were evaluated for the diagnostic positive and negative groups. In order to explore the probability that the IGDS9-SF would give the correct "diagnosis," we also calculated the positive predictive values (PPVs), the negative predictive values (NPVs), and the accuracy values for each possible IGDS9-SF cutoff scores. PPV was defined as the proportion of individuals with positive test results who are correctly diagnosed (11, 12). The NPV was defined as the proportion of individuals with negative test results who are correctly diagnosed (11, 12). The cutoff score of IGDS9-SF is optimal for diagnosis when the score is accompanied by the highest diagnostic accuracy and the optimal Youden's index.

To confirm the validity of the IGDS9-SF cutoff score proposed in this study, school sample were further classified into IGD and non-IGD groups according to the cutoff score of the IGDS9-SF. The demographic information and relevant characteristics of IGD were further compared between these two groups using a χ^2 test or t-test. A p value < 0.05 was considered statistically significant. The analyses were conducted using SPSS 25.0 for Windows.

RESULTS

Sample characteristics, including gender, levels of education, occupation, marital status, age, IGDS9-SF score, and weekly gaming (hours), were shown in **Table 1**.

TABLE 1 | Sample characteristics.

	Clinical sample (n = 131), n (%)	University students sample (n = 3,724), n (%)
Gender		
Male	98 (74.80)	1,638 (22.72)
Female	33 (25.20)	2,086 (51.63)
Education level		
Primary school and below	0	–
Middle school	109 (83.20)	–
High school and above	22 (16.80)	–
Occupational status		
Full time	22 (16.80)	–
Part time	10 (7.60)	–
Unemployed	99 (75.60)	–
Marital status		
Unmarried	127 (96.90)	3,605 (96.80)
Married	3 (2.30)	119 (3.20)
Divorced	1 (0.80)	0
	M (SD)	M (SD)
Age	18.70 (3.29)	20.31 (2.87)
IGDS9-SF score	29.51 (8.54)	14.67 (6.80)
Weekly gaming (hours)	26.00 (14.07)	8.14 (10.80)

Determination of Cutoff Score

To determine the cutoff score of IGDS9-SF, structured interviews based on the DSM-5 diagnostic criteria for IGD were used as the gold standard. Fifty-seven of the 131 participants met the DSM-5 criteria for IGD. **Figure 1** presents the ROC curve that from the samples. The AUC was 0.998 (P < 0.001), evidencing the high diagnostic efficiency of IGDS9-SF.

The sensitivity, specificity, diagnostic accuracy, positive predictive value (PPV), and negative predictive value (NPV) of the IGDS9-SF at possible cutoff scores were calculated, as they are shown in **Table 2**. The maximum value of the Youden Index of 96.2% is achieved by setting the cutoff score for a positive IGD diagnosis at a score of 32 with sensitivity of 98.0% and specificity of 91.9%. Which means, only 2% of the non-disordered cases would be considered as disordered in the clinical setting, while less than 10% of the truly disordered gamers are not identified by this measurement. Additionally, the PPV is 91.9% and NPV is 100% at this cutoff score of 32. In other words, only 8.1% of the individuals with a positive test result would be mistakenly identified, while all individuals with negative test results would be identified correctly. The diagnostic accuracy was 96.1%. Increasing or decreasing the cutoff scores would result in more false negative or false positive cases, and correspondingly decrease the diagnostic accuracy.

At the cutoff score of 36 recommended by Pontes et al. (4), the sensitivity is 68.0%, the specificity is 100%; or at the point of five or more of the nine IGD criteria on the basis of answering "Very often" (4), the sensitivity is 73.6%, the specificity is 100%. Comparatively, the sensitivity of the cutoff of 21 recommended

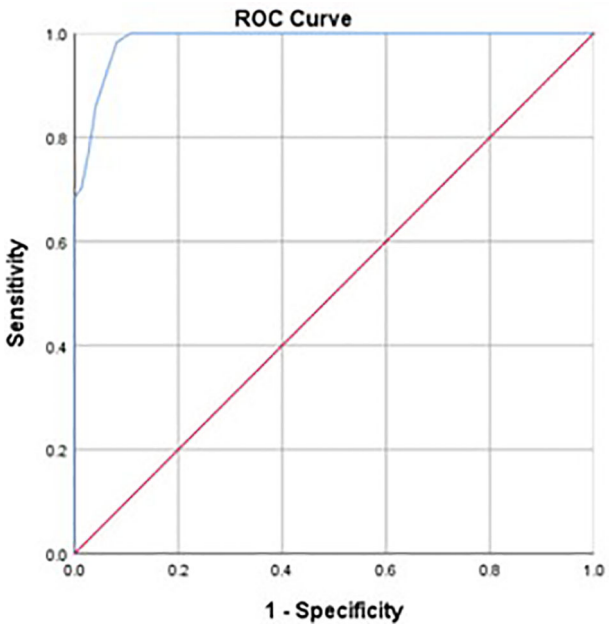

FIGURE 1 | ROC curve (blue line) obtained from clinical sample (AUC, 0.986). ROC, receiver operating characteristic; AUC, area under the ROC curve.

TABLE 2 | Cutoff points in IGDS9-SF between diagnostic positive and negative groups (N = 131).

Cutoff	True positive	True negative	False positive	False negative	Sensitivity (100%)	Specificity (100%)	PPV (100%)	NPV (100%)	Accuracy (100%)	Youden's Index (100%)
21	57	37	37	0	100	50.0	60.6	100	71.7	50
29	57	58	15	0	100	78.4	78.0	100	87.7	78.4
30	57	65	8	0	100	87.8	86.3	100	93.1	87.8
31	57	69	7	0	100	89.2	87.6	100	93.8	89.2
32	**57**	**74**	**5**	**0**	**98.0**	**91.9**	**91.9**	**100**	**96.1**	**90.1**
33	52	74	0	5	86.0	95.9	100	93.6	96.1	81.9
34	46	74	0	11	77.2	97.3	100	87.0	91.6	74.5
35	41	74	0	16	70.2	98.6	100	82.2	87.1	68.8
36	39	74	0	18	68.0	100	100	80.4	86.2	68.0
A*	42	74	0	15	73.6	100	100	83.1	88.5	73.6

IGDS9-SF, Nine-Item Internet Gaming Disorder Scale–Short-Form; sensitivity, the proportion of true positive rates; specificity, the proportion of the true negative rates; PPR, positive predictive rate; NPR, negative predictive rate; Youden's Index, defined as sensitivity + specificity − 1; AUC = 0.998.
A: indicated the cutoff point of five or more of the nine IGD criteria on the basis of answering "very often."*

by Monacis et al. (13) and Severo et al. (6) is 100%, while the specificity of 50.0%.

IGD Prevalence Estimate

Based on the cutoff score of 32 for classifying disordered gamers, IGD prevalence estimate in the present sample was 2.9% (95% CI = 2.4–3.4). The prevalence of IGD was 4.5% (95% CI = 3.5–5.5) in male students, which was higher than in female students (1.7%, 95% CI = 1.1–2.2, $\chi^2 = 25.16$, $p < 0.001$). **Table 3** presents the group comparisons of demographic data and relevant characteristics of IGD. Significant gender differences have been identified between the IGD and non-IGD groups. Compared with the non-IGD group, the IGD group exhibited significantly more weekly gaming time.

DISCUSSION

The study revealed a clinically optimal cutoff score of 32 for diagnosing IGD with the IGDS9-SF test in the Chinese context. This point represents the best balance between sensitivity (98.0%) and specificity (91.9%), and the highest diagnostic accuracy (96.1%). The cutoff point 32 in this study is lower than that in Pontes' study (with a sample of 1397 English-speaking gamers from 58 different countries) (4), which recommended a cutoff point of 36 or over, or five or more of the nine IGD criteria on the basis of answering "very often." In Pontes' study, the sensitivity of this two cutoff points is relatively low (68.0% and 73.6% respectively), although the specificity is 100%, which is the same as our study. The results from Pontes' study indicate that approximately one in three or four disordered cases would not be identified. This may be considered unacceptable in an epidemiological screening context.

TABLE 3 | Comparison between the IGD and non-IGD groups according to cutoff point of 32 in the IGDS9-SF (N = 3,724).

	IGD (*N*=108)		Non-IGD (*N*=3616)		χ^2
	N	%	*N*	%	
Gender					25.16**
Male	73	67.59	1565	43.28	
Female	35	32.41	2051	56.72	
	M	*SD*	*M*	*SD*	*t*
Age	20.44	3.34	20.31	2.85	0.49
Weekly game time	27.99	8.90	7.54	5.28	20.44**

IGD, Internet gaming disorder; IGDS9-SF, nine-item internet gaming disorder scale–short-form.
***p < 0.001.*

Monacis et al. (13) suggested that a score of 21 might be more appropriate for use in an Italian context. This point was confirmed by the study of Severo et al. (6) in a Brazilian context. The Italian sample recruited a sample of 687 participants, including 375 males and 312 females (mean age = 21.62 years, SD = 3.90), from schools, universities, and gaming halls. The receiver operating characteristics (ROC) analysis resulted in a cutoff point of 21 in determining IGD, and the AUC (area under the curve) of the Italian version of the IGDS9-SF was 0.935 (13). The Brazilian sample recruited a total of 555 participants, including 319 males and 236 females (mean age = 20.3 years, SD = 5.4), from a location where the individuals are highly exposed to computers and technology. ROC plot analysis also yielded a cutoff point of 21, with an AUC of 0.935 for IGD positive (6).

However, at the cutoff score of 21 suggested by Monacis et al. (13) and Severo et al. (6), the specificity is only 50%, meaning one half non-disordered cases would be considered disordered. The cutoff score of 21 from these two studies would be quite inappropriate if applied to the Chinese context, as it may lead to overestimation of the prevalence of IGD.

The prevalence estimate rate in this study is 2.9%, with relatively high consistency with earlier studies in Chinese context (14, 15). Our results also reveal a significant gender difference in IGD. Male students were more likely to meet the IGD criteria, which consistent with other culture studies (16–19). Furthermore, probable disordered gamers showed more weekly gaming time as expected. These results indicate that the cutoff score of 32 appropriately differentiated Chinese gamers.

These findings should be considered in the light of the limitations, that all participants were recruited on the basis of their availability (i.e., convenience sample). Consequently, the findings of prevalence rate need to be cautiously interpreted in terms of generalizability. Also, this study mainly recruited young adults (age ≤ 35 years old), and only a very small number of adolescents. Thus, we did not examine the differences of the prevalence of IGD between adolescents and young adults. Future studies should aim to reveal the prevalence of IGD in representative samples across different age groups. Furthermore, the sole use of self-report questionnaires in the sample from universities has some inherent disadvantages, such as memory bias.

CONCLUSIONS

In sum, the potential value of the IGDS9-SF as a screener for IGD is evidenced by the above results, including the high value registered for the area under the ROC curve and sensitivity and specificity figures. Based on our findings, we recommend the use of an IGDS9-SF score of 32 as the cutoff point for the positive diagnosis of IGD in a Chinese context.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding authors.

ETHICS STATEMENT

Informed consent was obtained from all participants, while parents' permission was also obtained for those less than 18 years of age. The procedures were carried out in accordance with the Declaration of Helsinki. The ethical approval for this study was also obtained from the Medical Ethics Committee of Tsinghua University.

AUTHOR CONTRIBUTIONS

TL and LQ conceived the study. TL, MH, QL, JT, and YL did the literature review, statistical analyses, and drafted the report. TL and LQ collected the data. All the authors interpreted the data and commented on the manuscript. All the authors contributed to and have approved the final manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Prevalence, Demographic, and Clinical Correlates of Comorbid Depressive Symptoms in Chinese Psychiatric Patients With Alcohol Dependence

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Background: Depressive symptoms are common among psychiatric patients with alcohol dependence (AD). However, the prevalence and clinical correlates of comorbid depressive symptoms are less well studied in Chinese Han patients.

Methods: In this hospital-based survey, we recruited 378 psychiatric patients diagnosed with AD according to the Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition (DSM-IV). All patients completed the Beck Depression Inventory (BDI) to evaluate depressive symptoms and the Alcohol Use Disorders Identification Test (AUDIT) to assess the severity of drinking.

Results: Compared to patients without depressive symptoms, 48.9% (185/378) of the patients with comorbid depressive symptoms were younger, had a more unstable marital status, had a higher AUDIT total score, and had a higher adverse consequences subscore (all $P < 0.05$). Further logistic regression analysis showed that unstable marital status (Odds ratios [OR] = 2.20, 95% confidence interval [CI] 1.21–3.99) and AUDIT total score (OR=1.07, 95% CI 1.03–1.11) were significantly associated with depressive symptoms.

Conclusions: Our findings indicate high comorbidity between AD and depressive symptoms in Chinese psychiatric patients. Moreover, some variables are correlates of comorbid depressive symptoms. Particular attention should be paid to the early detection and intervention for this comorbid condition and its risk factors.

Keywords: alcohol dependence, depressive symptoms, prevalence, Beck Depression Inventory, Alcohol Use Disorders Identification Test

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INTRODUCTION

Alcohol dependence (AD) and depression commonly co-occur (1, 2). The prevalence of their co-occurrence in individuals with AD fluctuates markedly, according to how depressive symptoms are measured or defined. Epidemiologic data showed that the comorbidity rate in the general population varied between 15% and 28% (1, 3–5), whereas in clinical samples, this rate was much higher, ranging from 43% to 48% (6, 7). Moreover, AD is significantly associated with depression. For example, Lai et al. conducted a meta-analysis of 22 community-based studies and found that the pooled odds ratio for AD and major depression was 3.09 (95% confidence interval [CI] 2.38–4.03) (8). In another hospital-based survey, alcohol abuse was strongly associated with depressive symptoms (OR 2.58, 95% CI 1.51–4.40) (9).

Comorbid depression in AD patients is associated with an increased risk of suicide attempts, relapse, treatment dropouts, and life dissatisfaction (10–12). For instance, earlier studies reported that compared with subjects with depression only, AD patients with depression had a suicide-specific standardized mortality ratio that was approximately two times higher, as well as more frequent episodes and more severe depression symptomatology (13–15). A more recent study showed that the co-occurrence of AD and depression negatively impacted quality of life and worsened physical functioning (16). Furthermore, AD aggravated the symptoms and prolonged the course of depression (17, 18). Two longitudinal studies revealed that problematic alcohol use increased the likelihood of persistent depression (19), and a reduction in drinking decreased the risk of depression (20). In turn, depressive symptoms heightened problem drinking and the risk of a relapse of AD (21). In summary, AD poses a potent risk for the development of depression and vice versa (22–24).

However, previous studies regarding comorbid depressive symptoms in AD patients were conducted in Western or African countries (6, 7, 25). To date, no published data on the prevalence and clinical correlates of comorbid depressive symptoms in AD patients in the Chinese Han population are available. Therefore, we conducted the present study to investigate the prevalence of and factors associated with depressive symptoms in AD patients in China.

MATERIALS AND METHODS

Subjects

This study was conducted in eight hospitals with the psychiatric department in China. These eight hospitals are as follows: the Second Xiangya Hospital of Central South University, Henan Mental Hospital, Peking University Sixth Hospital, Shanghai Mental Health Center, West China Hospital of Sichuan University, Nanjing Brain Hospital, Hubei General Hospital, and the First Affiliated Hospital of Xian Jiaotong University. They are located in northern, northwestern, southwestern, central-southern, and eastern China. Therefore, our sample can represent Chinese hospitals to some extent. Patients were recruited from consecutive admissions to the psychiatric department of these eight hospitals in China. The inclusion criteria for the study were that the patients aged≥18 years, and the patients had the diagnosis of AD which was made by trained psychiatrists according to the DSM-IV criterion for alcohol dependence. A total of 413 patients met the inclusion criteria. However, 35 patients were excluded because they refused to participate in the whole study and could not complete the whole interview. Finally, 378 patients were enrolled and interviewed. These patients completed our self-developed questionnaire for demographic characteristics and drinking patterns, the BDI for depressive symptoms and the AUDIT for the severity of drinking.

The Institutional Review Board at the Second Xiangya Hospital of Central South University approved this study and all patients gave informed consent for participating in the study.

Instruments

We used a self-developed questionnaire to assess 378 patients for demographic characteristics (gender, age, years of education, marital status, employment status, and monthly family income), and drinking patterns (age at first drink, daily consumption of pure alcohol, types of alcoholic beverages, and frequency of drinking).The Beck Depression Inventory (BDI) is a 21-item inventory that measures symptoms of depression. Based on the BDI total score, patients were classified into two groups: ≤13 = not depressed and ≥14 = depressed. Mild depression was defined as a total score of 14–19. Moderate depression was defined as a total score of 20–28, and severe depression was defined as a total score of 29 or above on the BDI (26). Suicidal ideation was measured using item I about suicide of the BDI (I don't have any thoughts of killing myself; I have thoughts of killing myself, but I would not carry them out; I would like to kill myself; I would kill myself if I had the chance). The Alcohol Use Disorders Identification Test (AUDIT) is a screening tool with 10 items about recent alcohol use, alcohol dependence symptoms, and alcohol-related problems. The maximum score of the AUDIT is 40 points. The AUDIT consists of two factors: a consumption factor (items 1–3) and an adverse consequence of drinking factor (items 4–10) (27, 28). The AUDIT has been standardized and validated for the Chinese population (29). The 10-item AUDIT-Chinese version was used in this study. Both the BDI and AUDIT were administered by trained and experienced psychiatrists. The diagnosis of AD was made for each participant by two trained psychiatrists according to the DSM-IV criterion for alcohol dependence, which requires three or more dependence items in twelve months (30).

Statistical Analysis

All data were analyzed by SPSS version 20.0 for Windows. Testing for normality was accomplished by Kolmogorov-Smirnov one-sample test. Continuous data were presented as the mean ± standard deviation. The daily consumption data of pure alcohol were described as the median and interquartile range because these data were non-normally distributed. Categorical data were shown as frequencies and percentages. Differences in continuous variables were compared using

Student's *t*-test, and categorical variables were compared using the chi-squared test. Logistic regression analysis with backward Wald method was used to compare depression and non-depression among the AD patients after controlling for the related variables (gender, age, marital status, years of education, employment status, monthly family income, age at first drink, and AUDIT total score). The significance level was set at $P < 0.05$.

RESULTS

The Prevalence of Comorbid Depressive Symptoms in AD Patients

A total of 185 (48.9%, 95% CI: 43.9–54.0%) of 378 AD patients met the criteria for depressive symptoms (BDI total score ≥14), and 193 (52.1%) did not. Eighty (43.2%) of the 185 patients met the criteria for mild depressive symptoms, and 105 (56.8%) of the 185 patients met the criteria for moderate or severe depressive symptoms. Importantly, 19.6% (74/378) of the sample reported the presence of suicidal ideation. A total of 37.3% (69/185) of patients with comorbid depressive symptoms had suicidal ideation; the rate was 2.6% in patients without depressive symptoms. The difference in suicidal ideation between these two groups was significant ($\chi^2 = 72.379$, $P < 0.001$) (**Table 1**).

Demographic Characteristics and Drinking Patterns of AD Patients

The whole sample consisted of 358 (94.7%) men and 20 (5.3%) women (male: female ratio=17.9:1.0). The mean age was 43.0 ± 10.0 years (men: 43.2 ± 9.7; women: 38.4 ± 13.4; $t = 2.140$, P=0.033), and the mean years of education was 11.0 ± 3.6 years (men: 11.0 ± 3.6; women: 11.9 ± 2.7; $t = 1.193$, $P = 0.234$). A total of 80.7% of the patients had a stable marital status, and 74.1% were employed.

Two hundred sixty-five (70.1%) of the patients drank alcoholic beverages at least once a day, 59 (15.6%) consumed 4–6/week, and 32 (8.5%) consumed 2–3/week. The median (interquartile range) daily consumption of pure alcohol was 100.8 g (59.4–168.0 g). A total of 378 patients consumed 61.4% of their alcohol in the form of strong distilled spirits (50% ethanol or above), 25.4% in the form of weaker distilled spirits (32%–45% ethanol), 9.8% in the form of beer, 2.1% in the form of rice wine, and 1.4% in the form of "other" beverages.

Mean scores on the AUDIT were as follows: consumption subscore, 11.1 ± 1.5; adverse consequences subscore, 17.0 ± 5.9; and total AUDIT score, 28.1 ± 6.5.

Factors Associated With Comorbid Depressive Symptoms in AD Patients

As presented in **Table 1**, compared with those patients without depressive symptoms, those with depressive symptoms were younger, had more unstable marital status, had higher scores for the adverse consequences factor and had higher AUDIT total score (all $P < 0.05$). After adjustments were made for related factors (gender, age, marital status, years of education, employment status, monthly family income, age at first drink, and AUDIT total score), further logistic regression analysis with backward Wald method indicated that unstable marital status (OR=2.20, 95% CI 1.21–3.99, Waldχ^2 = 6.670, P=0.010) and AUDIT total score (OR=1.07, 95% CI 1.03–1.11, Waldχ^2 = 12.207, $P < 0.001$) remained significantly associated with comorbid depressive symptoms (**Table 2**).

DISCUSSION

To the best of our knowledge, the present study is the first national hospital-based survey to investigate the prevalence and demographic and clinical correlates of comorbid depressive

TABLE 1 | Demographic and clinical features between the alcohol dependence (AD) patients with and without depression.

Characteristics	Depression (*n*= 185)	Non-depression (*n*= 193)	t/χ^2	*P*
Males/females	171/14	187/6	3.747	0.053
Age (years)	41.8 ± 9.9	44.1 ± 10.0	2.182	0.030
Education (years)	11.0 ± 3.5	11.0 ± 3.6	0.127	0.899
Marital status			10.233	0.001
Stable	137 (74.1%)	168 (87.0%)		
Unstable	48 (25.9%)	25 (13.0%)		
Employment status			0.508	0.476
Employed	134 (72.4%)	146 (75.6%)		
Unemployed	51 (27.6%)	47 (24.4%)		
Monthly family income(US$)			3.193	0.203
<$446	82 (44.6%)	102 (53.7%)		
$446~$744	77 (41.8%)	68 (35.8%)		
>$744	25 (13.6%)	20 (10.5%)		
Age at first drink (years)	18.6 ± 4.9	18.4 ± 4.7	0.395	0.693
BDI- suicidal ideation	69 (37.3%)	5 (2.6%)	72.379	<0.001
AUDIT score				
Consumption subscore	11.2 ± 1.4	11.0 ± 1.6	0.802	0.423
Adverse consequences subscore	18.1 ± 5.6	15.8 ± 6.0	3.713	<0.001
Total score	29.3 ± 6.2	26.9 ± 6.6	3.575	<0.001

TABLE 2 | Risk factors generated by logistic regression analysis with depression as dependent variable.

	B	*SE*	Waldχ^2	*P*	*OR*	95% CI
Marital status	0.786	0.304	6.670	0.010	2.20	1.21-3.99
AUDIT total score	0.068	0.019	12.207	<0.001	1.07	1.03-1.11
(Constant)	-0.601	1.165	0.266	0.606	–	–

symptoms in patients with AD in a Chinese Han population. We found that 48.9% of AD patients were depressed and that 56.8% of those depressed patients were moderate or severe depressed, suggesting a highly prevalent and serious depressive symptoms among AD patients. We also observed that the significant risk factors for depression in this population were unstable marital status and the AUDIT total score.

Our finding that the co-occurrence prevalence of depressive symptoms was 48.9% in AD patients closely corresponds with the findings of hospital-based studies conducted in other countries. For example, Ugochukwu et al. reported that the prevalence of major depression among 470 patients with alcohol use disorder was 45.8% in a tertiary hospital in Nigeria (6). Another study conducted by Odlaug and colleagues showed that the rate of depression among 2,979 AD patients was 43.1% in eight European countries (7). This consistency of results across multiple countries indicates a stable and high comorbidity of AD and depression. However, one recent survey employing the BDI revealed a much lower prevalence (24.7%) of depressive symptoms, with a cut-off of 21 points in Serbian patients with harmful alcohol use (31). This variation may be attributed to the inconsistent definitions of depression and alcohol use.

Importantly, many studies conducted in individuals from community populations also reported the comorbidity rate between AD and depression. For instance, according to the National Comorbidity Survey Replication, the prevalence of the co-occurrence of AD and major depression was 21.0% (1). In the Australian National Survey of Mental Health and Well Being, Burns and Teesson discovered that the prevalence of depression among residents with AD was 28% (5). One study conducted by Caetano in Puerto Rico reported that among those with alcohol use disorder, the rate of major depression was 23% (25). Another survey showed that the prevalence of depression in Nigerian college students with AD was 23.8% (4). In a prospective study, Briere et al. reported that the rate of cumulative alcohol use disorder and major depression comorbidity was 21% (12). The prevalence of depressive symptoms in AD patients was significantly higher than that in the general population, which might be explained by the greater severity of AD in hospital-based populations than in community-based populations. AD patients in psychiatric settings have more severe dependence symptoms, more life dissatisfaction, fewer family bonds, and worse social functioning and support than do community dwelling individuals (16). Therefore, the likelihood of AD patients developing depression is much higher than that of the general population. Further research conducted in both hospitals and communities at the same time is needed.

Recent evidence suggests that AD is associated with a significant risk of developing depression. According to Grant et al., the association between AD and depression was strong (OR=4.24, 95% CI 3.51–5.13) (3). Grant and colleagues updated this result in the National Epidemiologic Survey on Alcohol and Related Conditions, and found that the DSM-5 based alcohol use disorder was significantly correlated with major depression (OR=1.3, 95% CI 1.15–1.39) (32). Moreover, one study performed by Bazargan-Hejazi in a hospital in Los Angeles showed that alcohol abuse was associated with depressive symptoms, with an OR of 2.58(95% CI 1.51–4.40) (9). Furthermore, a meta-analysis of 22 studies conducted in non-clinical populations found that the pooled odds ratio for AD and major depression was 3.09 (95% CI 2.38–4.03) (8). Boden reviewed the literature and revealed that the presence of alcohol use disorder doubled the risk of major depression (33). Although the phenomenon of AD and depression comorbidity has been widely reported, the potential mechanisms underlying their association remain a controversial topic. Several possible explanations might account for the comorbidity between AD and depression. First, AD may precipitate the depressive symptoms caused by the pharmacological effects of alcohol. Moreover, AD patients may experience depressive symptoms as a result of a prolonged period of abstinence (31). Second, depression may occur as an emotional reaction to the adverse consequences of AD, such as unemployment (33–35). Third, individuals with depression drink alcohol for self-medication to alleviate depressive symptoms and eventually develop AD (36, 37). Finally, AD and depressive symptoms may occur simultaneously or develop independently due to shared biopsychosocial or genetic predisposition factors, such as poverty, genetic predisposition, or neuroimmune mechanisms (34). Many studies (38–42) found genetic overlap between AD and depression, suggesting that shared genetic liability possibly explains their comorbidity. For instance, Muench reported that the risk variant rs10514299 of major depressive disorder predicts the reward process of AD (39). Moreover, according to the survey from Zhou et al., SEMA3A variation was correlated with comorbid AD and major depression (41). Furthermore, studies have also found a neuroimmune interface in AD and depression comorbidity (43, 44). The mechanisms of comorbidity between AD and depression merit further investigation with a large sample size.

Previous studies (9, 25, 45–47) have revealed female gender, younger age, divorced or single status, unemployment, age at first drink, and severity of drinking problems to be independent predictors of comorbid depressive symptoms among patients with AD. In our study, gender, age, educational attainment, employment status, monthly family income, and age at first

drink were not significantly associated with comorbid depression in logistic regression analysis. The potential reasons might be due to the relatively small sample size or recall bias, which need to be further studied. Consistent with other research, we found that unstable marital status and AUDIT total score were risk factors for the comorbidity of AD and depressive symptoms. For instance, according to the survey from Caetano et al., the severity of alcohol use disorder was positively correlated with the likelihood of major depression, and the AUDIT total score indicates the severity of drinking problems (25). Regarding marital status, studies report that marriage disruption was a powerful risk factor for major depression (48). Moreover, Hasin et al. observed that being widowed, separated, and divorced increased the risk of depression (46). The present study indicates an urgent need to provide psychotherapy to AD patients who are unstable in marriage or drinking too much to prevent the development of depression.

The present study found that gender had no significant relationship with AD patients with and without depression due to the relatively small sample size of female patients. Earlier studies showed that male gender is a protective factor against depression (25). One survey in patients with AD indicated that female patients showed more depressive symptoms than male patients did (45). Moreover, Cornelius et al. reported that the proportion of women was greater among depressed patients with AD than among non-depressed patients with AD (49). Another study discovered that among individuals with AD, women reported more depression than men did (50). The meta-analysis conducted by Conner concluded that among individuals with alcohol use disorder, women experienced more severe depressive symptoms than men did (47). In the general population, the rate of depression was higher among women than that among men (46, 51). We will enlarge the sample size to better illustrate the gender difference between AD patients with and without depression in the future.

We found that 19.6% of AD patients had suicidal ideation, and the rate (37.3%) was much higher in patients with comorbid depressive symptoms. Our result was consistent with previous studies. For example, Ziolkowski et al. reported that 12% of 510 patients with AD had suicidal ideation, and comorbidity with depressive symptoms increased the likelihood of suicidal thoughts (52). According to the study from Cornelius et al., depressed alcoholics had a much higher risk of suicidality than non-depressed alcoholics (49). Furthermore, one survey revealed that more than half of the patients with alcohol use disorder had engaged in at least one suicide attempt (53). Hence, clinicians should pay particular attention to AD patients with depression and offer a variety of interventions to prevent potential suicidal behaviors.

We observed that AD patients were more common among males than females, and the male/female ratio of AD patients (17.9) was especially striking. Our findings corresponded to those of earlier studies (32, 54, 55). For example, Hasin et al. reported that AD was significantly more prevalent in male adults (55). Furthermore, men consumed more alcohol and were more likely than women to drink harmfully (56, 57). Interestingly, we also observed that female AD patients were younger than male AD patients. Many studies have confirmed that although women had later onset of drinking, they developed AD more rapidly than men (58–60). The possible explanations for such gender differences in AD patients are biological factors, such as different alcohol pharmacokinetics and their effects on the brain and different levels of sex hormones (57). Moreover, sociocultural factors might also play an important role, including different tolerances of social drinking, socioeconomic status and social networks (61, 62).

The strength of our study is that it is multi-centered and was conducted in eight hospitals in China. Therefore, it could represent hospitals at different levels from diverse parts of China. This study also has several limitations. First, although the DSM-IV criterion for alcohol dependence was used, the diagnosis of major depressive disorder was not established. Depressive symptoms were identified only based on the BDI. Second, this study was cross-sectional. Therefore, the potential causality and direction between AD and depression could not be ascertained. Longitudinal studies are needed to elucidate this relationship. Third, some of our data were abstained by self-report. Hence, recall bias was unavoidable. Fourth, information about clinical factors, such as psychiatric comorbidity, duration of treatment and history of hospitalization, were not collected. Further research on these clinical factors and their association with depressive symptoms will be needed.

In conclusion, comorbid depressive symptoms are quite common among AD patients in China. Moreover, depression is associated with unstable marital status and the AUDIT total score. Considering the potential adverse consequences, particularly the high rate of suicidal ideation in patients with depressive symptoms, more attention needs to be devoted to the early detection and intervention of comorbid depressive symptoms in patients with AD.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Institutional Review Board at the Second Xiangya Hospital of Central South University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

WH designed the study. HH, ZZ, HC, KN, RuZ, WS, BL, HJ, WW, JD, MZ, ZY, JL, RoZ, SL, SX, XW, WF, and CG collected the sample and performed the literature review. HH and ZZ conducted the analyses and wrote the initial version of this manuscript. All authors edited, read, and approved the last version of this manuscript.

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Childhood Trauma, Personality, and Substance Use Disorder: The Development of a Neuropsychoanalytic Addiction Model

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Background: While traditional psychoanalysis has been criticized as insufficient for the treatment of substance use disorder (SUD), recent progress in the field of neuropsychoanalysis has generated new and promising hypotheses regarding its etiology. However, empirical research applying this framework has been sparse.

Aim and Scope: The present overview aims at developing and empirically validating a neuroscientifically informed psychodynamic framework regarding the etiology of SUD. For this purpose, this review provides a concise overview of the most relevant historical and contemporary psychoanalytic theories on SUD etiology. Furthermore, the original research summarized in this paper consists of three studies investigating connections between childhood trauma, primary emotions, personality structure and attachment, as well as their relation to SUD development and treatment.

Conclusions: The results highlight the empirical validity of the neuropsychoanalytic approach towards SUD etiology. In particular, the findings underscore the conceptualization of SUD as a disorder related to dysfunctional attachment and affect regulation abilities especially linked to increased SADNESS and ANGER dispositions, which mediated the relationship between SUD and traumatic childhood relationships. Based on these findings, a refined model of SUD etiology is proposed, which should be tested in future studies.

Keywords: substance use disorder, psychoanalysis, neuropsychoanalysis, childhood trauma, affect regulation

INTRODUCTION

Substance use disorders (SUD), commonly referred to as addictions, are commonly defined as continuing, pathological, and compelling urges to consume one or more psychoactive substances, despite detrimental effects for oneself and others (1). Despite a "war on drugs", which has raged for almost 50 years with the aim of achieving a drug-free society, SUDs are still one of the most

common psychiatric disorders worldwide. As stated in the World Drug Report 2017, problematic substance use and SUD presently affects about 29.5 million people (2). The consequences of this disorder not only impact individual health, but also significantly strain the public health systems and inflict a toll upon society not often reported upon.

Considering the wide spectrum of psychoanalytic theories concerned with SUD etiology following Bilitza (1) and Rost (3), it is feasible to structure these approaches into the categories *drive theory*, *ego psychology,* and *object relations theory*. Furthermore, etiological models related to the work of Jacque Lacan, which have been traditionally neglected in reviews concerned with this topic, might be summarized under the umbrella term *structural psychoanalysis*. While all of these paradigms are associated content-wise, they often differ substantially in their emphases of specific etiological aspects.

The following will give a brief overview of the most important theoretical concepts of these schools of thought. Furthermore, this paper will review several contemporary SUD approaches of attachment theory and neuropsychoanalysis, which can be seen as further developments of classic psychoanalysis. Finally, we intend to give an overview of recent empirical research undertaken by our research group, which aimed to develop and validate an integrative neuropsychodynamic approach to SUD etiology.

Classical Drive Theory Approaches to SUD Etiology

Despite his publication on cocaine (4), Freud did not develop a comprehensive theory regarding the development of addiction disorders. This fact might not only be explained by Freud's serious nicotine addiction, leading to a lack of inner distance towards this topic, but also by his recommendation of cocaine as a cure for his morphine addicted friend Fleischl-Marxow, which significantly contributed to his friend's early death in 1891 (5). Therefore, Freud's notes on addiction are sparse and dispersed over several works. In his earliest remarks regarding addiction, Freud argues that chronic substance use might act as a substitute for sexual impulses and replace the "Ursucht" of infantile masturbation (6).

Later on, in his *Three essays on the theory of sexuality* Freud (7) highlights the importance of oral fixation regarding the etiology of addiction. Freud [(8), p.182] concludes: "It is not every child who sucks in this way. It may be assumed that those children do so in whom there is a constitutional intensification of the erotogenic significance of the labial region. If that significance persists, these same children when they are grown up will become epicures in kissing, will be inclined to perverse kissing, or, if males, will have a powerful motive for drinking and smoking." The idea that oral fixation and a consecutive disposition towards oral regression plays a significant role in the etiology of SUDs is reiterated by a great proportion of later psychoanalytic theorists, including Clark (9), Abraham (10), Rado (11), Knight (12), Fenichel (13), Rosenfeld (14), Limentani (15), Khantzian (16), and Wurmser (17). These authors agree on the predominance of a regressive oral defense mode in response to conflicts, which emerges due to an interplay of unresolved traumatic childhood experiences and constitutional factors. The ego of addicts is seen as insufficiently developed and therefore unable to withstand the anxiety. Hence, the relationship between the patient and the psychoactive substance is interpreted as a representation of the fulfillment of primitive and ambivalent oral demands and phantasies (18).

Finally, linked to his controversial introduction of the death drive—originally proposed by Sabina Spielrein (19)—Freud (20) shifts the emphasis regarding addiction development from direct masturbatory satisfaction of the pleasure principle to the avoidance of unpleasure. This establishes the function of drugs as artificial defense mechanisms, helping the individual to cope with external and internal stressors and establishes a psychological state comparable with mania, that temporally restores the phase of infantile narcissism. This approach is further elaborated by Simmel (21, 22), who conceptualizes addiction as a chemically evoked manic defense against melancholy, as alcohol attacks and ultimately disconnects the destructive super-ego of the addict.

The essay "The psychological relation between sexuality and alcoholism" (10), which was originally published in 1908, was the first psychoanalytic contribution exclusively discussing alcoholism. As claimed by Abraham, alcohol addiction is not only associated with oral fixation but also initially aims to increase sexual performance. Hence, he understands alcohol as a symbol of male fertility and the semen of the father. However, he observed a gradual decrease of virility in the later stages of addiction which is accompanied by increased castration anxieties, leading to a gradual replacement of the genital sexual aim by the alcoholic beverage. In this context, alcohol is conceptualized as a fetish-linking addiction to perversion (see also 23 for further discussion). Moreover, he proposes that chronic alcohol consumption dissolves mature defense mechanisms like inhibition, repression, and sublimation, which facilitates the release of unconscious homosexuality and sadistic partial drives, a trope which is repeatedly reiterated in the early psychoanalytic literature dealing with SUDs (18).

The contributions of the Hungarian analyst Rado (11, 23), might be the most influential drive-theoretical considerations concerned with addiction etiology. His first essay (11) focused predominantly on the significance of oral fixation, which he linked to the term "alimentary orgasm". This refers to orgasmic pleasure which is derived from the consumption and digestion of food. However, for the most part this concept gained little recognition from later theorists (24). Nevertheless, his second essay (23) is regarded as a seminal work for the later development of the ego-psychological conceptualization of this disorder (3). Rado's starting point is his observation of two kinds of effects linked to drug consumption: A sedative and analgesic effect, reducing reluctance and pain, and a euphoria inducing and stimulating effect, generating pleasure. The latter is seen as more problematic regarding addiction development, as it is prone to gradually compete with sexual desire, especially in the case of restricted opportunities for sexual satisfaction (11). What is more, he claims that his addicted patients are characterized by a specific predisposition towards an "initial depression" or "tense depression" in response to frustrations (23). He defines this as

the co-occurrence of both decreased tolerance of pain and extraordinary painful tensions. Therefore, drugs intervene in the insufficient affect regulation acting as an artificial stimuli barrier against both outwards and inwards frustrations. Referring to Freud's (25) theory of infantile narcissism he claims that "[i]n the pharmacogenic elation the ego regains its original narcissistic stature" [(23); p.57]. For the subject, this appears even more compelling as it can be achieved without effort and without the help of the other. However, as the effects of the drugs wear off the blissful state is replaced by an aggravated version of the initial depression, enhanced by a sharpened contrast between real-ego and ideal-ego, feelings of guilt and an exacerbated fear of reality. This intensifies cravings for the next drug hit, setting in motion a manic-depressive vacuous cycle and gradually shifting the ego organization from a realistic to a pharmacological regime. In line with Abraham (10), he observes decreasing genital drives as succumbing to pharmacological desires, which revive castration anxieties and hence are gradually displaced by fears of the psychoactive substance dissipating and increasing homosexual desires. Finally, a defusion of drives succeed in releasing aggressive impulses, which result in increased feelings of guilt and a secondary need for punishment (23).

Jouissance and Substance Use Disorder: The Lacanian Understanding of Addiction

While Lacan himself did not write much on addiction (26), in recent years scholars of Lacan contributed three major works on this subject [see: (27, 28)]. Following Freud's (4) writings on cocaine, Loose (26) highlights the significance of vulnerability within the subject in SUD development as opposed to the pharmacological effects of the drug itself. Despite not denying the addictive properties of specific psychoactive substances, he argues that the SUD patient receives a subject-unique effect from the substance, which people who are not addicted do not experience. In this context, the concept of *jouissance* is given a central place in the Lacanian etiology of SUD (26, 28).

Initially, Lacan used the term *jouissance*—usually translated as enjoyment of judicial rights, property, and sexual orgasm—to designate feelings of enjoyment tied to the satisfaction of biological needs like hunger and sexuality (29). Subsequently, referring to Kojeve (30), he developed a juxtaposition which contrasted jouissance and pleasure. In this context, Lacan argues that the pleasure principle acts as a restriction of the subjects enjoyment (31). Simultaneously, the subject attempts to transgress this limitation aiming to go beyond the pleasure principle. However, the transgression of the pleasure principle leads to pain and death (32). The prohibition of *jouissance* according to the pleasure principle is inherent to the symbolic order introduced by the "Law/Name-of-the-Father" (French translation: nom du père) at the end of the oedipal situation (31). This implies the forgoing of identification as the imaginary phallus for the mother due to symbolic castration. Loose (26) argues that many addicts suspend the structural obstruction of *jouissance via* the administration of psychoactive substances, aiming at a *jouissance* which is "non-phallic", non-linguistic, and independent of social attachments.

In correspondence to this, it is important to note that the Lacanian-framework proposes three different clinical structures which determine the function of the psychoactive substance for the addicted subject. Thereby, Lacan (33) differentiates between the (1) neurotic, (2) perverse, and (3) psychotic clinical structure, which is distinguished by their predominant mode of defense against the symbolic castration. In correspondence to this, (1) a neurotic structure is linked to repression; (2) a perverse structure is linked to disavowal; and (3) a psychotic structure is linked to dismissal of the symbolic order (31, 34). For neurotic and perverse subjects, these mechanisms result in a fundamental discontent with the pleasure principle. However, while what lies beyond the pleasure principle is "too much", the unavailability of that which is beyond generates a sense of "never enough" (28).

Based on this idea, SUD is understood as a technique to manage *jouissance*. For a structurally neurotic or perverse subject SUD is associated with the release of an surplus *jouissance*, which would otherwise be obstructed by the symbolic order or "symbolic castration" (26). Thus, drugs play the role of an "object-cause-of-*jouissance*", helping the subject to bypass the desire of the Other as a precondition for enjoyment, as required by the symbolic order. In contrast, for a psychotic subject the chronic consumption of psychoactive substances aims at limiting the surplus *jouissance*, which he/she experiences as invading, threatening, and often in the form of anxiety or pain (26). Therefore, within a psychotic structure drugs substitute the function usually executed by the symbolic order of language and the desire of the Other. Hence, the administration of psychoactive substances acts as a floodgate mechanism (28).

Ego Psychological Approaches Towards SUD

In contrast to drive theory, ego psychology prioritizes the ego and its functions regarding the etiology of psychopathological phenomena. The origins of this psychoanalytic school can be traced back to Anna Freud's (35) systematic categorization of defense mechanisms. This approach was then further elaborated upon in the writings of Heinz Hartmann (36), Margaret Mahler (37), Edith Jacobson (38), and Roy Schafer (39). In general, these authors agree that psychopathological symptoms are a result of defects within personality structure, which arise due to disruptions—like trauma, deprivation, or innate defects—during the development of the ego (40). In correspondence to this, personality structure is described as a set of ego-functions ensuring the maintenance of relationships to others and inner equilibrium (41). Thereby, personality structure and affects are compared to a container and its contents (42).

With regard to SUD, this "container" is too porous, causing the addicted patient to consume drugs to self-medicate unbearable affective states, iterating Freud's (20) and Rado's (23) writings on SUD etiology. Accordingly, Krystal and Raskin (43), Heigl-Evers (44), and Wurmser (17) underscore the significance of the addicts' inability to regulate overwhelming, unpleasurable, and often undifferentiated affects. This is often linked to a disposition towards depression and anxiety which is often somatized, unverbalized, and

experienced as physical pain. For example, Wurmser (17) emphasizes the role of narcissistic crises and intense feelings of depression, anxiety, rage, shame, and loneliness in SUD development, which he associates with traumatic childhood experiences and consequential conflicts between a malfunctioning ego and an archaic and sadistic super-ego. This results in an alliance between id and ego against the super-ego and the external world. This is in sharp contrast to neurotic disorders, which are understood as a pact between ego, super-ego, and the external word against the id. Furthermore, he proposed several defense mechanisms characteristic for SUD. These comprise splitting and denial of the internal and external reality, externalization of inner conflicts, and identification with the aggressor (17). However, drug abuse might also result from too-rigid affect defenses as well (43, 45). In this case, the addicted subject might take drugs in order to temporarily experience feelings of oneness with loved objects, otherwise unachievable in a sober state due to strict defenses against aggression towards the object.

What is more, Khantzian and Mack (46) emphasize the role of self-care in the etiology of treatment of SUD. This concept is related to reality testing and discernment and functionally acts as a protection against self-destructive tendencies and external dangers. Maltreatment experienced in childhood is able to disturb the development of this function, as feelings of anxiety and pain associated with early childhood trauma are often managed by the child neglecting its own feelings and self-worth. In adulthood, this internalized coping strategy might then predispose them to risky behavior like drug abuse (47, 48).

Object Relations Theory of SUD Etiology

Psychoanalytic object relations theory emphasizes the importance of early caregiver-child relationships and the early childhood environment, which contrasts with the often rather monadic approach of ego psychology. Moreover, while the classic oedipal paradigm of psychoanalysis focuses on the development from dyadic to triadic relationships, object relations theory is often more interested in the development from a primary symbiotic-like state between child and caregiver to more mature object representations (3). Major contributions to this school of thought were made by Melanie Klein and Roland Fairbairn. While Klein developed her theoretical framework around Freud's late drive/structure model, highlighting the role of aggression and the death drive, Fairbairn and his followers—like Guntrip and Winnicott—shifted the psychoanalytic paradigm to a relational/structure model, spotlighting the subjects' need for interpersonal relations rather than the desire for sexual gratification (49). In correspondence to this, Fairbairn's work is closely linked to the so-called Middle Group of British Analysts and strongly influenced John Bowlby's development of attachment theory (50).

Influenced by the Kleinian approach to object relations theory, early considerations regarding addiction were developed by Glover (51). He linked drugs to Klein's (52) concept of part-objects, defined as something which is perceived as both single body parts (breast, penis, feces, etc.) and their symbolic equivalents (e.g. persecutory, soothing, benevolent) (53), existing on the border between the internal and external world. What is more, he stresses the etiological significance of specific phantasies fueled by primitive sadism, hate and aggression, as well as associated paranoid states. The central phantasy system of addiction disorders is characterized by Glover [(51); p.38] as: "[…] a condensation of two primary systems, one in which the child attacks (later restores) organs in the mother´s body, and one in which the mother attacks (later restores) organs in the child´s body." In this context, the influence of aggression in addiction etiology is proposed as less severe than in psychotic disorders, but more intense than in neurotic disorders. Furthermore, Glover argues that the addicted patients' choice of drug is influenced by the intensity of destructive impulses, with more aggression linked to the selection of more hazardous substances. Therefore, in contrast to Abraham (10), Glover proposes that drugs of abuse symbolize not only (libidinously charged) semen but also (sadistically charged) feces. Consequently, the drug is perceived by the patient as an oscillating good and bad part-object. This is linked to an internalized and ambivalently loved mother (part-) object, which at its core is hated and felt as a hostile foreign body. Thus, the patient aims at destroying this bad and poisonous inner object by means of substance consumption, while the good representations of the self and others are projected onto the world, in an attempt to protect them against the individual's own sadistic impulses. Correspondingly, drug addiction is functionally understood as a simultaneous defense against psychotic fragmentation linked to regression towards the paranoid-schizoid position, as described by Klein (52), and a destructive self-medication against painful melancholic intrapsychic conflict associated with a progression towards the depressive position. Hence, Glover understands drug addictions essentially as a transitional state between psychosis and neurosis, and a compromise between the projected aggression linked to paranoid states and introjected aggression linked to depressive states.

The Kleinian approach is supplemented by Rosenfeld (14), who claims that addiction development is centered around a defensive manic formation involving idealizing, identification with ideal objects and denial of persecutory fears and depressive anxiety. Moreover, drug intake is interpreted as an identification with sadistic and destructive objects, which persecute the good representations of objects and the self in order to achieve omnipotent control of them. This enables the patient to act out sadistic impulses without concerns, feelings of guilt, or control by the super-ego. However, for Rosenfeld drug intake also serves a depressive and masochistic function, as the substance symbolizes a dead or damaged object which the patient feels obligated to incorporate and ultimately identify with, due to intense feelings of guilt.

In summary, the Kleinian model of SUD seems to be more pessimistic than the concepts of ego-psychology. In the view of Kleinian object relation theory, the inner landscape of the SUD patient is scattered and archaic. While ego psychology emphasizes the self-medication aspects of SUD, object relations theory highlights self-destructive impulses as the central driving

force in its etiology. In correspondence to this, both Glover (51) and Rosenfeld (14) describe a predominance of a rather primitive personality organization, fixated between the early paranoid-schizoid position and the later depressive position, and dominated by splitting and related mechanisms.

An attempt at approaching SUD etiology from an object relations theory perspective linked to the Middle Group of British Analysts was made by Krystal and Raskin (43) and Luerssen (54). These authors associated drug abuse with Winnicott's (55) concept of the transitional object. Winnicott described transitional objects as a materialistic object, bearing a significant role for infants—usually between 4 and 12 months of age—particularly while falling asleep. These objects (e.g. a blanket or towel) are important in the development of the infant's autonomy, as they substitute the calming qualities of the parents and, hence, are utilized in their absence. In turn, this permits a gradual transition between the early oral relationships with the caregiver and the later internalization of more mature object relations.

Accordingly, the drug abusing individual, who is unable to build stable inner object representations, employs the drug as a soothing and idealized object (43, 54). However, in contrast to the transitional object described by Winnicott, the psychoactive substance does not stay on the border between the external and internal world but oscillates. The addicted individual tries to incorporate the object in an attempt to overcome his severe fear of abundance. However, this introjection necessarily fails, due to the transformation of the drug into a persecutory object within the addict's body. In correspondence to this, Rost (3) argues that the relationship between SUD patient and drug mirrors the unintegrated ambivalence of the early infant-caregiver relationship, split between separation anxiety and need for autonomy, as well as love and hate.

Vital impulses in the advancement of object relation SUD theory can be drawn from the work of Otto Kernberg. Strongly influenced by Melanie Klein, Edith Jacobson, and Roland Fairbairn, he integrated findings of Kleinian object psychology, ego-psychology, and the object relations theory of the Middle Group into a cohesive theoretical framework (49). Furthermore, he argues for the necessity of (quantitative) empirical and neuroscientific research in order to further the advance of psychoanalysis as a science (56, 57).

Kernberg (58, 59) developed a model of personality organization which differentiates between three theoretically and empirically connected levels of functioning: (1) Coherence of identity, denoting the cohesion of differentiated and integrated representations of oneself and others; (2) Maturity of defense mechanisms, meaning the ability to cope with internal and external conflicts in a functional way; (3) Ability to test reality, describing the competence to differentiate between internal and external stimuli. A constellation of a predominance of primitive defenses like splitting and related mechanisms, severe identity diffusion a relatively functional capacity to test reality, is summarized in the concept of "borderline organization", which is closely related to Melanie Klein's concept of the paranoid-schizoid position (56), and theoretically related but not identical with the diagnosis of borderline personality disorder (BPD) (60). In contrast, a decreased facility for reality testing, due to fused representations of the self and objects, is linked to a psychotic personality organization, while individuals with a neurotic personality organization show only marginal deficits in all three areas of functioning (61).

Regarding SUD and impulse neurosis (kleptomania, psychogenic obesity, etc.), Kernberg (60) observed chronic repetitions of instinct driven behaviors, which are experienced as ego dystonic in most life situations, but very gratifying and ego syntonic during episodes of acting out. In line with the SUD theory of Kleinian object psychology (14, 51), SUD etiology was linked to the splitting defense mechanism, which for Kernberg (58, 60), is the essential defense mechanism of the borderline personality organization. According to Kernberg (60), splitting refers to the active separation of internalizations with opposite—meaning libidinal or aggressive—affective quality. This process obstructs the integration or fusion of introjections and identifications with contrary properties and serves as the main source of neutralization of aggression. Furthermore, he argues in line with Jacobson (38, 62), that the process of neutralization, which is linked to the prefrontal and anterior cingulate cortex (56, 63), provides crucial energy for ego development. Hence, an impaired ego is predisposed to use splitting, as this mechanism requires less energy than more mature forms of defense like repression. In turn, this triggers in a vicious circle of reciprocal reinforcement between a fragile ego and excessive use of splitting. This intrapsychic process is linked to substantial deficits in affect integration and regulation and, therefore, manifests in symptoms associated with low impulse control, like addictive behaviors or self-harming behavior, which at the time of their expression are experienced as ego syntonic. Like repression, splitting is not an isolated mechanism but occurs simultaneously with other so called primitive defense mechanisms (60). These are comprised of: Primitive idealization, projective identification, denial and omnipotence and devaluation.

Furthermore, the persistence of splitting obstructs the development of a coherent identity marked by an integrated ("good" and "bad") sense of self and others. This fosters the syndrome of identity diffusion, which is characterized by dissociated representations of the self and others into multiple segments of idealized and persecutory representations (64).

In correspondence to this, SUD can be seen as a coping strategy against painful and chaotic affective states, resulting from a borderline personality organization. However, this strategy creates a vicious circle, as the use of psychoactive substances further fosters the regression of ego functions and the fragmentation of its underlying object relations.

Recent quantitative-empirical studies, which investigated the relationship between SUD and personality organization, support the assumed association with a borderline personality organization (65, 66). Hiebler-Ragger et al. (65), applied the Borderline Personality Inventory [BPI; (67)] and compared healthy controls with inpatients diagnosed with SUD. These findings also suggest that SUD patients showed medium to large

differences in almost every personality structure dimension measured with the BPI, including increased "identity diffusion", "primitive defenses", "fear of fusion", and overall "structural deficits". In contrast, the dimension "reality testing", indicating a psychotic perception of reality, revealed only small differences between healthy and inpatient participants. Similarly, the study by Unterrainer et al. (66), which applied the Inventory of Personality Organization [IPO-16; (57)] to asses deficits in personality organization, revealed increased primitive defense mechanisms in SUD inpatients compared to healthy controls.

Summary of Psychoanalytic SUD Concepts

The psychoanalytic literature on the subject of SUD encompasses over 100 years and several paradigmatic shifts regarding attempts at understanding this disorder. Early analytics tried to frame SUD in the context of drive, oral regression, and perversion, while later theoreticians increasingly emphasized the importance of underlying object relations and ego structural deficits. However, most attempts at creating an etiological model of SUD are based on retrospective observations gained in qualitative case studies, which are prone to overgeneralizations of biographical findings and lack sufficient quantitative-empirical research validating the developed theories (68). Moreover, several large-scale quantitative longitudinal studies were unable to confirm central assumptions of the drive theory of SUD, like increased orality in childhood and homosexual tendencies predicting alcoholism in adulthood (69–71). In addition, the standard psychoanalytic treatment of SUD proved to be rather unsuccessful (72), which brought about the necessity of adapted psychotherapeutic strategies.

Furthermore, for most of the 20th century psychoanalysis ignored significant advances in neurobiology, leading to an increasing distance from natural science and academic psychology (73). However, this crisis of psychoanalytic theory fostered the development of the field of neuropsychoanalysis, which aimed at linking psychoanalysis with neuroscience and biological psychiatry.

Affective Neuroscience and Neuropsychoanalytic Approaches Towards Substance Use Disorder

At the beginning of his career, Freud (74) was already trying to develop a psychology grounded in neuroscience which, however, was aborted due to a lack of sufficient research methods. Nevertheless, the project to embed psychoanalysis within a neuroscientific framework had a resurgence in the late 20th century. This paradigmatic shift aimed on the one hand to counteract the increasing scientific deadlock and isolation of psychoanalytic theory and, on the other hand, to overcome the reductionist approach of biological psychiatry and neuroscience (75).

This approach is significantly influenced by the findings of Affective Neuroscience (AN), decisively developed by Jaak Panksepp (76), who emphasized an evolutionary perspective and affective cross-species similarities. His fusion of psychoanalysis and AN, labeled as neuropsychoanalysis, proposes a monistic relationship between mind and brain, which is expressed in the term *BrainMind* (77). The BrainMind is conceptualized as an interdependent and multi-layered dynamic structure comprised of primary, secondary and tertiary processes (see **Figure 1**) (77, 79).

As outlined in **Figure 1**, primary processes are comprised of largely subcortically (upper brainstem to the septal area) rooted networks including: (a) Homeostatic internal bodily drives like hunger or thirst; (b) Sensory affects like pleasurable taste or disgust; (c) Primary-process emotions, currently identified as networks for SEEKING, ANGER, LUST, FEAR, PANIC/GRIEF, CARE, and PLAY. These primary processes serve as the primary motivational system of behavior.

Secondary processes correspond with operations, linked to cognition and exteroceptive representations, which are largely based in the basal ganglia and upper limbic structures and mediate memory and the ability to learn behavior *via* classical, instrumental, and operant conditioning. Secondary processes include largely unconscious behavioral traits, like personality structure, attachment patterns, and object relations.

Tertiary processes are linked to more abstract cognitive operations and reflective awareness. These processes are predominantly neocortically and language based and summarize a broad spectrum of complex memory, cognitive, and executive functions like mentalization [meaning the ability to understand behaviors and mental states of oneself and others (80)], identity narratives, mindfulness, and spirituality. In each case, the different layers are reciprocally linked and therefore influence each other *via* bottom-up processes and top-down regulatory control.

At present, AN and neuropsychoanalytic researchers categorize seven primary emotion networks, which ascend from the PeriAqueductal Gray (PAG) into the limbic forebrain (77).

Four of those systems have evolutionary reptilian roots (81). These comprise SEEKING, which mediates appetitive foraging; FEAR, mediating freeze and flight behavior; LUST, mediating sexual and consummatory pleasure; and ANGER, mediating aggressive attack behaviors.

Furthermore, three basic emotion circuits specifically manifest in evolutionarily higher species like certain birds and mammalians. These systems consist of PANIC/GRIEF or SADNESS, which mediates separation distress; CARE, mediating nurturing behavior; and PLAY, mediating rough and tumble playing behavior (76, 77, 81).

The Role of Primary Emotions in SUD Development

All seven primary emotion networks are assumed to have relationships with a variety of psychiatric disorders (82). Regarding the etiology of SUD, the theory of neuropsychoanalysis focuses on the significance of SEEKING and PANIC/GRIEF (82–85).

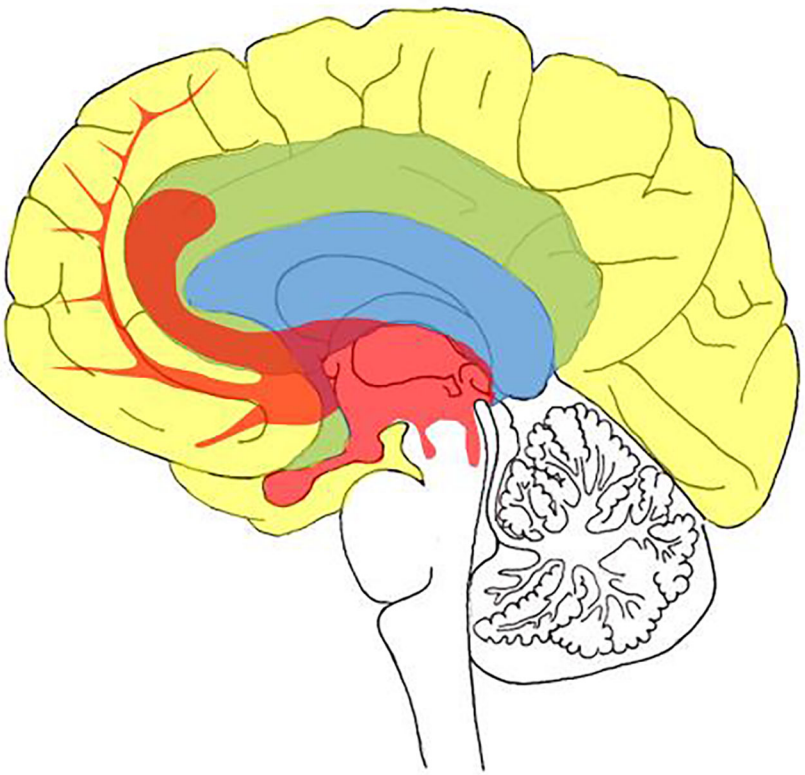

FIGURE 1 | Schematic visualization of the BrainMind as proposed by Panksepp (78): Primary process areas (red); secondary process areas (blue); tertiary process areas (yellow) processes.

SEEKING and SUD

Neuroanatomically, the SEEKING system largely corresponds to the medial forebrain bundle (MFB) (see **Figure 2**), and includes a complex network between the dorsal PAG, Ventral Tegmental Area (VTA), lateral hypothalamus, NAcc (which projects towards the amygdala), frontal cortex areas, and the Anterior Cingulate Cortex (ACC) (78, 81). Neurochemically, the SEEKING system is modulated largely by dopamine projected by the VTA, but also by descending Gamma-AminoButyric Acid (GABA) and glutamate systems and other ascending catecholamine systems (like norepinephrine and serotonin) as well as neuropeptides projected from source neurons within the lateral hypothalamus (86, 87).

Largely in consensus with Berridge (88, 89), AN theorists suggest that SUD is linked to pathological shifts within the SEEKING/wanting system. During the development of a SUD, SEEKING is increasingly and finally primarily activated in association with substance related appetitive memories, substance consumption, and the desire to alleviate negative affective states (83, 85, 90). Moreover, it is emphasized that several lines of evidence support the hypothesis that individuals predisposed to SUD might be vulnerable to the development of this disorder due to specific psychological and neural predispositions like depressiveness or hyperactivity of the brain stress system. Consequently, this might foster the restructuring of SEEKING towards substances of abuse or addictive behaviors like gambling (83). In correspondence to this, animals predisposed to SUD showed traits associated with the concept of "novelty seekers" or "sensation seekers" (91–93), like increased locomotion and explorative behavior in novel environments as well as a higher preference for novel environments (83, 94). Furthermore, animal models indicate that vulnerability to SUD is linked to increased approach behavior towards cues previously associated with rewards, in contrast to approach behavior towards the reward itself (95, 96). This enhanced conditioning power of environmental stimuli is described by the "incentive salience hypothesis" by Berridge and Robinson (97). In this model, it is proposed that dopamine activity predicts the reward gained from a specific object, which is called "incentive". In turn, this motivates the organism to approach this object, which is summarized in the term "salience". Regarding SUD, the drug induced increased dopamine release might lead to an overly strong incentive of the drug and associated environmental cues, which promotes their excessive salience (88, 97, 98).

This is underlined by findings indicating low basal dopamine levels and dopamine D2 receptor availability in humans predisposed to SUD and with a history of SUD (99–101). In line with this, AN-researchers argue that a predisposition towards SUD is linked to a general hypoactivity of the SEEKING/MesoLimbic-

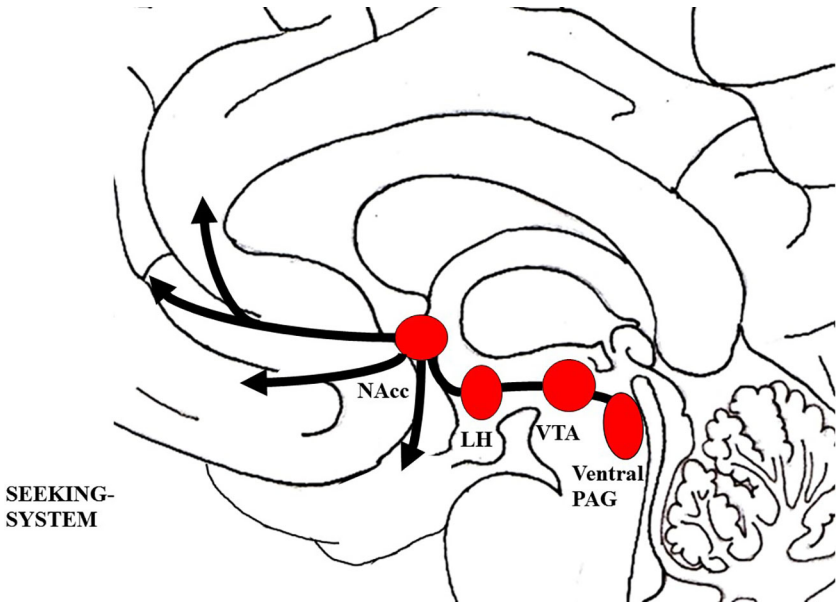

FIGURE 2 | Schematic visualization of the SEEKING system; The figure was drawn by J. Fuchshuber, based on theoretical concepts by Panksepp (78). LH, Lateral hypothalamus; NAcc, Nucleus accumbens; PAG, Periaqueductal gray; VTA, Ventral tegmental area.

DopAminergic (ML-DA) network (83–85). In turn, this is linked to a diminished capacity to seek rewards in the external world. This fosters a gradual development in which the individual learns that only excessive surges of dopamine D2 driven excitation, as triggered by addictive drugs, allows for the achievement of pleasurable objects in the external world (84). Therefore, it is proposed that the object of SUD is not the addictive substance, but rather the *possibility* of actual biological, social, or sexual rewards, facilitated by the effect of the substance (83–85). In correspondence to this, the SEEKING network would play a relevant etiological role especially regarding SUDs that involve stimulating drugs, like cocaine and amphetamines (84, 85). This is further emphasized by recent results indicating that striatal dopamine receptor availability is diminished in individuals suffering from stimulant and alcohol addiction, but not in patients suffering from opiate or cannabis addiction (102).

Another line of evidence which associates SEEKING with SUD is based on research focused on the so called "drug dreams" phenomenon found in SUD patients (103). With regard to Solm's (104, 105) neuropsychoanalytic dream theory—identifying the SEEKING system as essential for the generation of dreams—several studies observed that SUD patients frequently report dreams related to their craving of the drug they are addicted to (106–110). Usually, these dreams contain episodes in which subjects seek for drugs, and either use them or attempt to use them. It was also observed that these dreams are associated with increased drug-craving (106, 107, 111, 112). Hence, drug dreams are interpreted as hallucinatory wish fulfillment of drug cravings mediated by an upregulated SEEKING system (108).

PLEASURE/LUST, PANIC/GRIEF, and SUD

What is more, AN-theory emphasizes the significance of the PANIC/GRIEF and PLEASURE/LUST systems regarding the development of SUD (84, 85, 113).

The LUST system, which is linked to pleasure, sexual urges, and gratification, is reciprocally connected to the SEEKING network. Its activation diminishes SEEKING driven appetence behavior and triggers behaviors and feelings of satisfaction (114). Regarding its neuroarchitecture, the LUST network is not yet fully understood in humans and the theory behind its structural connectivity is inconsistent across different authors. Inferred from animal models it consists of a complex group of structures, descending from the hypothalamus to the posterior parts of the midbrain (see **Figure 3**) (81). Most authors agree that the LUST/sexuality system includes the Bed Nucleus of the Stria Terminalis (BNST), the central tegmental field, the preoptic area and the ventromedial hypothalamus, the NAcc shell, septum area, and the ventral PAG (77, 81, 115–117). Neurochemically, the LUST system is largely controlled by endorphins acting on mu-, delta-, and kappa-opioid receptors in the NAcc shell,

And hormones like vasopressin, testosterone, and oxytocin (77, 114, 116).

As shown in **Figure 4**, the PANIC/GRIEF system includes connections between the ACC, the BNST, the preoptic area, and the dorsomedial thalamus which descend to the PAG (see **Figure 6**) (77, 78). This system is predominantly controlled by endogenous mu-, delta-, and kappa-opioid-receptors. In correspondence to this, endogenous mu and delta opioid receptor ligands (like enkephalins and endorphins) deactivate the PANIC/GRIEF system, while

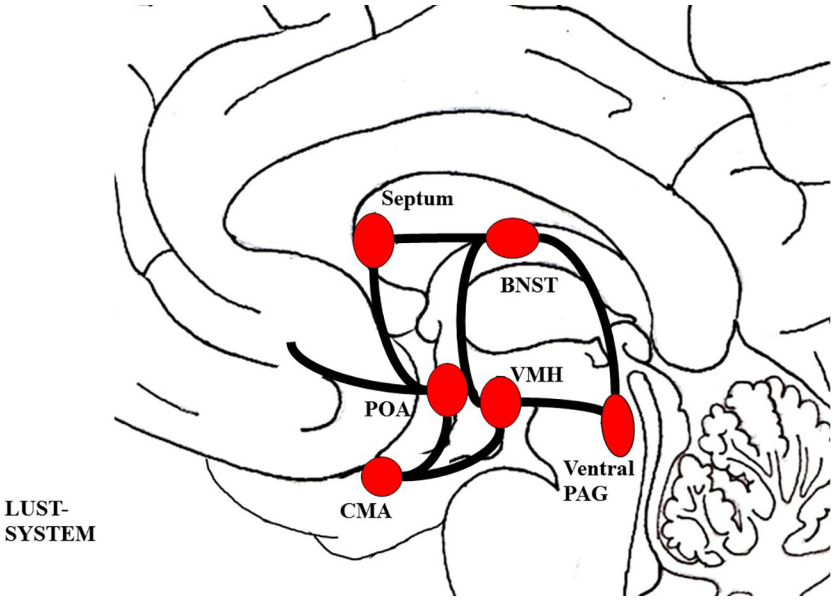

FIGURE 3 | Schematic visualization of the LUST system; The figure was drawn by J. Fuchshuber, based on theoretical concepts by Panksepp (78) and Solms and Turnbull (114). BNST, Bed nucleus of the stria terminalis; CMA, Cortico-medial amygdala; POA, Preoptic area; PAG, Periaqueductal gray; VMH, Ventromedial hypothalamus.

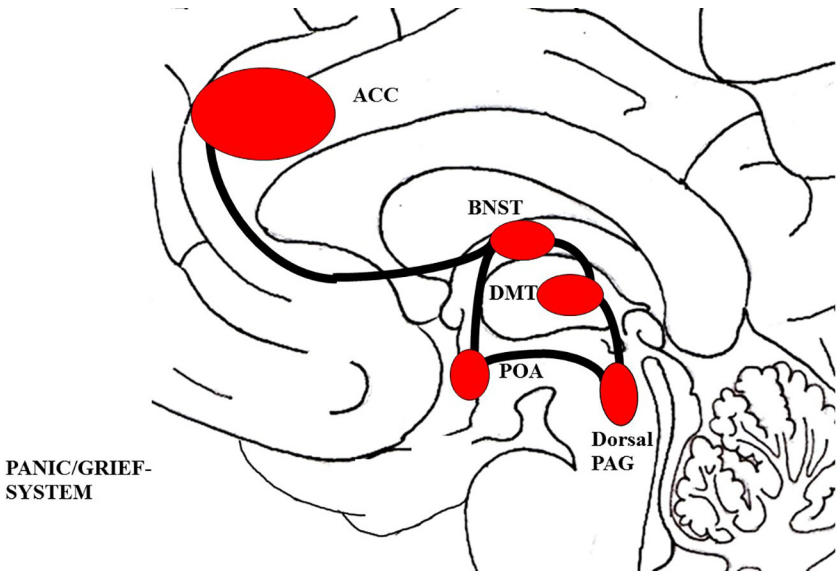

FIGURE 4 | Schematic visualization of the PANIC/GRIEF system; The figure was drawn by J. Fuchshuber, based on theoretical concepts by Panksepp (78) and Solms and Turnbull (114). ACC, Anterior cingulate cortex; BNST, Bed nucleus of the stria terminalis; DMT, Dorsomedial Thalamus; POA, Preoptic area; PAG, Periaqueductal gray.

kappa-opioid ligands (like dynorphins) increase PANIC/GRIEF activity (77, 119). Moreover, this system is deactivated by the hormones oxytocin and prolactin and activated by Corticotropin-releasing hormone (CRF) and the neurotransmitter glutamate (78).

Neuropsychoanalytic authors propose that it is not the dopamine surges linked to the artificially excited SEEKING system but the feeling of reward itself, mediated in large part by the primarily opioid controlled PLEASURE/LUST and

PANIC/GRIEF systems that are the dominant object of SUD. Moreover, it is proposed that there might be a clinically useful differentiation between SUDs primarily involving substances which stimulate the SEEKING system ("upper") and SUDs primarily involving substances which stimulate the PLEASURE/LUST and PANIC/GRIEF system ("downer"), with the latter being the clinically more severe disorder (84, 85).

What is more, the neuro-architecture of attachment in mammalians, predominantly mediated by the PANIC/GRIEF system and SUD, share remarkable resemblances, which are paralleled by a compelling overlap in the behavioral aspects of social dependence and SUD (76, 85, 120–122). Mutual neurochemical sites of action and change regarding attachment and SUD development include dopamine D1 and D2 receptors, μ-, Δ-, and κ-opioid receptors, and CRF (120). On a behavioral level similarities between SUD/withdrawal attachment/loss include: Drug dependence – social bonding; drug tolerance – alienation; and drug withdrawal – separation distress (76, 84, 85). Moreover, the phenomenology of opioid withdrawal show notable resemblances to separation distress, comprising somatic and mental pain, crying, loss of appetite, depression, insomnia, and agitation (76, 84, 85). In this context, SUD is seen as a dysfunctional attempt to compensate for overwhelming feelings of isolation, loss, and sadness mediated by an overactive PANIC/GRIEF system. Hence, the abuse of μ- and Δ-opioid receptor agonists might especially act as a replacement for secure love objects, substituting the warmth and comfort usually experienced in close relationships.

Despair as a Common Denominator of Depression and SUD

In correspondence to the proposed relation between SUDs, decreased SEEKING, and increased PANIC/GRIEF, the AN-framework sheds new light on the link between depression and SUD, already emphasized by Rado (11, 23). AN-theory frames depression as an evolutionarily conserved mechanism in which the hyperactive PANIC/GRIEF system shuts down the acute panic or protest phase of separation distress and triggers a state of *despair* which is characterized by sustained overactive PANIC/GRIEF and discontinuation of the SEEKING system, experienced as intense dysphoria (119). This mechanism seems to be mediated by increased dynorphin activity and reduced dopamine transmission within the ML-DA (123, 124). Similarly, SUD development is driven by the antagonist affective process triggered by the dysphoria of diminished SEEKING resources, either because of sustained artificial over-stimulation through drug consumption, a premorbid disposition towards SEEKING hypoactivity, or chronic hyperactivity of the PANIC/GRIEF network (85). Therefore, despair (increased PANIC/GRIEF and decreased SEEKING) is proposed as the common affective core of both disorders. However, individuals suffering from SUD self-medicate this painful affective state *via* the consumption of psychoactive substances. The plausibility of this assumption is underscored by recent progress in the neuroscientific research emphasizing the significance of dopamine and opioid systems in the etiology and treatment of depression and SUD (125–127) and the firmly established correlation between both disorders (128, 129). Furthermore, there has been a long tradition linking both SUD and depression to loss, mourning, and insecure attachment (8, 51, 130, 131).

Until recently, the influence of other primary emotion systems in the emergence of SUD cycles has been largely neglected in AN-theory and research. Nonetheless, the results of Unterrainer et al. (132) suggested increased SADNESS, FEAR, and ANGER dispositions in patients diagnosed with polytoxicomania compared to healthy controls. In addition, little is known about the role of PLAY and CARE in SUD etiology. Regarding the neurochemistry of PLAY, which partly relies on the endogenous cannabinoid system (77), it might be conceivable that PLAY is involved in cannabis SUD, however this hypothesis lacks empirical support (132). Similarly, there is no data suggesting the significance of CARE in SUD development in humans thus far. However, animal research has shown that lactating dams exhibit decreased activity in the ML-DA compared to virgin females if they had been exposed to cocaine (133). Therefore, the question remains if SUD should be understood as a self-medication strategy against negative affects in general, as proposed by authors of ego-psychology [e.g. (42)], rather than a distinct coping strategy against hyperactive PANIC/GRIEF and hypoactive SEEKING activity as suggested in AN-theory.

Trauma, Attachment, and SUD

In psychodynamic theory, the experience of childhood trauma has been assumed to play a crucial role in the etiology of psychiatric disorders since its beginnings at the end of the nineteenth century (134, 135). In a psychoanalytic context, trauma is defined as an event so intense and overwhelming that it is impossible for the subject to react functionally to it and results in an enduring pathogenic effect (53). Empirically, childhood trauma is frequently measured by retrospective assessments of emotional, physical, and sexual abuse, as well as emotional and physical neglect and deprivation (136, 137).

Meanwhile, contemporary research has gathered a considerable amount of evidence linking traumatic environments in childhood to a wide range of adult psychopathology (138). In correspondence to this, a recent review by Teicher and Samson (139) suggested that childhood trauma is substantially associated with structural changes in a number of brain regions linked with the processing and modulation of emotions. Specifically, this includes the anterior cingulate, dorsal lateral prefrontal and orbitofrontal cortex, the corpus callosum, and the hippocampus. Furthermore, childhood trauma is associated with increased amygdala response to emotional cues and conflict processing as well as a reduced striatal response to anticipated rewards. Hence, converging evidence indicates that the link between childhood trauma and adult psychopathology might be conveyed by disturbances in the neurobiological development linked to cognitive control and emotion regulation (140–142).

In correspondence to this, the development of secure attachment—which in line with AN-theory might be seen as a secondary order process (77)—has been linked to the development of emotional functioning (143). According to attachment theory, the emergence of emotion regulation relies

on the early nonverbal communication between infant and caregiver (144, 145). Ideally, primary caregivers perceive the nonverbal affective expressions of the infant and co-regulate these through symbolic mirroring and by providing physical and verbal comfort. This process supports the infant in tolerating its intense and unsymbolized affects. The repetition of this process leads to a gradual internalization of positive inner working models of the self and others. The positive inner working models serve as an internalized secure base which supports the individual in regulating emotions in a functional and relatively autonomous way, and enables him/her to explore the external world on his/her own (146). Moreover, secure attachment patterns facilitate the formation of stable and functional relationships, allowing the individual to additionally regulate emotions with the help of others (121). In contrast, internalized traumatic early experiences promote the development of corresponding negative inner working models and insecure attachment patterns that hamper the functional regulation of emotions and the formation of stable relationships (80, 145, 147, 148).

Due to the relationship between insecure attachment and affective dysregulation, insecure attachment has been repeatedly linked to the development of addiction disorders (149, 150). Largely in line with psychoanalytic object relations theory and ego-psychology, substance abuse is seen as a chemical affect regulation strategy, substituting a secure attachment figure and acting as an artificial "secure base" for the consumer (121, 151). Initially, this has a stabilizing effect on the self and its affect regulation capabilities. However, ultimately substance abuse further weakens attachment abilities and affect regulation, which triggers the vicious addiction circle of increased substance abuse, gradually leading to a complete loss of control. The assumed association between SUD and insecure attachment is well supported by a multitude of empirical studies applying psychometric attachment measures (149, 150, 152).

SUMMARY OF THE NEUROSCIENTIFICALLY INFORMED PSYCHODYNAMIC APPROACH TOWARDS SUD ETIOLOGY

In summary, all psychodynamic theories reviewed above share a common etiological structure explaining the emergence of SUD and other psychiatric disorders (1). Therefore, all psychodynamic approaches can be summarized thus: (1) An interaction between biography and hereditary dispositions determine (2) the individual's level of integration regarding his/her personality structure, which is comprised of the relationship between primary (including drives and affects), secondary, and tertiary processes (including ego and super-ego structures, object relations, and attachment working models). These developmental deficits are reflected on a neurobiological level and promote (3) a failing adaption to reality and its demands. In turn, this leads to (4) the manifestation of psychiatric disorders, which (5) in the case of maladaptation by the use of psychoactive substances leads to the development of addictive behaviors and ultimately to the manifestation of SUD (1, 41).

As outlined above, the historical development of the psychodynamic discourse on the phenomena of SUD might be described as a movement, starting from an emphasis of sexual and aggressive drives—sometimes crossing the border to moral judgment and stigmatization—to a broader and more empathetic understanding of SUD as a dysfunctional coping strategy, aimed at managing intolerable pain and suffering (153). This theoretical development is paralleled by a refinement of psychodynamic treatment strategies for subjects affected by SUD. Instead of the traditional psychoanalytic method, which is largely based on the interpretation of latent conflicts by an abstinent analyst (53), contemporary psychoanalysis stresses a more supportive, understanding, and active role of the psychotherapist (45, 154, 155). In correspondence to this, the psychodynamic therapist attempts to be perceived by the patient as a "sufficiently good" object, which might be gradually internalized. In turn, this is aimed at fostering increased interpersonal, affect regulation, and mentalization capabilities on the part of the patient (156, 157).

RESEARCH FOCUSED ON THE RELATIONSHIP BETWEEN CHILDHOOD TRAUMA, PERSONALITY, AND SUBSTANCE USE DISORDER

In correspondence to previous research conducted by our research group (65, 66, 132) which linked SUD to deficits in personality organization and primary emotions, we aimed at increasing the understanding of this association by including the impact of childhood trauma and applying path analytical and structural equation modeling techniques to extensive samples. For this purpose, the first study (158), tested assumptions proposed by Zellner et al. (85) and Solms et al. (84) focusing on the role of childhood trauma, despair, and personality organization in the development of SUD and depressive symptoms. (118), which was based on an extended sample of Fuchshuber et al. (158), examined the role of childhood trauma, adult attachment, and personality organization on overall primary emotion functioning in more detail. Finally, study three (159) conducted a secondary analysis of the sample described in study two (118), but focused on the relationship between primary emotions, symptoms of SUD, and mood pathology as well as gender. A brief overview of the methods and results of each study can be found in **Table 1**. Further information on the presented studies (e.g. detailed sample characteristics, statistical analyses) can be found in the respective publications (118, 158, 159) or obtained from the authors.

Study 1

With regard to (84, 85), we aimed at exploring the common neuropsychodynamic structure of SUD and depressive symptoms. Both disorders, which are two of the most frequently observed psychiatric disorders (160) are known to

TABLE 1 | Methods and results of studies on SUD.

Sample		Methods		Results
Study 1	n = 500	IPO-16, BSI-18, ANPS, ASSIST, CTQ	Structural equation modelling	**Correlation among latent variables:** Addictive behavior and depressive symptoms are significantly linked with more severe Childhood Trauma, increased Despair (decreased SEEKING and increased SADNESS) and Structural Deficit. **Structural equation modelling:** Influence of Childhood Trauma on Addictive Behaviors was mediated by structural deficit. Its influence on depressive symptoms was mediated by Despair (decreased SEEKING and increased SADNESS).
Study 2	n = 616	IPO-16, ANPS, ASSIST, CTQ	Path analysis	**General path analysis:** Childhood trauma predicted deficits in personality organization and insecure attachment. Increased structural deficit was associated with increased ANGER. Increased adult attachment security predicted primary emotion dispositions in general. **Mediation analysis:** The influence of childhood trauma on ANGER was mediated by structural deficit and comfort with depending on others. Its influence on other basic emotion dispositions was mediated by adult attachment security.
Study 3	n = 616	ANPS, ASSIST, BSI-18	Path analysis	**General path analysis:** Substance abuse was associated SADNESS and ANGER. Anxiety was linked to SADNESS, FEAR and PLAY. Somatization was associated with SADNESS, and PLAY. Depression was linked to SADNESS, SEEKING, FEAR and PLAY **Multi-group analysis:** Comparison between groups (female vs. male; "healthy" vs. participants reporting a lifetime psychiatric diagnosis) revealed no significant difference between paths.

AAS, Adult Attachment Scales; ANPS, Affective Neuroscience Personality Scales; ASSIST, The Alcohol, Smoking, and Substance Involvement Screening Test; BSI-18, Brief Symptom Inventory; CTQ, Childhood Trauma Questionnaire; IPO-16, Inventory of Personality Organization.

be considerably correlated (128, 129). As described above, AN-theory proposes a common primary affective core for both disorders, which is characterized by dysregulations within the SEEKING and SADNESS or PANIC/GRIEF system (84, 85). In correspondence to this, patients suffering from SUD and patients suffering from depression were expected to report increased SADNESS scores and decreased SEEKING scores. Therefore, the investigated model included the latent variable "Despair", which was composed of low SEEKING and high SADNESS. Furthermore, based on previous research (58, 65, 66), we expected significant associations between both disorders and impairments in personality organization. Moreover, with regard to a long line of research linking childhood trauma to impaired personality structure, increased negative affect, and adult psychopathology (134, 135, 138, 140, 145, 161), we assumed a mediating role for despair and personality organization in the relationship between childhood trauma, SUD, and depressive symptoms.

Methods and Results

This study investigated a sample of young adults aged between 18 and 39 years (M = 26 years; SD = 5.51), which included 500 (63.2% female) German-speaking participants. 37.4% of the investigated participants reported a lifetime diagnosis with a psychiatric disorder. A majority of these participants declared a diagnosis with depression (n = 129; 69%) and 9% reported a diagnosed form of SUD. All participants completed the German version of the *Affective Neuroscience Personality Scales* [ANPS; (162); German version by Reuter and Hennig (2014; see (163)], the *16-Item Inventory of Personality Organization* [IPO-16; (61)], the *Childhood Trauma Questionnaire* [CTQ; (137); German version by (164)], the "Depression" subscale of the *Brief Symptom Inventory* [BSI-18; (165); German version: (166)], and the *Alcohol, Smoking and Substance Involvement Screening Test* [ASSIST; (167)].

Correlation analysis among latent variables revealed significant correlations between every investigated factor ($p < 0.001$), which were in line with previous assumptions.

The initial standardized solution for the structural equation modeling (see **Figure 5**) exhibited an acceptable model fit: RMSEA = 0.06 (90% CI: 0.06, 0.07); TLI = 0.92; CFI = 0.93; AIC = 737.575. In this model, Addictive Behaviors is significantly associated with Structural Deficit ($\beta = 0.57$; $p < 0.001$) and male sex ($\beta = -0.27$; $p < 0.001$), while Despair significantly predicted Depressive Symptoms ($\beta = 0.92$; $p < 0.001$). Furthermore, Childhood Trauma did not show significant direct effects on either psychiatric variable but was associated with Structural Deficit ($\beta = 0.46$; $p < 0.001$) and Despair ($\beta = 0.52$; $p < 0.001$). Bootstrap analysis showed significant indirect effects of Childhood Trauma on Addictive Behaviors *via* Structural Deficit ($\beta = 0.22$; $p < 0.01$) and on Depressive Behaviors *via* Depressive Symptoms ($\beta = 0.44$; $p < 0.01$).

Study 2

Building upon insights gained in previous research (130, 132, 158, 168), Fuchshuber et al. (118) focused on mapping the relationship between childhood trauma, adult attachment, personality organization, and primary emotion functioning in more detail. We expected the influence of childhood trauma on primary emotion functioning to be mediated by adult attachment and personality organization, as recent psychodynamic literature assumes these concepts to be

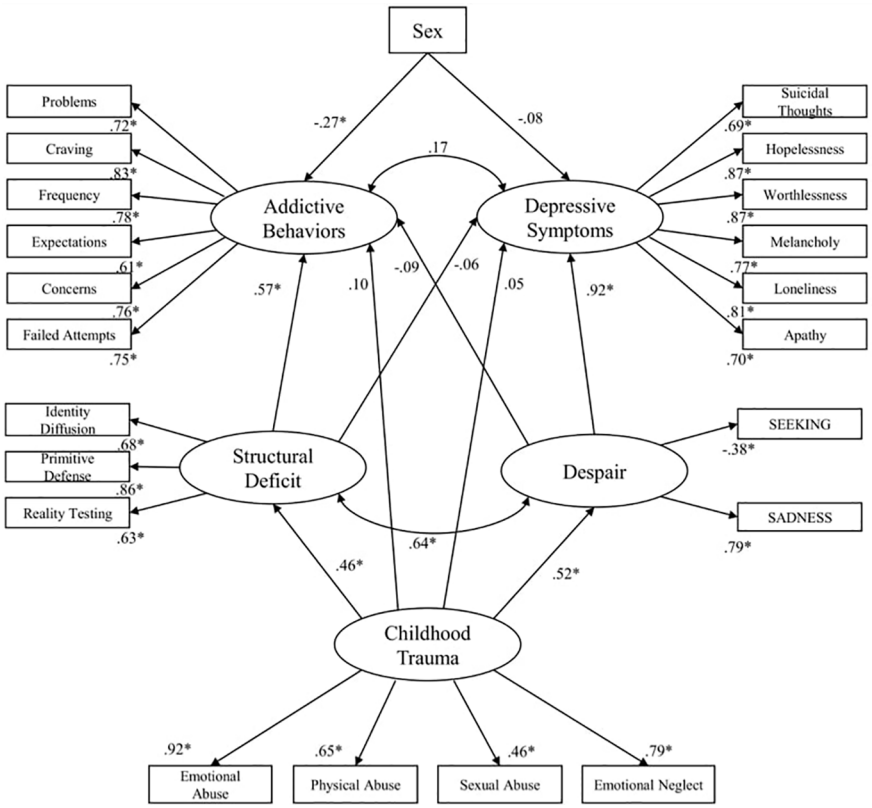

FIGURE 5 | Initial standardized solution for the structural equation model. *p < .001; Sex: Female = 1; Male =0 (158).

influenced by early childhood relationships and, in turn, associated with affect regulation in adults (56, 168).

Methods and Results

For this study, a community sample of 616 adults [61.9% female; extended sample of (158)], was investigated. The mean age of participants was M = 30 (SD = 9.53) and ranged between 18 and 69 years. 39.4% of the participants reported a psychiatric lifetime diagnosis. A majority (60%) of these participants were diagnosed with depression. AMOS 18.0 was used to estimate paths and fit indices of the path analytic models.

All participants completed the German version of the *Affective Neuroscience Personality Scales* [ANPS; (162); German version by Reuter and Hennig (2014; see (163)], the *16-Item Inventory of Personality Organization* [IPO-16; (61)], the *Childhood Trauma Questionnaire* [CTQ; (137); German version by (164)], and the *Adult Attachment Scale* [AAS; (169); German version: (170)].

The final model exhibited an excellent model fit: RMSEA = 0.03 (90% CI: 0.01, 0.05); TLI = 0.99; CFI = 1.00. As shown in **Figure 6**, this model suggested the following associations: Childhood Trauma was significantly associated with Depend (β = -0.56; p < 0.001), Close (β =-0.44; p < 0.001); Anxiety (β = 0.36; p < 0.001), and Structural Deficit (β = 0.39; p < 0.001). Structural Deficit was associated with ANGER (β = 0.21; p < 0.001) and PLAY (β = 0.12; p < 0.001). Depend was associated with SADNESS (β = -0.29; p < 0.001); ANGER (β = -0.21; p < 0.001) and SEEK (β = 0.29; p < 0.001); FEAR (β = -0.25; p < 0.001); PLAY (β = 0.41; p < 0.001) and CARE (β = 0.24; p < 0.001), while Close was associated with SEEK (β = 0.10; p < 0.02); PLAY (β = 0.33; p < 0.001); and CARE (β = 0.25; p < 0.001). Anxiety was associated with FEAR (β = 0.37; p < 0.001); CARE (β = 0.27; p < 0.001) and SADNESS (β = 0.47; p < 0.001). Finally, Structural Deficit was significantly correlated with every attachment scale (p < 0.001) and every attachment scale was significantly correlated with each other (p < 0.001).

What is more, bootstrap analysis revealed significant associations between Childhood Trauma and SEEK (β = -0.18; p < 0.01), CARE (β = -0.15; p < 0.01), FEAR (β = 0.29; p < 0.01) and SADNESS (β = 0.31; p < 0.01) which were solely mediated by adult attachment. Furthermore, the association between Childhood Trauma and PLAY (β = -0.31; p < 0.01) and ANGER (β = 0.20; p < 0.01) were mediated by Structural Deficit as well as adult attachment.

Study 3

Based predominantly on intracranial stimulation studies in animal models, AN-theory proposes perturbations in subcortical affective systems as a significant factor in the emergence of a variety of psychiatric disorders (77, 82). However, studies testing these hypotheses in human subjects

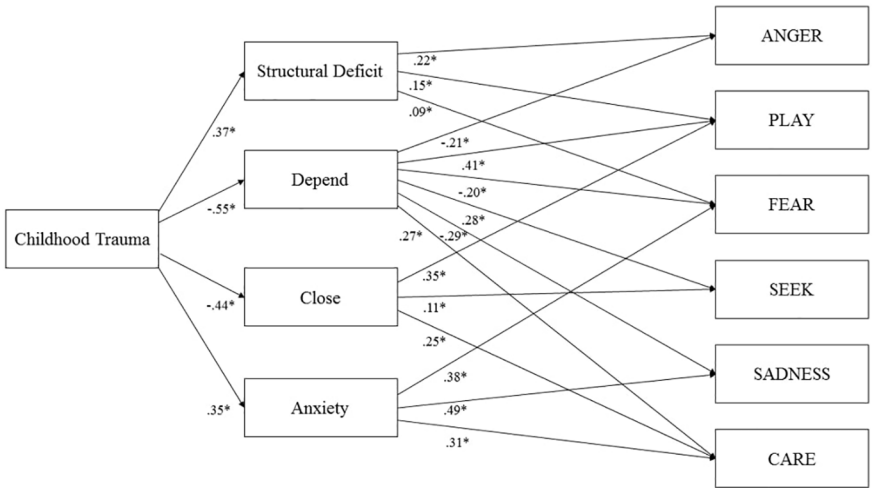

FIGURE 6 | Final model of Childhood Trauma, Structural Deficit, adult attachment, and primary emotions (118). *p < .001.

have been sparse. To investigate these claims within a quantitative-empirical framework, the third study (159) examined the relationship between psychiatric symptoms (SUD, depression, anxiety disorder, and somatization) and different dimensions of primary emotions (SEEKING, FEAR, ANGER, SADNESS, PLAY and CARE). Furthermore, by using the multi-group path analysis technique, this study was able to test the possible moderator effects of gender and psychiatric lifetime diagnosis.

Methods and Results

The final model showed an excellent fit: RMSEA = 0.05 (90% CI: 0.03, 0.08); TLI = 0.97; CFI = 0.99 AIC = 159.74, and suggested the following associations (see **Figure 7**): Global Continuum of Substance Risk was associated with SADNESS (β = .25), ANGER (β = .10) male sex (β = -.25). Depressive Symptoms were associated with increased FEAR (β = .10) and SADNESS (β = .53), and decreased dispositions to SEEKING (β = -.10) and PLAY (β = -.15). Anxiety Symptoms were related to increased SADNESS (β = .33), FEAR (β = .21) and decreased PLAY (β = -.10). Somatization was associated with elevated ANGER (β = .09) and SADNESS (β = .26) and diminished PLAY (β = -.12) (all p <.01). In summary, the final model was able to explain 14% of the variance of Global Continuum of Substance Use Risk, 52% of Depressive Symptoms, 32% of Anxiety symptoms, and 14% of Somatization.

Regarding possible moderation effects of psychiatric lifetime diagnosis and gender, additional multi-group analysis (female vs. male; "healthy" vs. participants reporting a lifetime psychiatric

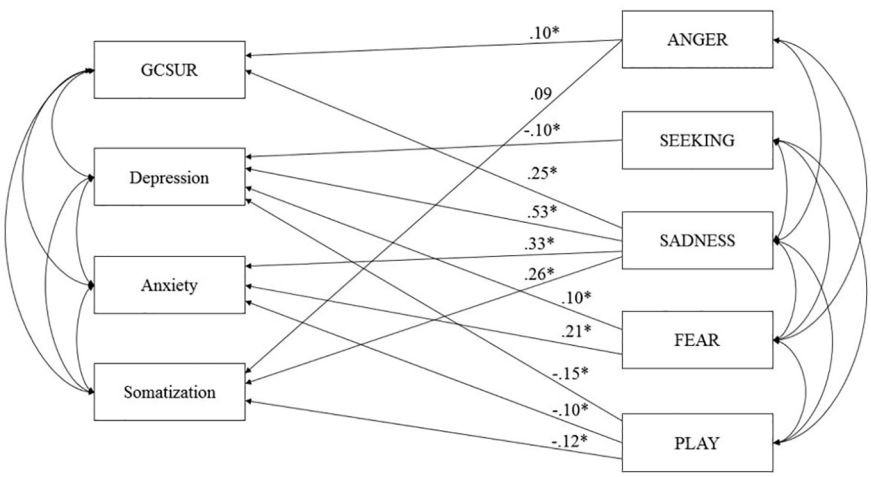

FIGURE 7 | Final model of primary emotions and psychiatric symptoms controlled for Age and Sex (159). GCSUR, Global Continuum of Substance Use Risk; *p < .001; curved arrows indicate significant correlations (p < .001).

diagnosis) revealed no significant difference between paths (all p = n.s.).

DISCUSSION

The research presented herein investigated the complex relations between childhood trauma, (neuro-) psychodynamic personality traits, and adult psychopathology. Largely in line with previous research (171–173) and theoretical assumptions in psychoanalytic literature (38, 56, 135, 145, 146, 174), our findings underline the harmful effects of early traumatic relationships on the development of personality organization, adult attachment, and emotional functioning. This result resonates with evidence gathered by Schimmenti and Caretti (172) and Granieri et al. (173) indicating that the effects of childhood trauma on adult psychopathology are partially mediated by dissociation, which is conceptualized as a primitive defense mechanism related to splitting (58, 175). In correspondence to this, our results expand this conceptualization by suggesting that deficits in emotional functioning and personality organization fully mediate the effect of childhood trauma in the emergence of SUD and depression in adults. Furthermore, results of the studies presented above (118, 158, 159), echo previous research, which suggested significant associations between personality organization, attachment, primary emotions, and SUD (65, 66, 85, 132, 149). On a neuroscientific level, this line of evidence is paralleled by recent findings linking childhood trauma to disturbances of neurobiological structures related to emotion regulation. As outlined above, this includes the anterior cingulate, dorsal lateral prefrontal and orbitofrontal cortex, the corpus callosum and the hippocampus (139), impairments in white matter fiber tracts, specifically in the cingulum and the superior longitudinal fasciculus (176) and chronic hyperactivity of the corticotropin-releasing factor systems (140). Moreover, very recent evidence suggests detrimental effects of childhood trauma specifically affect subfields of the hippocampus which are associated with emotion regulation like the bilateral presubiculum and subiculum (177).

With regard to study one (158), our results indicated that the underlying factors—primary emotions and personality organization—derogate the correlation between symptoms of depression and SUD. This is consistent with the assumptions made in AN-theory that assume a common etiological core for both disorders (84, 85). Therefore, it seems plausible that these underlying factors are the driving forces of the often-observed comorbidity of SUDs and depression. However, our findings suggest that Despair very precisely predicts depression, while its influence on addictive behaviors is diminished by deficits in personality organization. Therefore, addictive behaviors might be seen as a compensative strategy against gaps within a corrosive personality structure as proposed by authors linked to ego-psychology (48, 178). In contrast, our results highlight the fact that the influence of personality structure on depressive symptoms is repressed by the latent factor Despair, supporting the assumption that depression emerges due to hyperactive SADNESS and hypoactive SEEKING networks (119, 179). Nevertheless, this relationship might be more complex as both studies reviewed the above gathered evidence for a substantial interrelation between personality structure and primary emotion functioning (118, 158), which is consistent with basic assumptions of AN-theory (77, 79).

In correspondence to this, our second study (118) was able to gather new insights regarding the relationship between attachment security, personality organization, and primary emotion functioning. If computed in a single model, our results suggest that Kernberg's (59) concept of personality organization, which is linked to object relations theory, predominantly predicts ANGER, while adult attachment attitude is related to emotional functioning in general. With regards to our results, it might be concluded that deficits in personality organization and insecure attachment foster increased negative primary emotion dispositions (ANGER, FEAR, and SADNESS), while secure attachment plays an important role in increased positive primary emotion dispositions (SEEKING, PLAY, and CARE). This is with the exception of "anxiety of being rejected", which predicted increased CARE. This finding corresponds to the relationship between this concept and the preoccupied or insecure ambivalent attachment style, linked to exuberant clinging to attachment figures in relevant literature (180, 181). In conclusion, our results indicate empirical support for basic assumptions of object relations and attachment theory, assuming internalized object relations and attachment working models as crucial for the functional integration of affects (56, 62, 143, 168, 182).

Interestingly, the finding that compared to adult attachment, deficient personality organization is substantially related to increased ANGER, reflects theoretical differences between Kernberg's (58) conceptualization of object relations theory and attachment theory. While his theoretical assumptions, which are strongly influenced by Kleinian theory (175), emphasize the function of personality organization in the integration and neutralization of aggressive affects, aggression has considerably less significance in attachment theory (146).

What is more, in the study Fuchshuber et al. (118) we were able to shed new light on the relationship between adult attachment and personality organization. Thereby, results of the correlation analysis corresponded to previous research suggesting substantial links between both concepts (65, 146, 183, 184). More specifically, the strength of the correlations between structural deficits and attachment security scales ranged from negative medium correlations with "Comfort with Dependence" to large positive correlations with "Anxiety of being Rejected" (185), reflecting conceptual similarities of personality organization and attachment security.

Study three (159) further investigated the relationship between psychiatric disorders and primary emotions. In contrast to study one, which followed a confirmatory approach focusing on the role of despair (85), study three used path analysis to investigate the associations between SUD, mood disorders, and all primary emotion dimensions in a more exploratory manner. The results

indicate that SUD symptoms are linked to increased SADNESS and, to a lesser extent, to increased ANGER. This finding echoes recent results by Unterrainer et al. (132) suggesting increased FEAR, SADNESS, and ANGER dispositions in SUD patients. However, regarding the overall explained variance, SUD might be less related to primary emotions than previously expected. This is especially the case for SEEKING which—in line with Unterrainer et al. (132)—was not associated with SUD. While contradicting evidence from neuroscientific research (83, 90, 127), this finding might be the result of conceptual differences between functional aspects of the ML-DA or SEEKING system and the trait like disposition towards SEEKING assessed by the ANPS. Regarding its role in reinforcement learning, the ML-DA/SEEKING network seems significant the etiology of SUD, yet, this might not be reflected in decreased SEEKING personality traits. Moreover, our results were collected within a cross-sectional study, therefore it is impossible to infer causal conclusions based on our findings. Hence, it is plausible that many forms of SUD can be framed as dysfunctional coping strategies against diminished SEEKING network activity as outlined by Solms (84) and Zellner et al. (85). But, owing to the cross-sectional study design, we might have been unable to detect this association, as the abuse of drugs may have artificially increased the SEEKING disposition of participants (77). Thus, to sufficiently investigate the relationship between SEEKING and SUD, it will be necessary to conduct longitudinal studies assessing SEEKING dispositions before the onset of problematic substance use. By contrast, our findings highlight the role of SADNESS and ANGER in SUD. In line with the results of Unterrainer et al. (132), this partially supports assumptions of neuropsychoanalysis (84, 85) and also reaffirms early observations of object relations theory underscoring role of aggression in SUD etiology (14, 51). This observation further supports the notion of substance abuse as a function of artificial affect regulation. By taking drugs the addicted individual tries to seal gaps in a deficient personality structure (158), which is linked to increased negative affects (56, 57, 118). Specifically, addictive behaviors seem to be associated with increased feelings of loneliness and isolation in addition to feelings of rage and aggression, which are experienced as intensely unpleasurable and ultimately overwhelming (76, 77). The observed significant relationship between SUD and SADNESS further highlights the conceptualization of SUD as attachment disorder, specifically linked to dysregulations within the endogenous opioid system (120, 121, 186). This relationship is further underscored by recent findings which indicate that bipolar patients additionally diagnosed for alcohol use disorder (AUD) or SUD are significantly more likely to show a predominant depressive polarity in contrast to patients suffering from bipolar disorder without a comorbid AUD or SUD diagnosis (187).

Furthermore, the observed association between SUD and ANGER underlines theoretical considerations that relate substance abuse to auto-aggressive behavior against venomous inner self and object representations linked to traumatic childhood relationships (14, 51, 58).

What is more, our results indicate a differential role of primary emotions in the emergence of psychiatric disorders (159). Thereby, SADNESS seems to play a significant role in all assessed forms of mood disorders (depressive symptoms, anxiety symptoms, and somatization). However, contrasting with SUD, depressiveness was also predicted by increased FEAR and diminished PLAY and SEEKING, which is largely in line with findings from Montag et al. (163). Additionally, a similar pattern was observed for anxiety symptoms, which was linked to increased FEAR and SADNESS and decreased PLAY. Therefore, these results are in line with Panksepp's (82) hypothesis of the relevance of the SADNESS system in anxiety disorders but also underscore his theory regarding the clinical significance of PLAY, traditionally been neglected in psychiatry (77, 78). This assumption is further reaffirmed with regards to the significant link between PLAY and somatization symptoms. This association might reflect the relationship between SADNESS and the endogenous opioid system, as a hypoactivity of μ and Δ opioid system is known to promote feelings of bodily discomfort (78, 120).

Limitations

The present study reanalyzed the sample investigated in Fuchshuber et al. (118). Therefore, the results of our analysis should not be interpreted independently from either Fuchshuber et al. (158) and Fuchshuber et al. (118). Moreover, as there is no validated measure for the assessment of LUST presently available, we were unable to estimate the etiological relevance of this basic emotion network. Despite having a key role in neuropsychoanalytic theory, LUST was not included in the ANPS as its authors claimed that people would not be open enough to report about their sexuality (162). However, this assumption seems questionable, particularly with regard to the variety of self-report measures of sexuality already existing. Hence, future research should aim at developing a self-report measure for LUST to map the AN-framework and its role in etiology to a full extend.

In correspondence to that, the presented studies exclusively tested linear models regarding the relationship between primary and higher order processes, as well as regarding their influence on the development of SUD. However, with respect to the non-linear assumptions of AN-theory (82, 188), future studies should investigate non-linear relationships to describe the complexity of

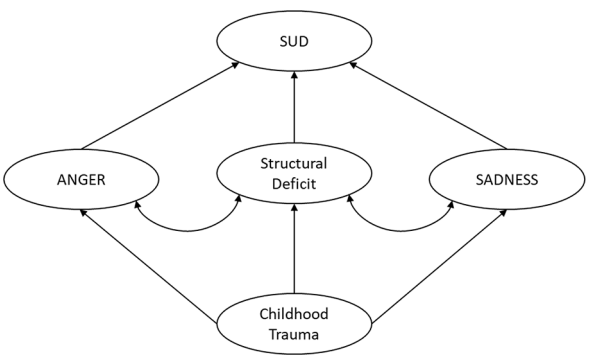

FIGURE 8 | Proposed neuropsychoanalytic model of SUD etiology.

these relationships in a more naturalistic manner (189). This might be achieved in future studies by the estimation of non-recursive structural equation models in longitudinal settings.

What is more, the validity of our findings is limited due to the assessment of psychiatric symptoms by self-rating measurements, which are vulnerable to distortions towards social desirability. Furthermore, it cannot be ruled out that some psychiatric symptoms and disorders were not detected in our sample due to a lack of self-reflection abilities, especially in participants suffering from psychotic disorders or severe personality disorders (190). Hence future studies should consider applying structured clinical interviews, preferably within a clinical setting, to improve the assessment of psychiatric symptoms.

The assessment of psychodynamic parameters relied on the use of self-rating measures which reflect consciously available representations of concepts that are at least partially hypothesized as unconscious. In order to strengthen the validity of our results, future projects should include other means of data collection including qualitative and semi-structured interviews.

Moreover, it should be considered that traumatic experiences in childhood are highly susceptible to splitting, dissociation or denial, and might therefore not be detectable to a full extent (172, 191).

CONCLUSION AND OUTLOOK

Despite these limitations, the studies reviewed above were able to preliminarily validate fundamental etiological assumptions proposed in psychoanalytic theory, which assume early relationships as building blocks or tuning variables of adult personality structure, ultimately determining psychopathological developments (1, 56, 174, 189).

With regard to Kernberg's (58, 59) conceptualization of personality organization, SUD treatment should thus focus on an increased availability of mature defense mechanisms and the development of more coherent identity narratives. Improvements in these areas might enable more autonomous and functional affect regulation abilities, which might be seen as a crucial factor in stabilizing the affected individual's process of weaning, abstinence, and rehabilitation (192, 193).

In correspondence to the role of primary emotions in SUD etiology, our research highlights the importance of increased SADNESS and ANGER. Thus, we propose the following model, which should be tested in future studies assuming the influence of childhood trauma on the development of SUD symptoms to be mediated by increased structural deficit as well as increased SADNESS and ANGER dispositions (see **Figure 8**):

In summary, it can be concluded that a neuroscientifically informed psychodynamic framework is able to contribute valuable insights regarding the underlying mechanisms of SUD development. However, many questions remain unresolved and future research should aim at expanding and refining the etiological model proposed in this overview. Hence, the development of improved standardized measures and the progressive embedding of psychoanalytic theories within empirical neuroscience might be rewarding goals for future research initiatives.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material; further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the ethics guidelines of the Medical University of Graz. The protocol was approved by the ethics committee of the Medical University of Graz. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

JF and HU conceptualized the manuscript. JF wrote the first draft of the manuscript. HU proofread the manuscript. All authors contributed to the article and approved the submitted version.

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A Meta-Analysis of Transcranial Direct Current Stimulation on Substance and Food Craving: What Effect Do Modulators Have?

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Substance addiction and food addiction are significant social problems worldwide. In previous studies of substance addiction, transcranial direct current stimulation (tDCS) has been used to influence craving of substance or food. However, the reported effects are not always consistent due to inconsistent experimental settings. The way modulators influence the effect of tDCS on substance addiction is worth exploring. This meta-analysis was conducted to estimate the effect size of tDCS on substance and food craving and to investigate the influence of potential modulators. We systemically identified and reviewed studies on substance/food craving using tDCS that were published between January 2008 to January 2020. A total of 32 eligible studies were identified. Hedges' g was computed as an indicator of the effect of tDCS and some potential moderators (substance type, stimulation sites, current intensities, number of sessions, duration of stimulation, and study design) were examined using subgroup analysis. Random effects analysis revealed a total medium effect size [Hedges' g = 0.536, 95% confidence interval (CI): 0.389–0.683, after adjusting Hedges' g = 0.416, 95% CI: 0.262–0.570] preferring active over sham stimulation to reduce craving. A significant difference was observed between the number of sessions (repeated stimulation was better than single stimulation). The duration of stimulation may have a positive influence on the effects of tDCS. No other significant differences were found in other subgroups analysis. In conclusion, our results provided evidence that tDCS can be an effective way to reduce craving of substance or food, and longer multiple stimulus durations in all can more effectively reduce craving; however, the influences of modulators still need be to be examined in depth in future.

Keywords: transcranial direct current stimulation, tDCS, craving, substance dependence, food addiction, dorsolateral prefrontal cortex

INTRODUCTION

Substance dependence (or substance addiction) is a chronic relapsing brain disorder leading patients to use a substance continuously despite the negative consequences that result from doing it (1). Many types of substances can cause addiction, such as addictive drugs including alcohol and tobacco. According to statistics released by the United Nations Office of Drug and Crimes, the percentage of individuals using cannabis, cocaine, and opioid worldwide were 3.8, 0.37, and 1.08%, respectively, in 2017 (2). Meanwhile, the harmful use of alcohol was estimated to cause 2.5 million deaths each year (3) and tobacco use causes more than five million deaths worldwide each year (4).

In the meantime, food addiction can be described as the dependence on refined foods that meets the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) substance use disorder criteria (5). Food addiction is significantly linked to obesity (6). Considering around 93.3 million US adults (7) and about 86 million people in China (8) could be classified as obese in 2016, food addiction is similar to substance addiction because food regulates body process by acting as the source of required energy and has a hedonic component that makes it an effective natural reward. Food-related rewards may promote increased intake and trigger withdrawal-related symptoms (e.g., overeating), suggesting that the behaviors parallel substance abuse (9). One possible explanation for food addiction (or overeating) is that sugar, fats, salt, caffeine, refined sweeteners, and refined carbohydrates in processed foods are addictive substances (5).

With substance addiction or food addiction, craving is dependent upon the past experience of an urge or desire to use substances (10). Reducing craving is an important aspect of treating substance dependence or food addiction. A direct method to reduce craving is pharmacotherapy (11–14), which is a long-term therapy focused on substitution or withdrawal. Because the use of medication carries a risk of damaging cardiopulmonary function (15), its application requires care. Cognitive treatments such as cognitive-behavior coping skills treatment (CBT) (16) and psychological counseling (17) are other mainstream treatments for helping patients recognize, avoid, and cope with the substance. It is often used with medications that interact with the type of psychotherapy provided (18, 19). Additionally, with the development of neural science, non-invasive neurostimulation techniques, including transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS), have been widely used to reduce substance cravings (20, 21). Electromagnetic brain stimulation could regulate activity in specific brain regions. Recent studies have explored the application of non-invasive neurostimulation for the treatment of substance dependence (22–25).

Among them, tDCS, which uses a weak safe current of 1–2 mA for 3–20 min to increase (anodal tDCS) or decrease (cathodal tDCS) cortical excitability (26, 27), has a significant effect on reducing cravings (28, 29). However, the results of the effect of tDCS on drug craving are mixed. Salling et al. (30) reviewed research on brain stimulation in addiction and found that tDCS has an acute effect on drug and alcohol cravings without consistent results. Coles et al. (31) found that tDCS reduced craving and consumption for alcohol and drugs while the results for tobacco were unclear because of different stimulation methods and parameters. Some studies indicated that a single stimulation of the left dorsolateral prefrontal cortex (DLPFC) with anodal tDCS significantly reduced cravings (32–35), but other findings did not support this conclusion (36–38). This kind of inconsistency may come from different study designs, stimulation parameters, and characteristics of participants in different studies.

Considering the inconsistent findings of empirical investigations and that only a medium effect of tDCS was found in a meta-analysis, it is essential to explore potential modulators separately. In 2013, Jansen et al. (39) performed the first meta-analysis in this field (both TMS and tDCS studies included). After comparing the effects on alcohol/nicotine users and people with a high craving for food, the results indicated that craving levels were decreased in substance dependence following non-invasive neurostimulation of the prefrontal cortex and further revealed a significant medium effect size of neurostimulation, but no difference between substances, stimulation technology, or side of stimulation. Recently, another meta-analysis from Song et al. (40) reported a medium effect size of TMS and tDCS treatment on craving and consumption, making a comparison between substance type/stimulation sessions/stimulated regions and found that multi-session stimulation had larger effects. Different from existing research, this meta-analysis evaluates tDCS based on separate stimulation parameters (e.g., the stimulation site, current intensity, number of sessions, duration of single-stimulation, and total-stimulation); the duration of stimulation, in particular, has seen little prior analysis. Substance type and study design may be important modulators that need to be investigated in depth. We aimed to conduct a meta-analysis focusing on the effects of tDCS in decreasing substance/food craving, and exploring the influence of potential modulators systematically. This should help find the optimal stimulation parameters in clinical settings for drug dependence and food addiction.

METHODS

An online search was conducted in the Web of Science, PubMed, and Google Scholar databases for articles published from January 2008 to January 2020. This search was implemented following the PICO-method (Patient/Population, Intervention, Comparison, Outcome) building a framework where "substance or food addiction" represented "P," "tDCS stimulation" represented "I," "active and sham stimulation" represented "C," and "craving" represented "O". The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines were followed in this meta-analysis.

Inclusion and Exclusion Criteria for the Selection of Studies

We searched for the combination of two sets of pre-defined terms in the title or abstracts. The following terms were set as the search terms: (transcranial direct current stimulation OR tDCS) AND (substance dependence OR substance abuse OR alcohol OR drug OR tobacco OR nicotine OR eating disorder OR food addiction).

All studies included in the meta-analysis met the following inclusion criteria: 1) tDCS was used as the stimulation tool; 2) substance craving/food craving changes were measured; 3) sham stimulation as a control condition; and 4) means, standard deviations, t, F, or p statistics and the number of participants in each intervention group were provided completely as basic data in order to calculate effect size.

Studies were excluded if: 1) they were meta-analyses, reviews, meeting abstracts, or case studies; 2) the subjects had other mental disorders other than substance addiction/food addiction; 3) tDCS was used combined with other intervention strategies (e.g., cognitive training or psychotherapy); or 4) the subjects were animals.

Data Extraction

Stimulation Parameters

The most important stimulation parameters were stimulation site (left DLPFC, right DLPFC, or other area), current intensity (2 mA or 1 mA), number of stimulation sessions (single-session or multi-session), duration of single-stimulation (10–30 min), and total-stimulation (10–200 min).

Substance Type

Four types of substance dependence were analyzed: drugs (e.g., cocaine, marijuana, methamphetamine, heroin, and opium), tobacco, alcohol, and food.

Study Design

Studies included in this meta-analysis were divided into double-blind or single-blind experiments, and into parallel experiments (i.e., more than two groups of participants and each group receives different treatments) and crossover experiments (all participants receive the same treatments but in a random order).

Craving Measures

The main outcome measures included in this meta-analysis were state craving scores from different questionnaires. For each study, craving scores pre-tDCS and post-tDCS of all participants (both active and sham groups) were obtained. Effect sizes on craving levels were standardized for the effect of stimulation.

Data Analysis

The Cochrane Collaboration's risk of bias tool was used to judge the risk of bias within individual studies (41). The publications identified through this search strategy were then examined by the three researchers (JQ, JC, and ZZ) individually to confirm eligibility. If there was any disagreement, it was resolved by discussion.

General Meta-Analysis

The Comprehensive Meta-Analysis 2.0 (CMA 2.0) computer program was used to process and analyze the data. Main analyses were implemented to examine the effects of tDCS on craving. To measure the difference in craving levels between active and sham stimulation, effect sizes (Hedges' *g*) were calculated. According to Borenstein et al. (42), Hedges' *g* is regarded as a conservative estimate, which could be applied to studies regardless of sample sizes. Basing on the value of *g,* the results may be defined as reflecting a small (g=0.2–0.5), medium (g=0.5–0.8), or large (g>0.8) effect.

Moderator Analyses

A random-effects model was used to analyze the difference between subgroups. Compared with the fixed effects model, the random effects model interval provides an unadventurous estimate of accuracy allowing for the existence of heterogeneity, which is more suitable for generalization (42). All comparisons used an alpha of 0.05.

The meta-regression was used to identify whether the duration of stimulation might influence the effect size estimates. Since every study was weighted by the precision of their respective effect estimates, the influence on the relationship depended on the size of study. This allows for residual heterogeneity among intervention effects rather than being modeled by explanatory variables (43).

Publication Bias

Publication bias analyzes whether the decision to publish or distribute a study was influenced by its findings (44). Publication bias was assessed using Egger's regression test (45). If publication bias was identified, a trim-and-fill procedure was applied to modify the effect size caused by publication bias (46).

RESULTS

Studies Included in the Meta-Analysis

The literature search identified 32 eligible studies (see **Table 1**). **Figure 1** shows the flow diagram of the inclusion and exclusion process. Of the included studies, 8 for nicotine, 10 for alcohol, 7 for food, and 7 for other drug cravings. There were four studies in which subjects received two types of active anodal stimulation at different sites in independent sessions (33, 34, 57, 68). The four studies were divided into eight "units of analysis." We adjusted for the interdependence of these data in the analyses by taking *study* as a unit of design instead of the *unit for analyses* because in this case it was difficult to distinguish differences in both design and stimulation locations (39). Therefore, there were 36 units of analysis included in total. The assessment of the risk of bias for all studies included is summarized in **Supplementary Table 1**.

TABLE 1 | Studies included in the meta-analysis.

Author	Stimulation parameters				Study design			Participant's characteristics	
	Stimulation site	Current intensity	Sessions	Duration	Crossover or parallel	Double or single	Addiction type	Subjects	Craving measure
Fregni et al. (34)	A-R/L DLPFC	2 mA	1	20 min	CO	Double	Nicotine	24	VAS
Boggio et al. (47)	A-L DLPFC	2 mA	5	20 min	PR	Double	Nicotine	27	VAS
Fecteau et al.(48)	A-R DLPFC	2 mA	5	30 min	CO	Double	Nicotine	12	sQSU
Kroczek et al. (36)	A-L DLPFC	2 mA	1	15 min	PR	Double	Nicotine	25	VAS
Yang et al. (49)	A-L DLPFC DLPFC	1 mA	1	30 min	CO	Single	Nicotine	32	VAS
Xu et al. (38)	A-L DLPFC	2 mA	1	20 min	CO	Single	Nicotine	24	UTS
Mondino et al. (50)	A-R DLPFC	2 mA	10	20 min	PR	Double	Nicotine	29	LTS
Hajloo et al. (51)	A-L DLPFC	2 mA	10	20 min	PR	Double	Nicotine	40	DDQ
Boggio et al. (33)	A-R/L DLPFC	2 mA	1	20 min	CO	Double	Alcohol	13	AUQ
Silva et al. (52)	A-L DLPFC	2 mA	5	20 min	PR	Single	Alcohol	13	OCDS
Klauss et al. (53)	A-R DLPFC	2 mA	5	26 min	PR	Single	Alcohol	33	OCDS
den Uyl et al. (54)	A-L DLPFC	1 mA	1	10 min	PR	Single	Alcohol	41	AAAQ
Wietschorke et al. (55)	A-R DLPFC	1 mA	1	20 min	PR	Double	Alcohol	30	VAS
Klauss et al. (56)	A-R DLPFC	2 mA	10	20 min	PR	Double	Alcohol	49	OCDS
Nakamura-Palacios et al. (37)	A-L DLPFC	1 mA	1	10 min	CO	Single	Alcohol	49	OCDS
Fregni et al. (57)	A-R/L DLPFC	2 mA	1	20 min	CO	Double	Food	21	VAS
Goldman et al. (58)	A-R DLPFC	2 mA	1	20 min	CO	Single	Food	19	VAS
Kekic et al. (59)	A-R DLPFC	2 mA	1	20 min	CO	Double	Food	17	FCQ-S
Lapenta et al. (60)	A-R DLPFC	2 mA	1	20 min	CO	Double	Food	9	VAS
Jauch-Chara et al. (61)	A-R DLPFC	1 mA	8	20 min	CO	Single	Food	14	VAS
Georgii et al. (62)	A-R DLPFC	1 mA	1	20 min	CO	Double	Food	42	FCQ-S
Montenegro et al. (63)	A-L DLPFC	2 mA	1	20 min	CO	Single	Food	9	VAS
Ray et al. (64)	A-R DLPFC	2 mA	1	20 min	CO	Double	Food	18	VAS
Ray et al. (65)	A-R DLPFC	2 mA	1	20 min	PR	Single	Food	74	FCT
Chen et al. (66)	A-R IFG	1.5 mA	1	20 min	PR	Single	Food	57	FCQ-S
Batista et al. (67)	A-R DLPFC	2 mA	5	20 min	PR	Double	Cocaine	36	OCCS
Boggio et al. (68)	A-R/L DLPFC	2 mA	1	15 min	PR	Double	Marijuana	25	VAS
Shahbabaie et al. (69)	A-R DLPFC	2 mA	1	20 min	CO	Double	Meth	30	VAS
Wang et al. (70)	A-OL	1.5 mA	1	20 min	PR	Single	Heroin	20	VAS
Shahbabaie et al. (71)	A-R DLPFC	2 mA	1	26 min	CO	Double	Meth	15	VAS
Taremian et al. (72)	A-R DLPFC	2 mA	10	20 min	PR	Single	Opium	60	DDQ
Anaraki et al. (73)	A-R DLPFC	2 mA	5	20 min	PR	Single	Meth	30	DDQ

A-R, anodal-right; A-L, anodal-left; DLPFC, dorsolateral prefrontal cortex; OL, occipital lobe; IFG, inferior frontal gyrus; CO, crossover; PR, parallel; Meth, methamphetamine; VAS, Visual Analogue Scale; sQSU, standardized Questionnaire of Smoking Urges; OCCS, Obsessive-Compulsive Cocaine Scale; LTS, Likert-type scale; UTS, urge to smoke; DDQ, The Desire for Drug Questionnaire; AUQ, Alcohol Urge Questionnaire; OCDS, Obsessive Compulsive Drinking Scale; AAAQ, Alcohol Approach and Avoidance Questionnaire; FCQ-S, Food Craving Questionnaire State; FCT, Food Craving Task.

General Effect Size (Hedges' *g*) on Craving

The test for heterogeneity was significant (Q=55.44, df=31, p=0.004, I^2=44.08%), showing that there was heterogeneity between the study findings. In order to address the heterogeneity problem, we used a random effects analysis for the meta-analysis because of its conservative estimate and appropriate nature for generalization (42). As shown in **Figure 2**, this analysis revealed a standardized effect size (Hedges' *g*) of 0.536 [95% confidence interval (CI): 0.389–0.683], indicating a medium effect size favoring active stimulation over sham stimulation (z=7.153, p<0.001). The variation caused by the true difference in the effect accounts for 44.08% of the total variation with medium heterogeneity in this study, which implies that there may be important potential modulating variables and subgroup analysis was needed to test for the moderating effects (74, 75).

Influence of Moderators

Stimulation Parameters (Stimulation Site, Current Intensity, Number of Sessions)

The left or right DLPFC was the anodal stimulation site in all studies except two (66, 70), which were excluded from this subgroup analysis, leaving 13 units for the left DLPFC and 21 units for the right DLPFC. Comparisons were made between the left and right DLPFC regardless of the cathodal site. The results showed that the difference between the left DLPFC and right DLPFC was not significant (Q=2.673, p=0.102) although Hedges' *g* for the left DLPFC was 0.402, while the Hedges' *g* for the right DLPFC was 0.636.

As far as current intensity was concerned, a comparison was made between 1 mA and 2 mA [six studies with 1 mA, 24 studies with 2 mA, two studies was not included because the stimulation current was 1.5 mA (66, 70)]. There was no significant difference

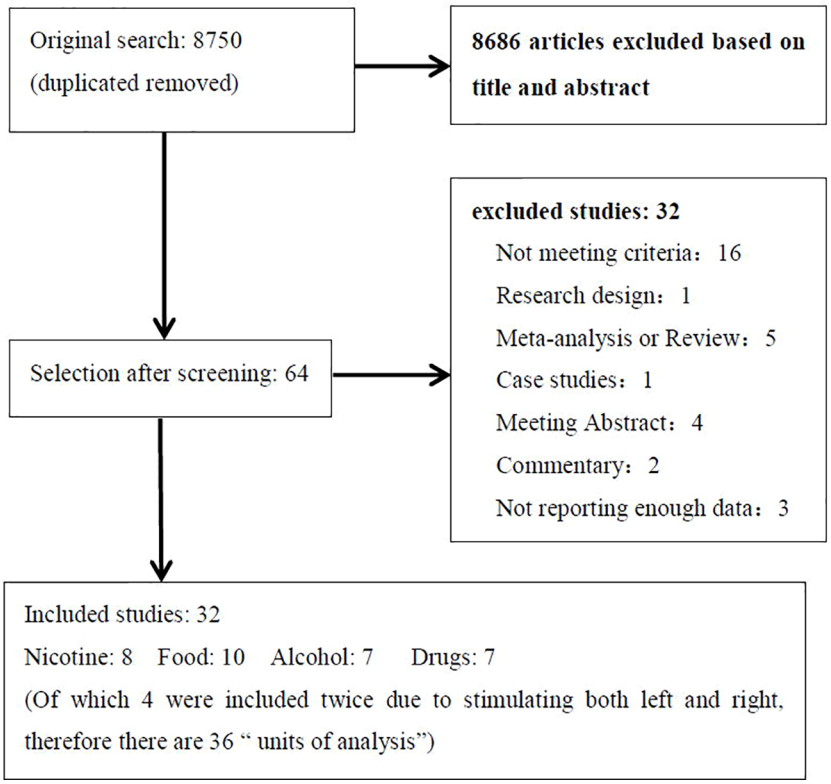

FIGURE 1 | Flow diagram showing the search and selection procedure that was used for this meta-analysis. It showed the reasons for exclusion in "not meeting criteria" and final numbers of study included in the meta-analysis.

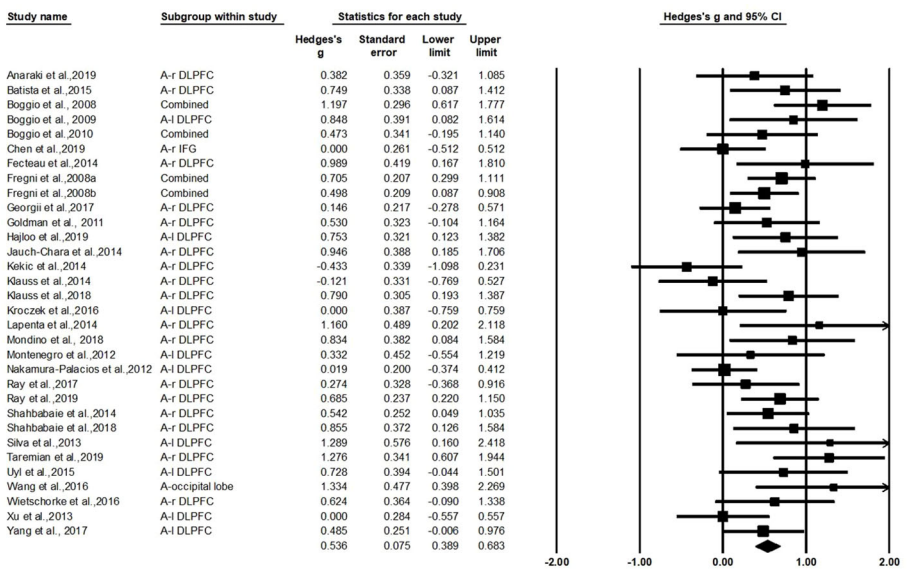

FIGURE 2 | The overall effect of transcranial direct current stimulation (tDCS) on craving.

in effect size between 1 and 2 mA (Q=1.635, p=0.201); the Hedges' g was 0.381 for 1 mA and 0.592 for 2 mA (**Table 2**).

A comparison was also made between the number of sessions (single-session *vs*. multi-session) (21 studies with one session, and 11 studies with multiple sessions). The Hedges' g for single-session stimulation was 0.443, while the Hedges' g for multi-session stimulation was 0.751 (see **Figure 3**). The results revealed a significant difference in effect size between the two subgroups (Q=4.261, p=0.039) (see **Table 2**). We found multiple session of tDCS intervention may have a better effect on craving.

Duration of Single Stimulation and All Stimulation

We performed a meta-regression analysis based on the duration of single stimulation and Hedges' g. Regarding the duration of single stimulation, we found a marginally significant result that the longer the stimulus lasts, the more the craving decreases (Q=2.832, β=0.023, p=0.092) (see **Figure 4**). Due to only the presence of only five values and the marginally significant result, we extended the analysis to include total stimulation time (duration of single stimulation × number of sessions) to obtain a more credible result. Regarding total stimulation duration, we found that longer stimulation was related to greater effect size (Q=8.505, β=0.003, p=0.004) (see **Figure 5**).

Substance Type

We compared the results for alcohol users, nicotine users, drug users, and food bingers. The results did not show a significant difference in effect size between substance types [Q (3)=4.121, p=0.249] (see **Table 2**).

When substance type was divided into two categories, substance and food, where alcohol, nicotine, and drugs were combined into substance, the Hedges' g for substance was 0.616, while the Hedges' g for food was 0.371. However, there was no significant difference between substance and food (Q=2.474, p=0.116).We also compared food with other substance types separately, and found that there was an obvious difference between drugs and food (Q=4.094, p=0.043), but no difference between alcohol and food nor nicotine and food.

TABLE 2 | Results of subgroup analysis (random-effects model).

Moderators	*k*	Hedges' *g*	95% CI	Heterogeneity	
				Q_B	*P*
Stimulation site				2.673	0.102
Left DLPFC	13	0.402***	[0.195, 0.609]		
Right DLPFC	21	0.613***	[0.446, 0.826]		
Current intensity				1.478	0.224
1 mA	6	0.381***	[0.102, 0.661]		
2 mA	24	0.583***	[0.417, 0.748]		
Number of sessions				4.261	0.039
Single-session	21	0.444***	[0.272, 0.615]		
Multi-session	11	0.751***	[0.515, 0.987]		
Substances type				4.121	0.249
Nicotine	8	0.555***	[0.309, 0.800]		
Alcohol	7	0.587***	[0.166, 1.008]		
Food	10	0.371***	[0.122, 0.620]		
Drugs	7	0.742***	[0.483, 1.000]		
Double or single				0.405	0.524
Double	18	0.582***	[0.404, 0.761]		
Single	14	0.483***	[0.236, 0.730]		
Crossover or parallel				0.913	0.339
Crossover	16	0.471***	[0.266, 0.677]		
Parallel	16	0.614***	[0.405, 0.823]		

***$p < .01$.

Study Design (Crossover-Design or Parallel-Design; Double-Blind or Single-Blind)

A comparison was made between study designs. No significant difference was found between crossover and parallel designs (Q=0.913, p=0.339) or between double and single blind designs (Q=0.405, p=0.524) (see **Table 2**). However, we found that the number of sessions may influence the results because most (8/11) multiple stimulation studies were parallel designs. Therefore, we compared the effects of the crossover design and parallel design by using the number of sessions as a covariate. The result confirmed no significant difference in effect size between crossover and parallel designs (Q=1.77, p=0.183).

Evaluation of Publication Bias

Egger's regression test was performed to empirically examine the presence of any publication bias. A publication bias was observed (p=0.031), as shown in **Figure 6**. A funnel plot was created (76) in which the measure of precision (standard error) of the effect size (Hedges' g) was shown with a trim-and-fill procedure applied (46). The results of the trim and fill method highlighted that there are seven "missing" effect sizes on the left side of the funnel plot. After adjustment, the analysis indicated an average effect size of 0.416 (95% CI: 0.262–0.570), which was comparable to the original result (Hedges' g=0.536), suggesting that the publication bias influenced our results lightly.

DISCUSSION

Based on 32 empirical studies, we performed a meta-analysis to review the effect of tDCS on substance craving and the influence of potential moderators. The results revealed a significant medium effect size (Hedges' g=0.536 or 0.416 after adjusting for publication bias) favoring real tDCS stimulation over sham stimulation in the reduction of craving. Furthermore, the number of sessions could significantly influence the effect of tDCS, favoring multi-session over single-session treatment. Other modulators appear to have no influence on craving reduction, such as stimulation site, current intensity, substances type, or study design.

Effect of Transcranial Direct Current Stimulation on Reducing Craving and Its Possible Mechanism

Our findings confirmed that there was significant decrease in craving after tDCS stimulation of the DLPFC, which supports previous findings (39, 40, 77, 78).

One possible mechanism by which DLPFC stimulation decreases craving is an increase in "cognitive control." As we

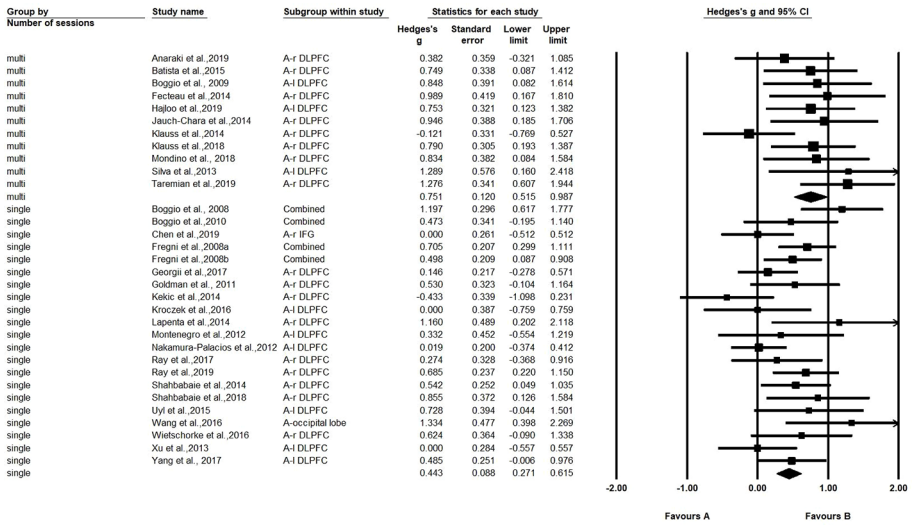

FIGURE 3 | The difference between single session and multi-session.

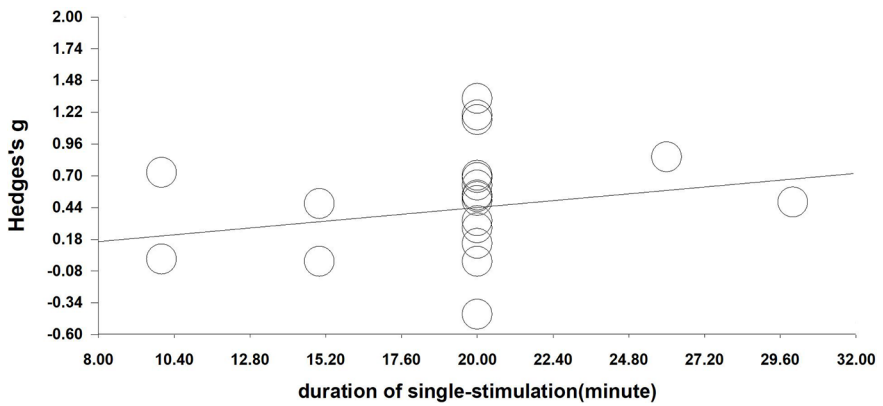

FIGURE 4 | Regression of the duration of single-stimulation on the effect size of neuromodulation of craving. (Q=2.832, β=0.023, p=0.092).

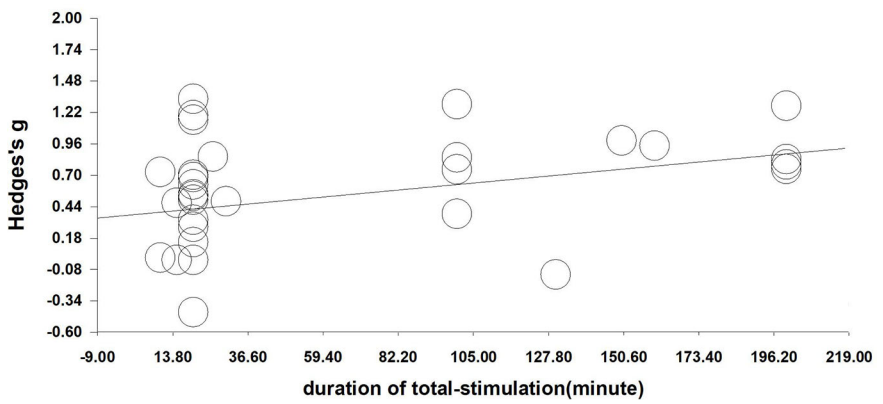

FIGURE 5 | Regression of sessions on the effect size of neuromodulation of craving. (Q=8.505, β=0.003, p=0.004).

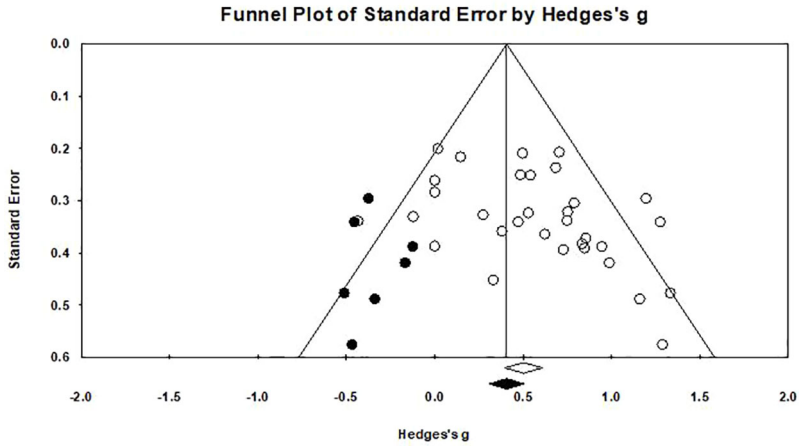

FIGURE 6 | Funnel plot for a model without moderators (random-effects model), the solid circles in the funnel plot represents the seven studies that were trimmed to the left. The solid diamond on the abscissa indicate that the correction effect size is 0.416, and the hollow diamond on the right represents the original effect size is 0.536.

know, the executive control network (ECN, including the DLPFC, orbitofrontal cortex, and anterior cingulate cortex) plays an important role in human executive control (79–81), including craving control (82, 83). tDCS can induce and regulate neural plasticity (84). Bilateral tDCS with anodal stimulation of the right DLPFC increases the intra-network functional connectivity (71). Cavaliere et al. (85) also showed that anodal tDCS over the DLPFC increases intra-network co-activation of the ECN. Thus, increased ECN activity and functional connectivity of the ECN could help individuals to decline or reduce craving—in other words, after changing the activity of the DLPFC subjects were able to better suppress their urges through its connections to the ECN.

Stimulation of the prefrontal cortex-stimulated dopaminergic pathways is another explanation. Addicted subjects were hardly roused by nondrug-related stimuli other than the substance they were addicted to, with decreased dopamine function disrupting frontal inhibition (86). Researchers have found that DLPFC stimulation alters the activation and functional connectivity of the cortical and subcortical reward systems in healthy individuals (87). Simultaneously, the membrane potential of pyramidal cells are regulated by anodal and cathodal tDCS, which alter the glutamate tone in the cortex, which is considered to correlate with GABA release, to restore the best excitation/inhibition balance to achieve the best steady-state plasticity in learning and cognition (88). Thus, tDCS stimulation of the DLPFC may improve the behavior of substance-dependent individuals by modulating the activities of brain regions such as the anterior cingulate cortex (ACC), the orbitofrontal cortex (OFC), and caudate nucleus.

However, it is not clear how tDCS affects substance and food craving. Further studies are needed to explore the psychological process and neural mechanisms underlying tDCS stimulation at the same time. A promising method can be seen in studies that use functional magnetic resonance imaging (fMRI) to explore the changes caused by tDCS stimulation (87, 89).

Influence of Moderators

Among all the stimulation parameters, our subgroup analyses showed that multi-session tDCS had a significant greater effect on reducing craving than single-session tDCS, which is in agreement with Song et al.'s (40) and Kim et al.'s (77) findings. This suggests that the effects of tDCS on craving reduction can be cumulative. However, it is worth noting that the multiple stimulations included in the meta-analysis were all performed separately and not on the same day, because one study reported there was no benefit of twice-daily stimulation over once-daily stimulation in increasing cortical excitability (90).

Although not significant, we found a trending positive influence of the duration of single stimulations (p=0.092). The non-significant results may be because there were sufficient data in the analysis. After calculating the total stimulation duration, there was a trend that longer durations were related to larger Hedges' g (p=0.004). The result is consistent with the finding that the duration of stimulation may enhance its efficacy in given applications (91). However, more evidence of the effect of stimulation duration is needed in future.

According to the substance type subgroup analyses, the effects on drug users were the biggest (Hedges' g=0.742), followed by alcohol and nicotine (Hedges' g=0.587 and Hedges' g=0.555), and then food (Hedges' g=0.371), suggesting that the process of food addiction may differ from substance addiction. In some study, food addiction has a bearing on stress exposure with damage to the hippocampus (92) and patients with food addiction were

easily affected by a sad mood (93). Stress, cognition, and emotion recognition may play a more important role in food addiction than other addictions meaning the treatment of food addiction may be more complex.

As for other factors, since the beneficial effects of tDCS vary with the pathology (94), we speculate that tDCS may be more effective for severely addictive substances than lightly addictive substances. That is to say that the severity of addiction may be a factor influencing the effect size of tDCS. However, the severity of addiction cannot be determined in the current study because there is no consistent standard for severity across various addictions and we have difficulty extracting effective data to distinguish the severity of addiction in the current literature.

Although we tried to explore various regulatory variables, there are still some possible influencing variables, which are not discussed in this paper, such as tDCS combined with other treatments (cognitive bias modification, emotional regulation). tDCS combined with cognitive bias modification has shown limited effect on treatment outcomes (95–97). Moreover, emotional regulation can help the curative effects of tDCS (98–101); after emotion regulation training, using tDCS has better results (100). Thus, different substance addictions may affect the participants' emotional management, in turn altering the effects of tDCS. Due to the limited number of studies and the great heterogeneity among the literature, they were not included in the current meta-analysis. However, these works are of great significance and may be a new trend for tDCS research that deserves further research.

Regarding stimulation site, we found that there was no difference between right/anodal, left/cathodal and left/anodal, right/cathodal protocols for substance, and food dependence, supporting the conclusion of Jansen et al. (39) that putting the anode on the left or right would not affect the results of treating addictive disorders. However, although not significant, we could not ignore that the right DLPFC may be a better choice for most individuals because of the higher Hedges' g (0.613) than that of the left DLPFC (0.402). Boggio et al. (68) showed that only the right anode + left cathode montage was significantly associated with a reduction of craving for marijuana. It seems there may have the possibility that the effect of stimulation site will depend on the health status of the subject and the type of substance dependence. Other parameters, including current intensity (1 *vs.* 2 mA), study design (crossover or parallel, and double blind or single blind) had no significant difference.

Limitations and Future Directions

In this meta-analysis, some limitations should be considered. First, the number of libraries and articles available was limited. Although we searched for accessible published articles, we still could not be sure that all relevant research was included. Second, the main effect of tDCS is only a medium effect size, suggesting more empirical studies are needed to explore the modulators of tDCS treatment of craving control, e.g., the substance type, addiction severity, and abstinence duration. Unfortunately, the majority of included studies did not report the abstinence duration or addiction severity; therefore, we could not examine its moderating effect in tDCS performed for craving control. Thus, an in-detail report of dependence-related data would be very important in the future. Third, the tools for measuring cravings need to be improved. In most studies, craving was measured using self-report questionnaires or visual analogue scales, which are subject to socially desirable answers. Some objective methods to measure drug craving (e.g., physiological measurement) would be very helpful in investigating the effect size of craving reduction.

CONCLUSION

The current meta-analysis provided evidence that tDCS protocols improved the symptoms of substance and food dependence as indicated by reduced craving, but some modulators does influence the effect of tDCS treatment on addiction. In general, our findings suggested that 2 mA stimulation may be better than 1 mA; multi-session interventions may be better than single-session. We also suggested that tDCS may be not ideal for the treatment of food addiction, implicating that compared with substance addiction, food addiction may be different in nature and needs complex treatment such as tDCS combined with psychological interventions. In clinic, the optimal treatment plan for any specific type of addiction still needs be to be examined in depth in future.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/ **Supplementary Material**.

AUTHOR CONTRIBUTIONS

Conceiving of the design framework: JC and ZZ. Writing the manuscript: JC and JQ. Revising the manuscript: JC, JQ, QH and ZZ.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2020.00598/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Increased Amplitude of Low-Frequency Fluctuation in Right Angular Gyrus and Left Superior Occipital Gyrus Negatively Correlated With Heroin Use

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Abnormal amplitude of low-frequency fluctuation has been implicated in heroin addiction. However, previous studies lacked consistency and didn't consider the impact of confounding factors such as methadone and alcohol. Fifty-one heroin-dependent (HD) individuals and 40 healthy controls underwent resting-state functional magnetic resonance imaging. The 'amplitude of low-frequency fluctuation' (ALFF) value was calculated and support vector machine (SVM) classification analysis was applied to analyze the data. Compared with healthy controls, heroin addicts exhibited increased ALFF in the right angular gyrus (AG) and left superior occipital gyrus (SOG). A negative correlation was observed between increased ALFF in the right angular gyrus and left superior occipital gyrus and the duration of heroin use (p_1=0.004, r_1=-0.426; p_2=0.009, r_2=-0.361). Moreover, the ALFF in the right AG and left SOG could discriminate the HD subjects from the controls with acceptable accuracy (Acc_1=64.85%, p_1=0.004; Acc_2=63.80%, p_2=0.005). HD patients showed abnormal ALFF in the brain areas involved in semantic memory and visual networks. The longer HD individuals abused heroin, the less the ALFF of associated brain regions increased. These observed patterns suggested that the accumulative effect of heroin's neurotoxicity overpowered self-recovery of the brain and may be applied as a potential biomarker to identify HD individuals from the controls.

Keywords: resting state fMRI, amplitude of low-frequency fluctuation (ALFF), heroin addicts, addiction, heroin use

INTRODUCTION

Heroin addiction is a chronic, relapsing brain disease characterized by addicts' compulsive heroin seeking and consuming in spite of serious negative consequences (1). Over the past decades, various neuroimaging studies have revealed extensive structural and functional disruption in heroin-dependent (HD) individuals. Structural MRIs have revealed the impaired white matter integrity

within the right frontal sub-gyral, corpus callosum, thalamic radiation, and inferior longitudinal fasciculus (2, 3), diminished regional homogeneity within the bilateral medial orbitofrontal cortex (OFC) and bilateral cuneus (4), and the reduced gray matter volume within the precuneus, cuneus, and right dorsolateral prefrontal cortex (DLPFC) (5, 6). Functional MRIs (fMRI), a classic method for assessing hemodynamic changes after increased neural activity, have been widely applied in neuroimaging with its merits of ever-increasing availability, its noninvasive nature, and its relatively high spatiotemporal resolution (7). Resting-state fMRI could explore the correlation of spontaneous neural excitation activity between different brain regions under rest (subjects didn't undertake any language, cognitive, or motor tasks) (8). Resting-state fMRI disclosed increased functional connectivity (FC) between the anterior cingulate cortex (ACC) and nucleus accumbens and between the OFC and amygdala, and reduced FC between the ACC and prefrontal cortex (PFC) and between the OFC and PFC (9).

In 1995, Biswal and his co-workers first found that the spontaneous Low-Frequency (0.01–0.08 Hz) Fluctuations (LFFs) in resting-state fMRIs were most frequently found between the right and left primary motor cortex (10). After that, amplitude of low frequency fluctuation (ALFF) was further improved by Zang et al. (11). It was represented as the square root of the power spectrum in a low frequency range (0.01–0.08 Hz), which could evaluate the brain's physiopathological state by computing the regional intensity of spontaneous fluctuation in Blood Oxygenation Level Dependent (BOLD) signal at rest. Studies applied to various diseases such as attention deficit hyperactivity disorder (11), epilepsy (12), Parkinson's (12, 13), Alzheimer's disease with depression (D-AD) (14), schizophrenia (15), and somatic depression (16) suggested that ALFF was a reliable approach to explore regional spontaneous neural activity (SNA) in resting-state.

ALFF measurement was firstly applied on HD subjects by Jiang et al. to study heroin-use-related SNA alteration (17). They studied 24 HD subjects (all subjects were treated with methadone during abstinence) and 24 control nondrug-using (CN) subjects and found that, compared with controls, the HD group had abnormal ALFF in multiple regions. Moreover, increased ALFF in the bilateral parietal lobe had a significantly positive correlation with the methadone dose, which suggested reduced ALFF may arise from taking heroin and the increased ALFF in the bilateral parietal lobe from the methadone treatment. Subsequently, the relationship between disrupted local neural activity and its functional organization pattern in resting-state was studied by Wang et al. with 17 male HD individuals and 15 controls (18). Their studies demonstrated that the ALFF value of the right caudate was negatively correlated with heroin use and an abnormal lateral PFC-dorsal ACC connection in the HD group, which suggested an altered balance between local neuronal assemblies' activity. However, these studies above had some flaws. 1) Participant sample sizes were too small. The sample size of participants were less than fifty (HD individuals <30). Chen et al. assessed test-retest reliability and replicability of resting-state fMRI and found repeatability of studies with small sample sizes (<80) was low (19). 2) Participant samples were heterogenous. Alcohol abuse of participants wasn't matched. A previous study demonstrated the degree to which alcohol use affected the reduction of thalamic grey matter volume in opioid-dependent subjects (20). In Jiang et al's study, the use of methadone complicated the interpretation of their results. Methadone plays a vital role in the destruction of white matter integrity and resting state FC in heroin users under Methadone maintenance treatment (MMT) (21–24). In Wang et al's study, all participants were male, which cannot comprehensively reflect ALFF differences in HD individuals. Therefore, these flaws above limited the reliability of these results.

To avoid the flaws from previous research, relatively larger (51 HD subjects and 40 CN subjects) and more homogeneous (unmedicated, cigarette, and alcohol abuse matched with professional questionnaire) samples were collected in the current study to investigate the ALFF differences in the whole brain between the HD and CN groups. In addition, to investigate potential biomarkers for heroin addiction, the support vector machine (SVM) classifier, which had already been successfully used in fMRI studies about heroin addiction (25, 26), was adopted on clusters that showed significant ALFF differences to discriminate HD subjects from healthy controls.

MATERIALS AND METHODS

Subjects

We initially recruited 62 HD subjects (20-55 years old) and 44 age- and sex-matched healthy controls. Eleven HD subjects and four CN subjects were removed because of excessive head motion. Therefore, the present study included 51 HD subjects and 40 CN subjects. All HD subjects come from Pingtang Mandatory Detoxification in Changsha City, Hunan Province. All their diagnoses were confirmed with the fifth edition of Diagnostic and Statistical Manual on Mental Disorders (DSM-V) and after that had accepted a short-term (<6 months) (27) compulsory abstinence. During abstinence, the participants were treated without Methadone, only with education and physical exercise. Inclusion criteria included: ranging in age from 20 to 55 years old, receiving at least an elementary school education, being right-handed, and having no history of neurological and psychiatric disease other than drug addiction. Exclusion criteria for all subjects included: head trauma history, other substance use except nicotine and alcohol in the past 5 years, and contraindications to MR scanning. During the MRI examination interval, Fagerstrom Test for Nicotine Dependence (FTND) and Alcohol Use Disorders Identification Test (AUDI) were obtained for all participants (28, 29). The history of heroin use, including the duration of heroin use, dosage of heroin use, and abstinence periods, were also recorded from HD individuals.

This study was approved by the Ethics Committee of the Second Xiangya Hospital, Central South University. Informed consent was also obtained from each subject.

MR Imaging Acquisition

All MRI examination data were obtained on a 3T Siemens Skyra MRI scanner (Magnetom Skyra, Siemens, Germany) with a 32-channel head coil. During the scans, participants were instructed to remain still, keep their eyes closed, and not think of anything in particular. The scanning sessions included: (i) localization; (ii) T1-weighted three-dimensional magnetization-prepared rapid acquisition with gradient echo (3D MPRAGE) (176 sagittal slices, slice thickness=1 mm, gap=0 mm, field of view (FOV)=256 mm×256 mm, repetition time (TR)=1450 ms, echo time (TE)=2.03 ms, inversion time (TI)=900 ms, flip angle=30°, and voxel size=1×1×1mm^3); and (iii) resting-state fMRI sessions (36 axial slices, thickness=4 mm, FOV=220 mm×220 mm, TR=2000 ms, TE=30 ms, flip angle=80°, and 225 volumes). Participants stayed supine with foam padding between their head and the coil to minimize head movements.

Data Processing and ALFF Calculation

Image data preprocessing was performed with Statistical Parametric Mapping (SPM 8) (SPM8, http://www.fil.ion.ucl.ac.uk/spm) and GRETNA (30). We firstly discarded the initial 10 scan volumes to allow for steady-state magnetization and then corrected for slice timing and head motion. We excluded participants whose head motion exceeded 2 mm or rotation exceeded 2°. After the resting state image registered with the T1 structure image, all images were reoriented into the AC-PC axis and then spatially normalized to Montreal Neurological Institute (MNI) space with the diffeomorphic anatomical registration through the exponential lie algebra (DARTEL) registration method (31). All images were smoothed using a 6mm half-height full-width Gaussian kernel. After that, linear detrending was conducted and covariates including white matter, cerebrospinal fluid, and 24 head movement parameters were subsequently removed.

The ALFF analysis was carried out with the Data Processing Assistant for Resting-State fMRI (DPABI, 2.3, Advanced edition) (32). The filtered time series of each voxel was transformed into the frequency domain with a Fast Fourier Transform and the power spectrum was then obtained. By measuring the square root of the signal across 0.01-0.08 Hz for each voxel (11), we obtained ALFF values. To reduce the influence of individual variation in ALFF values, the ALFF of each voxel was further divided by the global mean of ALFF values for each participant within the default brain mask from the DPABI. This made a standardized whole-brain ALFF map.

Statistical Analysis

We performed two-sample *t*-tests to assess the differences in age, years of education, cigarette smoking, and alcohol use, and a chi-square test to assess the difference in gender between the HD and CN group with SPSS (version 22.0). The significance level was set at $p<0.05$. Two-sample *t*-tests were performed to compare ALFF data between the HD and CN group with DPABI to find significantly different brain regions. The significance level was set at $p<0.001$, cluster size>119 voxels (Gaussian random field corrected (GRF)).

Correlation Analyzes

To identify the relationship between ALFF and history of heroin use, the average ALFF values of all abnormal clusters detected by group comparisons were extracted separately in every HD subject, and then partial correlation was applied to reveal the relationships between these ALFF values and the duration of heroin use, dosage of heroin use, and abstinence periods. Covariables included age, gender, years of education, smoking, and drinking. Significance levels were set at $p<0.05$ (two-tailed).

Support Vector Machine (SVM) Analysis

To test whether the ALFF values of significant clusters could highlight potential biomarkers to identify HD individuals from the controls, SVM was conducted with matlab. A five-fold cross-validation method was used to conduct the SVM. The Classification Learner Tool svmtrain and svmclassify function in MATLAB was performed to classify ALFF values of the nine sub-ROIs using 10-fold cross validation. (https://stackoverflow.com/questions/13804833/using-matlab-svmtrain) The SVM kernel used was linear. This framework applies the whole data set split into 10 folds/subsets randomly; each subset consisted of 1/10 HD subjects and 1/10 healthy controls. One subset was used as test dataset, and the remaining nine subsets were used as a training dataset. The training dataset was used to train a classifier, which was then used to predict the test dataset. The training set and the test set are independent for each cross-validation. Accuracy, sensitivity, and specificity were computed to quantify the cross-validated prediction performance of these classifiers. After that, a permutation test with 1000 permutations was applied to test the significance of the classification results.

RESULTS

Demographics and Clinical Characteristics of the Participants

Fifty-one HD subjects and 40 CN subjects were included in this study. We performed two-sample *t*-tests and chi-square tests to assess the demographics and clinical characteristics differences between the HD group and CN group. The significance level was set at $p<0.05$. There is no significant difference between the HD group and CN group in age (mean±SD) (42.90 ± 6.49 for HD group; 41.85 ± 8.13 for CN group; $t=0.686$, $p=0.494$), gender (36 male and 15 female for HD group; 28 male and 12 female for CN group; $\chi^2=0.007$, $p=0.951$), the years of education (9.33 ± 2.20 for HD group; 9.98 ± 1.84 for CN group; $t=1.477$, $p=0.143$), Fagerstrom Test for Nicotine Dependence (6.10 ± 2.21 for HD group; 5.53 ± 2.63 for CN group; $t=1.128$, $p=0.262$), or Alcohol Use Disorders Identification Test (1.50 ± 3.57 for HD group; 3.25 ± 4.39 for CN group; $t=1.128$, $p=0.157$) as shown in **Table 1**.

Two-sample *t*-tests were performed to compare ALFF data between the HD and CN group with DPABI. Group differences are shown in **Table 2** and **Figure 1**. In comparison with the CN

TABLE 1 | Demographic information and characterization.

	HD(n=51)	CN(n=40)		*p*
Age (years)	42.90±6.49	41.85±8.13	*t*=0.686	0.494[a]
Gender (male/female)	36/15	28/12	χ^2=0.007	0.951[b]
Education (years)	9.33±2.20	9.98±1.84	*t*=-1.477	0.143[a]
FTND	6.10±2.21	5.53±2.63	*t*=1.128	0.262[a]
AUDI	1.50±3.57	3.25±4.39	*t*=-1.573	0.120[a]
Duration of heroin use (years)	15.65±7.92	N/A	N/A	N/A
Dosage of heroin use (g/day)	1.25±3.85	N/A	N/A	N/A
Abstinence periods (days)	26.73±28.56	N/A	N/A	N/A

[a]*Two-sample* t-*test;* b *Chi-square test. Significance level was set at* p<*0.05. There are no statistically significant differences between the HD and CN group based on demographic information and characterization; HD, heroin-dependent; CN, control nondrug-using; FTND, Fagerstrom Test for Nicotine Dependence; AUDI, Alcohol Use Disorders Identtification Test; N/A, not applicable.*

TABLE 2 | Regions with increased ALFF in HD group compared with CN group.

Brain region (AAL)	Peak *t*-value	Cluster Size (voxels)	Peak MNI Coordinates		
			X	Y	Z
Occipital_Sup_L	3.7223	156	-12	-81	21
Angular_R	3.6889	146	39	-63	27

Statistical threshold was p<0.05 corrected for multiple comparisons by GRF. A combination threshold of voxels' p<0.005 and cluster size>119 voxels was considered significant; Coordinates are located in MNI space; L, left hemisphere; R, right hemisphere; AAL, Anatomic-Automatic-Labeling template.

group, the significant ALFF increases in the HD group were in the right angular gyrus (AG) and left superior occipital gyrus (SOG). No significant decrease cluster was found.

Correlation Analyzes

Partial correlation was performed to assess relationships between above-significant differences ALFF values and the duration of heroin use, dosage of heroin use, and abstinence periods in HD individuals. Covariates included age, gender, years of education, smoking, and drinking. The length of duration of heroin use in HD subjects is significantly negatively correlated with ALFF in the right AG as shown in **Figure 2A** (p=0.004, r=-0.426). The length of duration of heroin use in HD subjects is also significantly negatively correlated with ALFF in the left SOG as shown in **Figure 2B** (p=0.009, r=-0.361).

SVM Results

The SVM results were conducted with the five-fold cross-validation. The average accuracy for ALFF values in right AG and left SOG were 64.85% and 63.80%. The average sensitivity were 60.94% and 67.21%. The average specificity were 70.82% and 58.30%. The SVM classifier was then validated with a permutation test. After running1000 times with a 95% confidence interval, the average accuracy of the right AG and left SOG were 0.2747–0.6043 (p =0.004) and 0.2067 - 0.6044 (p =0.005).

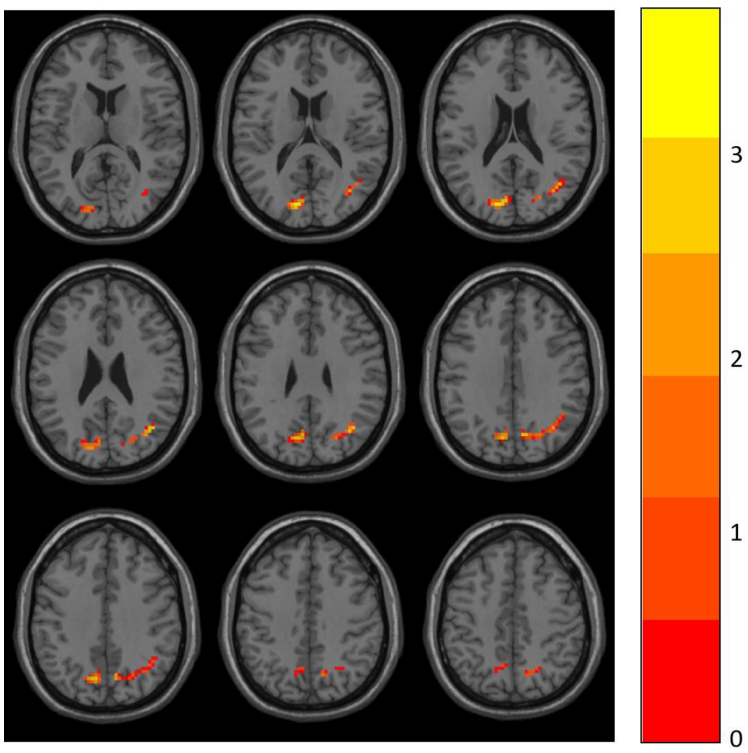

FIGURE 1 | ALFF differences between the HD and CN group. The differences map at the given threshold (p<0.001, cluster size>119 voxels, Gaussian random field corrected) was shown. Blue indicates HD individuals had decreased ALFF compared with controls and the red indicates the opposite.

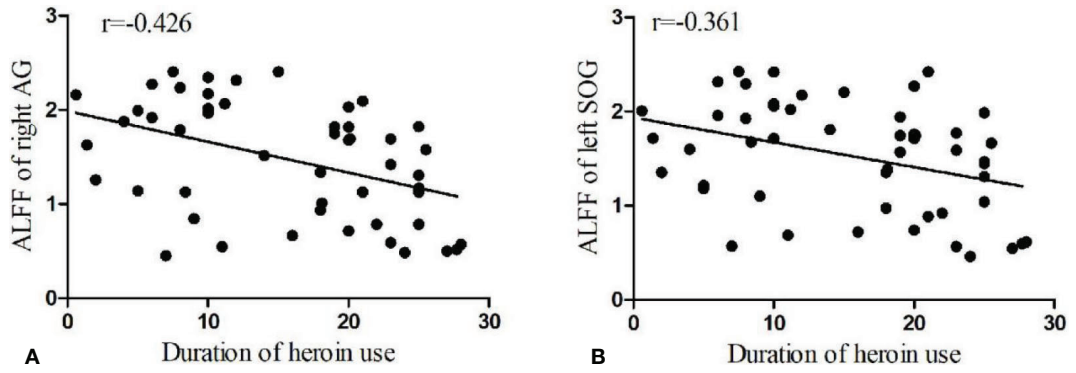

FIGURE 2 | Bivariate scatter plots. A shows the negative correlation between the duration of the heroin use and the ALFF of the right AG (p=0.004, r=-0.426), and B the negative correlation between the duration of heroin use and the ALFF of the left SOG (p=0.009, r=-0.361).

DISCUSSION

In the present study, we compared ALFF between the abstinent HD group and the CN group and revealed that the HD group showed significantly increased ALFF in the right AG and left SOG. In addition, in the HD group, the length of duration of heroin use was significantly negatively correlated with the ALFF values of right AG and left SOG. Meanwhile, increased ALFF in the right AG and left SOG allowed for correct identification of the HD individuals from the controls with relatively high sensitivity, specificity, and accuracy.

The AG plays an important role in semantic memory (33, 34), which is involved in a series of tasks from single word recognition (35) to sentence-level comprehension (36). Semantic memory refers to a major division of long-term memory that includes knowledge of facts, events, ideas, and concepts. Little is known about the effects of chronic and excessive heroin consumption on the organization or extent of the pre-existing semantic networks. Previous studies used semantic memory association tasks to assess the implicit cognition of substance abusers (37). Implicit means that it cannot be recognized by introspection. It is a trace of people's past experiences. Implicit cognition regulates people's emotions, cognition, and reactions to likes and dislikes in society. Positive comments on drugs increased the likelihood of drug abuse, while negative evaluations increased the likelihood of avoiding drugs. The researchers believed that the current memory activation mode often dominated human behavior, and this activation mode was implicit. In other words, this activation mode was at a relatively spontaneous level. The experience of taking drugs many times will make it easier for people to establish and consolidate related memory connections. The next time they encountered drug-related cues, the idea of the drug-using experience in the addict's brain will be automatically activated, which then triggers a memory-based concept-linked reaction. Therefore, researchers can use the semantic memory association task to assess the strength of drug-related memory connections between addicts. Previous research found that the results of the semantic memory association task can effectively predict alcohol and cannabis abuse in high-risk adolescents (38). A study found that, compared to the control group, cannabis and other drug abusers were more likely to have positive associations with clue topics, but non-abusers' associations with drugs were usually harmful or negative. After receiving drug treatment, drug abusers will have more negative associations with the consequences of drug abuse (39). The above research showed that the research method of semantic memory association tasks involved in spontaneous and drug-related cognition provided individual differences' indicators of associative memory. These indicators could help predict corresponding behaviors, including relapse. An early observational study (in the form of self-rating and observer-rating questionnaires) demonstrated that opiate addicts showed an increase in semantic affective memory (40). Semantic memory and novel semantic encoding impairment could result from alcohol dependence, which implied that specific impairment in category and feature learning may reflect a genuine deficit of new learning in the semantic memory (41, 42). In addition, angular gyrus is part of the default mode network, which is impaired in heroin addicts (43, 44). The increased ALFF in this region observed in our study was consistent with the previous report (17). In the present study, abnormal ALFF observed in the right angular gyrus may reveal pathological activation of semantic memory. After short-term withdrawal, heroin addicts will have more associations with drug-related cues, but whether this association was positive or negative remained unclear because of a lack of semantic memory associative tasks in our current study. To the best of our knowledge, there is no more research about the semantic memory of HD individuals in the literature. Therefore, further neuroimaging research is needed to clarify the relationship between heroin addiction and semantic memory.

The ALFF was also increased in the left SOG in our study, which is consistent with previous studies (18). The left SOG was an important part of the dorsal extrastriate cortex involved in higher level visual association processes (45). Many neuroimaging studies reported that the activity in visual brain

regions was significantly linked to heroin-related cues exposure, a therapeutic effect, and prediction of relapse (46–49). Our result that the ALFF of the visual related brain cortex of the HD group increased implied that drug cue-induced craving, which is one of the most stable elements that leads to continued consumption and relapse across substances (50), is vital among HD individuals after short-term abstinence.

The SVM results indicated that increased ALFF in the right AG and left SOG can be used for differentiating the HD individuals from the controls with a relatively high sensitivity, specificity, and accuracy. Although previous studies suggested that a sensitivity of less than 70% is not so excellent (51), significance of the classification outcomes suggested that the result is acceptable in the present study. Hence, we inferred that increased ALFF in the right AG and left SOG can be used as a potential biomarker to identify the HD individuals from the controls.

In the current study, we first found that the length of duration of heroin use is significantly negatively correlated with the increased ALFF values of the right AG and left SOG. Previous studies only demonstrated that decreased ALFF in the caudate was in negative correlation to both the duration of the heroin use and the heroin daily dosage, and average ALFF in the elevated regions may respond to the effectiveness of methadone (18). In our study, the increase in ALFF value could rule out the effect of treatment because the HD subjects didn't receive methadone maintenance treatment. Interestingly, the longer HD individuals abuse heroin, the less the ALFF in the right AG and left SOG increase. Since increased ALFF may be due to the compensatory response and part recovery of associated brain function after withdrawal of heroin (17, 18), we speculated that the effect of heroin's neurotoxicity overpowered self-recovery of the brain after short-term abstinence. Further study could explore ALFF changes of HD individuals after long-term abstinence.

Advantages and Limitations

The current study explored HD subjects' brain ALFF changes after short-term abstinence. To the best of our knowledge, this is the first study to demonstrate that the length of duration of heroin use is negatively correlated with the ALFF values of specific brain regions which show increased ALFF with respect to control. ALFF changes on semantic memory and visual network regions reveal that these areas play vital roles during heroin addiction and abstinence. This study provided a new perspective for understanding the pathological changes of ALFF in short-term abstinence HD individuals. Finally, ALFF values in the right AG and left SOG may be used as a potential biomarker to identify HD individuals from the controls.

There are some limitations in the current study. 1) This is a cross-sectional study, in which the neuroimaging data of HD subjects before abstinence was not obtained. Therefore, the causal relationship of abnormal ALFF values and the abstinence status could not be determined. 2) Some of the subjects have experienced no less than one period of abstinence. Repeated abuse and abstinence may affect the stability of ALFF in different brain regions. Further study with a first abstinence subgroup would help to solve this problem. 3) There are more men in compulsory detoxification, so the proportion of gender in the present study is not very even. Further study could recruit more women from HD individuals.

DATA AVAILABILITY STATEMENT

The datasets analyzed in this article are not publicly available. Requests to access the datasets should be directed to junliu123@csu.edu.cn.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Second Xiangya Hospital, Central South University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JL, WY, JZ, and JL conceptualized and designed the research. JL, WY, JZ, and JC performed the experiments. JL and RY undertook the statistical analysis. JL and RY wrote the first draft of the manuscript. JL, RY, and JL contributed to the final manuscript. CD and YD provided funding support. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Dynamic Neuroimaging Biomarkers of Smoking in Young Smokers

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Objective: To examine potential changes in the dynamic characteristics of regional neural activity in young smokers and to detect whether the changes were associated with smoking behavior.

Methods: The dynamic regional homogeneity (dReHo) and dynamic amplitude of low-frequency fluctuations (dALFF) in 40 young smokers and 42 nonsmokers were compared. Correlation analyses were also performed between dReHo and dALFF in areas showing group differences and smoking behavior [e.g., the Fagerström Test for Nicotine dependence (FTND) scores and pack-years].

Results: Significantly differences in dReHo variability were observed in the inferior frontal gyrus (IFG), superior frontal gyrus (SFG), medial frontal gyrus (MFG), insula, cuneus, postcentral gyrus, inferior semi-lunar lobule, orbitofrontal gyrus, and inferior temporal gyrus (ITG). Young smokers also showed significantly increased dALFF variability in the anterior cingulate cortex (ACC) and ITG. Furthermore, a significant positive correlation was found between dALFF variability in the ACC and the pack-years; whereas a significant negative correlation between dReHo variability in the IFG and the FTND scores was found in young smokers.

Conclusion: The pattern of resting state regional neural activity variability was different between young smokers and nonsmokers. Dynamic regional indexes might be a novel neuroimaging biomarker of smoking behavior in young smokers.

Keywords: dynamic, amplitude of low-frequency fluctuation (ALFF), young smokers, resting-state functional magnetic resonance imaging (rs-fMRI), regional homogeneity (ReHo)

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INTRODUCTION

There are more than one billion smokers globally according to the report in 2017 published by the Word Health Organization (https://www.who.int/). It has been demonstrated that the age period from late adolescence to adulthood is a crucial period of time of continued brain development (1). Numerous findings showed that people at this age have high prevalence of smoking cigarettes, and people who start smoking cigarettes at this age is more susceptible to nicotine addiction (2, 3).

Therefore, the elucidation of neural mechanisms underlying smoking cigarettes in young smokers might be helpful for preventing nicotine addiction.

Although the exact mechanism of nicotine addiction is still unknown, recent neuroimaging findings have advanced our understanding (4–8). Resting-state functional magnetic resonance imaging (rs-fMRI), measuring task-independent spontaneous brain activity in low-frequency blood oxygenation level dependent (BOLD) fluctuations (9), has been widely applied for delineating functional neural abnormalities in smokers (10–17). Previous studies have demonstrated that regional spontaneous neural characteristics are very important for understanding neuropathological and neurophysiological conditions (18). For example, abnormal regional energy consumption indicates decreased or excessive resting metabolic rates (19). Currently, the widely used approaches for characterizing spontaneous regional neural properties are the regional homogeneity (ReHo) and amplitude of low-frequency fluctuation (ALFF). Specifically, ReHo measures the temporal synchronization of regional neural activity among spatially adjacent regions (20); whereas ALFF reflects the intensity of regional neural activity (21). These two approaches have been widely applied to evaluate regional neural function in neuropsychiatric diseases and neurologic disorders (21–24).

In the context of nicotine addiction, numerous rs-fMRI studies have focused on the properties of regional neural activity (12, 25–28). For example, compared with nonsmokers, Yu and his colleague discovered that heavy smokers exhibited decreased ReHo in the prefrontal regions and increased ReHo in the insula and posterior cingulate cortex (PCC) (12). Another study found decreased ReHo in the inferior frontal cortex and increased ReHo in the superior parietal lobe (25). Chen et al. further found decreased ReHo in the superior frontal gyrus (SFG) and precuneus, and the decreased ReHo value in the precuneus was positively correlated with the Fagerström Test for Nicotine dependence (FTND) scores, an important index for clinical evaluation of smoker's dependence on nicotine addiction (26). Using fractional ALFF technology, enhanced neural spontaneous activity was found in the caudate of young smokers (28). Another study focused on regional spontaneous neural activity intensity in heavy smoker and discovered decreased spontaneous activity in the precuneus and inferior temporal gyrus (ITG) and increased spontaneous activity in the caudate (27). These findings indicated that static ReHo and ALFF were different at rest between smokers and nonsmokers.

However, the above-mentioned investigations ignored the dynamic characteristics of spontaneous neural activity, and they usually assumed the BOLD signal during scanning was stationary. In fact, it has been suggested that the brain dynamically responds and adjusts to external or internal stimuli over multiple time-scales (29, 30). By characterizing temporal changes in the brain's resting state functional connectivity, the intrinsic neural activity was also found to be time-varying in nature (31–34). Moreover, high-resolution spatiotemporal functional neuroimaging studies have confirmed substantial temporal variations in regional neural activity (35, 36). Consequently, elucidation of the temporal variability in oscillation amplitudes and synchronization of regional neural activity may help us better understand the effects of smoking cigarettes on brain function. Fortunately, the time-varying characteristics of resting state regional neural activity may be examined by the sliding window approach. This approach has been shown to work well for evaluating dynamic properties of regional neural activity (37), and it has been widely applied due to its simplicity and ease of implementation (30, 38).

Taken together, considering there are substantial temporal fluctuations in regional neural activity (35), and until now, there have been very few studies concerned with the dynamic characteristics of regional neural activity in young smokers. Currently, a sliding window technology combined with the dynamic ALFF (dALFF) and dynamic ReHo (dReHo) approaches were applied to investigate whether young smokers would present with abnormal dynamic characteristics of regional neural activity. In addition, correlation analyses were also performed to ascertain the relationships between the altered regional indexes (the dALFF and dReHo) and smoking behavior. We hypothesized that the temporal characteristics of regional neural activity may be altered in young smokers, and dynamic indexes may be associated with smoking behavior. It is hoped that the present study advances our understanding of nicotine addiction.

MATERIALS AND METHODS

Ethics Statement

The present study was approved by the Ethical Committee of Medical Research in First Affiliated Hospital of Baotou Medical College, Inner Mongolia University of Science and Technology. All study procedures were conducted in accordance with the Declaration of Helsinki. After explaining the aims and steps in the procedure of the current study, all the participants and their guardians signed written informed consents.

Subjects

The potential participants were male undergraduates from local universities recruited through advertisements. To screen for potential participants, a semistructured interview and questionnaires were used to assess psychiatric conditions, medical conditions, medication use, history of claustrophobia, history of substance use, and the presence of metal implants in their body. The inclusion criteria for smokers were 1) meeting DSM-V criteria for nicotine dependence, 2) reporting smoking ≥ 10 cigarettes per day in the last 6 months, 3) carbon monoxide (CO) > 6 parts per million (p.p.m.) in expired air (Smokelyzer, Bedfont Scientific, Kent, UK), and 4) having no period of smoking abstinence longer than 6 months in the past few years. We used the FTND and pack-years to assess nicotine dependence (39). Specifically, the FTND measured the severity of nicotine dependence (40), and a pack-years was defined as twenty cigarettes smoked per day for one year. The nonsmokers were included as those who smoked less than five cigarettes in their lifetime. Additional requirements for nonsmokers were CO concentrations below 3 p.p.m. in expired air. Exclusion criteria for

both groups were 1) any physical illness such as epilepsy, brain tumor, obstructive lung disease, or hepatitis, 2) urine test revealing any other substance or drug abuse (except nicotine), 3) existence of a neurological disease, or 4) claustrophobia. A total of 520 potential subjects completed the semi-structured interview and questionnaires, and among them, 46 participants were recruited in the young smoker group. The incidence was 8.8%. Forty-eight nonsmokers were recruited in healthy controls group. All participants were right-handed and 17–24 years of age. This study only focused on differences in regional neural activity between nondeprived young smokers and nonsmokers. Therefore, young smokers were asked to refrain from smoking in the 30 min immediately preceding the scan to exclude withdrawal symptoms (41).

Data Acquisition

The study was performed on a 3T magnetic resonance imaging (MRI) scanner (Achieva, Philips Healthcare, Best, the Netherlands) equipped with an 8-channel SENSE coil. The scanning parameters were repetition time (TR) = 2,000 ms, echo time (TE) = 30 ms, slice thickness = 5 mm, number of slices = 30, flip angle = 90°, field of view (FOV) = 220 mm × 220 mm, voxel size = 3mm × 3 mm × 3mm, matrix = 64 × 64, and 180 volumes. During the entire scanning procedure, a standard head coil and foam pads were used to minimize head movement. All subjects were instructed to close their eyes and stay still during scanning.

Data Analysis

The rs-fMRI data were preprocessed using the Data Processing Assistant for Resting-State fMRI Analysis Toolkit (DPARSF). The details included 1) deleting the first 10 images, 2) correcting slice-time delay, 3) realigning to correct for head motion, 4) normalizing to the standard Montreal Neurological Institute (MNI) template provided by SPM using linear and nonlinear transformations, 5) resampling (3 mm × 3 mm × 3mm), and 6) regressing out nuisance covariates (24 head motion parameters, white matter signal, cerebrospinal fluid signal and linear trend). The head motion of all participants was examined. If the head motion displacement exceeded 2.0 mm or rotation exceed 2.0°, the corresponding data will be excluded from the current study. Six nonsmokers and six young smokers were excluded because of excessive head motion. Eventually, 40 young smokers and 42 nonsmokers were included in further analysis. Group differences in the mean framewise displacement (FD) were also compared. The results showed that there were no significant group differences (p = 0.781) between the two group groups. Additionally, each participant's mean FD was included as a covariate in the follow-up, group-level analysis to reduce the effect of head movement. For ReHo calculations, the data were further filtered using a bandpass temporal filter of 0.01–0.08 Hz to reduce noise and low frequency drift.

The dALFF and dReHo analyses were performed using Temporal Dynamic Analysis (TDA) toolkits based on DPABI (42). Specifically, a temporal rectangular window was first chosen. Then, the ALFF and ReHo values in each window were calculated. Previous window-based analyses usually adopted a window length as small as 10 s (43) and as long as 180 s (44, 45). Ideally, the window size should be small enough to detect potentially transient signals, and yet large enough to analyze the lowest frequencies of interest in signals (45, 46). To avoid the introduction of spurious fluctuations, the minimum window length should be larger than $1/f_{min}$, where f_{min} is the minimum frequency of time series (47). Since there was currently no formal consensus regarding the window length, here a window length of 32 TR was selected according to previous studies (45), which was considered as the optimal parameter to maintain the balance between capturing a rapidly shifting dynamic temporal characteristics and obtaining reliable estimates of the regional neural activity (48). The preprocessed data of each individual were segmented into 139 windows. The ALFF and ReHo indexes were calculated in each window. In detail, the ReHo index was calculated using the Kendall's coefficient of concordance (KCC) to characterize the similarity of the time series of a given voxel and its 27 neighboring voxels (20). The ALFF index was calculated as the amplitude interval over a frequency range of 0.01–0.08 Hz, reflecting the intensity of spontaneous neural activity (21). To obtain the dALFF and dReHo maps, the standard deviation (SD) of ReHo and ALFF values at each voxel across all windows were calculated. Then, the obtained dReHo and dALFF maps were further normalized by dividing dReHo and dALFF maps by the mean dReHo and dALFF values. Finally, an isotropic Gaussian kernel of 4 mm full-width-at-half-maximum (FWHM) was used to smooth the maps.

Statistical Analysis

Group comparisons of demographic data were performed using two-sample t tests ($P < 0.05$). Group analyses of dALFF and dReHo maps were performed using the general linear model (GLM) in SPM12 with mean FD as a covariate. The results were thresholded at $P < 0.001$ (uncorrected) and a cluster size of 50 voxels, corresponding to a familywise error (FWE) correction for multiple comparisons of $P_{FWE} < 0.05$. Correlation analyses were conducted in young smokers between smoking behavior (FTND and pack-years) and mean dReHo/dALFF values in regions showing group differences with mean FD as a covariate. In the current study, the potential relationship between dynamic regional indexes and smoking behavior were surveyed in an exploratory investigation. Therefore, the threshold for statistical significance was set at $P < 0.05$ (uncorrected).

RESULTS

Smoking Behavior

Forty young male smokers and 42 male nonsmokers were included in the current study. The detailed demographic information was presented in **Table 1**.

Group Differences in dReHo and dALFF Maps

Compared with nonsmokers, young smokers demonstrated significantly decreased dReHo variability in the inferior frontal gyrus (IFG), medial frontal gyrus (MFG), SFG, inferior semi-lunar lobule, postcentral gyrus, cuneus, and insula, whereas significantly increased dReHo variability was observed in the

TABLE 1 | Demographic and smoking behavior.

	Young smokers (*n* = 40)	Nonsmokers (*n* = 42)
Age, years	19.70 ± 2.02	19.05 ± 1.72
sex(M/F)	40/0	42/0
FTND	6.73 ± 1.48	–
pack-years	2.44 ± 0.93	–

Data represent mean ± standard deviation; M, male; F, female; FTND, Fagerström Test for Nicotine Dependence; Pack-years, Years of smoking × Cigarettes smoked per day/20.

orbitofrontal gyrus and ITG (**Figure 1** and **Table 2**). Relative to the nonsmoker group, young smokers showed significantly increased dALFF variability in the ITG and anterior cingulate cortex (ACC) (**Figure 1** and **Table 3**).

Correlation Analysis

The results showed that the temporal variability in dALFF in the ACC was positively correlated with pack-years (r = 0.3268, p = 0.0396, uncorrected; **Figure 2A**), and the temporal variability in dReHo in the IFG was negatively correlated with FTND (r = -0.3147, p = 0.0479, uncorrected; **Figure 2B**).

DISCUSSION

Previous studies have probed static regional neural activity in smokers. However, to the best of our knowledge, very few studies have tried to assess nicotine-related dynamic regional neural activity changes in young smokers. In the current study, dynamic regional neural activity between young smokers and nonsmokers was examined using sliding window analysis. Differences in dReHo variability were observed in the IFG, SFG, MFG, insula, cuneus, postcentral gyrus, inferior semi-lunar lobule, orbitofrontal gyrus, and ITG, whereas differences in dALFF variability in the ACC and ITG distinguished young smokers from nonsmokers. Further correlation analyses showed that the pack-years/FTND scores were related to abnormal dALFF variability in ACC/dReHo variability in IFG in young smokers. The current findings about the dynamic neural characteristics in young smokers may expand our understanding of nicotine-related neural mechanisms.

The aberrant reward function is believed to play a key role in the progress of addiction. To date, the findings in this field showed that subcortical regions are crucial to facilitating such progress (49, 50). However, recent studies indicated that the frontal-cingulate regions are also heavily involved in addiction progress (51). For example, the ACC was found to be hyperactivated to drug-related reward and hypoactivated to natural rewards (52, 53). Recent studies further confirmed the function of ACC linking reward to goal-directed behaviors (54). One study focused on substance dependence found that compared with drug-related stimuli, substance-dependent individual exhibit decreased reward to stimuli related to

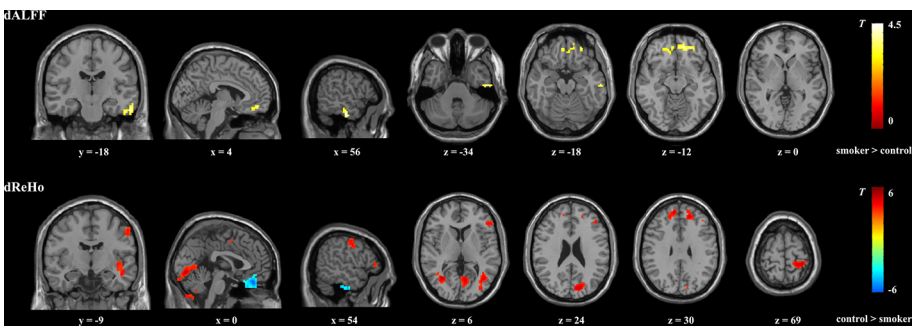

FIGURE 1 | Brain regions showing significant group differences in dALFF and dReHo between young smokers and nonsmokers using familywise error-corrected analysis (P_{FWE} < 0.05 with a minimum cluster size of k = 50 voxels and a cluster-defining threshold P < 0.001). dALFF, dynamic amplitude of low-frequencey fluctuation; dReHo, dynamic regional homogeneity.

TABLE 2 | Brain regions showing significant differences in dynamic ReHo between groups.

	Brain region	BA	MNI coordinates			Cluster size	Peak *t* value
			X	Y	Z		
HC > Smoker	Inferior semi-lunar lobule	–	-9	-72	-51	660	6.25
	Cuneus	18/30	12	-81	24	502	5.71
	Postcentral gyrus	5	30	-36	69	130	5.16
	Inferior frontal gyrus	46	51	33	6	62	4.9
	Superior frontal gyrus	8/9	-12	51	30	90	4.87
	Medial frontal gyrus	8	15	42	33	74	4.77
	Insula	13	48	-9	-12	69	4.4
HC < Smoker	Orbitofrontal gyrus	11	0	39	-24	141	6.19
	Inferior temporal gyrus	20	51	-15	-39	60	5.72

ReHo, regional homogeneity; MNI, Montreal Neurological Institute; BA, Brodmann's area; HC, healthy controls.

TABLE 3 | Brain regions showing significant differences in dynamic ALFF between groups.

	Brain regions	BA	MNI coordinates			Cluster size	Peak *t* value
			X	Y	Z		
HC < Smoker	Inferior temporal gyrus	21	57	-18	-27	50	4.82
	Anterior cingulate cortex	32	15	45	-12	141	4.72

ALFF, amplitude of low frequency fluctuation; MNI, Montreal Neurological Institute; BA, Brodmann's area; HC, healthy controls.

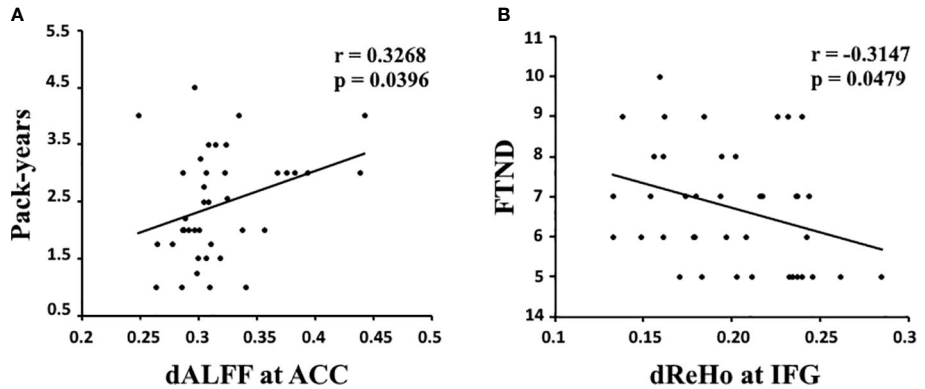

FIGURE 2 | (A) The dALFF variability in the ACC was positively correlated with pack-years in young smokers (r = 0.3268, p = 0.0396, uncorrected). **(B)** The dReHo variability in the IFG was negatively correlated with FTND scores in young smokers (r = -0.3147, p = 0.0479, uncorrected). dALFF, dynamic amplitude of low-frequency fluctuation; dReHo, dynamic regional homogeneity; ACC, anterior cingulate cortex; IFG, inferior frontal gyrus; FTND, Fagerström Test for Nicotine dependence. Pack-years of smoking were calculated by multiplying the average number of packs of cigarettes smoked per day by the number of years the participant smoked.

monetary gains. They speculated that monetary rewards may be devalued relative to drug-related reward, and such aberrant reward function may be related to abnormal goal-directed behavior (55). In the context of nicotine addiction, studies have shown that nicotine-related stimuli can elicit overvalued rewards than nonnicotine stimuli in abstinent smokers. This finding may reflect that the ACC would assign a larger reward value to nicotine-related behaviors in a state of craving (56, 57). The current findings of increased ALFF temporal variability in ACC further confirmed the abnormal function of the ACC in young smokers. Considering the importance of ACC in evaluating reward value related to goal-directed behaviors, we tend to speculate that the abnormal temporal characteristics of dALFF in the ACC might reflect reward processing impairment in young smokers, which may facilitate action-selection mechanisms to converge on nicotine addiction.

Substantial evidence demonstrated that the ACC and prefrontal regions are primary nodes of a putative "cognitive control network" (58). Specifically, the ACC plays an important role in detecting salient events, especially to erroneous or error-prone actions (59). Once detecting such events or actions, the ACC further signals reorientation of attention implemented by the prefrontal regions (60). Previous studies have indicated that abnormalities in the ACC and prefrontal regions were associated with impaired cognitive function (executive, inhibitory, and decision-making) in smokers (61, 62). Abnormal neural activity in these regions in smokers during abstinence may suggest inefficient cognitive control in abstinent smokers (63). Taken together, the abnormal dALFF and dReHo in the ACC and prefrontal regions and correlations between the changes in dynamic regional indexes and smoking behavior may provide novel neuroimaging biomarkers of smoking in young smokers.

The current study has several limitations. First, the sample size was small. Larger samples will be needed in future studies. Second, physiological noise from respiratory cycles and cardiac activity were not monitored during fMRI scanning. Finally, the current findings are cross-sectional, and longitudinal studies are needed to elucidate the dynamic characteristics of regional neural activity.

CONCLUSION

To the best of our knowledge, very few studies have tried to assess dynamic regional neural activity in young smokers. We found increased dynamic ALFF variability in the ACC and decreased ReHo variability in frontal regions. Correlation analyses showed that the changes were related to the severity of nicotine addiction. This finding is indicative of the importance of cingulate and frontal regions in nicotine addiction in young smokers. Dynamic regional indexes might be used as a powerful supplement to static regional indexes, helping us obtain a more comprehensive understanding of neural activity in young smokers.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because of the privacy of all the participants. Requests to access the datasets should be directed to fmydh@imust.edu.cn.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical Committee of medical research in First Affiliated Hospital of Baotou Medical College, Inner Mongolia University of Science and Technology. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

TX, DY, and KY were responsible for the study concept and design. FD, RH, YH, MZ, ZT, JT, XL, HJ, and YC contributed to the acquisition of fMRI data. TX and RH performed the data analysis. DY and KY provided critical revision of the manuscript for important intellectual context.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Reduced Cortical Thickness in the Right Caudal Middle Frontal Is Associated With Symptom Severity in Betel Quid-Dependent Chewers

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Background: Findings from brain structural imaging studies on betel quid dependence have supported relations between betel quid chewing and alterations in gray matter volume and white matter integrity. However, the effect of betel quid chewing on cortical thickness and the link between cortical thickness and symptom severity remains unascertained.

Methods: In this observational study, we compared cortical thickness measures from 24 male betel quid-dependent chewers with 27 male healthy controls. Using FreeSufer, we obtained three-dimensional T1-weighted images that were used to compute the thickness of the cerebral cortex throughout the cortical layer.

Results: Compared to healthy controls, betel quid dependent chewers displayed significant decreased cortical thickness in the precuneus, entorhinal, right paracentral, middle temporal, and caudal middle frontal gyri. Betel quid dependence scale scores negatively correlated (r = -0.604; p = 0.002) with reduced cortical thickness in the right caudal middle frontal of betel quid-dependent chewers.

Conclusion: The findings provide evidence for cortical thickness abnormality in betel dependent chewers and further propose that the severity of betel quid symptoms may be a critical aspect associated with the cortical alterations. The observed alterations may serve as potential mechanisms to explain why BQ chewing behavior is persistent among individuals with betel quid dependence.

Keywords: cortical thickness, symptom severity, betel quid dependence, right caudal middle frontal, dorsal lateral prefrontal cortex, decision-making, executive functions, memory

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Abbreviations: CT, Cortical thickness; BQ, Betel quid; BQD, Betel quid dependence; HC, Healthy controls; GM, Grey matter; rSTG, Right superior temporal gyrus; dlPFC, Dorsolateral prefrontal cortex; vmPFC, Ventral medial prefrontal cortex; OFC, Orbital frontal cortex; MTG, Middle temporal gyrus; MFG, Middle frontal gyrus; BQDS, Betel quid dependence scale; WM, White matter.

INTRODUCTION

Betel quid (BQ) is the fourth frequently used psychoactive substance after caffeine, alcohol, and nicotine (1), consumed by more than 600 million people worldwide (2). Prevalence of BQ dependence (BQD) among users in mainland China ranged from 20.9–33.3% (3), making it a substance of interest in addiction research. BQ consists of four main natural alkaloids including arecoline, arecaidine, guvacoline, and guvacine (4). Arecoline is the main active ingredient (5) assisting the release of dopamine (6) through binding to M5 muscarinic acetylcholine receptors in the ventral tegmental area (VTA) (7). Addictive drugs are thought to increase DA concentration in the major reward network (the VTA, nucleus accumbens, and the prefrontal cortex) (8). Arecoline is also known for its binding properties on GABA receptors in the brain, and influence on smooth muscles, therefore, supporting the reported psychoactive effects (1). For chronic users, BQ has been associated with reduced thinking capacity, mental processing problems, a greater sense of wellness, improved alertness, relaxation, and motor responses right after chewing (9). Some of the pleasant experiences (e.g. arousal, improved alertness, relaxation) have been linked with the observed repetitive BQ chewing behavior that leads to dependence (10). Fresh BQ users on the other hand reported more intense effects than chronic users, suggesting that continual use leads to tolerance (1). Furthermore, individuals with BQ dependence often experience tolerance, craving, BQ seeking behavior, and withdrawal symptoms (3, 11–13) that satisfy the characteristic features for substance abuse (14). Such features stem from continuous exposure of G protein-coupled receptors to arecoline that leads to weaker receptor sensitivity (15), therefore, supporting the occurrence of tolerance and habitual use of substances (10).

Substance dependence is characterized by compulsive intake of drugs, loss of inhibitory control, and negative emotions (such as anxiety, irritability, and dysphoria) portraying withdrawal syndrome in the absence of drugs (16). Previous studies have demonstrated a link between psychoactive substance use and alterations in brain functioning (17–19) and structure (20, 21). Advancement in neuroimaging studies has revealed various approaches used to investigate the effects of psychoactive substances in the brain structure and function of individuals with BQD. Studies have specifically examined the brain function and morphology of BQ dependent chewers by focusing their utmost attention on functional connectivity, white matter integrity, and grey matter (GM) volume alterations. For instance, functional magnetic resonance imaging (fMRI) studies have revealed that betel quid dependence is linked with structural and functional changes in the brain areas responsible for impulsivity, reward, and cognitive processing (22). Specifically, results from BQD resting-state fMRI studies found functional connectivity alterations in orbitofrontal, frontoparietal, occipital/parietal, frontotemporal, medial frontal/anterior cingulate cortex (ACC) networks (23, 24), anterior default mode network (DMN) (23, 25), bilateral precuneus, right insula and right hippocampus (26, 27), temporal and occipital lobe, primary motor cortex (28), ACC-brainstem, and ACC-DMN connectivity (27). Using structural magnetic resonance imaging (sMRI), studies have specifically examined white matter integrity. For example, increased diffusion anisotropy was observed in the right ACC, midbrain, bilateral angular gyrus, right superior temporal gyrus (rSTG), bilateral superior occipital gyrus, left middle occipital gyrus, bilateral superior and inferior parietal lobules, bilateral postcentral, and the precentral gyrus of BQ dependent chewers (29). Furthermore, compared with HC, a higher mean diffusivity and a lower fractional anisotropy were exhibited in anterior thalamic radiation of BQD individuals (30). BQD individuals also demonstrated reduced grey matter (GM) volume in the midbrain, right rostral ACC, bilateral dorsal lateral prefrontal cortex (dlPFC)/insula, and rSTG, while greater GM volume was displayed in the right hippocampal and precuneus (31). Similarly, decreased GM volume was observed in the bilateral ventral medial prefrontal cortex (vmPFC), bilateral dlPFC, and left orbitofrontal cortex (OFC) (32). To date, there is scarcity of studies which have looked into cortical thickness of BQ dependent chewers.

Psychoactive substances are known for their effects on the brain. Progress in neuroimaging research has revealed the roles of various substances in the human brain structure. For example, studies have investigated cortical thickness (CT) abnormalities in individuals dependent on psychoactive substances. For instance, compared with healthy controls (HC), individuals with heroin dependence had decreased CT in the bilateral superior frontal, left caudal middle frontal, right superior temporal, and right insular regions, while increased CT was observed in the left superior parietal, bilateral lingual, left temporal pole, right inferior parietal, right lateral occipital, and right cuneus regions (33). A thinner cortex was observed in the reward regulation and attention function areas of cocaine-dependent patients compared to their matched controls (34). Similarly, compared to controls, alcohol abstainers demonstrated diminished CT in the brain reward system (35), superior frontal, precentral, postcentral, middle frontal, middle/superior temporal, middle temporal, and lateral occipital cortical regions (36). On the other hand, significant decreased CT was observed in the frontal cortex, left caudal ACC, right lateral OFC, left insula, left middle temporal gyrus (MTG), right inferior parietal lobule, and right parahippocampus of young adult smokers (37). Meanwhile, experienced methamphetamine users exhibited thinner CT in the left middle frontal cortex, fronto-polar region, and closeby the central sulcus, than drug naïve controls. However, when compared with low-exposure users, experienced users displayed cortical thinness in the right middle frontal cortex (38). Decreased CT was also displayed in the right caudal middle frontal, bilateral insula, and bilateral superior frontal cortices of adolescent marijuana users compared to controls, whereas the thickness was greater in bilateral lingual, right superior temporal, right inferior parietal, and left paracentral brain regions (39). Additionally, compared to non-cannabis users, reduced CT was observed in the caudal middle frontal gyrus (MFG) of individuals with schizophrenia and bipolar disorder who used cannabis (40). Like other

psychoactive substances, exposure to BQ has previously been associated with brain structural anomalies. However, a few studies have examined CT abnormalities in BQ chewers. In one study, individuals with BQD exhibited reduced CT of the dlPFC compared to HC. The observed CT alterations in the dlPFC mediated executive function deficit in BQ dependent individuals (41).

Studies have also advocated the use of symptom severity as an important aspect of understanding the effects of psychoactive drugs on CT abnormalities. For instance, abstinent alcoholics displayed reduced CT which was associated with the severity of alcohol abuse (36). Additionally, the CT of the right dorsal lateral prefrontal cortex (dlPFC) and OFC were associated with the severity of nicotine dependence in young adult smokers (37). Studies have also observed that symptom severity detected by betel quid dependent scale (BQDS) may explain the effects of BQ chewing on brain structural abnormalities (30). Moreover, in a diffusion tensor imaging study, increased mean diffusivity and reduced fractional anisotropy predicted the severity of BQ dependence (30).

Various studies have proposed that dependence on different types of psychoactive substances has similar brain mechanisms (42–44). Basing on this idea, we hypothesized that BQ dependent chewers would display altered CT in some parts of the brain particularly, the caudal MFG (33, 37, 39), paracentral regions (39), and middle temporal cortices (36), of BQ dependent chewers. We also explored if CT in certain parts of the brain could predict symptom severity in BQ dependent chewers. Examining the CT alterations provides further understanding and evidence on the neurobiology of BQ chewing, which explains why individuals with BQD exhibit persistent BQ chewing behavior. Findings from this study may provide possible suggestions for the prevention and treatment of BQD.

MATERIALS AND METHODS

Aim, Design, and Setting of the Study

This observational neuroimaging study examined CT abnormalities and their association with symptom severity in individuals with BQD. Participants' recruitment and data collection were executed between January 2015 and March 2016 at the Second Xiangya Hospital of Central South University, in Changsha city, Hunan Province, China.

Characteristics of Participants

Ethical approval for this study was acquired from the Ethics Committee of the Second Xiangya Hospital of Central South University. Before inclusion into the study, each potential participant was asked to provide a signed written informed consent form. The authors had no access to any participant's identifying information during or after data collection.

The participants of this study were all male. Using the Structured Clinical Interview, participants were assessed to discern if they met the DSM-IV criteria for substance dependence (45). The subsequent inclusion and exclusion have been described before (23, 24, 46). Twenty four individuals with BQD were included in this study. Briefly, participants with BQD had to satisfy the following criteria: 1) 18–40 years of age; 2) Han Chinese ethnicity; 3) completed 9 or more years of education; 4) right-handed; 5) a diagnosis of BQD (a score of ≥5 on the Betel Quid Dependence Scale (BQDS); 6) using BQ at least 1 day at a time for more than 3 years. The BQDS was specifically designed to measure BQD according to the DSM-IV criteria (11). The BQDS is a 16-item self-report instrument encompassing three parts: physical and psychological urgent need, increasing dose, and maladaptive use. The scale has an optimal cut-off score of 4, main sensitivity of 0.926 and specificity of 0.977, and predictive accuracy of up to 99.3%. Furthermore, the BQDS showed high internal consistency ($\alpha = 0.921$) and exhibited good degrees of validity and reliability for both Chinese and English-speaking BQ chewers (11, 47). Exclusion criteria included: 1) a history of neurological disorder or other serious physical illness; 2) a history of any DSM 5 Axis-I mental disorders; 3) a history of substance abuse other than betel quid; 4) a contraindication to MRI, or 5) a history of electroconvulsive therapy. We recruited 27 HC from the community in Changsha City. The inclusion and exclusion criteria for HC were equivalent to the criteria in the BQD group, except for an additional requirement that; HC did not meet the BQD diagnostic criteria and did not have a family history of psychiatric illness among their first-degree relatives. Individuals with BQD and HC were requested not to use any psychoactive substance in the 24 h preceding scanning. We used Beck Depression (48) and Anxiety Inventories (49) to measure the emotional status of each subject before MRI scanning.

Images Acquisition

All participants were requested to move as little as possible and foam pads were applied to minimize head motion. Three-dimensional T1-weighted images (3D-T1WIs) were obtained sagittally on a 3.0-Tesla Philips Achieva whole-body MRI scanner (Philips, Netherlands) using turbo field echo sequence with the following parameters: repetition time= 7.5 ms, echo time= 3.7 ms, flip angle=8°, the field of view= 240×240 mm^2, acquisition matrix= 256×200, slice thickness= 1 mm, gap= 0, number of slices= 180.

Preprocessing of MRI Data

We carried out a CT analysis of the whole brain through a surface-based approach by employing FreeSurfer tools (version 5.3.0, http://surfer.nmr.harvard.edu) (50, 51). The 3D images were utilized to compute the thickness of the cerebral cortex throughout the cortical layer. In short, the processing stream included a Talairach transform of each of the subject's native brain, exclusion of non-brain tissue, and separation of grey matter (GM)/white matter (WM) tissue. The GM/WM boundary was tessellated to create multiple vertices across the whole brain. The cortical surface of each hemisphere was enlarged to an average spherical surface to locate the pial surface and the GM/WM boundary. The whole cortex of each subject was visually scrutinized, and topological defects were modified manually, blind to subject identities. CT was measured as the shortest distance between the pial surface and the

GM/WM boundary at each vertex across the cortical mantle. The mean CT of each of the 68 regions was calculated using FreeSurfer software (52). The surface maps were smoothed on the normalized CT through a Gaussian kernel with a full-width-half-maximum (FWHM) of 10 mm.

Statistical Analyses

Statistical Package for the Social Sciences 19.0 (IBM SPSS Inc., USA) was used to compare demographic and clinical characteristics between the two groups. An independent two-sample t-test was used to compare age, years of education, total intracranial volume (TIV), Beck depression, and Beck anxiety inventory scores. A P-value of <0.05 was considered statistically significant.

FreeSurfer's QDEC 1.4 (query, design, estimate, contrast) application was employed to create statistical maps. QDEC uses the Desikan-Killiany cortical atlas that parcellates the cerebral cortex into 34 cortical regions in each hemisphere (52). It fits a general linear model (GLM) at each surface vertex to describe the data from all participants in the study. Age and education are known to impact brain structure and function (53, 54). Additionally, TIV is also known for its impact on brain structure analysis (55). Basing on that knowledge, these three variables were accounted for as covariates, and regional CT differences were compared between BQD and HC. Using the CT maps, statistical difference maps were created based on the GLM. A Monte Carlo simulation analysis with 10,000 repetitions was performed to correct for multiple comparisons at a threshold of $p<0.05$ with a cluster size of at least 20 voxels.

Correlation Analysis

Pearson's correlation was calculated to examine the relationship between the mean values of CT and BQDS scores. Using SPSS 19.0 ($p<0.05$), correlation analysis was implemented to the mean value of the regions showing significantly altered CT between BQ dependent users and HC and BQDS scores.

RESULTS

Demographics and Clinical Characteristics

Fifty-one male participants (24 BQD and 27 HC) were involved in the present study. A significant difference in years of education (15.13±1.73 for individuals with BQD; 16.00±0.00 for HC; p=0.01) was observed between individuals with BQD and HC. No significant differences in age and TIV were detected between the groups. Individuals with BQD showed that they had been chewing BQ for a mean duration of 7.75±4.28 years, range 3.25 to 18 years. The average score of BQDS in the BQ dependent group was 7.42±1.86. Individuals with BQD had significantly higher Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI) scores than the HC. However, the scores on the BDI (10.58±6.69, range 0 to 24 for the BQ dependent group; 3.89±4.63, range 0 to 14 for the HC group) and BAI (28.50±6.20, range 21 to 45 for the BQ dependent group; 23.19±2.66, range 21 to 32 for the HC group) showed that none of the participants were found with depression or anxiety symptoms. The demographics and clinical characteristics of BQ dependent and HC participants are summarized in **Table 1**.

Cortical Thickness Differences Between Individuals With BQD and HC

Compared with HC, individuals with BQD exhibited decreased CT in left precuneus, left entorhinal, right paracentral, middle temporal, and caudal middle frontal brain regions (two-sample t-test, p<0.05, FDR corrected), (**Table 2**), and (**Figure 1**). Compared to the HC group, no brain regions were found with increased CT in the BQD group.

Correlation Analysis

The BQDS scores negatively correlated with the decreased mean value of CT in the right caudal middle frontal in the BQD group (r = -0.604; $p = 0.002$), (**Figure 2**). The correlation results of the mean value of all regions showing significantly altered CT between the BQD users and HC and BQDS scores are displayed in **Table 3**.

DISCUSSION

The hypothesis in our study was supported and we found significant cortical thinning in several areas of the brain. An important finding in the current study is that, compared to HC, individuals with BQD demonstrated reduced CT in the right caudal middle frontal gyrus (MFG) which negatively correlated with symptom severity.

Our results are consistent with findings of a cortical reduction in the caudal MFG among cannabis users (39, 40), and heroin-

TABLE 1 | Demographics and clinical characteristics of individuals with betel quid dependence (BQD) and healthy controls (HC).

	BQD (Mean ± SD)	HC (Mean ± SD)	t/χ^2	*P*-Value
Age (years)	23.54 (3.87)	24.52 (1.45)	-1.22[a]	0.23
Gender (male/female)	24/0	27/0		
Education (years)	15.13 (1.73)	16.00 (0.00)	-2.64[a]	0.01*
Total Intracranial Volume (cm^3)	1104.76 (103.7)	1209.59 (229.4)	-2.058	0.05
Betel Quid Dependence Scale	7.42 (1.86)	N/A		
Duration of Betel Quid (years)	7.75 (4.28)	N/A		
Beck Depression Inventory	10.58 (6.69)	3.89 (4.63)	4.20[a]	0.00*
Beck Anxiety Inventory	28.50 (6.20)	23.19 (2.66)	4.06[a]	0.00*

*SD, standard deviation; N/A, not applicable; BQD, betel quid dependence; HC, healthy controls; a, independent-samples t-test; *P < 0.05.*

TABLE 2 | Brain regions showing group differences in CT ($p < 0.05$, FDR corrected).

Brain region	Side	Cluster size (voxels)	MNI coordinates			Peak T value
			X	Y	Z	
BQD<HC						
Precuneus	Left	181	-16	-43	65	-4.27
Entorhinal	Left	49	-26	2	-35	-3.22
Paracentral	Right	71	17	-44	47	-3.33
Middle temporal	Right	38	58	-57	6	-2.96
Caudal middle frontal	Right	21	34	5	55	-2.95
BQD>HC						
Negative	–	–			–	–

BQD, betel quid dependence; HC, healthy control; CT, cortical thickness; MNI, Montreal Neurological Institute.

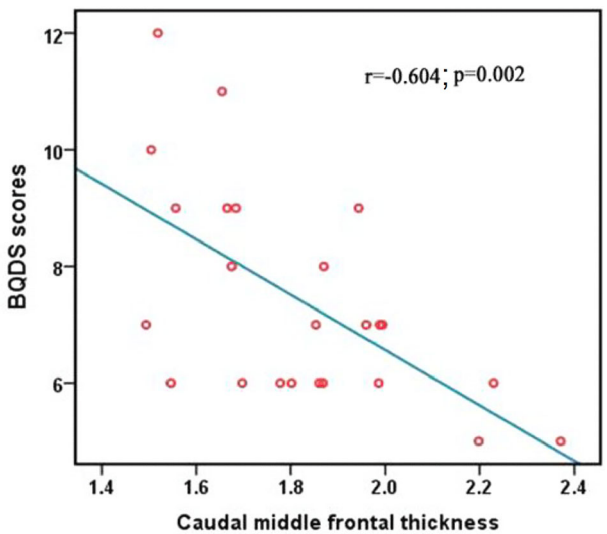

FIGURE 1 | Differences in CT between individuals with BQD and HC. Regions with decreased CT in BQ dependent individuals include the left precuneus, entorhinal, right paracentral, middle temporal gyrus, and caudal middle frontal. The color bars represent the range of t value. L, left; R, right; CT, cortical thickness; BQD, betel quid dependence; HC, healthy control.

dependent individuals (33). The MFG (encompassed in the dlPFC) located in the PFC, is known for its role in reorienting attention (56). Dysfunctions in the PFC have been linked with drug compulsivity, craving, denial of illness, and lack of motivation to seek treatment for drug addiction (42). A very recent study found that individuals with BQD displayed reduced CT of the dlPFC compared to HC (41). Moreover, compared with low-urge BQ dependent individuals, high-urge chewers displayed greater activation in areas of the brain concerned with cravings, such as the ACC, medial orbital frontal gyrus, and superior frontal gyrus (57). The dlPFC interacts with various cortical structures and has widely been mentioned in addiction studies. For example, the dlPFC assesses and joins emotional related information from the amygdala and nucleus accumbens and interacts with the dorsal ACC to exert top-down control and metacognitive functions (42). The dlPFC also participates in memory processing (58, 59), executive functions (60), and decision making (61, 62). A previous study has reported that CT of the dlPFC participated in mediating executive function deficits in individuals with BQD (41). The dlPFC has also been linked with cue-induced craving in substance use disorders (63) emanating from drug-related memories (42) that influence reward-related decision making (64). Additionally, the dlPFC is known for its role in conflict resolution. For instance, the dlPFC was associated with improved cognitive control and better ability to resolve conflicts among individuals who had resumed smoking (65). Similarly, methamphetamine-dependent subjects displayed reduced activation in the right middle frontal cortex when presented with conflict trials as a measure of behavior regulation (66). We argue that reduced CT in the right caudal MFG within the dlPFC may compromise decision-making abilities, enhances reward-related memories, leading to a craving, and compulsive BQ chewing behavior. Previous studies have also shown that longer duration of BQ chewing significantly correlated with a reduction in GM volume in the MFG, such that compared with HC, reduced GM volume (31) and short-range functional connectivity (27) were displayed in the dlPFC of BQ dependent individuals.

Our results demonstrated decreased CT in the entorhinal cortex of BQ dependent chewers. Inconsistent with our findings is a study that found marijuana adolescent users with increased CT in the entorhinal cortex (67), suggesting a different neuro-mechanism exerted by different types of psychoactive substances (8). The entorhinal cortex embedded in the anterior medial temporal lobe (68) has numerous projections to the hippocampus and neocortical regions (69) and is presumed to play an important role in learning and memory (70). Drugs of abuse can seize ordinary learning and memory structurethrough pharmacological mechanisms on numerous neurotransmitter systems (71). The mechanism of reward-related learning and memory in addiction is represented by pathologies in gene and protein expression alterations, changes in neuronal activity, and morphological alterations in the neural circuits (72). Addictive drugs can create artificial learning signals that are stronger and last longer than what is normally seen neurochemically as a result of natural events. Positive experience from the substance leads to enhanced learning that results in more repetitive behavior (73), typical of what is demonstrated in BQ dependent chewers.

Although other studies have reported increased CT in the precuneus (74), our study found a significant decrease. The precuneus is known for its involvement in visual imagery, attention, and memory retrievals. It is also linked with visual processing and integrating related memory (75). We argue that reduced CT observed in the precuneus of BQ dependent individuals may be associated with neurodegeneration emanating from chronic BQ chewing. We also observed reduced CT in the right paracentral lobule. Similar to our findings, more alcohol consumption was associated with a thinner paracentral lobule (67). Conversely, increased paracentral CT has been

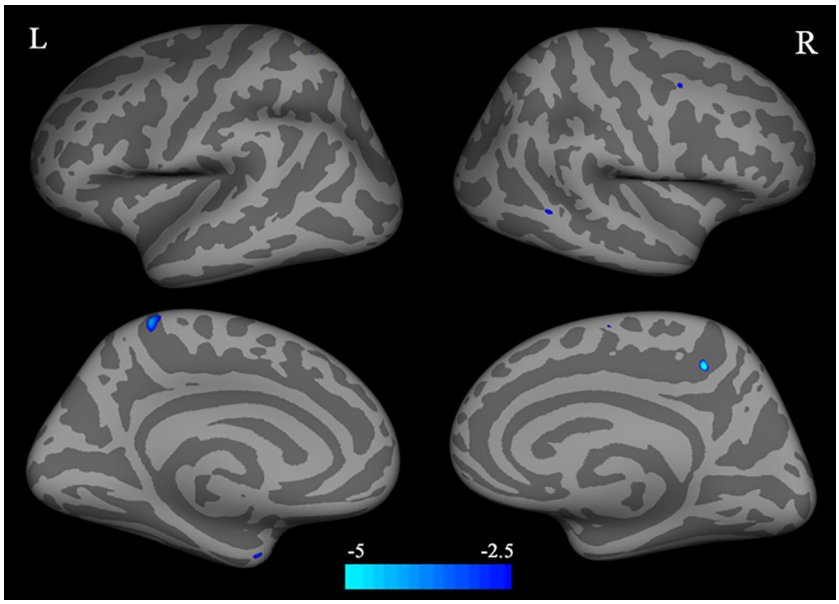

FIGURE 2 | Correlation between BQDS scores and the mean values of CT of the right caudal middle frontal. BQDS, Betel Quid Dependence Scale; CT, cortical thickness.

TABLE 3 | Pearson's correlation coefficients between the mean value of cortical thickness (CT) and betel quid dependence scale (BQDS) scores in betel quid dependent individuals (n=24, $^*p < 0.05$).

Cortical thickness	Betel Quid Dependence Scale scores	
	r	p-value
Precuneus (left)	0.341	0.103
Entorhinal (left)	-0.103	0.632
Paracentral (right)	0.032	0.881
Middle temporal (right)	0.121	0.572
Caudal middle frontal (right)	-0.604	0.002*

reported in adolescent marijuana users (39), signifying that different addictive substances may exhibit diverse mechanisms in the brain (8).

Consistent with previous studies that examined adult smokers (37), and abstinent alcoholics (36), our study found significant cortical thinning in the middle temporal gyrus. The decreased degree of centrality in the middle temporal gyrus has been associated with language expression (76), attention, and cognitive deficits in addicts (77, 78), suggesting a weakening of cognitive control in individuals with BQD.

There are several limitations to this study. First, the use of a small sample size consisting of only male participants may have influenced the interpretation of our results. Future studies with larger samples comprising male and female subjects may provide robust results. Second, although our observations show that CT abnormalities are associated with BQD, being a cross-sectional study, we cannot infer a cause and effect relationship between CT alterations and BQD. Therefore, future longitudinal neuroimaging studies may be more appropriate to establish the cause and effect phenomenon. Third, nicotine and alcohol are substances commonly used by most Chinese men (79, 80). Even though individuals with alcohol and nicotine abuse were excluded from this study, the influence of such substances in the observed cortical alterations cannot be ascertained. To overcome the possibility of confounding results, it would be useful to design future studies that will examine the potential differences in CT abnormalities between subgroups of individuals with BQD defined by their concomitant use of alcohol and other substances (21).

CONCLUSION

Our results provide further compelling evidence for CT abnormalities in BQ dependent chewers and propose that symptom severity is a critical factor linked with brain morphological alterations. CT loss in the right caudal MFG is a significant biological correlate for symptom severity in BQ dependent chewers. We presume that the caudal MFG participates in retrieving BQ reward-related memories which in turn influences one's decision making about whether to continue chewing BQ, leading to the perpetuation of BQ dependent behavior. However, the observed CT alterations may have preceded BQD and therefore act as risk factors for BQ use and BQD or they may have stemmed from chronic BQ use. Future longitudinal BQ neuroimaging studies may be useful to elucidate these conclusions.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Second Xiangya Hospital of Central South University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XH designed the study, collected and analyzed the data. AS prepared the first draft of the manuscript. AS and XH actively participated in writing and revising the manuscript for publication. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Cigarette Smoking, Health-Related Behaviors, and Burnout Among Mental Health Professionals in China: A Nationwide Survey

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Objectives: Mental health professionals (MHPs) play an important role in treating patients with nicotine addiction. However, data on MHPs' cigarette smoking habits are scarce. This survey aimed to collect such data and to examine the correlates of smoking among MHPs working in China.

Methods: A cross-sectional survey was conducted in all 41 provincial, tertiary psychiatric hospitals, and MHPs from these hospitals were targeted. An anonymous questionnaire was designed to collect socio-demographic and occupational factors, and the Maslach Burnout Inventory-Human Service Survey was used to assess burnout. Data about smoking and other health behaviors were also collected.

Results: In total, 13,614 MHPs were included in the analysis. The overall rate of current smoking was 8.6% (31.3% in males, and 1.1% in females). A substantial proportion (28.0%) of life-time smokers had abstained for more than 3 months. Those who were male (OR=37.73), older (OR=1.02), divorced or widowed (OR=1.72), working in West (OR=1.45), and Northeast China (OR=1.65), were nurses (OR=1.44), had a high income (OR=1.31), experienced burnout (OR=1.29), frequent insomnia (OR=1.39), and used alcohol (OR=2.76) were significantly more likely to be smokers, while those who had a higher level of education (OR=0.67, 0.47, and 0.43 for college, master, and doctorate degrees, respectively), and exercised regularly (OR=0.73) were significantly less likely to be smokers.

Conclusion: Although lower than that of the general population in China, smoking is still relatively high among MPHs. Efforts to lower smoking rates among MHPs in China should continue and should incorporate strategies that target burnout, sleep, alcohol use, exercise and other factors associated with smoking.

Keywords: smoking, mental health professionals, burnout, health behaviors, China

INTRODUCTION

Tobacco use is one of the most serious public health problems in the world. Six million deaths per year are associated with tobacco use worldwide, and more than 1 million of those occur in China (1, 2). In the past few decades, China has accounted for 40% of the world's production and consumption of cigarettes, far more than any other country (2). According to the 2019 WHO report on the tobacco epidemic, 27.7% of people in China aged 15 years and older were current smokers (52.1% of males, and 2.7% of females, men/women ratio = 19.3) (3).

The role of healthcare professionals in antismoking campaigns and patients' smoking cessation efforts is uniquely important. First, people often consciously or subconsciously view healthcare professionals as role models (4, 5). So, if the health professional smokes, then the patient might be less concerned about the dangers of nicotine use and might feel no urgency to quit. Second, healthcare providers' own smoking behavior may affect how they approach their patients' cigarette smoking (6), leading them to be more sympathetic to their patients' need to smoke and tenuous in their efforts to convince the patient to give up nicotine. Multiple studies (7–10) have found high rates of smoking rates among patients with mental illness with the highest rates seen among those with bipolar disorder and schizophrenia (over 60%). Given this high prevalence and the influence health care professionals have on their patients' smoking cessation efforts, it is important to characterize the smoking behavior of mental health professionals (MHPs), which include psychiatrists, psychiatric nurses, and psychologists.

So far, there have been several large sample surveys conducted outside of China (New Zealand, Spain, Japan, U.S., Ireland and Italy) assessing smoking behavior among doctors and nurses (11–16). The findings showed that the rates of smoking rates ranged widely, from 2%-31% for doctors and 2%–41% for nurses depending on the country studied and the criteria used. Related factors such as sex, age, profession, and health behaviors/lifestyles (such as alcohol use, skipping breakfast, general health and poor sleep) were found to be significantly associated with smoking (11, 15–17). Additionally, several studies also showed a significant association between smoking behavior and burnout among healthcare workers in Italy (18), and physicians and nurses in Saudi Arabia (19). Smoking can be seen as an option for healthcare workers to deal with feelings of exhaustion related to work problems (18). On the contrary, another study in Kazakhstan showed that smoking did not predict high burnout among doctors and nurses (20). The relationship between smoking and burnout in Chinese healthcare workers, especially in MPHs has never been explored.

Similar surveys have also been conducted in China. For example, a national survey involving 39,248 Chinese physicians in hospitals of different levels found that 20.4% overall were current smokers (38.7% in males and 1.1% in females), but the results did not include data on psychiatrists' smoking behaviors. Regarding smoking among nurses in China, two recent studies showed the current smoking rates were from 0.9% to 1.2% among nurses in general hospitals (21, 22). One of the studies reported a smoking rate of 13.4% among psychiatric nurses in China, with a relatively small sample size of 387 (21). To date, no studies have specifically investigated cigarette smoking among MHPs in a national sample in China. This study aimed to investigate the prevalence, sociodemographic and occupational correlates of smoking among Chinese MHPs, and to explore the relationship of smoking with burnout and other health behaviors of MHPs.

METHODS

Study Design and Participants

This cross-sectional study was part of a national survey, which was conducted in March 2019. Forty-one tertiary psychiatric hospitals from 29 provinces were selected as targets and all doctors, nurses and psychologists in these hospitals were invited to participate. Each Provincial Health Commission issued a notice to the selected hospitals, and then hospital administrators organized and facilitated healthcare professionals to participate in this survey. Each MHP completed a smartphone-based questionnaire anonymously and voluntarily throughout the process. Gansu and Tibet provinces had no provincial tertiary psychiatric hospitals and were excluded in the survey.

The research protocol was approved by the Ethics Committee of Chaohu Hospital of Anhui Medical University and an electronic consent form was obtained from each participant.

Questionnaires

The questionnaire consisted of three parts. Clear instructions were provided to participants before each section. The first part involved socio-demographic and occupational characteristics such as age (years), sex (male/female), marital status (married, single, divorced or widowed), region (East, Central, West, or Northeast China), education (degree), profession (doctor, nurse, or psychologist), and monthly income (low: ≤5,000, medium: 5,000–10,000, or high: >10,000 RMBs). We regrouped the monthly income levels based on the most recent Chinese income standards (23). We followed the current classification of economic regions in China according to the National Bureau of Statistics (24).

In part 2, the Maslach Burnout Inventory-Human Service Survey (MBI-HSS) (25) was used to measure burnout among MHPs. The Chinese version of MBI-HSS has been validated in many studies (26–28). This is a 22-item scale scoring the following three domains of burnout: emotional exhaustion (EE), involving ninr items; depersonalization (DP), involving 5 items; and reduced personal accomplishment (PA), involving eight items. These items were scored on a 7-point scale from 0 to 6 according to frequency of symptoms. Participants with high EE (≥ 27) and/or DP (≥ 10) scores were defined as "having burnout" (27).

The third part included health-related behaviors (29), such as physical exercise, sleep, alcohol use, and cigarette smoking. In this study, we defined "regular exercise" as exercising at least 3 times per week according to the recommendations of the National Fitness Guideline (30), "frequent insomnia" as having sleep disturbances (difficulty falling asleep, difficulty maintaining sleep, or waking up early) at least 3 times per week (31), and

"alcohol use" as having an alcoholic drink at least twice a month during the past year.

Questions about smoking in this study only refer to cigarette smoking, since it is by far the most common type of tobacco use in China (current cigarette/electronic-cigarette use ratio =54.6) (3). We asked about cigarette smoking status and there were three answers: never, past, and current smoking. We provided a descriptive definition for each answer: "smoking" was defined as cumulative smoking of at least 100 cigarettes (13), and "past (former or ex) smoking" was defined as a continuous cessation of smoking for more than 3 months (14). If participants answered "current smoking", then they were asked another question, "how many cigarettes did you smoke every day in the past month?" Individuals who smoked at least 20 cigarettes daily were defined as "heavy smokers" (14). Furthermore, the duration of smoking was recorded. Finally, current smokers' intention to quit was also investigated.

Data Analysis

Since the numerical variables between different groups were not normally distributed (Kolmogorov-Smirnov test, all $p < 0.05$), we compared the differences between groups using the Kruskal-Wallis H test (3 groups) or Mann-Whitney U test (2 groups) for continuous variables and chi-square test for categorical variables. Then, we compared the prevalence of burnout, regular exercise, frequent insomnia, and alcohol use between smoking (current) and non-smoking groups using logistic regression analyses to control for confounding factors. We applied Bonferroni correction to the comparisons of these rates to adjust for multiple tests. Finally, binary logistic regression analysis was used to determine the independent factors of smoking within the whole sample. As sex is considered to be an important factor associated with smoking, we conducted a further logical regression analysis of male participants. Since there were only 110 smokers among the female participants, the same analysis was not performed. We conducted statistical analyses using SPSS version 23.0 with the significance level at p value of 0.05 (2- tailed).

RESULTS

Participant Characteristics

A total of 21,858 MHPs, including 6,986 doctors (73.3% were psychiatrists), 13,867 nurses (78.8% were registered psychiatric nurses), and 1,005 psychologists were invited to participate in this survey. 14,666 responded (response rate = 67.1%) and 13,614 (62.3%) (4,345 doctors, 9,112 nurses and 257 psychologists) completed the questionnaire with no logical errors and were included in the statistical analysis. The mean age of the total sample was 35.8 ± 8.8 years (range, 19–90 years). Two thirds (66.9%) were nurses and three quarters were female (75.1%) and married (75.3%). Significant group difference was found in several socio-demographic and occupational variables, such as age, education, marital status, region, profession, and monthly income (all $p < 0.05$) (**Table 1**).

The Prevalence of Current and Past Smoking

The overall prevalence of current and past smoking was 8.6% (31.3% in males and 1.1% in females, same hereafter) and 3.3% (11.4% and 0.7%) in the entire sample, respectively. **Table 2** showed the prevalence of current and past smoking by gender, profession, and age. Using the sum of current and past smokers as life-smokers, we calculated the percentages of past smokers among life-time smokers. It was 28.0% in the whole sample, 26.5% among doctors, 28.9% among nurses, and 33.3% among psychologists. The highest prevalence of current and past smoking was found in the age group, ≥ 50 years (14.0% and 6.3%). The prevalence of heavy smoking was 27.5% in current smokers (28.9% and 14.5%). The mean duration of smoking was 13.9 ± 8.8 years in current smokers and 9.3 ± 7.7 years in past smokers. In current smokers, 20.8% (244/1170) reported an intention to quit within 1 year, and 29.7% (347/1170) reported no intention to quit.

Burnout and Other Health Behaviors

We found significant differences in scores on EE, DP, and PA between smokers and non-smokers (all $p < 0.01$) (**Figure 1**). The rates of burnout, regular exercise, frequent insomnia, and alcohol use in the whole sample were 38.1%, 7.2%, 26.9%, and 10.2%, respectively. Compared with non-smokers, smokers had higher rates of burnout (45.6% *vs* 37.4%, $\chi^{2} = 31.05$, $p < 0.001$), frequent insomnia (36.8% *vs* 25.9%, $\chi^{2} = 63.73$, $p < 0.001$), and alcohol use (41.9% vs 7.2%, $\chi^{2} = 1397.34$, $p < 0.001$). After Bonferroni correction (α=0.05/4 = 0.0125), all differences in these rates remained significant (all $p < 0.0125$). After controlling for the socio-demographic and occupational confounders, including age, education, marital status, region, profession, and monthly income, significant associations were found between smoking and burnout (OR = 1.45, 95%CI: 1.27–1.67, $p < 0.001$), frequent insomnia (OR = 1.56, 95%CI: 1.35-1.81, $p < 0.001$), regular exercise (OR = 0.72, 95%CI: 0.56–0.91, $p = 0.007$), and alcohol use (OR = 2.79, 95%CI: 2.39–3.26, $p < 0.001$).

Factors Associated With Smoking

We conducted a multiple logistic regression analysis with current smoking as the dependent variable and the other related variables as covariates. The reference category for each categorical covariate is shown in **Table 3**. The results showed that an older age, male sex, being divorced or widowed, working in West and Northeast China, being nurses, having a high income, burnout, frequent insomnia, and alcohol use were all significantly associated with a higher risk of smoking, while higher levels of education and exercising regularly were associated with a lower risk of smoking (all $p < 0.05$). The regression analysis of male participants showed that except income levels ($p > 0.05$), other factors were still significantly associated with smoking, which was consistent with the results in the whole sample (all $p < 0.05$).

DISCUSSION

This was the first national survey to investigate the prevalence of current and past cigarette smoking and their correlates among Chinese MHPs. We examined the demographic correlates as well as the association between smoking and burnout, and other health-related behaviors such as exercise, sleep and alcohol use. A few

TABLE 1 | Sociodemographic characteristics of the participating mental health professionals.

	Allparticipants	Current smokers	Pastsmokers	Neversmokers	Statistics	
	n=13,614	*n*=1,170	*n*=454	*n*=11,990	H or χ^2	*p* value
Age (years) (Mean ± SD)	35.8 ± 8.8	37.7 ± 9.6	38.4 ± 10.2	35.5 ± 8.6	79.24[a]	<0.001
Sex (%)					4058.39[b]	<0.001
Male	3,392 (24.9)	1,060 (90.6)	386 (85.0)	1,946 (16.2)		
Female	10,222 (75.1)	110 (9.4)	68 (15.0)	10,044 (83.8)		
Education level (%)					65.71[b]	<0.001
Associate degree or less	3,199 (23.5)	365 (31.2)	142 (31.3)	2,692 (22.5)		
College degree[c]	8,888 (65.3)	699 (59.7)	276 (60.8)	7913 (66.0)		
Master's degree	1285 (9.4)	86 (7.4)	30 (6.6)	1169 (9.7)		
Doctorate degree	242 (1.8)	20 (1.7)	6 (1.3)	216 (1.8)		
Marital status (%)					25.21[b]	<0.001
Married	10,258 (75.3)	895 (76.5)	370 (81.5)	8,993 (75.0)		
Single	2,868 (21.1)	219 (18.7)	63 (13.9)	2,586 (21.6)		
Divorced or widowed	488 (3.6)	56 (4.8)	21 (4.6)	411 (3.4)		
Region (%)					49.63[b]	<0.001
East China	5,148 (37.8)	364 (31.1)	158 (34.8)	4,626 (38.6)		
Central China	2,523 (18.5)	219 (18.7)	77 (17.0)	2,227 (18.6)		
West China	3,537 (26.0)	317 (27.1)	113 (24.9)	3,107 (25.9)		
Northeast China	2,406 (17.7)	270 (23.1)	106 (23.3)	2,030 (16.9)		
Profession (%)					104.24[b]	<0.001
Doctors	4,245 (31.2)	501 (42.8)	181 (39.9)	3,563 (29.7)		
Nurses	9,112 (66.9)	657 (56.2)	267 (58.8)	8,188 (68.3)		
Psychologists	257 (1.9)	12 (1.0)	6 (1.3)	239 (2.0)		
Income (%)[d]					23.06[b]	<0.001
Low	5,269 (38.7)	425 (36.3)	153 (33.7)	4,691 (39.1)		
Middle	6,832 (50.2)	573 (49.0)	243 (53.5)	6,016 (50.2)		
High	1,513 (11.1)	172 (14.7)	58 (12.8)	1,283 (10.7)		

[a]*Kruskal-Wallis H test;* [b]*Chi-square test;* [c]*Including medical school in China;* [d]*Monthly income, low: ≤5,000, medium: 5,000–10,000, or high: >10,000 RMBs, one dollar is approximately equal to 6.95 RMBs at the time of study.*

TABLE 2 | The prevalence of current and past cigarette smoking by gender, professions and age.

Profession	Doctors	Nurses	Psychologists	Total	χ^2	*p* value
Male	*n*=1,786	*n*=1,566	*n*=40	*n*=3,392		
Current smoker[a]	490 (27.4)	559 (35.7)	11 (27.5)	1060 (31.3)	26.77	<0.001
Past smoker[a]	176 (9.9)	206 (13.2)	4 (10.0)	386 (11.4)	8.98[b]	0.01
Female	*n*=2,459	*n*=7,546	*n*=217	*n*=10,222		
Current smoker[a]	11 (0.4)	98 (1.3)	1 (0.5)	110 (1.1)	14.64[b]	0.001
Past smoker[a]	5 (0.2)	61 (0.8)	2 (0.9)	68 (0.7)	12.75[b]	0.002
Age	**19–29, years**	**30–39, years**	**40-49, years**	**≥50, years**	χ^2	*p* value
Male	*n*=758	*n*=1,503	*n*=650	*n*=481		
Current smoker[a]	232 (30.6)	432 (28.7)	219 (33.7)	177 (36.8)	13.24	0.004
Past smoker[a]	73 (9.6)	152 (10.1)	83 (12.8)	78 (16.2)	17.09	0.001
Female	*n*=2,810	*n*=4,569	*n*=1,961	*n*=882		
Current smoker[a]	30 (1.1)	47 (1.0)	19 (1.0)	14 (1.6)	2.48	0.48
Past smoker[a]	15 (0.5)	36 (0.8)	9 (0.5)	8 (0.9)	3.82	0.28

[a]*Data are presented as number of professionals (percentage);* [b]*Fisher's Exact Test.*

strengths of this study should be mentioned. First, our sample size is by far the largest involving MPHs and the online survey format and anonymity likely encouraged participants' honest responses. We targeted 41 top-tier psychiatric hospitals from 29 provinces and the participation rate at the hospital level was 100%. MHPs in these hospitals accounted for approximatively one-fifth of the total registered doctors and nurses working within psychiatric hospitals in China (32). Second, we collected data on both current and past smoking behavior and we used clear descriptive definitions for all items. Third, we collected data on burnout and health-related behaviors so we could examine their associations with smoking.

We found the rate of current smoking was 8.6% in the whole sample (31.3% in males, and 1.1% in females) and the rate of past smoking (smoking cessation ≥3 months) was 3.3% (11.4% in males and 0.7% in females). We found a striking sex difference with males being 37.7 times more likely to be a smoker than females. We also found cigarette use was significantly associated with demographic factors such as older age, being male, divorced

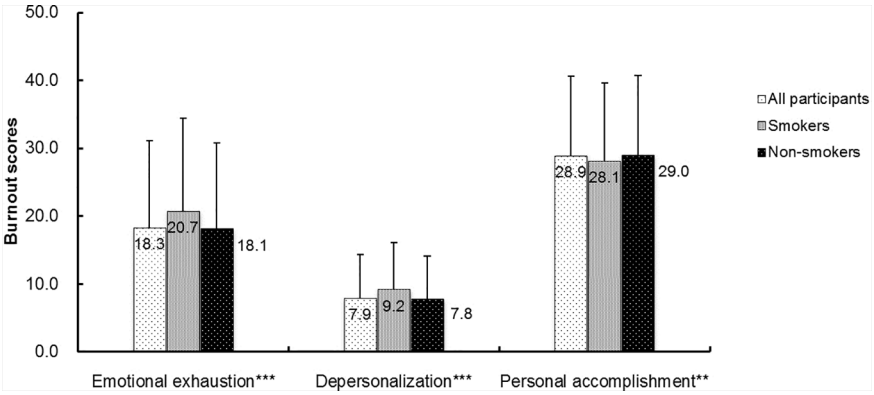

FIGURE 1 | The comparisons of burnout scores between smokers and non-smokers among the participating mental health professionals. $^{**}p < 0.01$; $^{***}p < 0.001$ by Mann-Whitney U-test. The *p* values were comparison between smokers and non-smokers.

TABLE 3 | Multiple logistic regression of factors associating with smoking in mental health professionals.

Variables	Coefficient	Std. Error	Wald $\chi 2$	*p* value	OR (95% CI)
Age (years)	0.02	0.01	9.62	0.002	1.02 (1.01-1.03)
Male (ref. female)	3.63	0.11	1099.99	<0.001	37.73 (30.45-46.76)
Education level (ref. associate degree or less)					
College degree	-0.41	0.10	18.28	<0.001	0.67 (0.55-0.80)
Master's degree	-0.76	0.17	20.41	<0.001	0.47 (0.34-0.65)
Doctorate degree	-0.85	0.29	8.83	0.003	0.43 (0.24-0.75)
Marital status (ref. married)					
Single	0.01	0.11	0.11	0.91	1.01 (0.82-1.25)
Divorced or widowed	0.54	0.19	8.33	0.004	1.72 (1.19-2.48)
Region (ref. East China)					
Central China	0.10	0.11	0.95	0.33	1.11 (0.90-1.37)
West China	0.37	0.10	13.75	<0.001	1.45 (1.19-1.77)
Northeast China	0.50	0.12	18.80	<0.001	1.65 (1.32-2.07)
Profession (ref. doctors)					
Nurses	0.37	0.10	13.68	<0.001	1.44 (1.19-1.75)
Psychologists	0.22	0.35	0.39	0.53	1.24 (0.63-2.44)
Income (ref. low income)					
Middle	0.06	0.09	0.43	0.51	1.06 (0.89-1.26)
High	0.27	0.13	3.96	0.047	1.31 (1.00-1.70)
Burnout (ref. no)	0.26	0.08	11.04	0.001	1.29 (1.11-1.50)
Regular exercise (ref. no)	-0.31	0.13	5.90	0.02	0.74 (0.57-0.94)
Frequent insomnia (ref. no)	0.33	0.08	16.20	<0.001	1.39 (1.18-1.63)
Alcohol use (ref. no)	1.02	0.08	160.89	<0.001	2.76 (2.36-3.23)
Constant	-5.63	0.29	366.08	<0.001	0.004

R^2 = 0.436; CI, confidence interval.

or widowed, and working in West and Northeast regions. Importantly, we also found cigarette smoking was significantly associated with burnout, insomnia and alcohol use.

In this study, the prevalence of cigarette smoking among MHPs was lower than that in the general population of China (31.3% vs 51.4% in males; 1.1% vs 2.7% in females) (3). On the other hand, we found relatively high rates of past smoking (i.e. successfully quit smoking for at least 3 months) in our sample across different professions and age groups: it was 13.2% in male nurses, 0.8% in female nurses, and 16.2% in male participants older than 50 years. The ratio of male past smokers to current smokers in our study (11.4% to 31.3%) is higher than that reported in previous study (8.4% to 54.0%) (33). Furthermore, compared with the general population of China, MHPs have a lower proportion of heavy smokers (27.5% vs 51.3%), and a higher proportion of smokers with an intention to quit (20.8% vs 7.9%) (34). These findings suggest that healthcare professionals' overall awareness of smoking risks and access to smoking cessation treatments (35) may be better than that of the general population, or there may be greater pressure among MHPs to quit because of their role as healthcare workers.

The striking sex difference in smoking is consistent with most studies from China (21, 36–38), and reflects a cultural stereotype around smoking and alcohol use (39, 40). Traditionally, smoking

and alcohol use have been considered masculine behaviors and women who engage in them are often strongly discouraged or stigmatized for doing so. However, recent data show that there has been a slight increase in cigarette use among women (from 2.5% in 2010 to 2.7% in 2015) (41). Additionally, smoking is considered a symbol of showing-off and independence (42). Our data revealed that, although still very low compared to their male counterparts, the rate of smoking in female nurses was more than three times the rate in female doctors (1.3% vs 0.4%), and the ratio between female and male nurses (1:27.5) was higher than that in doctors (1:68.5).

The rates of smoking in male and female doctors in our sample were 27.4% and 0.4%, respectively, and they were both lower than those seen among doctors working in general tertiary hospitals in China (35.9% in males, and 1.1% in females) (36). There are two possible explanations for this discrepancy. First, about 10 years ago, China initiated a series of public campaigns against smoking in public places, including restaurants, schools and hospitals (43). The Jiang et al. (36) study was conducted before the launch of a major public antismoking campaign, so the rates were expected to be higher. Second, the samples in the Jiang study included surgeons (41.4% and 2.7%) and dentists (42.2% and 0.8%), two specialties known for their high rates of smoking. The rate of smoking in male doctors in this study is significantly higher than that in New Zealand (2%) (11) and Spain (8.9%) (15), and it is similar to that in Japan (27.1%) (16) and Italy (29%) (14). It is worth noting that the samples from these countries were not limited to health professionals in psychiatric hospitals. Of note, the smoking rate among male nurses in our sample was particularly high (35.7%). This is higher than that seen among male nurses working in Ireland (25%) (12), but lower than the rate reported among 283 Italian professional nurses (40%) (14). This elevated rate may be due to the high levels of stress experienced by male psychiatric nurses in China. Our previous study showed that within a 12-month period, one-third of psychiatric nurses in China experienced patient-initiated violence (44).

Furthermore, we found that an older age, a lower level of education and being divorced or widowed were associated with smoking among MHPs. Young health professionals in China are provided universal education on the harms of smoking and tend to have positive attitudes towards smoking bans (45), both of which may account for their lower smoking rates. Perhaps for the same reasons, those with higher levels of education have been more successful in quitting smoking than those with lower levels of education (46).Previous studies found that being divorced or separated was more likely to be associated with lower life satisfaction and predicted a higher risk of smoking than being married (29, 47). In addition, we found significant regional differences in smoking, with higher rates in West and Northeast China than those in East China. This may reflect the cultural differences that exist between different regions of the country (48).

One new finding in our study is the significant association between smoking and burnout. We found that a MHP meeting criteria for burnout had a 1.3 greater odds of being a smoker than a non-smoker. This finding is consistent with the results of previous studies conducted in other countries (18, 19). Burnout of health professionals often coexists with low job satisfaction (26), and leads to negative emotions (28) and negative lifestyle choices, including smoking (18). Smoking is also commonly used as a coping method for anxiety symptoms and negative affect (49, 50).

The substantial link between alcohol and tobacco use has been repeatedly confirmed (51, 52). Our results showed that drinkers are more likely (OR=2.76) to smoke than non-drinkers. Especially in some areas in China, such as the Northeast, the co-use of tobacco and alcohol increases the smoking rate of the local people, in that they often only smoke when using alcohol (48). This, along with our findings associating smoking with other health-related behaviors and symptoms (e.g. drinking, lack of regular exercise, insomnia) suggests that strategies targeting smoking cessation must incorporate additional health-promoting interventions. For example, a previous study showed that people with poor sleep may self-medicate and try to improve their mood using nicotine (53). Another study found that among heavy smokers who also drank alcohol, reducing their alcohol use helped them quit their daily smoking habit (54). An earlier study showed vigorous exercise facilitates short- and longer-term smoking cessation in women when combined with a cognitive-behavioral smoking cessation program (55). As these behaviors are all inter-related with each other, targeting only one aspect is unlikely to succeed on its own.

Several limitations in our study should also be noted. First, we only surveyed cigarette smoking and other types of tobacco use (although relatively rare) were not included in our questionnaire. Second, we collected data based on self-report and we did not use validated instruments (such as the Fagerström Test for Nicotine Dependence) or objective measures for smoking (such as cotinine levels). In addition, possible biases may exist due to the nature of self-report measurements, and due to the fact that some MHPs declined to participate or did not complete the survey. Third, as is the case in most cross-sectional surveys, our data were not able to address the direction of causality. Finally, the participants in our study were MHPs working in top-tier psychiatric hospitals and our findings may not generalize to other populations of MHPs in China.

CONCLUSIONS

In conclusion, based on this survey of a large, national sample of MHPs from 41 tertiary psychiatric hospitals across China, we found the overall rate of cigarette smoking was 8.6% (31.3% in males, and 1.1% in females), which was lower than that seen in the general population. Additionally, cigarette smoking among MHPs was associated with a number of socio-demographic factors, burnout levels and health-related behaviors, including alcohol use, insomnia, and lack of regular exercise. Longitudinal studies using validated questionnaires or objective measures for

smoking are necessary to further clarify the relationship of cigarette smoking with these factors in Chinese MHPs. Given their work as healthcare professionals who often address nicotine addiction and the dangers of smoking with their own patients, efforts to lower smoking rates among MHPs in China should continue and should incorporate strategies that target burnout, sleep, alcohol use, exercise and other factors associated with smoking cigarettes.

DATA AVAILABILITY STATEMENT

The data used for this study are available from the corresponding authors on reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Chaohu Hospital of Anhui Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FJ, HL, YL, and Y-LT conceived and planned the survey. LX, YZ, and KZ contributed to the implementation and acquisition of data for this study. LX and FJ did all data analyses and drafted the manuscript. Y-LT and JR gave many useful suggestions and amended the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Reciprocal Substitution Between Methamphetamine and Heroin in Terms of Reinforcement Effects in Rats

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Heroin and methamphetamine are both popular illicit drugs in China. Previous clinical data showed that habitual users of either heroin or methamphetamine abuse the other drug for substitution in case of unavailability of their preferred drug. The present study aimed to observe whether heroin can substitute the methamphetamine reinforcement effect in rats, and vice versa. Rats were trained to self-administer heroin or methamphetamine (both 50 μg/kg/infusion) under an FR1 reinforcing schedule for 10 days. After having extracted the dose–effect curve of the two drugs, we administered methamphetamine at different doses (12.5–200 μg/kg/infusion) to replace heroin during the period of self-administration, and vice versa. The heroin dose–effect curve showed an inverted U-shaped trend, and the total intake dose of heroin significantly increased when the training dose increased from 50 to 100 or 200 μg/kg/infusion. Following replacement with methamphetamine, the total dose–effect curve shifted leftwards and upwards. By contrast, although the dose–effect curve of methamphetamine also showed an inverted U-shaped trend, the total dose of methamphetamine significantly decreased when the training dose decreased from 50 to 25 μg/kg/infusion; conversely, when the methamphetamine training dose increased, the total dose did not change significantly. The total dose–effect curve shifted rightwards after heroin was substituted with methamphetamine. Although heroin and methamphetamine had their own independent reward effects, low doses of methamphetamine can replace the heroin reward effect, while high doses of heroin can replace the methamphetamine reward effect. These results demonstrated that heroin and methamphetamine can substitute each other in terms of reinforcement effects in rats.

Keywords: amphetamine, opiate, drug abuse, conditioning reward, addiction

INTRODUCTION

Drug abuse remains a significant public health concern in China and worldwide. The 2016 Annual National Drug Report of China counted about 2.95 million drug abusers registered in China; moreover, real drug users were estimated to be more than 14 million (1). Before 2014, heroin was the most common illicit drug in China, followed by methamphetamine (MA), either in the form of crystals or pills; henceforth, the number of MA users increased significantly coinciding with the reduction of heroin users (1), suggesting dual drug use epidemics in China. In addition, issues related to the combined utilization of MA and heroin were also identified (2). Further, a 2014 survey conducted in five major Methadone Maintenance Treatment (MMT) clinics in the Yunnan province showed that the urine of about 9.2% of participants was positive for both morphine and MA (3). Previous clinical data showed that among current heroin users, half of them used amphetamine during the preceding 6 months while, in the case of amphetamine users, two-thirds of them used heroin in that period; besides, although transitions from amphetamines to heroin are more common than those in the other way round, they are relatively common in both directions (4). A 2015 survey from the National HIV Behavioral Surveillance (NHBS) system in Denver, Colorado, showed that half of the participants injected both MA and heroin during the past 12 months (5). Recently, the last-month use of MA among treatment-seeking opioid users significantly increased from 18.8% in 2011 to 34.2% in 2017 (6). Since the global emergence of combining MA with heroin may have serious public-health implications, understanding the mechanisms underlying co-morbidity of MA and heroin use disorders is crucial.

Heroin is a μ-opioid receptor agonist that mimics the effects of endogenous opioids, resulting in euphoria, analgesia, and sedation (7). On the other hand, MA — a psychostimulant — principally affects the monoamine neurotransmitter system, resulting in alertness feelings, increased energy, and euphoria (8). Heroin and MA greatly differ in many aspects, including their pharmacological features, behavioral or neurobiological mechanisms, and clinical effects (9, 10); however, heroin and MA share the ability to increase dopamine levels in the nucleus accumbens (NAc), one of the terminal regions of the mesocorticolimbic dopamine projecting system; the activation of this system and the subsequent dopamine release play a key role in the rewarding effects of the drugs (11). Interestingly, heroin is an attractive option for MA users due to its high purity and relatively low price (12). Similarly, opioids users prefer MA when it is easily accessible or cheap (6). Nonetheless, the reasons for the combined utilization of MA and heroin are still unclear.

The present study investigates whether heroin and MA can substitute each other in terms of reinforcement effect in rats. First, we observed whether MA could replace heroin reinforcement effects in heroin intravenous self-administered rats. Second, we evaluated whether heroin could substitute MA reinforcement effects in MA self-administered rats.

MATERIALS AND METHODS

Animals and Drugs

Male Sprague–Dawley rats initially weighing 260 to 300 g (Zhejiang Experimental Animal Center, Hangzhou, China) were individually housed in a temperature-controlled and ventilated colony room, with food and water available *ad libitum*. To simulate the rodents' natural state, all experiments were conducted during the dark phase of the light–dark cycle (lights on from 7:30 am to 6:30 pm). We performed the experiments following the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health (8th edition).

We used heroin (diacetylmorphine HCl) and D-methamphetamine — obtained from the National Institute of Forensic Science (Beijing, China) — dissolved in sterile 0.9% physiological saline.

Surgery

The rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and implanted with chronically indwelling jugular catheters as previously described (13, 14). The catheter was flushed daily with 0.3 ml saline containing heparin (5 units) and penicillin B (20,000 units) to prevent bacterial infection and maintain catheter patency. Following the surgery, the rats were allowed 7 d to recover.

Intravenous Self-Administration Training

The rats were trained to self-administer heroin or MA in operant chambers following an FR1 schedule for 4 h/d during 10 consecutive days. Every session started with a blue light inside the "active" nose-poke hole. The rat received a single infusion following a nose-poke inside the "active" hole based on an FR1 schedule. Each infusion was paired with 5 s of illumination at room light, as well as with the noise from the infusion pump, as a discrete conditioned stimulus (CS). Further, we set a timeout period of 20 s during which the response produced no programmed consequences but was still recorded. Illumination of the blue light in the "active" nose-poke signaled the end of the timeout period. Nose-poking in the "inactive" hole produced no programmed consequences.

Special Experiments

Experiment 1: MA Substitution of the Heroin Reinforcement Effects

Heroin self-administration: All rats (n=19) were trained to self-administer heroin for 10 days (4 h/d; 100 μg/kg/infusion for the first 3 days, 50 μg/kg/infusion for last 7 days) based on an FR1 reinforcement schedule.

Dose–effect response in heroin self-administration: On the 11th day, the rats were randomly divided into three groups (n = 6–7/group). Each group self-administered heroin for 4 h with doses of either 5, 12.5, or 25 μg/kg/infusion instead of the original dose of heroin (50 μg/kg/infusion). On the 12th day, all rats recovered with the original dose of heroin. On the 13th

day, the rats were randomly divided into two groups (n = 9–10/group) and each group was given either 100 or 200 μg/kg of heroin for 4 h.

MA substituted for heroin reinforcement: Following another recovery day, on the 15th day, the rats were randomly divided into two groups (n = 9–10/group) with the order mixed and counterbalanced. Each group self-administered MA doses of either 12.5 or 50 μg/kg/infusion for 4 h. Then, MA doses of 25 or 100 μg/kg/infusion were given for 4 h on alternate days, using the original heroin dose for recovery on interval days. Then in one groups (n=9) MA dose of 200 μg/kg/infusion were given for 4 h on alternate days.

Experiment 2: Heroin Substitution of the MA Reinforcement Effects

MA self-administration: All rats (n=16) were trained to self-administer MA for 10 days (4 h/d; 50 μg/kg/infusion) as described above.

Dose–effect response in MA self-administration: On the 11th day, the rats were randomly divided into two groups (n=8/group). Each group self-administered MA doses of either 25 or 100 μg/kg/infusion instead of the original dose of MA (50 μg/kg/infusion) for 4 h. On the 12th day, all rats recovered with the original dose of MA (50 μg/kg/infusion). On the 13th day, the rats were randomly divided into two groups (n = 7–8/group) and each group was given either 50 or 200 μg/kg of heroin for 4 h. One rat was abandoned due to an unexpected physical condition.

Heroin substituted for MA reinforcement: After 1 day for recovery, the rats were randomly divided into two groups (n = 5–6/group) with the order mixed and counterbalanced. Each group used a dose of either 12.5, 25 μg/kg/infusion heroin to replace the original dose of MA (50 μg/kg/infusion) for 4 h; then, the heroin doses changed to either 50 or 200 μg/kg/infusion on alternate days, using the original heroin dose (50 μg/kg/infusion) on recovery interval days.

Data Statistics

Data are expressed as mean ± SEM. All statistic tests were performed using SPSS (IBM, Armonk, New York, United States). The mean amount of "active" and "inactive" pokes, infusions, and total dose during self-administration were analyzed *via* One-way ANOVAs with Bonferroni *post hoc* testing when appropriate. And the t test was used to compare between the two groups. A P-value< 0.05 was considered statistically significant.

RESULTS

The Dose–Effect Curve of Heroin Self-Administration

Rats were successfully trained to self-administer heroin under an FR1 reinforcing schedule (**Figure 1A**). When in dose–effect response in heroin self-administration session, One-way ANOVA revealed a significant difference in the active pokes ($F_{5, 41}$ = 7.6, P<0.01), infusions ($F_{5,41}$ = 10.4, P<0.01), and total dose of heroin intake ($F_{5,41}$ = 18.1, P<0.01). The *post hoc* comparisons indicated that at the number active pokes and infusions at the dose of 25 μg/kg/infusion was significantly increased compared to other doses. The dose–effect curve of heroin showed an inverted U-shaped trend, whose peak likely being 25 μg/kg/infusion. Nevertheless, the active pokes and the infusions of high dose of heroin changed not so significantly (**Figures 1B, C**). Besides, the total dose of heroin intake showed a linear trend, being higher at high doses (i.e., 100 or 200 μg/kg/infusion) (**Figure 1D**).

The Dose–Effect Curve of MA Substitution for Heroin Reinforcement

When the rats were allowed to self-administer several MA doses instead of heroin, they exhibited significant changes in terms of active pokes ($F_{5, 47}$ = 2.7, P<0.05) and infusions ($F_{5, 47}$ = 7.1, P<0.01). However, *post hoc* comparisons indicated that MA significantly increased the active pokes and the infusions only at low doses (**Figures 2A, B**).

Moreover, multiple comparisons revealed that, when replaced with MA, the total intake infusions only significantly increased above the training MA dose of 50 μg/kg/infusion, and 25 μg/kg/infusion could equally instead of 50 μg/kg/infusion heroin self-administration at total dose (**Figure 2C**). The total dose curve of MA substitution shifted leftwards and upwards compared to the total dose–effect curve of heroin (**Figure 2D**).

The Dose–Effect Curve of MA Self-Administration

Rats were successfully trained to self-administer MA under an FR1 reinforcing schedule as well (**Figure 3A**). When in dose–effect response in MA self-administration session, the statistical analyses indicated significant differences in terms of active pokes ($F_{3, 27}$ = 17.5, P<0.01), infusions ($F_{3,27}$ = 16.4, P<0.01), and total intake dose of MA ($F_{3,27}$ = 8.5, P<0.01) at different training doses. *Post hoc* comparisons showed that active pokes and infusions decreased significantly at high MA doses (100 or 200 μg/kg/infusion) compared to 50 μg/kg/infusion doses (P<0.01). The dose–effect curve of MA also showed an inverted U-shaped trend distribution (**Figures 3B, C**). However, only the total intake dose of MA decreased at low MA doses (i.e., 25 μg/kg/infusion) (P<0.01) (**Figure 3D**).

The Dose–Effect Curve of Heroin Substitution for MA Reinforcement

When the rats were allowed to self-administer different doses of heroin instead of 50 μg/kg/infusion of MA, the statistical analyses showed a significant change in the number of active pokes ($F_{4, 28}$ = 5.8, P<0.01) and infusions ($F_{4, 28}$ = 6.8, P<0.01). However, *post hoc* comparisons showed a significant decrease in the active pokes and infusions following heroin substitution at doses of 50 or 200 μg/kg/infusion (**Figures 4A, B**).

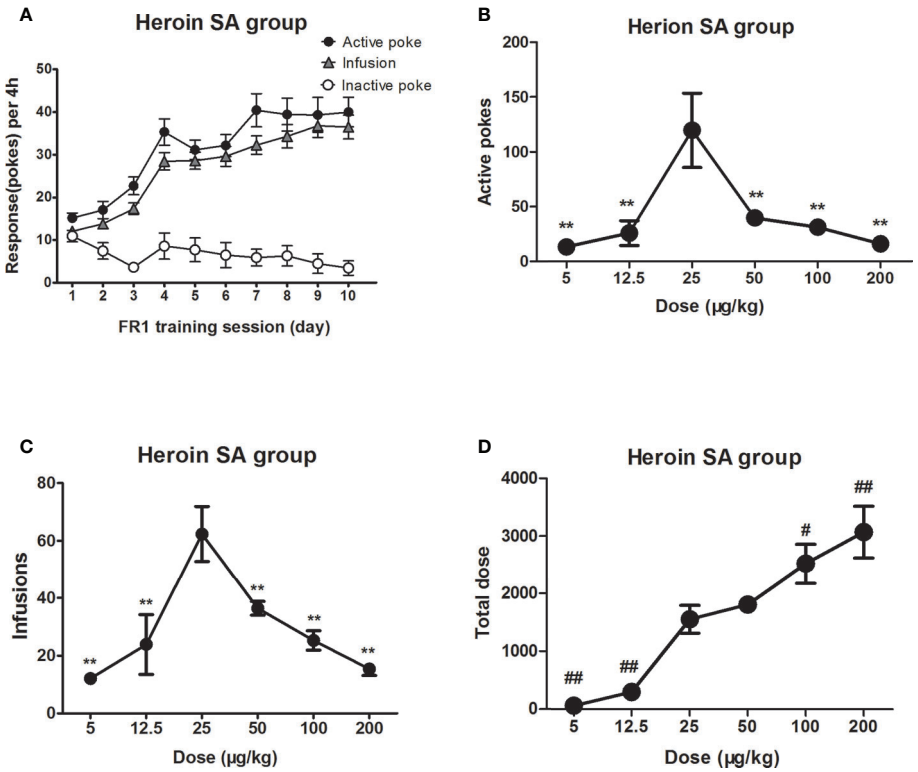

FIGURE 1 | Dose–effect response in heroin self-administration. Rats (n = 19) were successfully trained to self-administer heroin 4 h/d under an FR1 reinforcing schedule within 10 days **(A)**. When the heroin trained rats were tested in different dose (5 μg/kg/infusion–200 μg/kg/infusion) of heroin to self-administer 4 h in the following days (n=6-10/group). The active pokes and the infusions were changed and showed an inverted U trend, whose peak likely being 25 μg/kg/infusion **(B, C)**. Rats took more heroin when doses increased to wither 100 or 200 μg/kg/infusion and less heroin intake when doses highly decreased to either 5 or 12.5 μg/kg/infusion, but maintained the total dose unchanged following the dose decreased to lower at 25 μg/kg/infusion **(D)**. $^{**}P < 0.01$, compared to 25 μg/kg/infusion; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared to 50 μg/kg/infusion. Error bars = SEM.

Moreover, the total dose of heroin showed a progressive increase in MA trained rats. *Post hoc* comparisons indicated the total intake dose of heroin significantly decreased at 50 μg/kg/infusion or below when replaced with heroin. Only heroin doses of 200 μg/kg/infusion could equally substitute MA doses of 50 μg/kg/infusion (**Figure 4C**). Finally, compared to the total dose–effect curve of MA, the substituted curve shifted rightwards (**Figure 4D**).

DISCUSSION

According to these results, heroin and methamphetamine could replace each other during the self-administration training, suggesting that both opiates and psychostimulants may have a common rewarding mechanism. Besides, higher heroin doses seem to be necessary to replace the MA rewarding effects, whereas lower MA doses seem to be sufficient to replace the heroin rewarding effects.

With 4 h/d drug access, heroin or MA self-administration were stable both over time and across doses after 10-d training (15–18). The dose–effect curve of heroin showed a negative linear trend, and the active response decreased following the dose increase, consistently with the reinforcement theory (19).The limbic dopaminergic system was proposed as the neurobiological substrate for the rewarding effects of both opiates and psychostimulants. More specifically, heroin indirectly activates dopaminergic neurons in the ventral tegmental area (VTA), through inhibition of GABAergic interneurons (20), whereas MA directly redistributes dopamine from synaptic vesicles to the synaptic cleft, blocks dopamine reuptake, and inverts the direction of dopamine transport (8). According to the existing literature, both MA and heroin injection produce the release of dopamine in the NAc, as analyzed by microdialysis (21, 22). Further, more selective studies of drug-induced neuronal plasticity recently highlighted that these two drugs promote an overall reward circuitry signaling overlap, particularly through the modification of excitatory synapses in the NAc (23). Therefore, the common dopamine mechanism could explain the reciprocal substitutability between heroin and MA.

When heroin was replaced with MA, the total dose–effect curve shifted leftwards and upwards, possibly meaning that

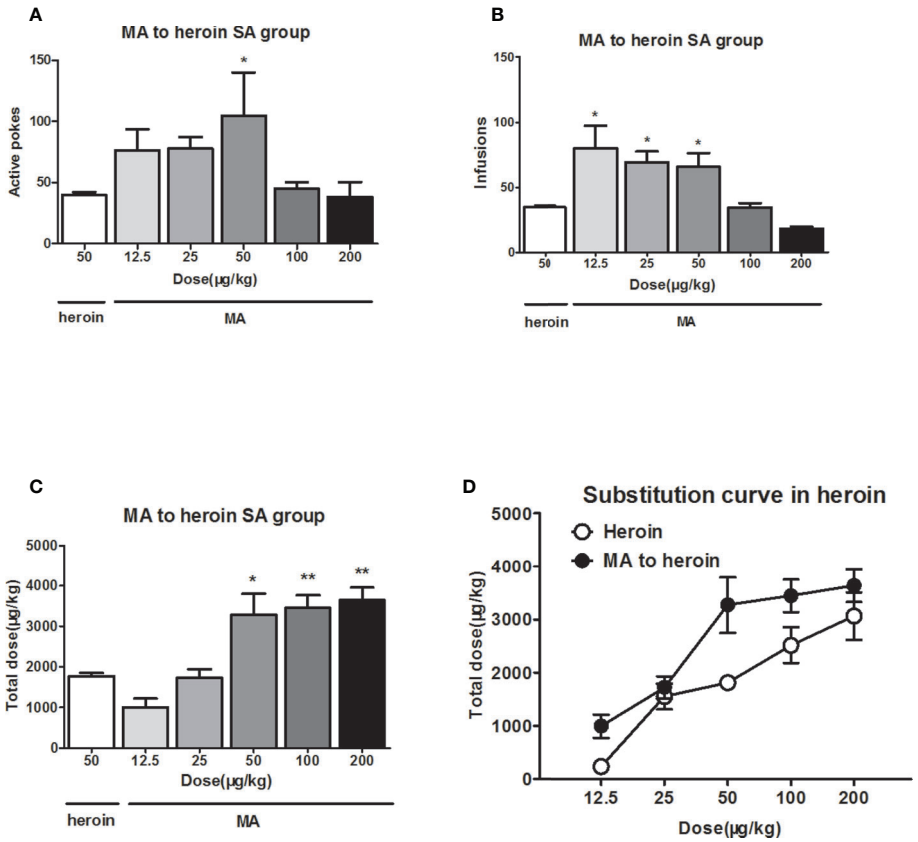

FIGURE 2 | MA substituted for heroin reinforcement. As substitution with different dose of MA to self-administer 4 h in heroin trained rats (n=9-10/group), only dose of 50 μg/kg/infusion of MA significantly increased the active pokes and the lower doses of 12.5, 25, 50 μg/kg/infusion of MA increased the infusions **(A, B)**. The total dose significantly increased above the training MA dose of 50 μg/kg/infusion, and 25 μg/kg/infusion could equally instead of 50 μg/kg/infusion heroin self-administration at total dose **(C)**.The total dose–effect curve of MA replacement showed shifted leftward and upward compared to the total dose–effect curve of heroin **(D)**. *$P < 0.05$, **$P < 0.01$, compared to 50 μg/kg/infusion heroin. Error bars = SEM.

MA served as a heroin substitute; in addition, lower MA doses seem to be required to produce an identical or even more sensitive rewarding effect of heroin. Likewise, heroin could be replaced by a relatively lower dose of MA to reach are warding effect. On the other hand, when MA was replaced with heroin, the dose–effect curve shifted to the rightward, indicating that MA rewarding effect required a higher dose of heroin to be replaced.

Combining the substitution curves, the original heroin dose–effect curve was very similar to the dose–effect curve of MA substitution; likewise, the original MA and the curve of heroin substitution was also identical. The same trend in two separate experiments suggested that heroin and MA substitution was symmetrical, and their rewarding effect was independent. The phenomenon was supported by self-administration heroin and cocaine on alternate days, the escalated levels of cocaine self-administration fail to generalize heroin when change the heroin self-administrate back later (24). Similarly, the total dose of heroin or cocaine was not different from the total dose the rats intake which trained by single drug (heroin or cocaine) directly (25). The term "substitution" is typically used in drug discrimination procedures to determine if drugs share discriminative stimulus effects. For example, cocaine can partially substitute the discriminative stimulus effects of heroin in rats, and when heroin–cocaine combinations are administered to the heroin-trained rats, cocaine did not significantly alter the mean heroin dose–effect curve (26). Nonetheless, in another study with rhesus monkeys, opioids substituted the cocaine discriminative stimulus in some, but not in all of them (27). Some evidence showed that only small subsets of rats preferred either cocaine or heroin instead of the food alternative in the choice procedure, indicating the substance-specific pattern of drug preference (28). In several experiments, it was repeatedly shown that when the heroin trained rats resumed heroin training after being given MA instead of heroin, their heroin use was the same as before replacement with MA, indicating that the MA fully met the heroin demand during replacement days. Overall, these results suggest that MA could at least partly substitute heroin, and vice versa. Taken together, although

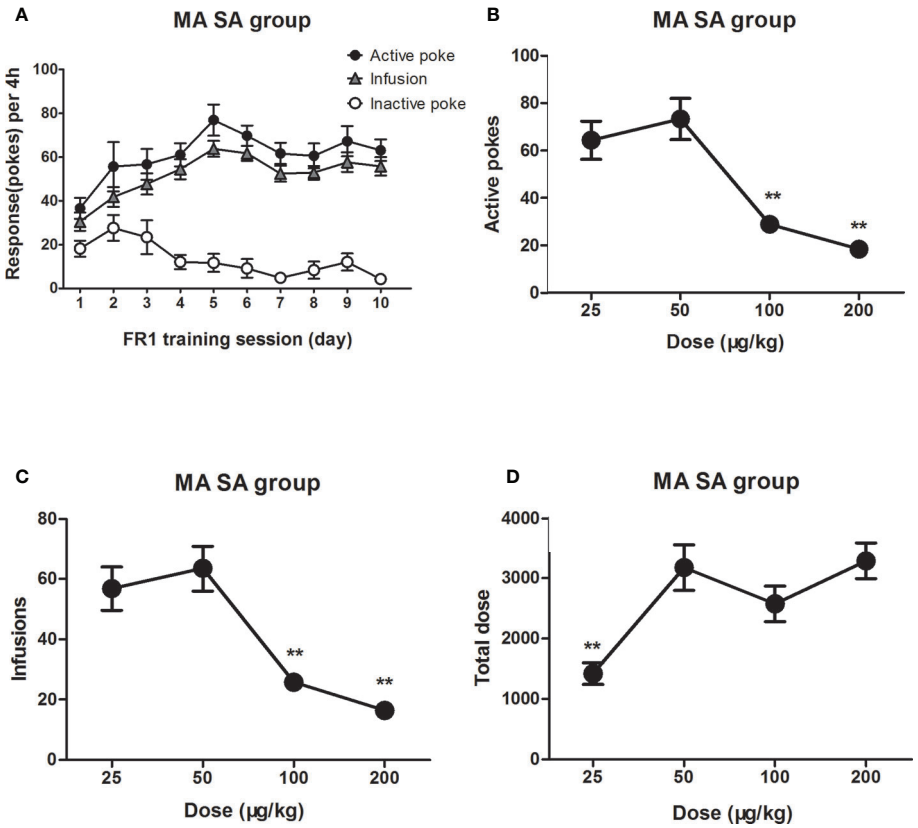

FIGURE 3 | Dose–effect response in MA self-administration. Rats (n=16) were successfully trained to self-administer MA 4 h/day under an FR1 reinforcing schedule within 10 days **(A)**. When the MA trained rats were tested in different dose (25 μg/kg/infusion–200 μg/kg/infusion) of MA to self-administer 4 h in the following days (n=7-8/group). The active pokes and the infusions were changed and showed an inverted U trend. The number of active pokes and infusions decreased significantly at high MA doses (100 or 200 μg/kg/infusion) compared to 50 μg/kg/infusion doses **(B, C)**. Rat took less MA when dose decreased to 25 μg/kg/infusion, but maintain the total dose unchanged as dose increased (100, 200 μg/kg/infusion) **(D)**. $^{**}P < 0.01$, compared to 50 μg/kg/infusion MA. Error bars = SEM.

psychostimulants and opioids seem to have their own independent way to substitute, MA and heroin did not affect the self-administration of the other.

Heroin and MA greatly differ in terms of pharmacological features as well as in clinical *manifestations* following their utilization (9, 10). The preclinical data indicated that the increase in the amount of dopamine induced by MA in the NAc is more than that induced by heroin. For example, when MA doses of 1 mg/kg were injected subcutaneously in rats, the levels of dopamine in their NAc increased up to 698% (29); however, when heroin doses of 0.2 mg/kg were injected intravenously in rats, the levels of dopamine in their NAc only increased up to 300% (30). Besides, gamma-vinyl GABA — an inhibitor of irreversible GABA-transaminase — only partially blocks the dopamine increase in the NAc caused by MA, yet completely inhibits the dopamine increase in the NAc caused by heroin (31). By contrast, heroin could enhance its rewarding effect through the CB1 receptor and other mechanisms independents of the dopamine levels in the NAc (21); further, MA also released large amounts of serotonin through its actions (22). Concerning cocaine, serotonin acts on the 5-HT_{1b} and 5-HT_{2c} receptors with bidirectional feedback to regulate the levels of dopamine in the NAc (32, 33). This indicates that the downstream reinforcement mechanisms of the two drugs are not the same. Therefore, MA and other psychostimulants may increase the levels of dopamine in NAc more significantly than heroin. That explained in part why low-dose MA could effectively replace heroin.

Previous clinical data showed a rather high frequency of combined utilization of MA and heroin in both habitual heroin and MA users (2, 5). Further, preclinical studies demonstrated that MA–heroin combinations ("speedballs") produce greater rewarding effects (34); for example, urine of MMT patients in Yunnan tested positive for both morphine and MA (3), and half had used amphetamine among current primary heroin users while two-thirds of primary amphetamine users had used heroin (4). The main reasons for transition in drug use reside in either obtaining synergistic high or balancing out the effects of each other and

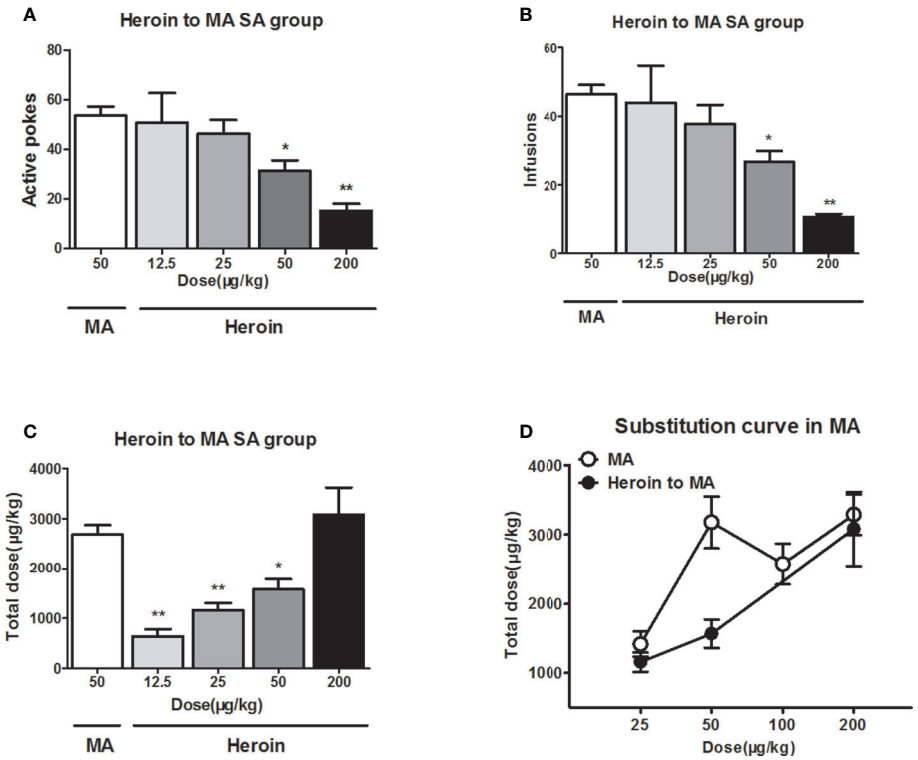

FIGURE 4 | Heroin substituted for MA reinforcement. As substitution with different dose of heroin to self-administer 4 h in MA trained rats (n = 5–6/group), the higher doses (50, 200 μg/kg/infusion) of heroin significantly decreased the active pokes and the infusions **(A, B)**. The total intake dose of heroin significantly decreased at 50 μg/kg/infusion or below when replaced with heroin, and 200 μg/kg/infusion heroin could equally instead of 50 μg/kg/infusion MA self-administration at total dose **(C)**. The total dose–effect curve of heroin replacement showed shifted rightward and downward compared to the total dose–effect curve of MA **(D)** $^{*}P < 0.05$, $^{**}P < 0.01$, compared to 50 μg/kg/infusion MA. Error bars = SEM.

keeping body functions as normal as possible (6). Another reason for drug transition is related to the price and availability of drugs: when MA prices increase, co-users choose to consume more heroin, and vice versa (35). The drug-market period in which injecting initiation occurred sensitively influenced the kind of first injected drug, thereby influencing some aspects of subsequent drug use in Australia (36).The present results provided the first direct evidence for the transition between MA and heroin use due to the reciprocal substitutability of their rewarding effects. The crisis of the global emergence of combining MA with heroin use is merging, and attention should be paid to their negative effects on public health (37). In conclusion, the present study demonstrated that heroin and MA can partly substitute each other; further, heroin and MA may follow their independent dose–effect mechanism during the substitution process.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Animal Ethics Committee of Ningbo University.

AUTHOR CONTRIBUTIONS

D-SM designed and performed research, analysis the data, and the writing of the paper. Y-JC, F-MW, and B-MM performed research and data collection. H-FL were responsible for guarantee of animal and experimental conditions. W-HZ and J-PX were responsible for the study concept and design, revising the paper, and study supervision.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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Gender Differences in Sleep Problems Among Drug Users

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Background: Illicit drug use has been recognized as a major problem. Clinical studies demonstrated that poor sleep quality was associated with increased frequency of drug use and relapse. However, few studies addressed the issue of sleep quality and gender differences in illicit drug dependent subjects. The present study aimed to explore the gender differences in sleep problems in drug users.

Methods: In this cross-sectional study, a total of 2,178 illicit drug users, including 1,875 male users [884 methamphetamine (MA) users and 991 heroin or other drug users] and 303 female users (78 MA users and 225 heroin or other drug users, 13.9%), from drug rehabilitation centers in Changsha, and 2,236 non-drug-using subjects, including 1,910 males and 326 females (14.6%) completed the self-report Pittsburgh Sleep Quality Index (PSQI).

Results: We found that the prevalence of suggestive sleep problems (PSQI>5) between male and female was 67.4 and 75.2% in overall illicit drug use sample ($p<0.001$), 52.4 and 75.6% in MA users (<0.001), 80.8 and 75.1% in heroin or other drugs users ($p=0.054$), 26.0 and 28.8% in healthy controls ($p=0.287$). For sleep quality, the mean of PSQI total score (M±SD) between male and female was 7.8±4.42 and 8.9±4.15 in overall illicit drug users ($p<0.001$), 6.4±4.45 and 9.1±4.00 in MA users (<0.001), 9.1±3.96 and 8.9±4.21 in heroin or other drugs users ($p=0.394$), 4.2±2.46 and 4.4±2.51 in healthy control sample ($p=0.090$). These results indicated that only MA users, rather than heroin or other drugs users and healthy controls, showed gender differences in sleep problems.

Conclusions: In this study, female users reported higher frequency of sleep problems and poorer sleep quality than male users in MA users' group, but not in heroin or other drug users group. Future studies aim at quantifying the benefits of treatment interventions should not neglect the influence of sleeping problems and its gender differences.

Keywords: sleep quality, sleep problems, drug use, healthy control, gender differences

INTRODUCTION

Sleep disorders are prevalent problems in the general population and are closely connected with medical, psychological, and social disturbances (1). An early study from the Los Angeles metropolitan area showed that approximately half of people from general population displayed sleep disorders (2). Insomnia is the most common sleep disorder. It is characterized by the presence of an individual's report of difficulty falling and/or staying asleep. About one-third of the adult general population experience one or more of the symptoms of insomnia, such as difficulty initiating sleep, difficulty maintaining sleep, waking up too early, and nonrestorative or poor quality of sleep (3, 4).

Insomnia is associated with substantial impairments in emotional and mental health, and quality of life (5, 6). Sleep problems are much more likely to affect patients with mental disorders, such as anxiety, depression, bipolar disorder, and attention deficit hyperactivity disorder (ADHD), alcohol, and other substance use disorders, than people in the general population (7–9).

Sleep problems may increase risk for developing substance use disorders, as well as result from chronic substance use. An ongoing longitudinal study showed that early childhood sleep problems (3–5 years of age) significantly increased the likelihood of an early onset of any use of alcohol and illicit drugs, as well as an early onset of occasional or regular cigarette smoking in adolescence (12–14 years of age) (7). Heroin and methamphetamine (MA) are the most commonly used illicit drug in China (10). Our previous study showed that a majority of heroin users displayed poor sleep quality, especially those long-term users (11). Other chronic drug users [such as MA users and ketamine users (12)], and individuals with behavior addiction [such as Internet addiction (13) and Instagram addiction (14)] were also reported high prevalence of sleep problems. The illicit drugs, such as methamphetamine, cocaine, ecstasy, and marijuana, have pronounced impact on sleep quality by polysomnography (PSG, a test that electronically transmits and records specific physical activities while in sleep). For example, cocaine administration suppresses rapid eye movement (REM) sleep and increases wakefulness; abstinent heavy 3,4-methylenedioxymethamphetamine (MDMA; "ecstasy") users display altered sleep architecture (15).

It is quite evident that sleep problems are more common in women than in men in the general population (16, 17). Our previous study with a large sample from the general population in China also shows that women reported poorer sleep quality than men did, but little gender difference in the prevalence of insomnia (4). However, gender differences of sleep problems were found to be associated with socio-economic patterning, people with low income, low education have more chance to suffer from sleep problems (17). Our previous study found that smokers, especially heavy smokers had more sleep problems than nonsmokers (18). However, gender differences of sleep problems among drug users, especially the mainly used MA and heroin, are not fully understood.

The mechanism of action of MA involves blockade of presynaptic reuptake and displacement of vesicular stores of various neurotransmitters, mainly affected dopamine, norepinephrine, and serotonin. Central nervous system effects of acute MA abuse include insomnia, anxiety, tremors, hallucinations, paranoia, chronic effects include paranoia, hallucinations, paranoid psychosis, memory loss, depression (19). Heroin is an opioid (narcotic) drug made from morphine. Acute heroin abstinence changes various states of sleep, waking and related behaviors (20), causing sedation and daytime drowsiness (side effects of opioid analgesics) (21). Chronic heroin use also linked to poor sleep quality (11, 22).

The present study sought to explore them in a sample of drug users and healthy controls. It is hypothesized that the female drug users, either MA users or heroin or other drug users, would report more sleep problems and poorer sleep quality than the male ones. It is also hypothesized that, compared with healthy controls, gender differences of sleep problems and poorer sleep quality would be more significant in drug users.

METHODS

Subjects

A total of 2,390 healthy nondrug users and a total of 2,178 illicit drug users (after detoxification-treatment) from voluntary drug rehabilitation centers (the Kangda Voluntary Drug Rehabilitation Centre in Hunan Province and the Department of Addiction Medicine, Hunan Brain Hospital) in Changsha completed the self-report Pittsburgh Sleep Quality Index (PSQI) to explore sleep quality among them. The data of drug users was collected after their completion of a 10-day detoxification-treatment. The date of healthy controls was collected by visiting demographically matched households. Healthy controls were excluded if they reported any drug use, major medical, or psychiatric disorders. More details about subjects see (12).

Assessment Measures

Socio-demographic and sleep-related information were gathered for all participants. Drug used related information were collected for all drug users. The Chinese version of Pittsburgh Sleep Quality Index (PSQI) (23) was used to assess sleep quality and sleep problems. PSQI total score > 5 was an indicator of suggestive poor quality of sleep (poor sleeper).

Procedure

The study and oral informed consent were approved from the Second Xiangya Hospital of Central South University Institutional Review Board (No. S117, 2009). All participants were informed that they can withdraw from the study at any point without penalty or adverse consequences. Issues of confidentiality and anonymity were discussed with them. Oral informed consent was obtained from all participants. Drug dependent patients were invited to answer the short self-reported questionnaire on the first week of the administration about their drug use. Eligible participants were invited to participant the survey after their completion of a 10-day detoxification-treatment.

Statistical Analysis

Statistical analysis was performed using the SPSS for Windows (Version 22, SPSS Inc., Chicago, IL, USA) software package. Descriptive statistics were used to examine demographic and drug use characteristics, and pooled responses. Mann-Whitney U-test and χ^2 (Chi-square) were conducted to determine gender differences of demographic characteristics, sleep problems (including subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, need for sleep medications, daytime dysfunction), sleep quality (by the mean of PSQI total score), and poor sleepers (suggestive insomnia) in each group (healthy control group, overall drug use group, MA use group, heroin, or other drugs use group). Analysis of variance (ANOVA) was applied to explore gender and group differences in sleep quality (by the mean of PSQI total score). First, one-way ANOVA was used to compare each two-group differences across three groups (healthy control group, MA use group, heroin, or other drugs use group) of males and females. Then, further explored whether there were differences by gender (male, female)/drug class (MA, heroin or other drug) differences (either main effect or an interaction with gender) with general linear model (GLM) of univariate ANOVA. A *post-hoc* test was conducted using the Fisher's least significant difference (LSD). In addition, χ^2 was conducted to compare the percentage of poor sleepers (suggestive insomnia with PSQI > 5) among healthy controls, heroin, or other drug users and MA users in samples of overall, male, and female, respectively. A p-value threshold of 0.01 was set to determine statistical significance.

RESULTS

Demographic characteristics between male and female in healthy controls and drug users (MA users and heroin or other drug users) are shown in **Table 1**. Cigarette smoking and alcohol drinking are the most significantly different characteristics between males and females in control group. Only a very small proportion of healthy females were cigarette smokers (1.9%) or alcohol drinkers (2.8), but approximately 90% and over 10% of female drug users were cigarette smokers and alcohol drinkers, respectively. The MA users used an average of 36 (mean ± SD: 36.2 ± 31.44) months of MA, and heroin users 82 (82.1 ± 60.81) months of heroin. In the last 3 months before administration, MA users used an average of 0.6 g (0.6 ± 0.91) MA with an average of two (2.3 ± 1.13) times per day, heroin users used 1.7 g (1.7 ± 0.98) heroin with an average of one (1.1 ± 0.34) time per day.

The PSQI components' scores and PSQI total score between male and female in healthy control group and drug use (methamphetamine, heroin, or other drugs) group are shown in **Table 2**. The prevalence of suggestive sleep problems (PSQI>5) between male and female was 67.4 and 75.2% in overall illicit drug use sample (p<0.001), 52.4 and 75.6% in MA users (<0.001), 80.8 and 75.1% in heroin or other drugs users (p=0.054), but little gender difference was found in healthy control sample (26.0% in males and 28.8% in females, p=0.287). The mean of PSQI total score (M±SD) between male and female was 7.8±4.42 and 8.9±4.15 in overall illicit

TABLE 1 | Demographic characteristics between male and female in groups of healthy controls and drug users.

Variables	Healthy controls (n=2,236)			Drug users (n=2,178)								
				Overall sample			MA users (n=962)			Mainly heroin users[#] (n=1,216)		
	Male (n=1,910)	Female (n=326, 14.6%)	p*	Male (n=1,875)	Female (n=303, 13.9%)	p*	Male(n=884)	Female (n=78, 8.4%)	p*	Male(n=991)	Female (n=225, 18.5%)	p*
Age (yrs), M±SD	30.7±11.23	30.2±10.66	0.486	30.0±7.13	30.1±7.16	0.776	29.1±6.79	26.7±5.95	0.003	30.9±7.33	31.3±7.18	0.428
Education (yrs), M±SD	12.2±3.23	11.7±3.19	0.008	10.7±2.96	10.4±3.05	0.070	11.1±2.89	10.2±2.99	0.004	10.2±2.96	10.5±3.07	0.298
Drug use duration (month), M±SD	–	–	–	59.0±51.73	62.3±55.38	0.581	36.6±32.03	31.9±23.49	0.205	78.9±57.47	72.9±59.25	0.157
Ethnicity (Han Chinese, %)	96.7%	95.1%	0.146	98.7%	99.7%	0.135	98.6%	98.7%	0.955	98.7%	100%	0.084
Employed %	62.6%	52.5%	0.001	55.1%	61.9%	0.026	47.0%	66.7%	0.001	62.2%	60.3%	0.589
Married %	50.8%	55.4%	0.125	45.2%	67.7%	<0.001	41.9%	24.4%	0.002	48.2%	35.1%	<0.001
Cigarette smoking%	46.8%	1.9%	<0.001	97.8%	90.1%	<0.001	98.0%	88.5%	<0.001	97.7%	90.7%	<0.001
Alcohol drinking%	35.7%	2.8%	<0.001	38.3%	13.9%	<0.001	53.0%	16.7%	<0.001	25.2%	12.9%	<0.001

M, mean; SD, standard deviation; n, number; %, the percentage of subjects.
Cigarette smoker was defined as smoked more than 100 cigarettes in the life time.
Alcohol drinking was defined as drunk no less than 30 g alcohol (equal to 900 ml beer) per week.
Difference in percentage or mean differences analyzed between male and female drug users by the Chi-squared test and the Wilcoxon rank sum test (Mann-Whitney U-test) respectively.
[#]Mainly heroin users (heroin or other drug users, including 1,012 heroin users in a total of 1,216 subjects, 83.2%), and some poly-substance users who mainly used "club-drug".
**Significantly different from male group, p < 0.01.*

TABLE 2 | Score of the Pittsburgh Sleep Quality Index (PSQI) components and PSQI total score between male and female in groups of healthy controls and drug users.

	Healthy controls (n=2,236)			Drug users (n=2,178)								
				Overall sample			MA users (n=962)			Mainly heroin users (n=1,216)		
	Male	Female	P*	Male	Female	P*	Male	Female	P*	Male	Female	P*
Subjective sleep quality, M±SD	0.8±0.64	0.7±0.64	0.004	1.4±0.90	1.6±0.84	<0.001	1.2±0.93	1.6±0.89	<0.001	1.6±0.83	1.6±0.82	0.615
Sleep latency, M±SD	0.7±0.69	1.0±0.85	<0.001	1.4±1.13	1.6±1.08	0.003	1.2±1.13	1.7±1.17	<0.001	1.6±1.11	1.6±1.05	0.960
≤15 min, %	42.9	29.1	–	27.5	17.2	–	35.4	16.7	–	20.5	17.3	–
16–30 min, %	46.5	49.1	–	29.7	34.3	–	27.8	33.3	–	31.3	34.7	–
31–60 min, %	9.3	15.0	–	18.2	19.1	–	16.7	9.0	–	19.6	22.7	–
>60 min, %	1.3	6.7	–	24.6	29.4	–	20.0	41.0	–	28.7	25.3	–
Sleep duration, M±SD	0.8±0.82	0.5±0.74	<0.001	0.8±0.99	1.0±1.06	0.007	0.6±0.93	1.2±1.12	<0.001	1.0±1.01	0.9±1.04	0.475
>7 h, %	46.0	62.0	–	51.1	45.2	–	61.5	41.0	–	41.8	46.7	–
5–7 h, %	52.1	37.7	–	40.6	42.9	–	31.9	44.9	–	48.4	42.2	–
<5 h, %	1.9	0.3	–	8.3	11.9	–	6.6	14.1	–	9.9	11.1	–
Habitual sleep efficiency*, M±SD	0.3±0.70	0.4±0.62	0.168	0.6±1.03	0.8±1.14	0.023	0.5±0.92	1.0±1.24	0.001	0.8±1.10	0.7±1.10	0.621
≥85%, %	80.9	71.5	–	65.9	60.7	–	72.6	55.1	–	59.9	62.7	–
75–84%, %	12.0	23.0	–	15.3	14.2	–	13.6	12.8	–	16.8	14.7	–
65–74%, %	3.5	4.3	–	7.4	9.2	–	5.8	10.3	–	8.9	8.9	–
<65%, %	3.5	1.2	–	11.4	15.8	–	8.0	21.8	–	14.4	13.8	–
Sleep disturbance, M±SD	0.7±0.53	0.8±0.55	0. 003	1.1±0.72	1.2±0.79	0.028	1.0±0.70	1.4±0.70	<0.001	1.2±0.72	1.2±.81	0.181
Need for sleep medications, M±SD	0.1±0.27	0.1±0.34	0.271	0.9±1.23	1.0±1.30	0.151	0.6±1.07	0.7±1.19	0.663	1.1±1.32	1.1±1.33	0.963
Not during the past month, %	95.4	93.9	–	62.7	60.7	–	71.8	74.4	–	54.5	56.0	–
Less than once a week, %	3.7	5.2	–	8.4	5.0	–	8.0	2.6	–	8.8	5.8	–
Once or twice a week, %	0.7	0.3	–	8.3	9.6	–	7.4	5.1	–	9.2	11.1	–
≥3 times a week, %	0.1	0.6	–	20.6	24.8	–	12.8	17.9	–	27.5	27.1	–
Daytime dysfunction, M±SD	0.9±0.85	1.0±.85	0.010	1.6±1.10	1.7±1.14	0.091	1.3±1.10	1.6±1.07	0.017	1.9±1.03	1.8±1.16	0.159
PSQI total score, M±SD	4.2±2.46	4.4±2.51	0.090	7.8±4.42	8.9±4.15	<0.001	6.4±4.45	9.1±4.00	<0.001	9.1±3.96	8.9±4.21	0.394
Poor sleepers (PSQI > 5), %	26.0	28.8	0.287	67.4	75.2	<0.001	52.4	75.6	<0.001	80.8	75.1	0.054

M, mean; SD, standard deviation; n, number; %, the percentage of subjects; PSQI, Pittsburgh Sleep Quality Index.

Difference in percentage or mean differences analyzed between male and female drug users by the Chi-squared test and the Wilcoxon rank sum test (Mann-Whitney U-test) respectively.

**Significantly different from male group, $p < 0.01$.*

The total score range ranges from 0 to 70 and each score ranges from 0 to 3, with higher scores indicating poorer functioning.

Habitual sleep efficiency = total hours of sleep/(get-up time–bedtime) × 100%.*

drug use sample (p<0.001), 6.4±4.45 and 9.1±4.00 in MA users (<0.001), 9.1±3.96 and 8.9±4.21 in heroin or other drugs users (p=0.394), 4.2±2.46 and 4.4±2.51 in healthy control sample (p=0.090). These results indicated that only female MA users, rather than heroin or other drugs users and healthy controls, had overall poorer sleep quality or more poor sleepers than males MA users. Noticeably, all drug users, either male or female, MA users or heroin or other drug users, had significantly poorer sleep quality and more sleep problems than that in healthy controls.

Table 3 showed that samples of overall, male, and female were all significantly different in sleep quality (mean of PSIQ total) among healthy controls, heroin, or other drug users and MA users (p<0.001), expect for comparing female heroin or other drug with female MA groups (p=0.61). Then, further explored whether there were differences by gender [male and female, nondrug or drug class (MA and heroin or other drug) differences (either main effect or an interaction with gender), and showed significant effects of gender (F=26.742, p<0.001)], group (F=440.705, p<0.001) and gender*group (F=19.809, p<0.001). **Table 4** showed that samples of overall, male, and female were all significantly different in poor sleeping (PSQI>5) among healthy controls, heroin, or other drug users and MA users, expect for comparing female heroin or other drug with female MA groups (p=0.926).

DISCUSSION

To our knowledge, this is the first study to assess gender differences in sleep problems among illicit drug users. The current study found that gender differences in sleep problems occurred only in MA users, but not in heroin or other drug users or healthy controls.

The overall prevalence of suggestive sleep problems or disturbances (PSQI>5) between male and female was 67.4 and 75.2% in overall illicit drug use sample, with 52.4 and 75.6% in MA users, 80.8 and 75.1% in heroin or other drugs users, and 26.0% in healthy males and 28.8% in healthy females. For sleep quality, the mean of PSQI total score between male and female was 7.8 and 8.9 in overall illicit drug users, 6.4 and 9.1 in MA users, 9.1 and 8.9 in heroin or other drugs users, 4.2 and 4.4 in healthy control sample. Compared with healthy controls, all drug users, especially male heroin or other drug users, had much worse sleep quality. Our previous study already showed higher prevalence of sleep problems in drug users, especially in heroin or other drug users, than healthy controls. Furthermore, heroin or other drug users experienced more sleep problems than MA users (12). However, this study found that female MA users experienced sleep problems similar to female heroin or other drug users. Only MA users showed gender differences in sleep problems, i.e., a higher proportion of female poor sleepers.

Previous studies showed that insomnia certainly had a more profound impact on females than on males (24). The gender difference in the prevalence of sleep problems, specifically the excess in females compared with males, was also observed from our previous large sample size study (4). This study, surprisingly, showed that female drug users had poorer sleep quality than those males only in MA users. Although with no statistically significant differences, females had a trend of poorer sleep quality than their counterparts in general population, and male had a trend of poorer sleep quality than female heroin or other drug users. The underline factors that contributed to these trends are worth exploring in the future studies.

One of the interesting findings is the differences in sleep duration between male and female drug users. Our previous study with a sample (n = 26,851) from the general population found that a total of more women (60.6%) than men (55.9%) reported more than 7 h sleep duration (4). In this study, consistent with the large sample survey, we found that more females had more than 7 h sleep duration in healthy controls and heroin or other drug users, but less female MA users (41%) had more than 7 h sleep duration than male MA users (61.5%). Furthermore, compared with male MA users, more than twice of female MA users (41%) reported sleep latency with more than 1 h.

Short sleep durations have been associated with negative health outcomes (25). For example, short self-reported sleep duration was associated with the incident diagnosis of hypertension, which may result from disrupting circadian rhythmicity and autonomic balance (26). Furthermore, the self-reported sleep duration was associated with interleukin-6 (IL-6) and high-sensitivity C-reactive protein (hs-CRP) in women but not in men (27). Further studies will be needed to establish whether the connection between short sleep duration and female gender in drug use, whether this connection can be demonstrated with other measures, and whether the relationship is causal.

Gender differences in acute effects of MA on subjective mood and reward-related behavior (28) may contribute to gender

TABLE 3 | Pittsburgh Sleep Quality Index (PSQI) total score comparisons among healthy controls, heroin users and MA users in samples of overall, male, and female, respectively.

Groups		N	Mean	SD	95% CI (lower, upper)	P*
Overall	Control	2,236	4.2	2.47	4.09, 4.30	<0.001
	MA	962	6.6	4.48	6.31, 6.88	
	Heroin	1,216	9.1	4.01	8.84, 9.29	
Male	Control	1,910	4.2	2.46	4.05, 4.27	<0.001
	MA	884	6.4	4.45	6.08, 6.67	
	Heroin	991	9.1	3.96	8.86, 9.36	
Female	Control	326	4.4	2.51	4.14, 4.68	<0.001
	MA	78	9.1#	4.00	8.18, 9.98	
	Heroin	225	8.9#	4.21	8.30, 9.40	

CI, confidence interval for mean.

**Significantly different from male group, p < 0.01.*

#No significant differences between female heroin and female MA groups, p = 0.61.

TABLE 4 | The percentage of poor sleepers [Pittsburgh Sleep Quality Index (PSQI) > 5] comparisons among healthy controls, mainly heroin users (heroin or other drug users) and MA users in samples of overall, male, and female, respectively.

	Controls	MA users	Mainly heroin users	P*
Overall	26.4%	54.2%	79.8%	<0.001
Male	26.0%	52.4%	80.8%	<0.001
Female	28.8%	75.6%#	75.1%#	<0.001

**Significantly different from male group, p<0.01.*

#No significant differences between female heroin and female MA groups, p = 0.926.

differences in sleep problems, i.e., compared to their counterparts, female MA (rather than heroin or other drug) users showed overall poorer sleep quality and high prevalence of sleep problems. In addition, MA users experienced significantly more depressive symptoms than healthy controls (29), and there is a gender differences in depression-females are more likely than males to present with depression (30).

The substance/gender interactions with sleep quality could be more complicated. Even a single intranasal methamphetamine dose could produce significant reductions on measures of subjective sleep quality and objective sleep (31). The gender differences of sleep quality between male and female MA users may thank to the gender-specific contribution of the specific genes in MA use disorder (32) or due to the fact that female MA users experience higher rates of depression than their counterparts (33). Furthermore, compared with male MA users, although female users seem more dependent to MA, they show diminished dopamine responses and fewer cases of emergency department-related deaths involving MA (34). Further explore the underline factors associated with gender differences in sleep quality between male and female MA users may provide new evidence-based effective treatment for MA use disorders, especially for these female MA users.

Previous research found that, compared with no sleep disturbance, poor sleep quality or insomnia were associated with higher frequency of cocaine use (35). On the other hand, disturbed sleep and daytime sleepiness occupies in adolescence were associated substance abuse and emotional outcomes, and associated with poor addiction treatment outcomes (36). Thus, sleep quality may serve as a predictor for drug treatment and relapse. Assessing and addressing insomnia and its gender differences among treatment seeking drug users may improve treatment outcome and prevent relapse.

Limitations

In China, men have much higher rates of use or dependence on illicit drugs than do women (37). Thus, the current sample included only 13.9% of female drug users. Furthermore, female drug users reported higher heroin use rate (18.5%) and lower MA use rate (8.4%) that male users. Another limitation is that the percentage of cigarette smoking and alcohol drinking was lower in female drug users than that in male users. However, previous studies showed that both cigarette smoking (38) and high-risk drinking behaviors (39) were more likely to report sleep problems. Furthermore, this study did not assess many other sleep problems related factors, such as socio-economic status, medicine, drug use patterns, severity of dependence. On the other hand, sleep problems in early childhood can predict the onset of alcohol-related problems and illicit drug use in adolescence (7). In the current study, even fewer women than men had cigarette smoking and alcohol drinking, they still reported more sleep problems than male MA users. In addition, this study only assessed substance use, but not internet or other types of behavior addiction, which may also associated with sleep problems (13). Some of the drug users may also try other drugs. However, this study excluded heroin users or MA users who also mainly used other drugs. Last, the minority population of LGBT (lesbian, gay, bisexual, and transgender) may have high risk of drug use (40, 41). This study, however, did not include the third gender.

CONCLUSION

In conclusion, our data suggest that female MA users reported poorer sleep quality by presenting higher PSQI total score than male users. This study found no gender differences in sleep problems in heroin or other drug users or in healthy controls. Female MA users also showed shorter sleep duration than that in male users. Future studies aim at quantifying the benefits of treatment interventions for MA users should not neglect the influence of sleeping problems and its gender differences. Gaining more insight into the impact of gender differences of sleep quality on the MA addiction treatment could also help to target future intervention measures more effectively for female users.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Second Xiangya Hospital of Central South University Institutional Review Board (No. S117, 2009). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

YL conceived the study. YL and HH did the literature review, statistical analyses, and drafted the report. YL and HH collected the data. YL took the lead in writing the manuscript. TL and JT interpreted the data and commented on the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Association Between Cognitive Function and Early Life Experiences in Patients with Alcohol Use Disorder

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Introduction: Early life experiences could be potential risk factors for the development of alcohol use disorder. In similar circumstances, it might also influence cognitive impairment in later life. However, the relationship between early life experience and cognitive function in people with alcohol use disorders is unclear. The current study examined the effects of early social environments and experiences on cognitive function in patients with alcohol use disorder.

Methods: A total of 37 adult male patients with alcohol use disorder and 30 healthy control (HC) subjects were enrolled. The MATRICS Cognitive Consensus Battery (MCCB) was used to evaluate cognitive function. The Childhood Trauma Questionnaire (CTQ) and the Revised Adult Attachment Scale (RAAS) were used to evaluate early life experiences of the participants. The RAAS was used to evaluate the attachment patterns of participants.

Results: Compared with the HC group, the alcohol use disorder group reported higher levels of childhood abuse and were more likely to form an insecure attachment style. Patients with alcohol use disorder who experienced trauma performed worse in terms of discrete cognitive parameters such as social cognition, reasoning and problem solving compared to patients without trauma. Importantly, emotional neglect and social comfort were significantly associated with individual social cognitive skills.

Conclusions: Our results suggest that the cognitive function of patients with alcohol use disorder, especially social cognitive function, is affected by early life experiences.

Keywords: early life experiences, attachment, cognitive function, alcohol use disorder, social cognitive function

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INTRODUCTION

The consumption of alcohol has increased substantially in recent years in developing countries (1). Long-term and excessive consumption of alcohol increases the risk of alcohol use disorder (AUD). Current estimates show that 4% of China's total population has AUD (2, 3). AUD is associated with various debilitating physical functions, including neurophysiological and cognitive changes (4–6), which can have lasting negative effects on cognitive function even when drinking alcohol infrequently (5). The pathogenesis of AUD is complex, and its occurrence and development may

be the result of the interaction between environmental effects and cognitive dysfunction (7, 8). which may lead to a worse prognosis for AUD (9, 10).

It is well known that the exposure to adversity in early life causes a series of negative consequences in later life. Exposure to adversity and poor attachment patterns in early life may lead to negative emotions, and drinkers may use alcohol to ease these negative feelings (11). Extreme stress in childhood may have a serious impact on brain development (12). Neuroimaging studies have shown that repeated exposure to childhood adversity affects the development of the cortical thickness and its surface area (13). Moreover, experiences of abuse in infancy or childhood can have a negative impact on the development of cognitive function in later years (14).

In the 1950s, John Bowlby, a British psychologist and founder of attachment theory, first proposed that attachment is generally defined as a special emotional relationship between a baby and its caregivers. In the process of growing up, the lack of interaction with the object of attachment (such as parents or stable guardians) will have disastrous consequences for a person's life. In addition to childhood maltreatment, a number of intra-familial influences, such as early separation with caregiver and insecure attachment patterns have also been associated with the development of cognitive function in later life (15).

Early experiences are associated with cognitive development in later life. Cognitive dysfunction may be associated with development of AUD (8). Research on exploring the relationship between social cognition and child trauma is generally conducted in other groups, not in patients with AUD. For example, some studies have examined the relationship between childhood trauma and cognitive function in clinical high-risk mental illness (16). In contrast, others have studied the relationship between childhood adversity and cognitive function in schizophrenia spectrum disorders (17). Some studies found that physical trauma in childhood is negatively associated with the development of working memory and executive function in patients with a psychotic disorder (18–21). The relationship between childhood trauma and cognition among patients with AUD remains unexplored, which is important for their treatment outcome. Hence, in this study, we aimed to investigate the early life experiences, attachment patterns, and their association with cognitive function among individuals with AUD. We investigated the correlations between childhood trauma, attachment styles, social cognition in patients with AUD, and then investigated the predictors of social cognition in patients with AUD.

MATERIALS AND METHODS

Participants

We recruited 37 adult male patients with AUD and 30 healthy controls (HC; all men), with an average age of 36.7 years. Patients were recruited from the Substance Dependence Treatment Center of Anhui Mental Health Center in China between January and June 2019. All HC individuals were selected from the patient caregiver cohort and conformed to the demographic characteristics.

Patients who met the following criteria participated in further testing: 1) confirmation of DSM-IV diagnosis of AUD, assessed by two trained psychiatrists independently using the Structured Clinical Interview for DSM-IV (AUD); 2) aged 18–60 years and Han Chinese to reduce any age related variance in cognitive capacity assessment and bias in the recall of childhood traumas; 3) with a course of disease greater than one year; 4) treated with a benzodiazepine during the acute detoxification period, with more than two weeks of withdrawal time and without any obvious withdrawal response; 5) were able to submit written informed consent and participate in psychopathological measurement. Moreover, we excluded patients satisfying any of the following criteria: 1) comorbid with lifelong psychiatric symptoms, substance abuse other than alcohol; 2) combined use of psychoactive drugs other than alcohol in the past few weeks; 3) abnormal baseline EEG and positive blood screening for benzodiazepines and history of head injury or loss of consciousness in the past 12 months. Furthermore, all individuals' demographic characteristics were investigated through the questionnaire including age, sex, race, and year of education of parents. Finally, we obtained a complete medical history from all participants, as well as the results of a physical examination. All participants signed an informed consent form. All surveyors had passed consistency training.

Measures

Childhood Trauma Questionnaire (CTQ)

The CTQ is a retrospective questionnaire used to assess adverse events in early life and comprises 28 items (22). Each item is scored on a 5-point-scale ranging from 1–5 (never true =1 to very often true =5). The total score of the CTQ ranges from 25–125. It assesses five types of trauma: physical abuse (PA), physical neglect (PN), emotional abuse (EA), emotional neglect (EN), and sexual abuse (SA). Using the cutoff point (physical abuse ≥ 10; physical neglect ≥ 10; emotional abuse ≥ 13; emotional neglect ≥ 15; sexual abuse ≥ 8), individuals can be divided into two subtypes: maltreated or not maltreated. The CTQ has been reported to have adequate validity and reliability in China (23). Cronbach's alpha for the factors related to each trauma type ranged from 0.75 to 0.89 in this study.

Revised Adult Attachment Scale (RAAS)

The early social environment and social activity were assessed using the RAAS (24), which is an instrument used to assess the attachment pattern of participants. It consists of 18 items, and is divided into three subscales. First, the Closeness subscale consists of six items: 1, 6, 8, 12, 13, and 17. This subscale evaluates the comfort feelings of individuals in close and intimate relationships. The Dependence subscale contains items 2, 5, 7, 14, 16, and 18. This measured the personal internalized feeling that they could rely on others to be available and that others would be in proximity when needed. The Anxiety subscale comprised of six items: 3, 4, 9, 10, 11, and 15, which assess the levels of personal anxiety from interpersonal rejection by others. Each item is rated on a 5-point scale ranging from 1 (strongly disagree) to 5 (strongly agree). Attachment security was defined as a combined score of <36 on the attachment avoidance

measures (Closeness and Dependence scales) and <18 on the attachment anxiety measures (Anxiety scale). Attachment insecurity was defined as a combined score of >36 on the attachment avoidance measures and/or >18 on the attachment anxiety measures. The coefficient alphas in this study's sample were 0.77 and 0.83 for attachment avoidance and anxiety. The RAAS has demonstrated adequate validity and reliability in China (25).

Neurocognitive Function Assessments

Cognitive performance in participants was assessed using the MATRICS Cognitive Consensus Battery (MCCB). The battery assesses seven cognitive domains through ten subtests. Speed of Processing tested by Trail Making Test Part A, Symbol coding and Category fluency; Working Memory tested by Wechsler Memory Scale Spatial span and Digital sequence test; Verbal Learning tested by Hopkins Verbal Learning Test Revised; Reasoning and Problem Solving tested by Neuropsychological Assessment Battery: Mazes; Attention/Vigilance tested by the Continuous Performance Test: Identical Pairs. Social Cognition tested by Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT): managing emotion. The MCCB battery was translated into a Chinese version, and clinical validity and test-retest reliability were established in the Chinese population (26).

Alcohol Use Disorders Identification Test(AUDIT)

AUDIT was used to identify harmful drinkers and chronic alcohol addicts. It contains ten items: the first three items measure the amount and frequency of regular and irregular alcohol consumption; the middle three items measure the symptoms of chronic alcohol dependence; and the last four items assess short-term and long-term problems related to alcohol consumption. 16 ≤ AUDIT < 20 is harmful to use; 20 ≤ AUDIT < 40 is alcohol dependence. The Chinese version of the AUDIT has good reliability and validity among Chinese students (27).

All neuropsychological assessments were performed in the morning with starting times between 9 am and 6 pm. Tests wer performed by trained psychologists who had received consistency training. Participants with AUD were assessed when they were clinically stable.

Statistical Analysis

In our study, all data analyses were performed using IBM SPSS 22.0. The data were expressed as mean ± standard deviation. We compared differences between the AUD and HC groups using an independent t-test, nonparametric test, and χ^2 test for continuous and categorical variables, respectively in the first step. The patients were then divided into maltreated versus non-maltreated, and the distinct variables of cognitive function domains and attachment dimensions were assessed between the groups. Next, bivariate Pearson correlation coefficients were used to explore the relationship between CTQ subtypes and attachment dimensions and cognitive function domain scores conferred. The variables displaying significant correlations within multiple linear regression analysis were tested to determine whether adult attachment and childhood trauma were significant predictors of cognitive function. All tests were 2-tailed, and a p-value of less than 0.05 was considered statistically significant.

RESULTS

Demographic and Clinical Parameters

A total of 51 patients were recruited in the current study; only 37 patients (age = 36.7 ± 8.5 years) met the inclusion criteria. There was no significant difference in the average age and education years (participants and their parents) between patients and HCs. All the selected AUD patients were treated with benzodiazepines during the acute detoxification period, drug withdrawal had reached more than two weeks, and there was no obvious abstinence reaction.

Early Life Experiences and Attachment Patterns of AUDs and HCs

A total of 56.8% of AUDs reported having experienced at least one childhood trauma. Physical neglect was most frequently reported (45.9%), followed by emotional neglect (35.1%), physical abuse (16.2%), emotional abuse (10.8%), and sexual abuse (10.8%). The frequency of physical abuse and emotional neglect in patients were higher than in HCs. AUDs presented higher CTQ emotional abuse, emotional neglect and sexual abuse estimates than HCs (8.14 ± 2.62 vs. 6.57 ± 1.92, 10.73 ± 5.36 vs. 8.57 ± 2.61, 5.78 ± 1.06 vs. 5.30 ± 0.70, p <0.05).

A total of 35.3% of AUDs demonstrated a secure attachment pattern, which was lower than that of HCs (64.7%). The levels of the Closeness and Dependence scales in patients were inferior to HC (19.97 ± 2.40 vs. 22.43 ± 2.43, 13.68 ± 2.26 vs. 18.37 ± 3.16, $p < 0.05$), and the levels of the Anxiety scale were higher among patients than the controls (17.05 ± 3.36 vs. 14.50 ± 5.19, $p < 0.05$) (**Table 1**).

Relationship Between Early Life Experiences and Cognitive Function of AUDs

AUDs with a history of maltreatment were found to perform poorly in reasoning and problem solving and in social cognition than the AUDs with no history of maltreatment. At the same time, the AUDIT score in AUDs with a history of maltreatment was relatively higher ($p < 0.05$) (**Table 2**). Further, while exploring the relationship between childhood trauma, attachment patterns, and cognitive function in patients, the bivariate Spearman's correlation coefficient was utilized. The scores of physical neglect, emotional neglect, and close attachment were highly correlated with social cognition (r = −0.33, r = 0.52, r = −0.33, $p < 0.05$), while the correlation between the AUDIT score and social cognition was not significant (**Table 3**). The data of physical neglect, emotional neglect, and close attachment were analyzed by stepwise linear regression analyses to explore the association between social cognition (**Table 4**). The regression equation was significant ($F_{[1,35]} = 7.02$, $p < 0.05$ with $adjR^2 = 0.30$). Stepwise

TABLE 1 | Comparison between patients with alcohol use disorders and healthy control groups.

Demographics		AUD (N=37, Mean ± SD)	HC (N=30, Mean ± SD)	$t/z/\chi^2$	p
	Age (years)	36.7 ± 8.5	35.0 ± 8.7	0.81	0.42
	Education (years)	11.76 ± 2.93	12.33 ± 3.22	0.42	0.75
AUDIT		25.24 ± 5.98	6.60 ± 5.13	13.51	< 0.001
CTQ					
	Childhood trauma,n (%)	21(56.8%)	12(40.0%)	1.86	0.22
	Emotional abuse score	8.14 ± 2.62	6.57 ± 1.92	1.47	< 0.01
	Emotional abuse, n (%)	4(10.8%)	0(0%)	3.44	0.12
	Physical abuse score	7.03 ± 2.54	5.80 ± 1.22	0.94	0.09
	Physical abuse, n (%)	6(16.2%)	0%	5.34	0.02
	Sexual abuse score	5.78 ± 1.06	5.30 ± 0.70	1.17	0.02
	Sexual abuse, n (%)	4(10.8%)	1(3.3%)	1.34	0.37
	Physical neglect score	9.62 ± 2.84	8.53 ± 2.61	1.61	0.11
	Physical neglect, n (%)	17(45.9%)	12(40.0%)	0.24	0.80
	Emotional neglect score	10.73 ± 5.36	8.57 ± 2.61	1.43	0.01
	Emotional neglect, n (%)	13(35.1%)	0(0%)	13.08	< 0.001
RAAS					
	Close scale score	19.97 ± 2.40	22.43 ± 2.43	-4.15	< 0.001
	Dependence scale	13.68 ± 2.26	18.31 ± 3.16	2.45	< 0.001
	Anxiety scale score	17.05 ± 3.36	14.50 ± 5.19	1.68	< 0.01
	Attachment, n (%)	12(35.3%)	22(64.7%)	11.08	< 0.01

Continuous variables are expressed as mean ± standard deviation (SD). AUDIT, Alcohol Use Disorders Identification Test; CTQ, Childhood Trauma Questionnaire; RAAS, Revised Adult Attachment Scale; AUD, patients with alcohol use disorders; HC, healthy control.

TABLE 2 | Description of cognitive function measured in patients with alcohol use disorders.

	Non-maltreated(N=16, Mean ± SD)	Maltreated (N=21, Mean ± SD)	z	p
Speed of processing	32.75 ± 14.71	25.71 ± 10.87	0.88	0.29
Attention/vigilance	39.81 ± 10.42	36.24 ± 11.32	0.69	0.57
Working memory	34.19 ± 8.59	31.95 ± 5.45	0.61	0.63
Verbal learning	41.56 ± 6.89	44.00 ± 11.21	0.82	0.36
Visual learning	39.25 ± 12.62	36.14 ± 13.62	0.74	0.50
Reasoning and problem solving	31.81 ± 4.83	27.67 ± 4.39	1.39	0.02
Social cognition	40.13 ± 10.66	30.71 ± 9.49	1.87	< 0.01
AUDIT	22.63 ± 5.64	27.24 ± 5.55	-2.04	0.04

Continuous variables are expressed as mean ± standard deviation (SD). AUDIT, Alcohol Use Disorders Identification Test.

linear regression analysis showed that emotional neglect (beta = −0.92; t = −3.12, $p < 0.05$) and close attachment (beta = 1.48; t = 2.24, $p < 0.05$) were significant predictors of variance in social cognition.

TABLE 4 | Predictors of social cognition in patients with alcohol use disorders.

Model	Unstandardized Coefficients		Standardized Coefficients	t	p
	β	s.e	Beta		
Constant	15.18	13.47		1.13	0.26
Emotional Neglectt	-0.92	0.29	-0.45	-3.12	0.004
Closeness Scale	1.48	0.66	0.32	2.24	0.032

$R^2 = 0.54$, $adjR^2 = 0.30$, $F_{(1,35)} = 7.02$ ($p < 0.05$).

DISCUSSION

This study aimed to explore the influence of the early living environment on the cognitive function of individuals with AUDS. The results showed that individuals with AUD were more likely to experience severe trauma at an early age and had more unsafe attachment patterns. Individuals with a history of emotional neglect in childhood perform poorly in social cognitive function, and a safe attachment model has a positive effect on social cognitive function. This may be

TABLE 3 | Correlations between childhood trauma and attachment styles and social cognition of patients with alcohol use disorders.

	EA	PA	SA	PN	EN	Closeness Scale	Dependence Scale	Anxiety Scale	AUDIT
Social cognition	-0.19	-0.23	-0.16	-0.33*	-0.52*	0.33*	0.26	-0.02	-0.17
Reasoning and problem solving	-0.25	-0.21	-0.29	-0.29	-0.17	-0.22	-0.20	-0.02	-0.08

EA, emotional neglect; PA, physical abuse; SA, sexual abuse; PN, physical neglect; EN, emotional neglect; AUDIT, Alcohol Use Disorders Identification Test.
**p < 0.05.*

illustrated by the link between early life experiences and cognitive function; that is, adverse experiences can lead to negative outcomes in adulthood.

Our study found that 56.8% of patients with AUD in rural areas of China reported exposure to at least one childhood trauma, and physical neglect was most frequently reported (45.9%), followed by emotional neglect (35.1%). This is inconsistent with the findings in developed areas (28); low-or-middle-income countries presented higher estimates of childhood neglect than the high-income countries (29). Patients with AUD with severe childhood trauma experiences presented poorer skills in reasoning, problem solving, and social cognitive functions. One study reported that individuals exposed to physical and emotional events in the initial stages of their life may have impaired verbal skills and social cognitive functions (17). A history of emotional neglect is an independent risk factor for AUD (28). Our findings demonstrated that exposure to emotional neglect in childhood has a negative association with social cognition function performance, as evidenced in the MCCB test in individuals with AUD. Deficits in social cognitive function are hallmarks of major psychiatric disorders, causing weakened social competence, impaired community functioning, and deterioration in quality of life (30, 31). Relevant studies have investigated the impact of childhood trauma on social cognitive function (32–34). Our findings concur with the findings of Kilian, Asmal (17) that emotional neglect is negatively associated with social cognition within MCCB. The effects of emotional neglect are transmitted based on the common environmental factors that arise from the parents and family environments (35). Children with experiences of emotional disturbances have a higher propensity to develop sensitivity toward social cues, which may cause failure to perceive the social information (36). Across different contexts (37, 38), it has been indicated that individuals, both in HC and psychiatric disorder patients, who are victims of emotional trauma, display worse performance in tasks involving emotional perception, theory of mind decoding, and social inference. Moreover, the experience of emotional neglect in early life (0–6 years) could contribute to deficits in theory of mind (39). Heleniak and McLaughlin (40) also reported that violence would alter the cognitive and affective theory of mind.

AUDs with characteristics of insecure attachment (41). Our study found that a higher level of closeness to an attachment figure was strongly associated with increased social cognitive function in patients with AUD. The structure of attachment formed in initial life could have a top-down effect on social information processing. Exposure to appropriate maternal mind-mindedness is beneficial for the evolution of infants' social understanding (42). Individuals with insecure attachment patterns tend to show lower levels of understanding of social information and social cognitive skills than those with secure attachment patterns (30). For example, anxious attachment is associated with a deficit in social cognitive function, and avoidant attachment showed a U-shaped association with social cognitive, individuals with medium scores on avoidant attachment displayed worse theory of mind than lower and higher individuals (43). Further, the exposure to insecure attachments in early life may interrupt the interaction with caregivers, decrease social interaction exposure throughout life, lead to a lack of adequate experiences to communicate an understanding of mental states during early development, and impair the development of the hippocampal neurons, resulting in a decline in cognitive function (44, 45).

It has been found that exposure to stress is negatively associated with long-term hypothalamus-pituitary-adrenal axis activity leading to glucocorticoid release and activation of the ascending dopamine system as well as impairment of specific brain regions (e.g., hippocampus and prefrontal cortex, major areas involved in learning, memory, and social information processing) (46). The stress also has regulatory effect on the function of neuropeptide. For example, Wagner and Echterhoff (47) et al. reported that the impact of oxytocin on memory accuracy relies on individuals' attachment patterns. It is positively affected in individuals who find dependence on others uncomfortable and negatively affected in individuals who find dependence on others comfortable. Additionally, Cancel, Comte (48) et al. found that the stability of connectivity among brain regions involved in emotional processing such as the amygdala and the precuneus, the posterior cingulate cortices, and calcarine sulcus, are affected by a history of childhood abuse. Moreover van Schie, van Harmelen (49) et al. also mentioned in the context of emotional trauma, the ability to understand the mental states of others is related to increased activation of the left inferior frontal gyrus.

Exposure to adversity in early life has a lasting effect on the development of brain function. Individuals with impaired cognitive function, especially impairment in social cognitive, had a poorer quality of life than the HCs (31). Furthermore, such patients are more vulnerable to psychological and physical violence (50). Any kind of misery (viz. domestic violence, childhood abuse and neglect, and economic adversity) contributes to the deterioration in quality of life and are potential risk factors for the onset of early drinking problems (51). Individuals exposed to trauma in early life may adopt the use of alcohol to deal with trauma-related symptoms (52). In the context of stress, addicts are more likely to increase their alcohol consumption, which could further lead to more trauma exposure. Meanwhile, the effect of trauma might be transferred across generations and negatively affects an infant's cognitive and neurologic development. Molenaar, Tiemeier (53) et al. revealed that maternal psychopathology and stress during pregnancy are associated with elevated cortisone in the hair of the offspring, which might affect the function of the cortex. Thus, the transmission of unsolved traumatic experiences could be intergenerational (54). Therefore, protecting patients who are vulnerable to exposure to violence and helping post-traumatic patients make them aware of the impact of trauma to avoid being affected by violence is a necessary practice in clinical intervention.

In our results, patients with AUD drink more alcohol, have more social cognitive impairment and more childhood maltreatment. Therefore, is the social cognitive impairment of people with AUD caused by alcohol use or childhood maltreatment? Our results show that, among AUDs, people with childhood maltreatment have more cognitive impairment than those without childhood maltreatment. In the correlation analysis,

there was no significant correlation between social cognitive impairment and AUDIT score, while the correlation between social cognitive impairment and childhood maltreatment was significant. Our results are supported by previous research reports (55–58). One study found that, in men, there were no differences in cognitive decline among alcohol abstainers, quitters, and light or moderate alcohol drinkers (< 20 g/d) (55). A two-year longitudinal study that enrolled 12,408 participants by Stampfer et al. (56) showed that moderate drinkers had better mean cognitive scores than nondrinkers; up to one drink per day does not impair cognitive function and may actually decrease the risk of cognitive decline. A prospective cohort study (57) found that alcohol consumption was U-shaped in all areas of cognitive function, and low to moderate alcohol consumption was associated with a better overall cognitive score.

There are several limitations to the current study. First, participants had diffused and unclear memories of the early adverse experiences, which could introduce a risk of recall bias. Second, when we designed the experiment, we did not collect the subjects' alcohol use characteristics and other general demographic information. Third, the study used cross-sectional data and small samples, excluding any causal inference. Moreover, in the present study, no female patients were enrolled. Loi, Mossink (59) et al. confirmed that females may be more resilient to early life stress. Further studies are required to investigate the relationship between early life experiences and social cognitive abilities, focusing on sex-based analyses.

CONCLUSION

The main findings of the current study include an enhanced understanding of the relationship between early social environment, early life adversity, and cognitive function in individuals with AUD. In particular, the association of emotional neglect and attachment figures could be significant predictors for development of social cognitive function. Therefore, early intervention in trauma, good parent-child relationships, and modification in the deficit internal working models of social interaction are immensely important to influence the recovery of cognitive functions and psychological adjustment.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Chaohu Hospital, Anhui Medical University (No. 201901-kyxm-02). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FC and SC contributed equally to this article. (I) Conception and design: XZ. (II) Administrative support: XZ, KZ. (III) Provision of study materials or patients: FC, SC, LZ, CZ, QY, CH, LW. (IV) Collection and assembly of data: FC, SC, LZ, CZ. (V) Data analysis and interpretation: FC, CS, KZ. (VI) Manuscript writing: All authors. (VII) Final approval of manuscript: All authors.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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A Novel Treatment of Opioid Cravings With an Effect Size of .73 for Unilateral Transcranial Photobiomodulation Over Sham

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Background: Opioid use disorders (OUDs) are an epidemic causing catastrophic consequences to individuals, families, and society despite treatments including psychotherapy, substitution therapy or receptor blockers, and psychoeducation. We have developed a novel treatment that combines unilateral transcranial photobiomodulation (t-PBM) to the hemisphere with a more positive valence by Dual Brain Psychology (DBP).

Methods: We used a randomized, double blind, placebo-controlled protocol in which 22 patients with significant opioid cravings and a history of recent or current OUD attended three 1-h weekly sessions. After baseline measures of opioid craving and other psychometrics, subjects received two unilateral t-PBM applications (810 nm CW LED, 250 mW/cm^2, 60 J/cm^2, 4 min) or a sham (foil-covered LED) at F3 or F4. Prior to any treatment we used two tests to determine which hemisphere was more associated with a negative outlook and cravings and treated that side before the more positive hemisphere. Primary outcome measure was an opioid craving scale (OCS). Secondary outcomes were weekly Hamilton Depression (HDRS) and Anxiety (HARS) Rating Scales prior to treatments and at follow-up.

Results: Immediately after treatment the OCS improved significantly for both the sham and active treatments, but one week later the active treatment showed a 51.0% (SD 33.7) decrease in OCS while a week after the sham treatments there was a decrease of only 15.8% (SD 35.0) (by Wilcoxon Sign Rank Test, p = 0.004) and by a mixed model it was p = 0.0071. The effect size for the differences between active and sham was 0.73. For the active treatment from before and after treatment the effect size was 1.51 and for the sham, 0.45. The HDRS improved from a baseline of 15.1 to 8.8 (SD 10.3) a week after the active treatment and to 13.3 (SD 12.9) after the sham (p = 0.0071). HARS improved from 14.7 to 8.0 (SD 13.2) after the active treatments and to 14.3 (SD 16.0) after the sham, p = 0.08. Active treatment of the positive hemisphere after the negative hemisphere significantly improved the OCS, but there was no significant difference after the sham treatment. One

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patient complained of 2 h of abdominal bloating and dropped out; no other adverse effects were observed.

Discussion: Unilateral t-PBM to the hemisphere with a more positive hemispheric emotional valence was an effective and safe treatment for opioid cravings as well as for depression and anxiety. Our results also lend support to the underlying premises of DBP.

Keywords: opioid craving, depression, anxiety, photobiomodulation, brain hemispheres, laterality, dual-brain psychology

INTRODUCTION

According to the National Institute on Drug Abuse, "The combined healthcare, crime-related, and productivity costs of tobacco, alcohol, and illicit drugs exceed $700 billion a year, but dollars only poorly approximate the devastating human cost of substance use disorders" (1). In the US in 2015, there were over 52,000 drug overdose deaths, an increase of 11% over 2014 (2–4). Drug abusing patients demonstrate mental distress, health complications, loss of productivity, and increased criminality, which are injurious not only to patients and their families, but also to society as a whole. We have obtained preliminary data (5) that a simple, painless, inexpensive, and safe treatment called unilateral transcranial photobiomodulation (tPBM) using near-infrared light (NIR) may effectively benefit patients with a history of opiate dependence by improving their psychological well-being.

The physiological benefits to the brain from tPBM have been the subject of a rich literature of >400 papers on PubMed and several reviews (6–10). The reviews describe in detail how NIR light is absorbed by cytochrome-C oxidase, which stimulates ATP formation in the mitochondria (11). tPBM also increases neurotrophic factors in the brain, increases blood flow, and decreases inflammation (8, 12).

Hemispheric emotional valence has generally been considered in three hypotheses. The first is the right brain hypothesis, which posits that the right hemisphere is specialized for all emotional responses (13, 14). The second is the motivational hypothesis (15, 16), which suggests that approach emotions (including anger) are associated with the left hemisphere and that withdraw emotions are associated with the right. The third hypothesis, the valence hypothesis (17, 18), proposes that the left hemisphere is associated with positive emotions and the right with negative emotions.

Schiffer originally proposed Dual-brain Psychology (DBP) in 1997 (19–22), which posits that one brain hemisphere tends to be relatively mature and healthy, while the other hemisphere may be more affected by past traumas and supports a personality that is more prone to immature and/or destructive beliefs and/or behaviors. Schiffer has been concerned not simply with positive or negative emotions, but with entire personalities that differ in that one associated with one hemisphere tends to be associated with a disposition that is more mature and grounded in present reality and that the other, associated with the opposite hemisphere, seems to be more affected by past traumas, especially childhood traumas, and has a more childlike disposition that is more strongly influenced by past adversities. His hypothesis came out of his clinical observations, his rereading of the split-brain studies (23), studies by Wittling (24), and a number of published experiments by him and his associates on split-brain patients (25), fMRI imaging during lateral visual field stimulation (26), probe auditory evoked potentials during emotional memories (27), the use of HEV to successfully predict rTMS outcome in two studies on depression (28, 29), and a study using tPBM to treat anxiety and depression (5). The present study combined the physiological benefits of tPBM with the use of Schiffer's HEV to guide the treatment to the positive hemisphere.

Contrary to the established models that have generally associated negative emotions with the right hemisphere, several findings from WADA studies (30, 31), metanalyses of functional imaging studies (32, 33), and studies of post-stroke patients (34–36) indicated that negative emotions can often be left hemisphere dominant. Schiffer has reported that the side that is less symptomatic, which thereby has a positive HEV varies between individuals, but strongly tends to be a trait for any individual. Schiffer's HEV is determined simply by asking the patient to look out of either the left or the right lateral visual field by blocking his vision with safety glasses taped to allow vision out of one visual field and reporting his feelings and thoughts. He might be asked to look at a provocative photograph of an angry person while looking out that visual field, based in part on the pioneering work by Wittling and associates (24). The patient might be asked to rate this level of depression from 0 to 10. Then he is asked to look out the other lateral visual field and repeat the requests. In about 70% of patients there is at least a 2-point difference between the sides. Further, the patient is apt to see the therapist as critical (perhaps, as his mother was), to dislike himself, and to develop drug or gambling cravings while looking out of the negative side, all of which are reversed out of the other visual field. The visual field that is less symptomatic relates to the contralateral hemisphere which is then considered positive. See **Figure 1**. Recently, we have developed a computer test for HEV that correlates with lateralized volumes of the n. accumbens and amygdala. In two studies of rTMS, those patients who by this simple visual test had a positive left hemisphere had a far superior outcome compared to those with a negative left hemisphere from a course of two weeks rTMS, which only by tradition is only given to the left side of the head (28, 29).

Schiffer found that in his practice using unilateral tPBM to the positive hemisphere at either F3 or F4 gave very superior results compared with bilateral tPBM at F3 and F4 (5). See **Table 1**.

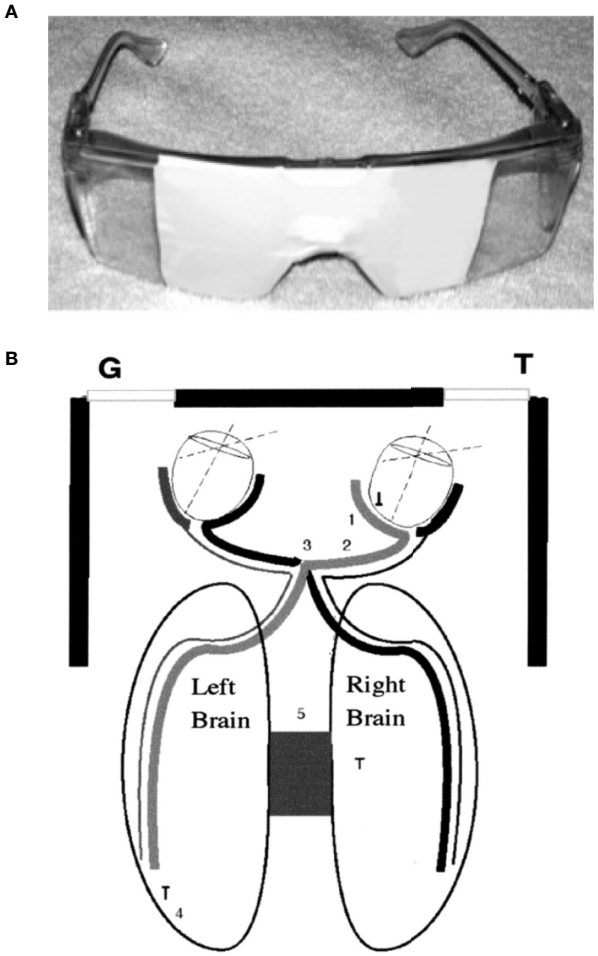

FIGURE 1 | (A) Taped safety glasses used in the experiment. **(B)** A diagram of how looking to the left allowed vision primarily out of the LVF and looking to the right allowed vision primarily out of the RVF. (1) Nasal retina; (2) optic nerve; (3) optic chiasm; and (4) occipital cortex. The diagram shows how the medial retina receives light from the lateral visual field and transfers information to the contralateral hemisphere. These neurological facts were used extensively in the split-brain studies, and we assume that the personality changes described here and in our other publications use a similar mechanism (26).

AIMS OF THE STUDY

Firstly, to test whether unilateral tPBM to stimulate the positive hemisphere in patients with a current or recent history of opioid use disorders (OUD) could significantly reduce opioid cravings in a randomized, double blind, placebo-controlled design.

Secondly, to evaluate the effects of unilateral tPBM on affect as measured by the Hamilton Depression Rating Scale (HDRS) and the Hamilton Anxiety Rating Scale (HARS).

Thirdly, to test the hypothesis that treating the positive hemisphere is superior to treating the hemisphere with a more negative HEV using active treatment. Applying the sham treatment to the positive hemisphere should not be superior to applying it to the negative hemisphere. We wanted this aim to further test Schiffer's DBP hypothesis. Schiffer had found clinically that applying tPBM to the negative hemisphere before the positive resulted in the patient's still having the positive benefit of the treatment of the positive hemisphere.

Our fourth aim was to confirm that there were no significant side effects of tPBM as found in other reports (5, 6, 8–10, 37, 38).

TABLE 1 | A comparison of bilateral tPBM and unilateral tPBM.

Bilateral tPBM	Unilateral tPBM
No obvious improvement; requires HDRS to observe improvements	Immediately after Rx report 84% of patients report profound or moderate improvements which on average last for about 3 days
Modest improvements 2 weeks after of Rx	Immediate observable improvement
No long-term clinical experience, except for one case report	Two years of experience in clinical practice with opiate disorders with more than 1,000 treatments given as an off-label, adjunctive treatment
No serious side-effects observed	No serious side-effects observed
Not related to any psychological theory	Integrated with Dual-Brain Psychology

MATERIALS AND METHODS

This single site, double blind, placebo-controlled study was approved by the New England IRB, Needham, MA. Twenty-two patients who reported active opioid cravings and had a current or recent opioid use disorder were recruited from online advertising between July and September 2019. Each went through the consent procedure and gave written consent for the study, following which they were given a clinical medical and psychiatric history.

Inclusion and Exclusion Criteria

Inclusion criteria were that the patients complained of opioid cravings and were between the ages of 18 and 65. Each met the criteria for a history of opioid dependence by DSM V. Patients who were receiving treatment for opioid dependence or other psychiatric disorders, either psychological or pharmacological treatments, could continue their treatment during the time of the study but were asked to try not to alter their treatment from the onset of the experiment until its conclusion. No patient requested to alter his regular treatment. Subjects were recruited without regard to gender or ethnicity and were enrolled on a first come basis. Exclusion criteria were past history of psychotic disorder (including schizophrenia or schizoaffective disorder), a history of violent behavior, a history of a past suicide attempt, a history of current suicidal ideation, a history of a neurological condition (*e.g.* epilepsy, traumatic brain injury, stroke), pregnancy, any current acute or chronic medical condition that might confound the study. Any patient judged by an investigator to have an impaired decision-making capacity would have been excluded. In actuality, no patient seeking recruitment was excluded after a screening phone call or clinical interview.

Study Design and Treatment

The patients attended the laboratory for three consecutive weekly visits. Patients were not allowed to reschedule a missed session so that the timings of the treatments would be consistent. At the first visit the experimenters gave the patients two tests for HEV to determine the positive hemisphere. The two tests for HEV are the lateral visual field test (LVFT) and a novel computer test for HEV (CTHEV), which has been submitted for a US Patent (patent application number: 16703937). The LVFT consists of asking each patient to put on taped safety goggles that allowed vision out of only one visual field, when the patient looked as far as possible to the left or the right (5, 26, 27). See **Figure 1**. While looking out of each visual field the patient was asked to rate from 0 to 10 his level of distress and his level of opioid cravings while he looked at a photograph of a very angry man, designed so that each half of the face was identical. The CTHEV, showed a video of alternating, symmetrical photographs of angry men to one visual field (by having the patient fixate on a central dot for 1 min) after which he was asked to rate from 0 to 10 his level of distress and his level of opioid cravings. As with the LVFT, the CTHEV was then repeated to the other visual field and the side with the lower scores was considered the positive visual field, which suggested that the contralateral hemisphere was the positive hemisphere. The HEV with either test was the numeric difference between the recorded scores from each visual field.

At each of the three weekly visits before treatment each patient was also given a urine drug screen, and if female, a pregnancy test, as well as each of the baseline outcome measures, which were the Hamilton Depression Rating Scale-17 (HDRS) (39), the Hamilton Anxiety Rating Scale (HARS) (40), and the Opioid Craving Scale (OCS) (41). The OCS was our primary endpoint and was given before and after each treatment and the follow-up session. It consisted of a mean score from three questions each rated from 0 to 9. (1) Please rate how strong your desire for an opiate is right now. (2) Please rate how strong your urges would be for an opiate if something in the environment had reminded you (examples; seeing a spoon), a needle, a mirror, or an alcohol advertisement), and (3) Please imagine yourself in the environment in which you previously used drugs and/or alcohol (a bar, your dealer's house, a shooting gallery, or whatever situation reminds you most strongly of active drug use). If you were in this environment right now, what is the likelihood that you would use an opiate?

At the beginning of each of the three weekly sessions, we obtained a detailed verbal report from the patient of his daily use of opioids over the previous week. We used this data (which we felt was honestly and accurately reported and was consistent with the urine drug screen) to calculate a timeline follow-back score using a typical unit of opioid use for the patient multiplied by the number of units and days of use.

After the baseline measures each patient then was given either a sham treatment or an active treatment (unilateral tPBM) in subsequent weeks on a randomized basis. That is, on the first visit the patient was treated with either an active device or a sham device. On his second visit, he was treated with the opposite device. On the third weekly visit, he received only a follow-up evaluation. See **Figure 2**. Before and after each treatment (first to the negative hemisphere and then to the positive hemisphere), the patient was administered the OCS. At the follow-up session we gave the patients the OCS, UDS, HDRS, HARS, and a report of opioid use during the prior week. Using preliminary data from his private practice FS found that treating the positive hemisphere after the negative relieved any negative effects that the first treatment may have caused and left the patient in a more positive psychological state, while allowing a comparison of the effects of treatments to the negative and the positive hemispheres under active and sham conditions. See **Figure 2**.

Randomization and Blinding

All treatments were randomized by a research assistant (EF) who also administered all the patient treatments, which were blinded to the two data recorders (HM) and (FS). The randomization procedure used computer generated random numbers blocked so that 60% would be given the sham condition before the active to try to avoid crossover effects.

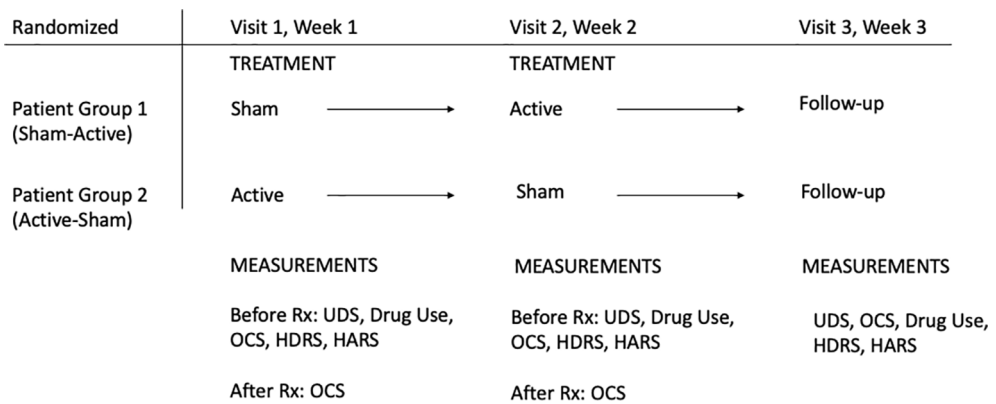

FIGURE 2 | A diagram of the randomization and timing of the study treatments and measurements.

Device

We used the same light source that was used in our 2009 study (6). The active treatment consisted of applying tPBM from a light emitting diode (LED) array (Marubeni America Corp, Santa Clara, CA) with a peak wavelength of 810 nm (+40 nm), delivering 250 mW/cm^2 when applied to the skin. tPBM for 4 min (total delivered fluence per site of 60 J/cm^2) at each of two sites on the forehead that correspond to the 10–20 EEG sites, F3, and F4. Based on a penetration of 3.7% of the light to the dura, we calculated that 2.1 J/cm^2 was delivered to each of the treated areas of the brain. The level of light exposure at the skin was well below the irradiance allowed by the ANSI standard of 320 mW/cm^2. The New England IRB currently and the Partners IRB in 2009 both determined that the device posed no significant risk. In other published tPBM studies no significant side effects have been reported to date (5, 8, 9, 12, 37, 42–47).

The LED was attached to a heatsink and a fan for cooling. If the patient wanted further cooling, he could tilt the device so that the LED was a few millimeters from the skin and allowed more air flow from the fan. The power supply was constructed by a product engineer so that the LED would deliver 250 mW/cm^2 at the skin. The sham device was the same device, except that the LED was covered with aluminum foil so that the patient felt the same warmth but received no light as verified by a photon detector. In this and other trials, subjects could not detect whether they were receiving active or sham treatment other than by their psychological response.

Analysis

A biostatistician (WR) selected and conducted all of our statistical tests using R (48). Our main statistical tests were mixed model analyses and Wilcoxon Sign Rank Tests, depending on the statistical need. Because our hypothesis was that the active condition would obtain better clinical results, we used one-sided tests. A copy of all study data and of the statistical tests performed and their results are contained in the **Supplementary Material (SM)**. In the *Results* section, we present the results extracted from the SM that we feel most accurately present our findings without repetition or data overload. In the SM, we noted which tables were included in the main paper as well as their table numbers which were different from the numbers in the SM.

RESULTS

Baseline Characteristics

We initially enrolled 22 patients whose overall baseline characteristics are described in **Table 2** and a more complete description is in the SM. We categorized those patients who received the active treatment the first week and the sham the second week as Active–Sham and the other group as Sham–Active. We attempted to enroll female subjects but were only able to enroll two, one of whom dropped out after the two treatments but before the follow-up week because she got a job with a conflicting schedule. For males, one patient dropped out before the follow-up session. Three others, who were all in the Active–Sham group, dropped out after the first treatment. Of the five patients who dropped out, four gave reasons that we felt, and the patient reported, were unrelated to the study. For example, one patient reported that he dropped out because he "had to get out of town." Only one patient dropped out because of a possible adverse reaction. He had what was described in a phone conversation, initiated by (FS), as "bloating in his abdomen," which resolved quickly with self-treatment with Pepto-Bismol the evening of the study.

TABLE 2 | Baseline demographic and clinical characteristics.

Characteristic	Active–Sham (n = 9)	Sham–Active (n = 13)	All Patients (n = 22)
Age Mean (SD)	52.6 (9.8)	46.5 (9.2)	49.0 (9.7)
Gender			
Male	8 (88.9%)	12 (92.3%)	20 (90.9%)
Female	1 (11.1%)	1 (7.7%)	2 (9.1%)
Race/Ethnicity			
Black	6 (66.7%)	5 (45.4%)	11 (50.0%)
White	3 (27.3%)	8 (72.7%)	11 (50.0%)
Handedness			
Left	2 (22.2%)	1 (7.7%)	3 (13.6%)
Right	7 (77.8%)	12 (92.3%)	19 (86.4%)

All Randomized Patients with Complete Data.

Immediate Results From Treatment

Immediately after the active and the sham treatments we found large and significant decreases in the OCS in both groups. Active had a mean (SD) percent decrease from immediately before treatment of 44.1% (39.6), and the sham had a decrease of 45.5% (35.6), both of which had a p value < 0.0001, by a one-sided Wilcoxon Test. The difference between conditions was not significant, p = 0.445. However, we found that when we compared the percent decrease in OCS one week after the treatments, there were significant differences between the active and the sham treatments.

One Week Post-Treatment Results

When we compared the active and sham conditions for all remaining 17 patients we found, as shown in **Table 3** and **Figure 3**, a percent decrease in OCS of 51.0% (33.7) for the week following the active treatment and only 15.8% (35.0) for the week following the sham, p = 0.0040, one-sided Wilcoxon test. For this analysis we chose using the week 1 baseline because the values were common for all patients and were measured before receiving any study intervention. More patients (71%) received the active treatment in week 2, because we wanted to avoid follow through effects with a blocked randomization. For the active over the sham the effect size, Cohen's ∂, was.73. For the active treatments from their baseline the Cohen's ∂ was 1.51, while for the sham treatments it was 0.45.

Nine of 17 (53%) in the active group had at least a 60% improvement in the OCS, using the week 1 baseline. Two additional patients who did not have a sham treatment also

TABLE 3 | Comparison of mean OCS scores (percent change) between active and sham treatments one week after treatment.

Statistic	Baseline (Week 1)	Active	Pct Difference (Active – Baseline)	Sham	Pct Difference (Sham – Baseline)	Difference of Pct Differences (Active – Sham)
N	17	17	17	17	17	17
Mean (SD)	6.9 (1.3)	3.5 (2.7)	–51.0 (33.7)	5.8 (2.6)	–15.8 (35.0)	–35.2 (48.2)
Median	7.0	3.3	–60.0	6.7	–4.2	–31.8
Min, Max	4.3, 9.0	0.0, 8.3	–100, 0.0	2.0, 9.0	–72.7, 33.3	–115, 52.4
95% CI	(6.2, 7.6)	(2.1, 4.9)	(–68.3, –33.7)	(4.5, 7.2)	(–33.7, 2.2)	(–60.0, –10.5)
p-value (paired t-test)			<.0001		0.0408	0.0041
p-value (signed-rank test)			<.0001		0.0898	0.0040

All Randomized Patients with Complete Data.
[1] Wilcoxon signed-rank tests were performed to assess percent change from baseline in the OCS score one week after active treatment and sham treatment, separately.
[2] A Wilcoxon signed-rank test was performed to compare the percent change from baseline in the OCS score on active treatment versus sham treatment (Difference of Percent Differences column). A positive value for the difference of percent difference favors sham treatment, while a negative value favors active treatment.
[3] One-sided lower p-values were calculated for all tests.

had greater than a 60% improvement, 11/19 (58%). In the sham group, including one patient who did not have an active treatment, only three of 18 (17%) achieved at least a 60% reduction in the OCS. As shown in **Table 4**, by McNemar's test, a paired, one-sided test of N = 17, we compared the paired percent differences and found that it was significant, p = 0.029.

Table 5A shows the results of a mixed model analysis of the percent change in mean OCS prior to treatment each week that included treatment, treatment sequence and week as fixed effects and subject level as a random effect. The active treatment *versus* the sham treatment had a one-sided p value of 0.0078. **Table 5B** shows that the active treatment had an adjusted mean of –51.93% (95% CI –69.21, 34.64), and the sham treatment had an adjusted mean of –22.17% (95% CI –41.22, –3.12).

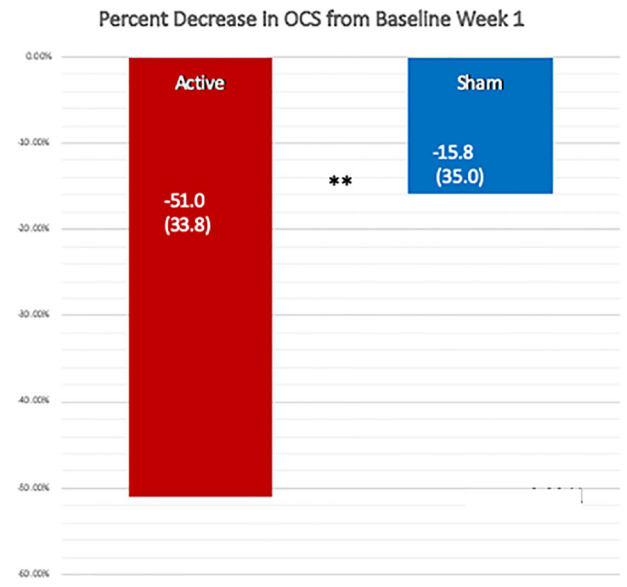

FIGURE 3 | Comparison of the percent reduction in the Opioid Craving Scale one week after the active and the sham treatments, using the OCS from week 1 before treatment as the baseline. The change after the active treatment, –51.0% (SD 33.7) was significantly greater than that following the sham treatment, –15.8% (SD 35.0), by a one-sided, Wilcoxon Test, **p = 0.004. Cohen's ∂ was 0.73 for the active treatment over sham.

Table 6 shows the data from a mixed model for the percent change in mean HDRS, and the active treatment had an adjusted mean of –7.97 (95% CI –57.50, 41.56), and the sham had 52.53 (95% CI –0.55, 105.60) with a one-sided p value = 0.0078. **Figure 4** shows a comparison of the raw HDRS scores at baseline and after the active and sham treatments and the p values from the one-sided Wilcoxon tests, including an active *versus* sham difference with a p value of 0.0053.

Table 7 shows the data from a mixed model for the percent change in mean HARS and the active treatment had an adjusted mean of 2.96 (95% CI –79.13, 85.06), and the sham had 53.96 (95% CI –33.54, 141.46) with a one-sided p value = 0.039. **Figure 5** shows a comparison of the raw HDRS scores at baseline and after the active and sham treatments and the p values from one-sided Wilcoxon tests, including an active *versus* sham difference with a p value of 0.0124.

Table 8 shows that when the more positive hemisphere (HEV by the LVFT and/or CTHEV tests) was treated during the active treatments, the OCS scores were lower (better) than when the hemisphere with the more negative HEV was treated. When the positive hemisphere was treated there was an improvement of –2.3 (SD 2.6) on the 9-point OCS compared with an improvement of –1.6 (SD 2.2) points when the negative hemisphere was treated, which by a one-sided Wilcoxon test had a p = 0.0087. During the sham treatments there were some placebo improvements on both the correct and the incorrect hemispheres, but the difference between them was not significant by a one-sided Wilcoxon test, p = 0.446. The hemispheric differences between the active and the sham conditions were significant, p = 0.0437, (**Figure 6**).

TABLE 4 | Comparison of Clinically Meaningful Improvement in Mean OCS Between Active and Sham Treatments One Week After Treatment.

–	Active (n = 17)	Sham (n = 17)	P-value
Patients with decrease in OCS of at least 60% from baseline, n (%)	9 (52.9%)	3 (17.6%)	0.0289

All Randomized Patients with Complete Data.
[1] Clinically meaningful improvement in mean OCS was defined as a percent decrease from baseline (pre-treatment week 1) of at least 60%.
[2] McNemar's test was performed to compare paired percentages and one-sided p-value was estimated.

TABLE 5A | Summary of Mixed Model for Percent Change in Mean OCS.

Parameter	Beta Estimate (Std Err)	95% CI	P-value
Intercept	–35.14 (11.74)	–59.80, –10.48	0.0078
Active–Sham Sequence (*vs* Sham–Active Sequence)	5.22 (13.12)	–22.74, 33.18	0.6962
Active Treatment (*vs* Sham Treatment)	–29.76 (10.92)	–53.04, –6.48	0.0078
Week 2 (*vs* Week 3)	20.73 (10.92)	–2.56, 44.01	0.0772

All Randomized Patients Non-Missing Data at Particular Visit.
Summary of Parameter (Beta) Estimates from Mixed Model.
[1] A mixed model for percent change in mean OCS assessed prior to treatment each week that includes treatment, treatment sequence, and week as fixed effects and a subject level random effect was constructed.
[2] Active Treatment (vs Sham Treatment) is a one-sided p value.

TABLE 5B | Summary of Mixed Model for Percent Change in Mean OCS.

Treatment	Adjusted Mean (95% CI)
Active	–51.93 (–69.21, –34.64)
Sham	–22.17 (–41.22, –3.12)

All Randomized Patients Non-Missing Data at Particular Visit.
Summary of Adjusted Means by Treatment from the Mixed Model.
[1] A mixed model for percent change in mean OCS assessed prior to treatment each week that includes treatment, treatment sequence, and week as fixed effects, and a subject level random effect was constructed.

TABLE 6A | Summary of Mixed Model for Percent Change in Mean HDRS.

Parameter	Beta Estimate (Std Err)	95% CI	P-value
Intercept	16.54 (30.67)	–47.89, 80.97	0.5963
Active–Sham Sequence (*vs* Sham–Active Sequence)	120.73 (42.72)	29.11, 212.36	0.0135
Active Treatment (*vs* Sham Treatment)	–60.49 (21.59)	–106.81, –14.18	0.0071
Week 2 (*vs* Week 3)	–48.76 (21.59)	–95.08, –2.45	0.0404

All Randomized Patients Non-Missing Data at Particular Visit.
Summary of Parameter (Beta) Estimates from Mixed Model.
[1] A mixed model for percent change in mean HDRS assessed prior to treatment each week that includes treatment, treatment sequence, and week as fixed effects and a subject level random effect was constructed.
[2] Active Treatment (vs Sham Treatment) is a one-sided p value.

TABLE 6B | Summary of Mixed Model for Percent Change in Mean HDRS.

Treatment	Adjusted Mean (95% CI)
Active	–7.97 (–57.50, 41.56)
Sham	52.53 (–0.55, 105.60)

All Randomized Patients Non-Missing Data at Particular Visit.
Summary of Adjusted Means by Treatment from the Mixed Model.
[1] A mixed model for percent change in mean HDRS assessed prior to treatment each week that includes treatment, treatment sequence, and week as fixed effects and a subject level random effect was constructed.

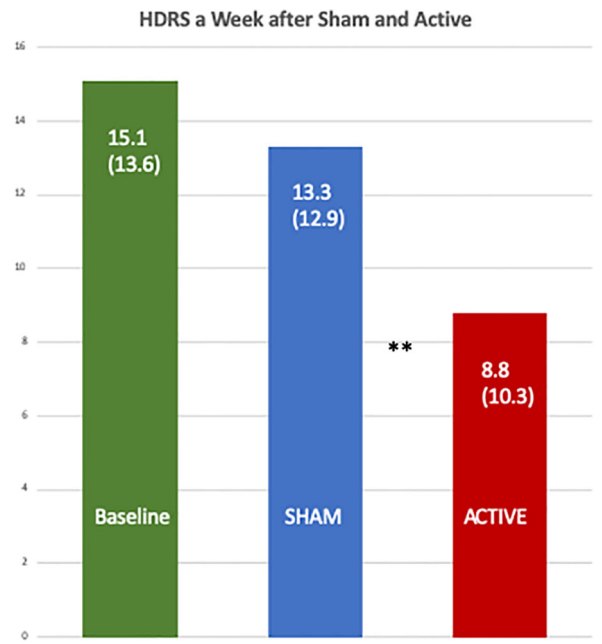

FIGURE 4 | Comparison of the baseline Hamilton Depression Rating Scales with those one week after the active and the sham treatments. The differences between the scores after the active [8.8 (SD 10.3)] and sham treatments [13.3 (SD 12.9)] were significant by a one-sided, Wilcoxon Test, **p = 0.0053.

TABLE 7A | Summary of Mixed Model for Percent Change in Mean HARS.

Parameter	Beta Estimate (Std Err)	95% CI	P-value
Intercept	41.46 (50.34)	–64.29, 147.22	0.4209
Active–Sham Sequence (*vs* Sham–Active Sequence)	51.37 (71.44)	–101.84, 204.59	0.4839
Active Treatment (*vs* Sham Treatment)	–51.00 (34.00)	–123.91, 21.92	0.0779
Week 2 (*vs* Week 3)	–26.39 (34.00)	–99.30, 46.53	0.4506

All Randomized Patients Non-Missing Data at Particular Visit.
Summary of Parameter (Beta) Estimates from Mixed Model.
[1] A mixed model for percent change in mean HARS assessed prior to treatment each week that includes treatment, treatment sequence, and week as fixed effects and a subject level random effect was constructed.
[2] Active Treatment (vs Sham Treatment) is a one-sided p value.

TABLE 7B | Summary of Mixed Model for Percent Change in Mean HARS.

Treatment	Adjusted Mean (95% CI)
Active	2.96 (–79.13, 85.06)
Sham	53.96 (–33.54, 141.46)

All Randomized Patients Non-Missing Data at Particular Visit.
Summary of Adjusted Means by Treatment from the Mixed Model.
[1] A mixed model for percent change in mean HARS assessed prior to treatment each week that includes treatment, treatment sequence, and week as fixed effects and a subject level random effect was constructed.

Opioid Use

We counted the number of urine drug screens that were positive for morphine or opioids and found five were positive for either at baseline, and of these four were in the Active–Sham group and none of the five were positive at either week 2 or 3. Two patients who were negative at baseline were positive at week 2 or at week 3. One was after an active treatment and one after a sham.

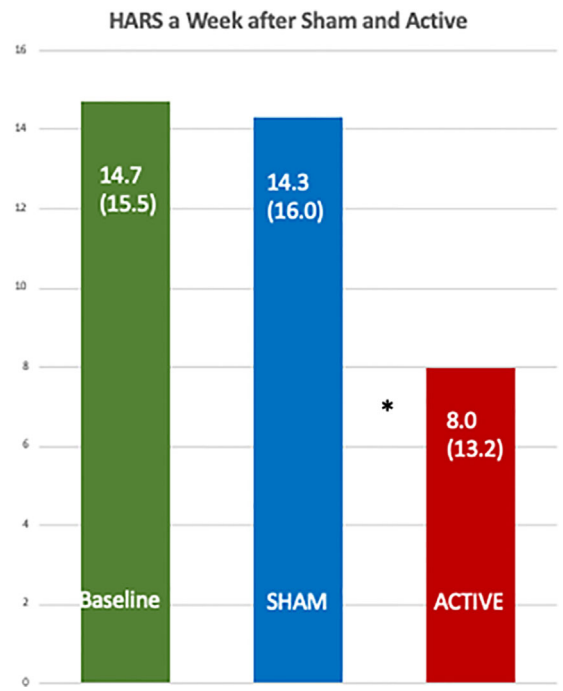

FIGURE 5 | Comparison of the baseline Hamilton Anxiety Rating Scales with those one week after the active and the sham treatments. The differences between the scores after the active [8.0 (SD 13.2)] and sham treatments [14.3 (SD 16.0)] were significant by a one-sided, Wilcoxon Test, *p = 0.012.

Using the timeline follow-back scores we found:

- 14/22 patients had zero opioid use at baseline over the preceding 7 days
- two of the 14 patients had on-study usage—both at week 2 after receiving sham treatment at week 1. They returned to zero at the week 3 assessment after receiving active treatment at week 2
- 12 of the 14 had no on-study usage
- Among the eight users at baseline (numbers in parentheses are quantitative data calculated from the followback timeline, not patient numbers)
- one patient was a "heavy" user at baseline (126). He received the active treatment at week 1 and saw his usage drop to (1.5) after the active treatment and then (zero) the following week
- four patients were "moderate" users at baseline (7, 14, 14, 8). All received active treatment at week 1, and all saw a decrease in usage at the week 2 assessment. Two of the four saw another decrease at the week 3 assessment. Another patient did not have a week 3 assessment while the fourth patient increased (8 at week 1, 6 at week 2, 10 at week 3, after sham at week 2).
- three patients were "low" users at baseline (1 or 2) and remained low users at each of the subsequent assessments

Adverse Side-Effects

We purposely did not use a formal side-effect questionnaire to avoid negatively influencing the patients. All but one patient had a very positive relationship with the treatment team. The one exception was the patient mentioned above who complained of abdominal bloating the night of the active treatment. We know of one other report of a patient treated with t-PBM experiencing abdominal bloating possibly in response to t-PBM (37), who recovered rapidly.

DISCUSSION

The opioid crisis is well known and widely appreciated (1–4), but while current treatments can be effective when skillfully applied by both the patient and caregivers, they still leave a profound need for further improvements. We have developed a novel treatment that attempts to address the underlying depression and anxiety symptoms likely arising from unappreciated (or appreciated) past traumas and which can be both promoters of the addiction and consequences of it. The treatment tested in this study is based

TABLE 8 | Comparison of Absolute Change in Mean OCS Scores Between Active and Sham Treatment by Correct *vs.* Incorrect Hemisphere.

Statistic	Active Correct	Active Incorrect	Active Difference (Correct - Incorrect)	Sham Correct	Sham Incorrect	Sham Difference (Correct - Incorrect)	Difference of Differences (Active - Sham)
n	20	20	20	20	20	20	20
Mean (SD)	−2.3 (2.6)	−1.6 (2.2)	−0.7 (1.3)	−2.1 (2.2)	−1.9 (1.9)	−0.2 (1.4)	−0.5 (1.2)
Median	−2.7	−1.7	−0.3	−1.3	−2.0	0.0	−0.2
Min, Max	−8.0, 2.0	−6.0, 2.7	−4.7, 1.0	−7.3, 0.7	−6.7, 0.7	−5.0, 2.3	−2.7, 1.7
95% CI	(−3.5, −1.0)	(−2.6, −0.6)	(−1.2, −0.1)	(−3.1, −1.1)	(−2.8, −1.0)	(−0.8, 0.5)	(−1.0, 0.1)
p-value (paired t-test)			0.0155			0.2836	0.0431
p-value (signed-rank test)			0.0087			0.4462	0.0437

All Randomized Patients with Complete Data

[1] Wilcoxon signed-rank tests were performed to assess change from baseline in the OCS score on the correct and incorrect hemispheres after active treatment and sham treatment on the same day, separately. The correct and incorrect hemispheres were based on a computer test for emotional valance.

[2] The baseline score used to calculate absolute change corresponded to the pre-treatment score the week they received the assigned treatment. For patients who received active treatment at week 1, the baseline score from week 1 was used. For patients who received active treatment at week 2, the baseline score from week 2 was used. The baseline for sham treatment was derived in a similar way.

[3] A Wilcoxon signed-rank test was performed to compare the difference in absolute change from baseline in the OCS score on active treatment versus sham treatment (Difference of Differences column).

[4] One-sided lower p-values were calculated for all tests.

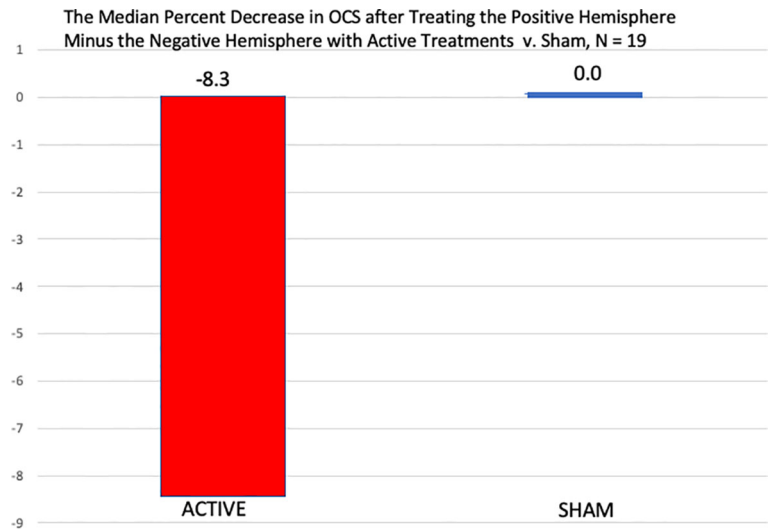

FIGURE 6 | Comparison of the median percent reduction in the OCS measures immediately after the treatment of the negative hemisphere followed by the treatment of the positive hemisphere in both the active conditions and the sham conditions. In the active conditions there was a significantly greater percent decrease in OCS when the positive hemisphere was treated than the negative, by a one-sided, Wilcoxon Test, p = 0.021, but after the sham treatments, there was no difference between the two hemispheric sham treatments.

on DBP which suggests that in a large majority of patients, one hemisphere (left or right) is a trait associated with a mental state that manifests a relationship to past traumas (5, 20, 22, 25) causing depression, anxiety, and an immature cognition that leads to adverse behavior, including substance abuse. DBP also suggests that the opposite positive hemisphere is less affected by past trauma and is less prone to negative affects and behaviors (20). Based on Teicher's (49, 50) work suggesting that the neuropsychiatric consequences of maltreatment relate to its type and timing (sensitive periods) in development, we suspect but have not demonstrated that the development of the negative, more traumatized hemisphere may also relate to these factors: type and timing. We have suggested that unilateral treatments to the positive hemisphere (5, 28, 29) might stimulate it and further promote its dominance thereby inducing a state of greater wellbeing. DBP therapy focuses on using the healthier hemisphere to assist the troubled side, like a parent, with love, insight, and limits. Our innovation is to combine DBP with unilateral tPBM to stimulate the healthy hemisphere with NIR light. Our results, documented in this paper, indicate that this new treatment, compared to sham, had an effect size of –.73, and the benefits were usually obvious to the patients and to our staff. A recent publication in the American Journal of Psychiatry described using CBD to reduce opioid cravings. They found a 3 to 5% decrease in craving in CBD group which was superior to placebo (51). We now have a follow-up blind, placebo-controlled trial offering patients twice a week treatments for 4 weeks underway, which was funded by a NIDA/SBIR grant (#1R43DA050358-01).

The mechanisms of tPBM have been well reviewed in many papers (5, 6, 8, 12) and include the absorption of photons by cytochrome-c to stimulate ATP formation (11) in the mitochondria, increased cerebral blood flow (5), decreased inflammatory factors (52), and increased brain neurotropic factors (53), but none of these mechanisms explains our observations of the often profound decreases in cravings, anxiety, and depression within minutes after a treatment has begun. Caldieraro and Cassano have speculated that tPBM might act through induced electromagnetic fields (54), and Schiffer has speculated that these changes might involve quantum effects due to alterations in biophoton emissions from tubulin molecules encoding brain information related to experiences (55), but these speculations have not yet been tested. What is clear, however, is that several studies have reported psychological benefits from bilateral tPBM (9, 37, 42, 44), including one case report of a 31-month treatment (56).

Because of his success in accurately predicting patients' responses to left-sided rTMS using his DBP hypotheses, Schiffer was motivated to treat the right hemisphere with 10 Hz in patients in whom the visual test suggested that their right hemisphere was more positive, but he was unable to find an interested collaborator. His first study of tPBM was a bilateral pilot study of 10 patients with a history of trauma, anxiety and depression, seven of whom have a past history of substance abuse. All had been in remission for at least 4 years, and none had active cravings (5). Immediately after treatment and in follow-up these patients did not notice the improvement that was observed on the Hamilton Scales. Schiffer then used unilateral t-PBM in his private practice as an off-label treatment and found that the unilateral treatment to the positive hemisphere seemed to be far superior to the bilateral application as shown in **Table 8**. This paper reports the first double-blind, placebo-controlled study of unilateral t-PBM to a hemisphere with a more positive HEV. That unilateral tPBM to the positive hemisphere is superior to that to the negative

hemisphere, and the fact that unilateral tPBM was effective in reducing cravings, depression, and anxiety offer support for Schiffer's hypotheses as expressed in DBP and for his clinical observations from his practice and his research indicating that unilateral tPBM is superior to bilateral tPBM. Further study needs to directly compare unilateral and bilateral tPBM.

Limitations

The main limitation of the study is its small number of patients and its short duration. t-PBM is a relatively new field, and many initial studies have had small numbers (9, 37, 56–58), and larger and longer studies are needed. We have completed 31 of 40 patients in a NIDA sponsored double-blind RTC study (Grant #1R43DA050358-01), which has been temporarily interrupted by the pandemic, but to date shows a highly significant treatment * time effect in regard to opioid craving reduction.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/ **Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by New England IRB, Needham, MA WIRB-Copernicus IRB Group. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FS made substantial contributions to the conception or design of the work as well as to the acquisition, analysis or interpretation of data for the work; drafting the work or revising it critically for important intellectual content. WR performed the statistical analyses for the work. EF and HM made substantial contributions to the acquisition of data for the work. MH made substantial contributions to the conception or design of the work; analysis or interpretation of data for the work and revising it critically for important intellectual content. All authors have provided approval for publication of the content, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2020.00827/full#supplementary-material

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Conflict of Interest: WR was a freelance statistician who was referred to MindLight by Horizon Data Science Applications. FS is Founder and President of MindLight, LLC, which has been awarded a NIDA/SBIR grant (1 R43 DA050358-01) for further research and commercialization of the methods and device described in the paper. FS has been issued 2 US patents which cover the method of unilateral tPBM to a positive hemisphere as described in this study: U.S. Patent No. 8303636, Issued 11/06/2012: Methods for treating psychiatric disorders using light energy; and U.S. Patent No. 8574279, Issued 11/05/2013: Methods for

treating psychiatric disorders using light energy. FS has filed on December 5, 2019, a US patent application, #16/703,937, Method and Apparatus for Determining Hemispheric Emotional Valence.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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Sensitivity to Morphine Reward Associates With Gut Dysbiosis in Rats With Morphine-Induced Conditioned Place Preference

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Gut microbiota has been found to establish a bidirectional relationship with the central nervous system. Variations of the gut microbiota has been implicated in various mental disorders, including opioid use disorders. Morphine exposure has been repeatedly found to disrupt the gut microbiota, but association between the gut microbiota and the sensitivity to morphine reward remains unknown. In this study the conditioned place preference (CPP) paradigm was used for morphine-treated rats and saline-treated rats. After the CPP procedure, the morphine-treated rats were divided equally into the low and high CPP (L- and H-CPP) groups according to the CPP scores. We adopted 16S rRNA sequencing for the fecal bacterial communities at baseline and post-conditioning. By comparing the morphine-treated group with saline-treated group, we found alterations of microbial composition in the morphine-treated group, but no significant differences in alpha diversity. The L-CPP group and H-CPP group differed in microbial composition both before and after morphine treatment. The relative abundance of certain taxa was correlated to the CPP scores, such as *Alloprevotella* and *Romboutsia*. This study provides direct evidence that morphine exposure alters the composition of the gut microbiota in rats and that microbial alterations are correlated to the sensitivity to morphine reward. These findings may help develop novel therapeutic and preventive strategies for opioid use disorder.

Keywords: morphine, gut microbiota, gut dysbiosis, conditioned place preference, sensitivity to morphine reward, rat model

INTRODUCTION

An estimate of over 10^{14} microorganisms, including over 1000 species and over 7000 strains, inhabit the human gastrointestinal (GI) tract, the quantity of which is far more than all other cells in human bodies (1). The composition of this mass microbiota can be affected by many contributors, such as genetic factors (2), age (3), diet and exercise (4), etc. Gut microbiota has been found to establish a

bidirectional relationship with the central nervous system, referred to as the gut-brain axis, possibly through immune, neural and endocrine pathways (5, 6). The variation of gut microbiota was demonstrated to be associated with a wide range of central nervous system disorders, including autism, anxiety, depression, and substance use disorders (7–9).

Morphine is a widely prescribed analgesic used for moderate to severe pain management, but its clinical use is impacted by side effects such as dependence and GI symptoms (10), including anorexia, constipation, nausea, vomiting, and bloating (11). Chronic morphine use could impair GI function and epithelial integrity (12–14). In humans, cirrhotic patients with long-term opioid use showed significant dysbiosis of gut microbiota, compared to cirrhotic patients without opioid use, especially, hepatic encephalopathy patients with chronic opioid use showed lower relative abundance of autochthonous taxa and *Bacteroidaceae* (15). In animals, after a slow release morphine pellet was implanted subcutaneously, mice with poly-microbial sepsis appeared elevated mostly in the *Firmicutes* phylum and Gram-positive bacterial species, and these disturbances were antagonized by the opioid receptor antagonist naltrexone (16). Evidence also showed that chronic morphine treatment significantly caused dysbiosis and preferential expansion of Gram-positive bacteria, and reduction in bile-deconjugating bacterial strains (17). Morphine-induced gut dysbiosis in mice exhibited a significant increase in communities related to pathogenic function and a decrease in communities related to stress tolerance (18). Unique alterations in gut microbiota composition were noted following intermittent or sustained morphine administration (19). The above mentioned studies provided a basis for the effect of morphine use on gut microbiota.

However, these studies mainly concentrated on the gut microbial comparison between the morphine group and control group to reveal the association between morphine treatment and gut microbiota; but ignored the influence of individual differences within each group. In fact, individuals report a variety of analgesia to the same doses of opioids, and not all opiate users develop addiction clinically (20). Certain individuals are more vulnerable to addiction than others because of higher sensitivity to drug effects. This individual sensitivity to substance could be affected by genetic, molecular and behavioral contributors (21). Drawing on discoveries in association between gut microbiota and substance use disorders, the role of gut microbiota in the sensitivity to the substance reward is worth further study. Researchers have adopted rat models of alcohol self-administration and divided models into vulnerable and resilient groups. The vulnerable group had increased impulsive and compulsive behaviors, and increased relapse after abstinence, and the taxa of gut microbiota presented a change in trend compared to the resilient group (22). With the treatment of non-absorbable antibiotics, animals showed reduced gut microbiota and enhanced sensitivity to cocaine reward and locomotor-sensitizing effects of repeated cocaine administration (23). Antibiotic treatment reduced opioid analgesic potency and impaired cocaine reward in morphine dependent models (19). These studies provided direct evidence of association between gut microbiota and rodents' sensitivity to alcohol or cocaine reward. It is well established that although various substances have different pharmacological actions, their final common pathway is to act on the midbrain limbic dopamine (DA) system, the center of reward system and promote DA neuron impulses and DA release (24). Therefore, we suspect that gut microbiota may also play a role in morphine reward.

Conditioned place preference (CPP) paradigm is one of the most popular measures of "reward" in rodents. During the paradigm, the rewarding property of a drug is assessed by its ability to promote the formation of a preference for an environment in which the drug has been repeatedly treated (25).The CPP scores could reflect the sensitivity to rewarding values of the drug (26, 27).

Our goal was twofold. First, we aimed to establish morphine-induced conditioned place preference (CPP) rat models, and adopted 16S rRNA sequencing of fecal bacterial communities before and after morphine exposure, with an attempt to describe the impact of morphine on the alterations in gut microbiota. Second, we compared the gut microbiota of rats with different CPP scores and examined the correlation between microbial alterations and the sensitivity to morphine reward.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats were purchased from Hunan Silaike Jingda Laboratory Animal, China. Their body weight ranged approximately between 180g to 200g. Each cage housed four rats. The temperature (20°C~25°C), humidity (50%~55%) and light/dark cycle (12h/12h) were controlled. The rats were offered enough food and water. All animals were maintained in pathogen-free facilities. The experimental protocol was approved by the Ethics Committee of the Second Xiangya Hospital of Central South University (ethical approval no. 2015-063).

Chemicals and Drugs

Morphine was purchased from Northeast Pharmaceutical group Shenyang NO.1 Pharmaceutical Co., Ltd. It was diluted in 0.9% sterile saline.

Conditional Place Preference

The CPP apparatus consisted of two larger compartments (60cm L × 40cm W × 40cm H) and one connection zone (40cm L × 6.5cm W × 60cm H), and the connection zone served as a connection between the two compartments. Each compartment was separated by an optional manual guillotine door. One floor had horizontal black and white stripes with metal bars, and the other had vertical black and white stripes with wire-mesh, providing the visual-cue difference between the two larger compartments. A video camera, transmitting the data to the analysis software (Jiliang Software and Instruments, Shanghai, China), was above the apparatus which recorded the locomotor activity of rats and the time they spent in each compartment. The

apparatus was illuminated with two spotlights mounted on either side of the camera. The CPP procedure was assigned into four phases: habituation, preconditioning, conditioning and post-conditioning.

All the Sprague-Dawley rats were randomly divided into two groups: morphine group (n=28) and saline group (n=7). For convenience, we named morphine group at baseline as the MB group, morphine group for post-treatment as the MP group, saline group at baseline as the SB group, saline group for post-treatment as the SP group. All the rats lived with unlimited food and water intake. Rats adapted to the environment for three days (day1, 2, 3). We initially placed the rats into the connection zone of the CPP apparatus with the door removed for 30 minutes to adapt to the apparatus and return it to the home cage (day4). The next day rats were placed into the connection zone with the door removed for 15 minutes to test for preference at baseline (day5). When we divided rats in the morphine group into two groups according to CPP scores, the rats either stayed in the connecting zone more than 70% of time or passed through the guillotine door less than ten times, or both at baseline were removed. Here we removed four rats in morphine group. During the conditioning phase, the rats were injected with morphine (0.9%; 10mg/ml, 1ml/kg, i.p. the morphine group) or equal saline (0.9%, 1ml/kg, i.p. the saline group) and confined to the non-preference side (morphine-paired side) for 15 minutes (day6, 8, 10, 12). On alternate days, all rats were injected with equal saline (0.9%, 1ml/kg, i.p.) and confined to the preference side for 15 minutes (day7, 9, 11, 13). The morphine group has four-cycle conditioning training. During the Post-conditioning phase (day14), the rats were allowed free access to the two compartments to test preference after treatment. The video camera and the CPP software recorded the time they spent in the bilateral compartments and locomotor activity of rats throughout Preconditioning and Post-conditioning phase. The CPP score means the difference between time spent in the drug-paired side after CPP training and time at baseline (28). The remaining 24 rats of the morphine group were divided into two groups according to the CPP score, one group of 12 rats with higher CPP scores was designated as the H-CPP group, while the other group of 12 rats with lower CPP scores was designated as the L-CPP group.

Fecal Sample Collection

Fecal samples were freshly collected at day 5 (Preconditioning) and day 14 (Post-conditioning) and stored at-80C until use.

DNA Extraction and PCR Amplification

E.Z.N.A.® Stool DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) was used to extract fecal microbial DNA from the fecal sample according to the manufacturer's instructions. The bacterial genomic DNA was amplified with 341F (5'-barcode-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC') specific for the V3 and V4 region of 16S rRNA, where the barcode was an eight-base sequence unique to each sample. PCR reactions were performed in triplicate 20 μL mixture containing adequate TopTaq DNA Polymerase kit (Transgen, China) and 10 ng of the template DNA using the 2720 Thermal Cycler (Thermo Fisher Scientific, USA) and setting the cycling parameters (94°C for 3 min, followed by 25 cycles at 94°C for 10s, 55°C for 15s, and 72°C for 30 s and a final extension at 72°C for 7 min).

Illumina MiSeq 16S rRNA Sequencing

We purified amplicons with AgencourtAMPureXPPCR Purification Beads (Beckman Coulter, USA) according to the manufacturer's instruction. Amplicons were quantified using Invitrogen Qubit3.0 Spectrophotometer (Thermo Fisher Scientific, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, USA). A master DNA pool was generated from the purified products in equimolar ratios. Purified amplicons from the samples were sent out for pyrosequencing on an Illumina MiSeq platform at Gensky Biotechnology (Shanghai, China).

Processing of Sequencing Data

All sequences in the FASTQ file format were filtered and merged following three criteria: (i) Using TrimGalore to truncate raw reads whose average quality are lower than 20, deleting the sequences that contain the adapter (ii) Exact barcode matching, two nucleotide mismatches in primer matching and reads containing ambiguous characters and primers were removed. (iii) Sequence assembling, using FLASH (v1.2.11) to assemble the paired end reads, reads that could not be assembled were discarded.

After filtering, the sequences were matched at the operational taxonomic unit (OTU) using UPARSE (version 7.1). OTU clustering was performed with a 97% similarity cut-off. Chimeric sequences were identified and removed using UCHIME. Taxonomic assignments were determined by Mothur with Ribosomal Database Project (RDP) (29), SLIVA 16S rRNA database (30) and UNITE database (31) set with a confidence threshold of 80%. All bacteria at family and genus levels analyzed were shown in **Table S1**.

Statistical Analysis

We used Mothur (version v.1.30.1 4) to calculate and analyze microbial community estimators, including richness estimators (the ACE index and Chao index) and alpha diversity estimators (the Simpson index and Shannon index). Rarefaction curve was generated for the observed OTU. All correlations were performed by Spearman correlation for data in accordance with bivariate normal distribution or Pearson correlation for data not in accordance with bivariate normal distribution. A Mann-Whitney test, Wilcoxon signed rank test and Student's t test were used to determine whether differences of median existed. An F test was assessed for equal variance, and normality was assessed by the Shapiro-Wilk normality test. All tests were two-sided. The statistical significance threshold was defined as p-value <0.05. Besides, when we compared the micrbiota of saline group with that of morphine group and made rarefarction curve, the whole 28 rats in morphine group were included in the analysis. While we compared the microbiota of H-CPP withe that of L-CPP group, four rats had been removed and the remaining 24 rats were included in analysis.

RESULTS

Morphine-Induced CPP Model Was Established and Rats Presented Individual Differences in Sensitivity to Morphine Reward

During the preconditioning phase, as we expected, there was no significant difference in the CPP scores between the morphine group and saline group (p=0.1616; **Figure 1A**). After the morphine or saline treatment, the CPP score of the morphine group was slightly higher than the saline's (p=0.0783; **Figure 1B**). According to this result, the rats in the morphine group were exposed to morphine. After the four-cycle conditioning training, the weights of rats all increased, but there was no difference in weight between the MP group and SP group (p=0.4587; **Figure 1C**).

Although rats in the morphine group were exposed to the same dose of morphine, rats presented individual differences in the CPP score. The CPP score served as a surrogate of rats' sensitivity to morphine, which can evaluate the rewarding and reinforcing properties of morphine (26). The H-CPP group showed a significant increase in the CPP scores compared to the L-CPP and SP (p<0.0001 for H-CPP versus L-CPP; p=0.0004 for H-CPP versus SP; **Figure 1D**), which implicated that the rats in the H-CPP group had prominent preference and higher sensitivity to morphine than L-CPP. The L-CPP group showed no significant difference compared to the SP (p=0.8229; **Figure 1D**).

Morphine Altered the Gut Microbiota Composition Pattern, but Not the Alpha Diversity of the Microbial Community

According to the rarefaction curve (**Figure 2**), the sequencing depth was satisfactory and represented the majority of bacterial species. We evaluated ecological features of fecal bacterial communities using several indices based on the OTU level. The richness estimators, such as the ACE index and Chao index, were used to extrapolate species richness. The diversity estimators, such as the Shannon index and Simpson index, were used to extrapolate

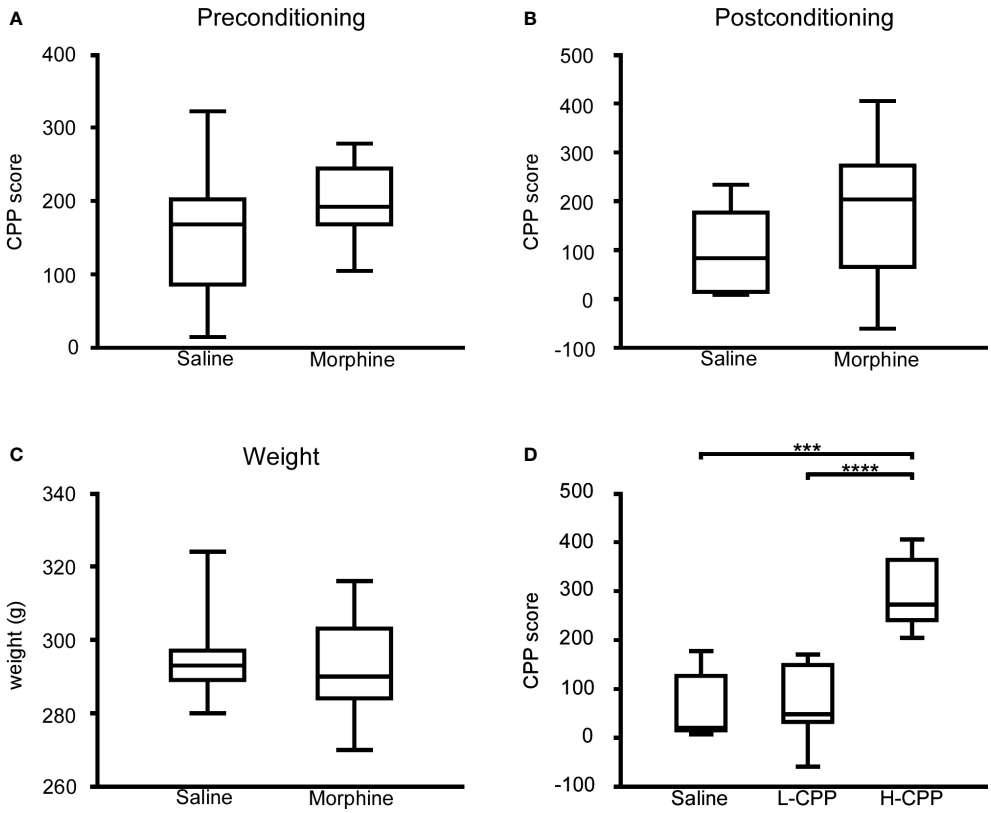

FIGURE 1 | (A) The conditional place preference (CPP) score of rats were compared between the morphine and saline group in the preconditioning phase, and there was slight difference appeared in the CPP score between the morphine group and saline group. **(B)** The CPP score of rats were compared between the morphine and saline group in the postconditioning phase, and there was slight difference appeared in the CPP score between the morphine group and saline group. **(C)** The weights of rats were compared between the morphine and saline group in the preconditioning phase. There was no significant difference between the weight of posttreatment for morphine and saline group. **(D)** The CPP score of rats were compared among the saline, L-CPP and H-CPP group in the postconditioning phase. The CPP score of H-CPP is significantly higher than L-CPP and saline group, and there was no difference between the CPP score of L-CPP and saline group. The CPP score means the difference between time spent in the drug-paired side after CPP training and time at baselinSe.t udent's t test was used to analyze the data. The central line shown in each box plot indicates the median of data. Whiskers extend to cover the whole range of values. Statistical significance was accepted at p<0.05. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

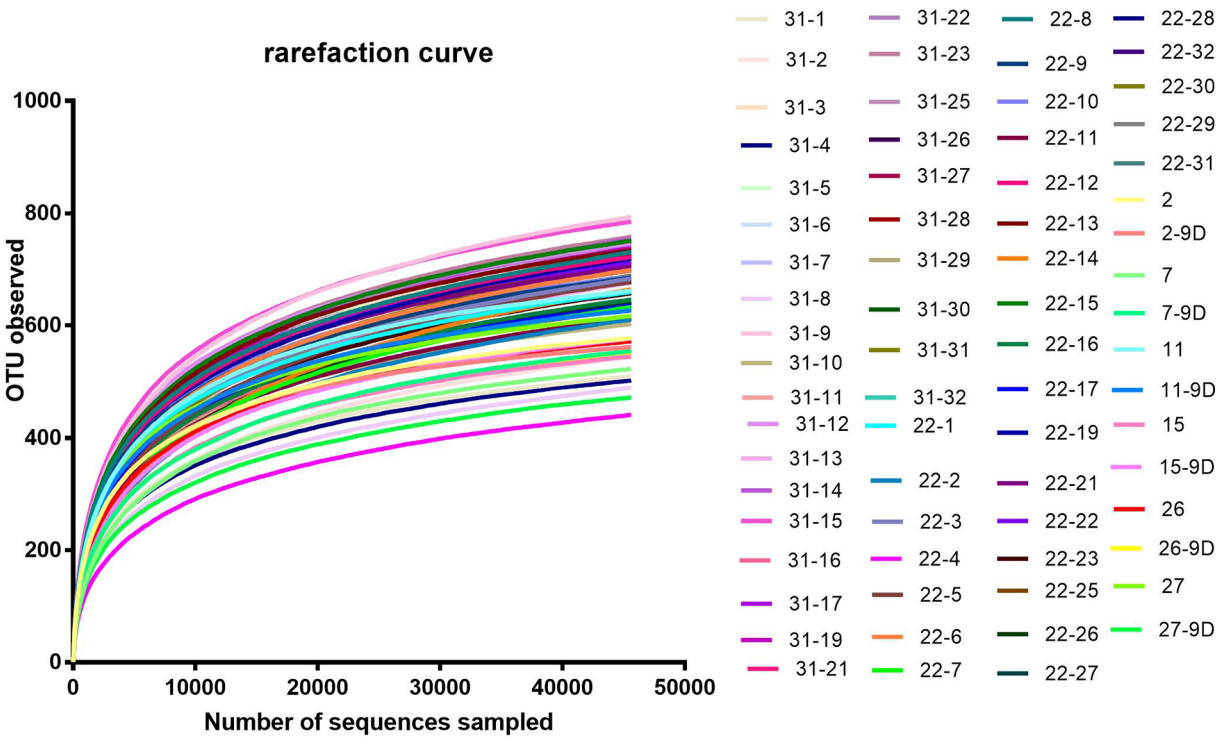

FIGURE 2 | Rarefaction curve. Rarefaction curves were based on the 16S rRNA gene sequencing of the all samples from baseline group and the post-treatment for morphine and saline group. The rarefaction curve suggested that the sequencing depth was satisfactory and represented the majority of bacterial species, because the curves became relatively flat as the number of sequences analyzed increased.

species richness and evenness. The ACE indexes (mean ± SD) were 867.4 ± 54.4, 857.6 ± 92.16, 801.6 ± 99.92 and 811.5 ± 133 for the SB, SP, MB and MP groups, respectively. Chao indexes were 886.8 ± 42.31, 869.4 ± 88.05, 815.6 ± 102.6 and 821.5 ± 136.3, Shannon were 4.301 ± 0.1692, 4.384 ± 0.2279, 4.243 ± 0.2999 and 4.295 ± 0.3134, and Simpson were 0.5655 ± 0.0202, 0.04586 ± 0.01522, 0.05043 ± 0.02189 and 0.04935 ± 0.02056 for the SB, SP, MB and MP groups, respectively (**Figures 3A–D**). These four indexes ACE, Chao, Shannon, and Simpson all showed no significant differences among the SB, SP, MB and MP groups. We also compared alpha diversity between L-CPP and H-CPP both at baseline and after treatment. It was found that alpha diversity did not appear significantly different in these comparisons (**Figures S1** and **S2**).

To assess whether morphine altered the gut microbial community, we compared the gut microbiota between the baseline and post-treatment for the morphine and saline group respectively (**Figures 4A–C**). At the genus level, when compared the MP to MB, *Allobaculum* (p=0.0002) and *Parasutterella* (p=0.0061) were more abundant, and *Alloprevotella* (p=0.0002), *Desulfovibrio* (p<0.0001) and *Rikenella* (p=0.0176) were less abundant in the MP. In the saline group, *Clostridium_XlVa* (p=0.0125), *Corynebacterium* (p=0.0156), and *Parasutterella* (p=0.0313) increased and *Desulfovibrio* (p=0.0156) decreased after saline treatment. At the family level, when compared the MP to MB, *Coriobacteriaceae* (p=0.0004) *and Peptococcaceae_1* (p=0.0034) were more abundant in the MP. In the saline group, *Coriobacteriaceae* (p=0.0181), *Peptococcaceae_1* (p=0.0156) and *Streptococcaceae* (p=0.0156) increased after saline treatment.

Additionally, we compared the MP to SP. It was found that the MP had lower relative abundance of *Corynebacterium* (p<0.0001), and *Clostridium_XlVa* (p=0.0117) at the genus level, *Enterococcaceae* (p=0.0325), *Staphylococcaceae* (p=0.0035), *Streptococcaceae* (p=0.0201) at the family level. All the taxa with significance are shown in **Table S2** and **Table S3**.

Differences in the Microbial Composition Between H-CPP and L-CPP After The Morphine-Induced CPP Training

In order to find the taxa that were associated with morphine preference, we compared microbiota in H-CPP to L-CPP. At the family level, the following bacterial decreased: *Spirochaetaceae* (p=0.0252), *Veillonellaceae* (p=0.0236), *Catabacteriaceae* (p=0.0087), *Elusimicrobiaceae* (p=0.0196) and *Christensenellaceae* (p=0.0474), while *Peptostreptococcaceae* (p=0.01) increased in the H-CPP group. At the genus level, *Alloprevotella* (p=0.0384) and *Romboutsia* (p=0.01) increased, whereas *Clostridium_IV* (p=0.0387), *Roseburia* (p=0.0036), *Schwartzia* (p=0.0236), *Catabacter* (p=0.0087), *Elusimicrobium* (p=0.0196), *Dorea* (p=0.0423), *Christensenella* (p=0.0471) and *Anaerofilum* (p=0.0185) decreased significantly in the H-CPP group (**Figures 5A, B**). All the taxa at the family and genus levels with significance are shown in **Table S4**.

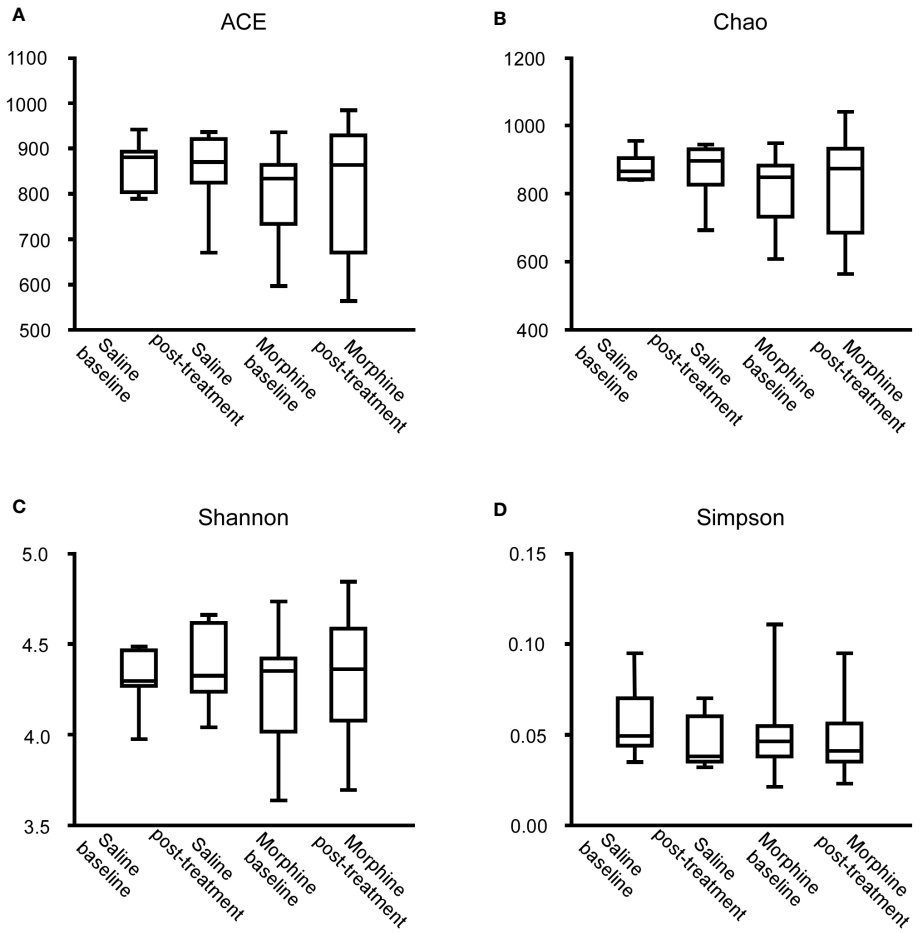

FIGURE 3 | Comparison of the community alpha diversity. Alpha diversity was measured by ACE **(A)**, Chao **(B)**, Shannon **(C)** and Simpson **(D)**. A Mann-Whitney test or student's t test was used to analyze the data. The central line shown in each box plot indicates the median of data. Whiskers extend to cover the whole range of values. Statistical significance was accepted at $p < 0.05$. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

To identify whether microbial taxa correlated with the CPP score, we performed Pearson correlation or Spearman correlation between the relative abundance of bacterial and the CPP scores. At the family level, we found that the relative abundance of *Lachnospiraceae* (r=-0.4946, p=0.0140), *Ruminococcaceae* (r=-0.5681, p=0.0038) and *Elusimicrobiaceae* (r=-0.4728, p=0.0196) were negatively correlated with the CPP scores. At the genus level, the relative abundance of *Alloprevotella* (r=0.4394, p=0.0317) and *Romboutsia* (r=0.5130, p=0.0104) were positively correlated with the CPP scores, while the relative abundance of *Roseburia* (r=-0.6261, p=0.0011) and *Elusimicrobium* (r=-0.4728, p=0.0196) were negatively correlated with the CPP scores (**Figures 6A–D**).

Differences in the Microbial Composition Between L-CPP and H-CPP at Baseline

After analyzing the differences in the microbial composition between H-CPP and L-CPP, we hypothesized that some specific bacteria might determine the sensitivity to morphine before the morphine administration. We analyzed the bacterial composition at baseline between H-CPP group and L-CPP group. At the family level, in H-CPP-baseline, *Helicobacteraceae* (p=0.0238) was more abundant, while *Puniceicoccaceae* (p=0.0237) was less abundant. At the genus level, the H-CPP-baseline appeared to have elevated *Helicobacter* (p=0.0238), and decreased *Olsenella* (p=0.0496) and *Rothia* (p=0.0097; **Figure 7A**). We also performed the Spearman correlation between the relative abundance of bacteria at baseline and the CPP scores. We found the relative abundance of *Rothia* (r=-0.6241, p=0.0011; **Figure 7B**) was negatively correlated with the CPP score.

Within-Group Differences in the Relative Abundance Between the Baseline and Post-Conditioning

We compared the within-group differences between the baseline and post-conditioning for the L-CPP and H-CPP group at the genus and family levels (**Table S5**, **S6**). According to the characteristics of results, we divided these taxa with significant differences into three types. In the first two types, the microbiota only showed significant difference in the L-CPP or H-CPP group. The last type was characterized by the microbiota with significant differences both in L-CPP and H-CPP groups.

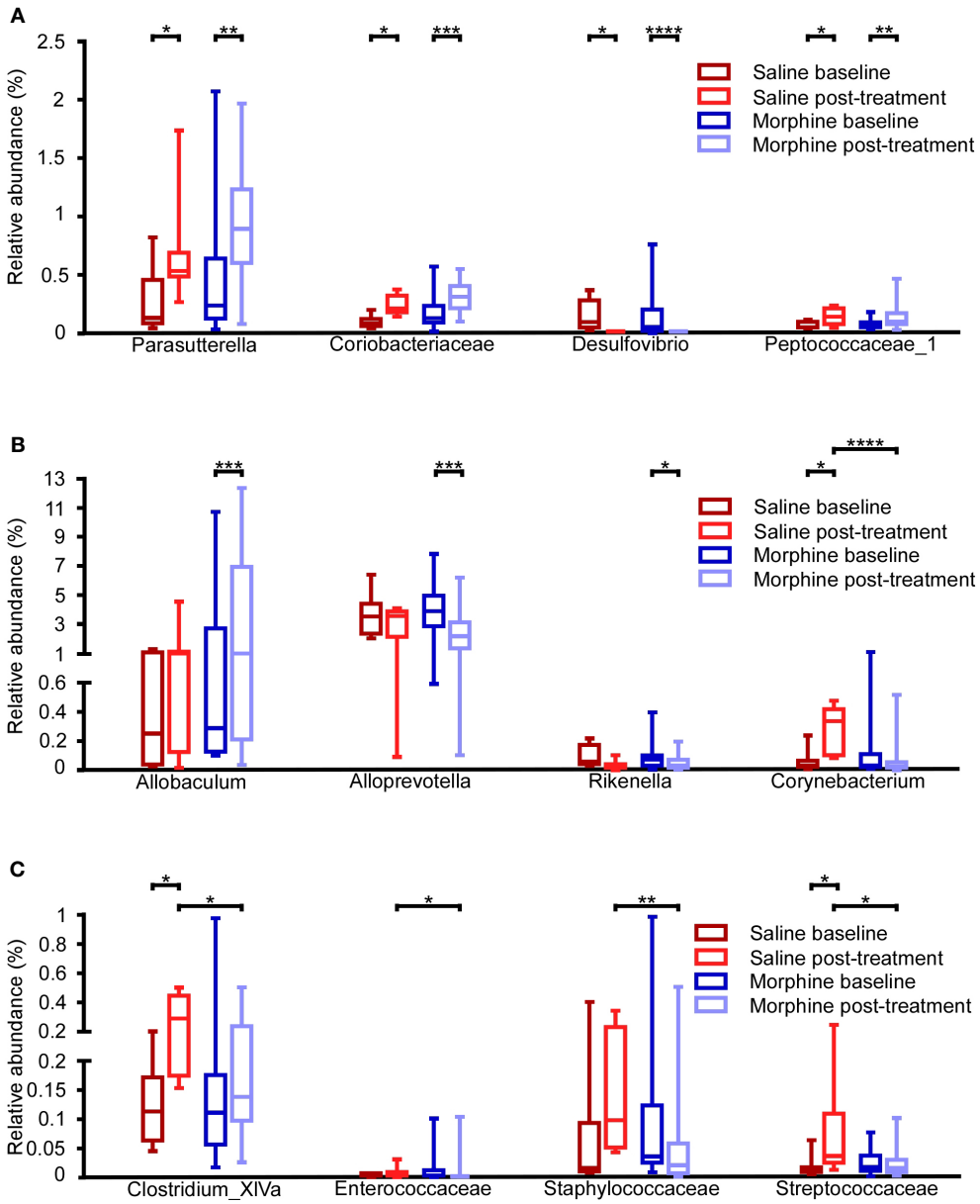

FIGURE 4 | Comparison of the gut microbiota composition among the baseline and post-treatment for the morphine and saline group. Comparison of the relative abundance of gut microbiota at the family and genus levels among the saline-baseline, saline post-treatment, morphine-baseline and morphine post-treatment groups. **(A)** The relative abundance of *Parasutterella*, *Coriobacteriaceae*, *Desulfovibrio* and *Peptococcaceae_1* were shown. **(B)** The relative abundance of *Allobaculum*, *Alloprevotella*, *Rikenella* and *Corynebacterium* were shown. **(C)** The relative abundance of *Clostridium_XlVa*, *Enterococcaceae*, *Staphylococcaceae* and *Streptococcaceae* were shown. A Mann-Whitney test and Wilcoxon signed rank test were used to analyze the data. A student's t test was used for the data with Gaussian distribution. The central line shown in each box plot indicates the median of data. Whiskers extend to cover the whole range of values.Statistical significance was accepted at $p < 0.05$. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

DISCUSSION

Our study is the first to find that rats with different sensitivities to morphine reward also showed a varying composition of gut microbiota. In the morphine group, although the rats belonged to the same species, received the same additional manipulations, and fed in the same environment, the rats presented individual differences in the CPP scores (32). The CPP score was used to assess the sensitivity to morphine reward (26, 27). Comparing the microbiota of the H-CPP and L-CPP groups, we found different gut microbiota compositions both at baseline and after morphine treatment. Recent studies indicated that gut dysbiosis played an important role in the sensitivity to morphine effects. Antibiotic cocktail or probiotics treatment

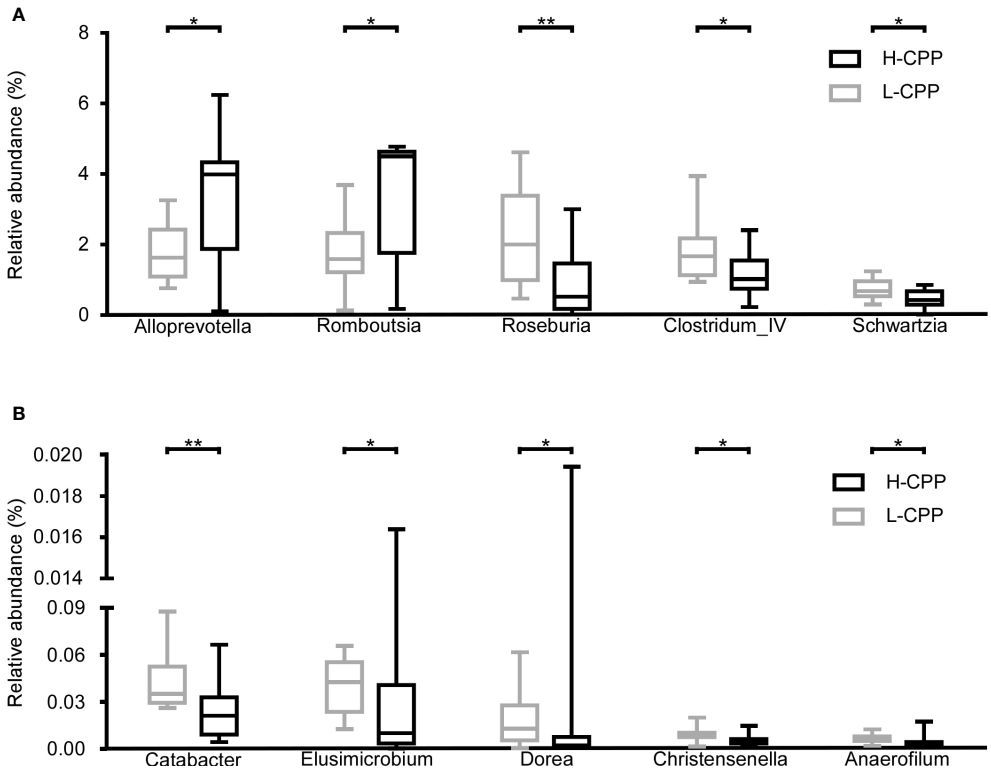

FIGURE 5 | Comparison of the gut microbiota composition between the low and high conditional place preference group (L-CPP versus H-CPP) after the morphine-induced CPP training. **(A)** The relative abundance of *Alloprevotella*, *Romboutsia*, *Roseburia*, *Clostridium_IV* and *Schwartzia*, were found to be different between L-CPP and HCPP. **(B)** The relative abundance of *Catabacter*, *Elusimmicrobium*, *Dorea*, *Christensenella* and *Anaeroflium* were found to be different between L-CPP and H-CPP. A Mann-Whitney test was used to analyze the data. The central line shown in each box plot indicates the median of data. Whiskers extend to cover the whole range of values. A student's t test was used for the data with Gaussian distribution. Statistical significance was accepted at $p < 0.05$. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

significantly reduced the morphine analgesic tolerance in morphine-treated mice (33, 34). Our results are the first to reveal the relationship between gut microbiota and the sensitivity to morphine reward.

We found that the relative abundance of specific bacterial taxa was in correlation to the CPP scores. Comparing the gut microbiota of H-CPP to L-CPP at baseline, we found significant difference in the *Olsenella*, *Rothia*, and *Helicobacter*. *Rothia* was negatively correlated with the CPP score. Intriguingly, researchers found that a maternal high-fat diet led to a shift of the offspring gut microbiota, including reduced *Olsenella* and *Helicobacter*, and this shift prevented long-lasting neural adaptation in the mesolimbic DA reward system (35). This result provided evidence for the association between these two taxa and the reward systems. Due to these differences showed before the morphine treatment, the differential relative abundance of these taxa may be the nature of rats with high/low sensitivity to morphine. These taxa may become the predictors of sensitivity to the morphine reward. Individuals with decreased *Olsenella* and *Rothia*, and increased *Helicobacter* may have a higher risk of addictive behaviors after morphine exposure. Additionally, researchers found that some common signal paths may be involved in the process of gut microbiota affecting the rewarding property of morphine. Gut microbial metabolites including short-chain fatty acids and bile acids putatively influence this process (9). Short-chain fatty acids, as histone deacetylase inhibitors, could contribute to the extinction of morphine-induced CPP (36). Reddy et al. conducted a bariatric surgery for mice to augment circulating bile acids, which appeared to decrease the rewarding properties of cocaine (37). Besides, microbial metabolites modulate production of inflammatory cytokines such as interferon-alpha and interleukin-10. Rats with interferon-alpha treatment showed potentiated reinstatement of the morphine CPP (38). However, interleukin-10 over expression reduced the morphine CPP scores and acquisition of self-administration (39, 40). Collectively, these results suggest that microbial metabolites are probably linked to morphine sensitivity.

We also found differences between the H-CPP and L-CPP groups after morphine treatment. The three taxa *Olsenella*, *Rothia* and *Helicobacter* which showed differences at baseline, showed no differences after the training. We speculated that because the H-CPP group was more sensitive to morphine reward, morphine exerted a stronger effect on gut microbiota in the H-CPP than the L-CPP group after the same morphine exposure. However, we found no significant difference in the alpha diversity of microbiota appearing both at baseline and post-treatment, which indicated that rats with different sensitivity

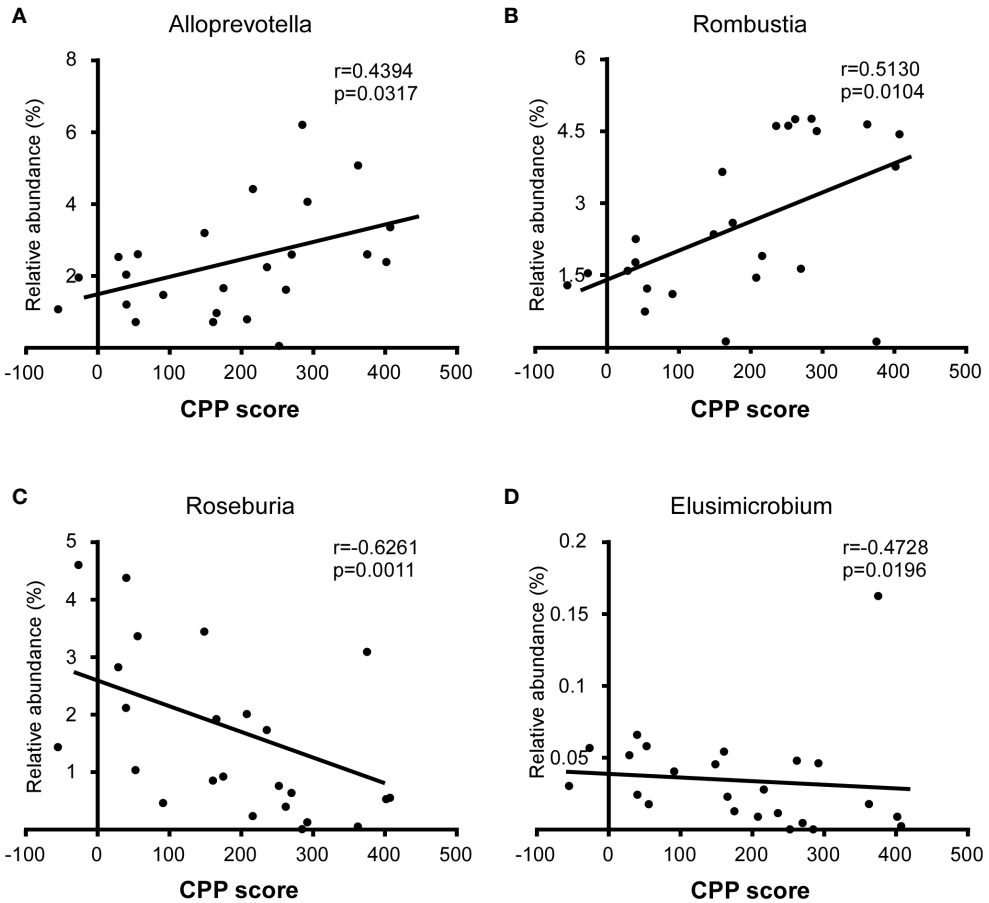

FIGURE 6 | Correlation between specific taxa and the CPP scores after the morphine-induced CPP training. The relative abundance of *Alloprevotella* **(A)** and *Romboutsia* **(B)** were positively correlated with the CPP score, while the relative abundance of *Roseburia* **(C)** and *Elusimicrobium* **(D)** were negatively correlated with the CPP score. A Spearman correlation was performed for *Romboutsia*, *Roseburia* and *Elusimicrobium*, and a Pearson correlation was performed for *Alloprevotella*. The r value and p value were used to evaluate statistical significance.

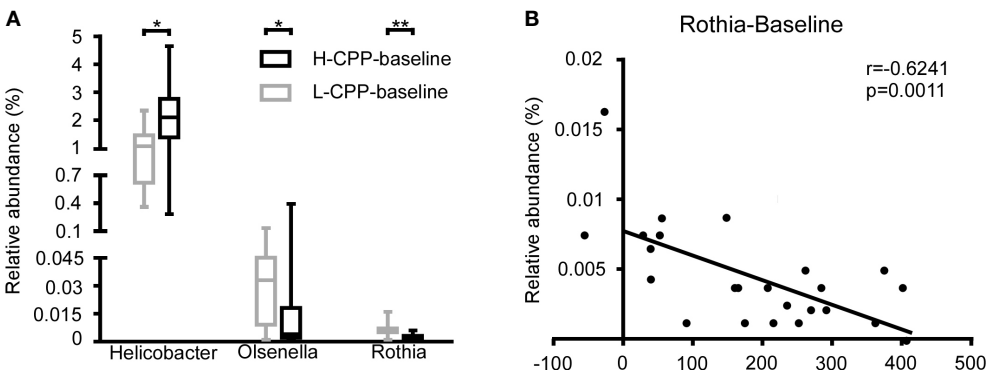

FIGURE 7 | Comparison of the gut microbiota composition between L-CPP and H-CPP groups at baseline and the correlation between the relative abundance of *Rothia* at baseline and CPP score. **(A)** The significant differences among *Helicobacter*, *Olsenella*, and *Rothia* were noted. A Mann-Whitney test was used to analyze the data. A Wilcoxon signed rank test was used to analyzed the data. A student's t test was used for data with Gaussian distribution. The central line shown in each box plot indicates the median of data. Whiskers extend to cover the whole range of values. Statistical significance was accepted at $p<0.05$. **(B)** The relative abundance of *Rothia* was negatively correlated with the CPP score. Spearman correlation was performed for *Rothia*. The r value and p value were used to evaluate statistical significance. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

to morphine reward showed no essential difference in richness and diversity of gut microbiota. To summarize, this study revealed the relationship between the composition of gut microbiota and sensitivity to the morphine reward. Given that sensitization to the drug effects is regarded as an important role in the development of drug addiction (41), our finding may provide basis for predicting the risk of addition and direct addiction treatment.

At last, we compared the MP with the SP in order to observe the morphine effect on microbiota. It is noted that some taxa changed both after morphine and saline treatment, including *Alistipes* and *Adlercreutzia* at genus level and *Coriobacteriaceae* and *Rikenellaceae* at family level. We guess that the additional manipulations mainly contributed to these alterations. The additional manipulations, such as grabbing, saline or morphine injection and even changed living environment, could become stimuli to rats and caused abnormality of physiology, pathological and mental state (8, 42). Several studies have described an increase in *Coriobacteriaceae* in rat models experiencing depression and stress (43–45). Maybe these stimuli become stressors, which can lead to stress or stress-related disorders (46). While other taxa changed in different ways after morphine or saline treatment, which may be induced by the morphine inherent effects. Prior studies showed that morphine itself could impair the GI function and epithelial integrity, and disrupt gut homeostasis (12–14). However, we found no significant difference in weight between the morphine group and saline group, so we ignored the possible effect of morphine on appetite and absorption. Anxiety, depression, and memory deficits are common adverse effects of morphine use or morphine addiction, whose relation with gut microbiota has been repeatedly identified (47–52). It is worth noting that morphine is a prescribed analgesic that can depress the central nervous system and induce sedative and antalgic actions (10). Thus morphine might offset some stress effects caused by additional manipulations, which caused the differences of some taxa in the comparison between the SB and SP but not in the comparison between MB and MP. Besides, the addictive property of morphine or the addiction-related behaviors might have an influence on gut microbiota. Our discovery was consistent with recent studies that found increased *Clostridium* and *Enterococcus* in morphine dependence models (17–19). Nevertheless, it is still uncertain that the above alterations in gut microbiota were caused by the effects of morphine itself or the addictive property.

This is the first study to discover that microbial alterations are connected to the sensitivity to morphine reward. Our study had several limitations. First, gut microbiota can be altered by many host factors, such as genes, physiological and pathological state and internal and external environment (53), which we did not control in the current study. Second, the causality between sensitivity to morphine reward and gut microbiota is not clear. Further studies on probiotics, antibiotics, and bacterial metabolites are needed to determine how manipulations of the microbiome may affect the rewarding effects of morphine. In this study, we only adopted rat models to prove the correlation between rewarding effects of morphine and gut microbiota, so the correlation in other species is to be determined. Specifically, although animal models allow for greater experimental control than human studies, there are great differences in microbiome compositions between humans and animal models, due to the large differences in size, metabolic rate and dietary habits (54). Additionally, it will be important to define whether gut dysbiosis responds to rewarding properties of other substance, like psychostimulants and alcohol. However, our findings do suggest gut dysbiosis may be a biomarker and a predictor to the risk of developing opioid use disorder. Gut microbiota may become a novel therapeutic target for opioid use disorder.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, PRJNA 559598.

ETHICS STATEMENT

The animal study was reviewed and approved by Ethics Committee of the Second Xiangya Hospital of Central South University (ethical approval no. 20150063).

AUTHOR CONTRIBUTIONS

XZ conceived and designed the experiments. JY, JZ, CY, JL, and FQ performed the experiments. JY and JZ analyzed data and contributed reagents, materials, and analysis tools. JY and JZ interpreted the results and wrote the paper. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Internet Addiction and Related Psychological Factors Among Children and Adolescents in China During the Coronavirus Disease 2019 (COVID-19) Epidemic

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Background: The Coronavirus disease 2019 (COVID-19) is an infectious disease presenting a major threat to public health. This study aims to assess Internet use characteristics and objectively examine the potential psychological factors associated with Internet addiction (IA) during the COVID-19 epidemic.

Methods: A cross-sectional, anonymized, self-reported survey was conducted among Chinese children and adolescents aged 6 to 18 years old. Participants completed questionnaires containing Young's Internet Addiction Test (IAT) and the Depression, Anxiety, and Stress Scale (DASS-21), and questions regarding demographic information and Internet use characteristics.

Results: A total of 2050 participants (mean age:12.34 ± 4.67 years old, female: 48.44%) were enrolled. Fifty-five (2.68%) participants met the criterion for addictive Internet use (IAT≥70), while 684 (33.37%) participants were classified as problematic Internet users (69≥IAT≥40). Internet usage had grown during the COVID-19 epidemic, including the frequency and duration of recreational Internet use, and the frequency of stay-up Internet use. A linear regression analysis showed female gender (β=-0.091, $p<0.001$), age (β=0.066, $p=0.001$), depression (β=0.257, $p<0.001$), and stress (β=0.323, $p<0.001$) were significantly correlated with the IAT total scores ($R=0.539$, $R^2 = 0.291$, $p<0.001$).

Conclusions: We observed excessive Internet use among Chinese children and adolescents during the outbreak of COVID-19. Age, gender, depression, and stress were the potential key factors affecting IA. Extended family and professional support should be considered for vulnerable individuals during these unprecedented times.

Keywords: Internet addiction (IA), children and adolescents, depression, anxiety, stress

INTRODUCTION

In December 2019, the Coronavirus disease 2019 (COVID-19) was first reported in Wuhan city, Hubei province, China (1). The COVID-19 is an extremely contagious disease with high infectivity, fast transmitting speed, susceptibility of all-age groups, and damage to public health. The emergency response and massive vigorous actions taken by the Chinese government have slowed down the epidemic. However, the Chinese people were facing enormous pressure and a grim challenge of prevention and control. The ministry of education of China issued notices in January, the extension of the opening of the spring semester in 2020, requiring all primary and secondary schools to delay school opening and students to stay at home (2) and learn *via* online courses (3). As an unprecedented, nationwide, even worldwide public health emergency, the epidemic of COVID-19 is bound to have a corresponding impact on the psychology and behaviors of school-age children and adolescents, who deserves more attention.

The rapid rise of the Internet age has popularized Internet use in China. More and more children and adolescents spend time on the Internet to study, play online games, shop, watch movies, use social media, and chat. These activities are often used to reduce stress and anxiety or to alleviate depressed mood. As there are 588 million Internet users in China (including 287 million teenagers) that account for 20% of all Internet users worldwide (4). Internet use in a reasonable way is beneficial, but excessive and uncontrolled Internet use may develop into Internet addiction (IA), which is defined as an individual's inability to control his/her use of the Internet. IA is a serious public health problem in the world, especially in Asia (5). In China, the prevalence of IA has been reported as 2.4% to 10% (6, 7).

The COVID-19 outbreak is an excellent opportunity to study the association between stressful life events, its consequent psychological responses, and addictive behaviors. Previous studies have confirmed stress, depression, and anxiety are correlated with IA (8–12). We hypothesized that the increased level of stress, anxiety, and depression caused by the crises of the COVID-19 outbreak might change Internet use behaviors. Research data are needed to develop evidence-driven strategies to reduce adverse psychological impacts on Internet use. So this population-based epidemiological study screened, described, and compared the Internet addictive behaviors and identified risk factors of IA among school-aged children and adolescents in China in response to the outbreak.

METHODS

Study Design

The popularization of Internet services and smartphones have enabled mental health professionals to provide online mental health investigation and assistance during the COVID-19 outbreak. As the Chinese Government recommended that the public to minimize face-to-face interaction, we conducted a cross-sectional study and electronically invited participants continuity through the WeChat-based 'SurveyStar' online platform (Changsha Ranxing Science and Technology, Shanghai, China). The 'SurveyStar' is a website providing online survey and statistical analysis functions equivalent to Amazon Mechanical Turk, from February 19, 2020 to March 15, 2020. The original sample was obtained from Hunan province, Shandong province, and Inner Mongolia Autonomous Region, based on geographic location (central, east, and north of China). There were 1019 confirmed COVID-19 cases and 4 deaths in Hunan province, 775 confirmed cases and 7 deaths in Shandong province, and 117 confirmed cases and 1 death in Inner Mongolia Autonomous Region (up to April 2, 2020). Official online accounts of hospitals released the survey.

Samples

School-age children and adolescents from these three areas were recruited through the anonymous online questionnaire, designed by the first author and reviewed by senior psychiatrists. The admission criteria: (1) 6-18 years old, male or female, (2) voluntary participation in this survey, (3) ability to complete the online survey or with the help of parents, (4) being a student in primary or middle school, (5) signing informed consent online. The exclusion criteria: diagnosed with cognitive impairment, organic brain diseases, or severe mental disorders. The research protocol and consent procedures were approved by the Human Ethics Committee of the Xiangya Hospital of Central South University (the ethical approval number: 202003034), which conformed to the principles embodied in the Declaration of Helsinki.

Measures

We obtained demographic data, including gender, date of birth, whether they were an only child or not, current study section, growth environment in the past three years, family income in the past one year, and educational level of participants' mother and father. Participants were asked if they had ever been diagnosed with cognitive impairment, organic brain diseases, or mental disorders (schizophrenia, depression, bipolar disorders, obsessive-compulsive disorder, anxiety disorder, or substance use disorders) in their lifetime. If the answer was yes, they were excluded from the study.

We included several measures of Internet use, the primary electronic device of Internet use (smartphones or tablets, Internet protocol television (IPTV), computers, others), the frequency of recreational use of electronic devices (several times per day, once per day, 4-6 times per week, 1-3 times per week, no use); the duration of recreational use of electronic devices (>6 hours per day, 4-6 hours per day, 2-4 hours per day, <2 hours per day, no use); the frequency of recreational use of electronic devices after 00:00/week (>4 times per week, 3 times per week, twice per week, once per week, no use); the frequency of recreational use of electronic devices overnight/week (>4 times per week, 3 times per week, twice per week, once per week, no use); the degree of addiction to electronic devices (self-rating, 0-100 scores) during and before the epidemic of COVID-19.

The Chinese version of Young's Internet Addiction Test (IAT) was used to screen symptoms of IA. It is a self-rated test with 20 items, and each item is scored on a scale of 1-5. According to Young et al.'s criteria (13), participants whose IAT total scores 70 or above were classified as addictive Internet users (AIU) who had encountered significant life problems due to Internet use. Participants with an IAT total score of 40-69 were classified as problematic Internet users (PIU) who had encountered general life problems due to Internet use. Participants with an IAT score of 39 or below were classified as normal Internet users (NIU), who only had some or no problems controlling Internet use. Adequate reliability of IAT in Chinese languages compared with English (Cronbach alpha≥0.82) (14, 15).

The mental state was assessed using the Chinese version of Depression, Anxiety, and Stress Scale (DASS-21). DASS-21 is a screening tool to measure depression, anxiety, and stress in the reference period of "past one week." Questions 3, 5, 10, 13, 16, 17, and 21 formed the depression subscale. The total depression subscale score was divided into normal (0–9), mild to moderate depression (13–27), and severe depression (28–42). Questions 2, 4, 7, 9, 15, 19, and 20 formed the anxiety subscale. The total anxiety subscale score was divided into normal (0–7), mild to moderate anxiety (8–14), severe anxiety (15-42). Questions 1, 6, 8, 11, 12, 14, and 18 formed the stress subscale. The total stress subscale score was divided into normal (0–10), mild to moderate stress (11–25), severe stress (26–2). The Chinese version of DASS-21 has been demonstrated to be a good psychometric screening tool with good validity and reliability in the Chinese population (16), even in adolescents (17, 18) and research during the severe acute respiratory syndrome (SARS) outbreak (19).

Statistical Analysis

Data online was exported in.sav format. Simple descriptive statistics were expressed as 'mean and standard deviation' for continuous variables, 'frequency and percentage' for categorical variables. Differences in demographics, Internet use, and DASS-21 variables among groups were tested using t-test, ANOVA test, Pearson's χ^2 test, or Fisher's exact test. The binary logistic regression with the 'enter' method was used to evaluate if factors significant in univariate analysis were strongly associated with AIU and PIU. We also used linear regression analysis to investigate the associations between demographic characteristics, the subscales of the DASS-21 (depression, anxiety, and stress) and IAT total scores. The odds ratios (OR), corresponding to 95% of confidence intervals (CI), standardized coefficient β values were generated for each variable. All tests were two-tailed, with a significance level of $p<0.05$. To adjust for multiple post-hoc test the significance level was set to $p<0.014$ ($\alpha'=(2*\alpha)/[n*(n-1)+1]$, n=3). Statistical analysis was performed using SPSS Statistic 21.0 (IBM SPSS Statistics, New York, United States).

RESULTS

Of the 2270 surveys administered, 39 surveys were interrupted because they did not agree to participate in this research, and 181 surveys were removed, belonging to respondents who were less than 6 years old or more than 18 years old. The final participants were 2050 children and adolescents aged 6–18 years old from three different areas (660 from Hunan province, 651 from Shandong province, and 739 from Inner Mongolia Autonomous Region). The mean age of participants was 12.34 (SD: 4.67) years old (males, 12.44 (SD: 5.45) years old, n=1057; girls, 12.24 (SD: 3.661) years old, n=993).

Incidence of Addictive, Problematic Internet Use and Demographic Characteristics

The mean score of total IAT was 36.83 (SD, 13.80); Male, 37.86 (SD, 14.53); Female, 35.72 (SD, 12.89)). Based on total IAT scores, 2.68% (Male: 3.50%; Female: 1.81%) and 33.37% (Male, 35.10%; Female, 31.52%) of the participants were classified as addicted and excessive Internet users, respectively. The mean IAT score was 78.96 (SD,7.303) in the AIU, 49.38 (SD, 7.603) in the PIU, and 28.51 (SD, 6.275) in the NIU. We found age, gender, and education status were significantly different among AIU, PIU, and NIU ($p<0.001$). There were no significant differences in the rate of the only child, residence, annual family income, or mother's or father's education level in the three groups (see **Table 1**).

Electronic Device Use During and Before the Epidemic of COVID-19

Smartphones or tablets were the primary electronic devices (AIU, 90.91%; PIU, 91.23%; NIU, 83.30%), followed by IPTV (AIU, 3.64%; PIU, 4.97%; NIU, 9.31%), computers (AIU, 3.64%; PIU, 3.51%; NIU, 5.95%) and other devices (AIU,1.82%; PIU, 2.92%; NIU, 1.45%%). For AIU, PIU and NIU groups, the frequency of recreational use of electronic devices online, the duration of recreational use of electronic devices online, the frequency of the use of electronic devices after 00:00/week, and the degree of addiction to electronic devices (self-rating, 0-100 scores) during the epidemic of COVID-19 were all increased than before. However, the frequency of use of electronic devices overnight/week increased only in NIU, but not in AIU and PIU (**Table 2**, **Figure 1**).

Prevalence of Depression, Anxiety, and Stress During the Epidemic of COVID-19

The prevalence of depression, anxiety, and stress were found to be 17.66% (n=362), 15.54% (n=298), and 7.07% (n=145), respectively. The prevalence of different levels of depression, anxiety, and stress in the past one week differed significantly among the three groups ($p<0.001$). Post-hoc analyses respectively showed differences between the AIU and PIU, AIU and NIU, as well as the PIU and NIU in **Table 3** ($p<0.014$).

Associations Between Demographic Characteristics, Depression, Anxiety, Stress, and Addictive, Problematic Internet Use

We used two statistical models to explore the factors which may contribute to Internet addiction. As shown in **Table 4**, the binary logistic regression analysis shows that male gender (OR=1.491,

TABLE 1 | Comparison of demographic characteristics among subsamples of addictive, problematic, and normal Internet use.

	Addictive Internet Users (n=55)	Problematic Internet Users (n=684)	Normal Internet Users (n=1311)	$\chi 2$	*p*
Age, *mean (SE), n (%)*	12.95 (3.297)	12.78 (3.791)	12.09 (5.101)		
6~9	7 (12.7)	118 (17.3)	295 (22.5)	38.692	<0.001
10~14	29 (52.7)	318 (46.5)	706 (53.9)		
15~18	19 (34.5)	248 (36.3)	310 (23.6)		
Gender, *n (%)*					
Male	37 (67.3)	371 (54.2)	649 (49.5)	994.610	<0.001
Female	18 (32.7)	313 (45.8)	662 (50.5)		
The only child, *n (%)*				2.439	0.295
Yes	29 (52.7)	322 (47.1)	663 (50.6)		
No	26 (47.3)	362 (52.9)	648 (49.4)		
Education status, *n (%)*				70.601	<0.001
Primary school	14 (25.5)	207 (30.3)	624 (47.6)		
Junior middle school	30 (54.5)	296 (43.3)	479 (36.5)		
Senior middle/Technical secondary school	11 (20.0)	181 (26.5)	208 (15.9)		
Residence, *n (%)*				7.722	0.259
First- and second-tier cities	8 (14.5)	121 (17.7)	231 (17.6)		
Third- and fourth-tier cities	36 (65.5)	363 (53.1)	758 (57.8)		
Villages and towns	9 (16.4)	151 (22.1)	247 (18.8)		
Rural area	2 (3.6)	49 (7.2)	75 (5.7)		
Annual family income (RMB yuan), *n (%)*				4.123	0.660
<5000	19 (34.5)	174 (25.4)	375 (28.6)		
5000-10000	23 (41.8)	332 (48.5)	602 (45.9)		
10000-30000	9 (16.4)	132 (19.3)	239 (18.2)		
>30000	4 (7.3)	46 (6.7)	95 (7.2)		
Mother's Education Level, *n (%)*				9.294	0.158
Primary school or below	12 (21.8)	70 (10.2)	156 (11.9)		
Junior middle school	13 (23.6)	172 (25.1)	355 (27.1)		
Senior middle/Technical secondary school	10 (18.2)	183 (26.8)	317 (24.2)		
College/Bachelor degree or above	20 (36.4)	259 (37.9)	483 (36.8)		
Father's Education Level, *n (%)*				9.105	0.168
Primary school or below	8 (14.5)	57 (8.3)	96 (7.3)		
Junior middle school	15 (27.3)	168 (24.6)	384 (29.3)		
Senior middle/Technical secondary school	12 (21.8)	183 (26.8)	318 (24.3)		
College/Bachelor degree or above	20 (36.4)	276 (40.4)	513 (39.1)		

Categorical variables were compared using Chi-square test.

p<0.001), age (OR=1.073, p<0.001), severe depression (OR=3.672, p=0.003), mild to moderate depression (OR=2.881, p<0.001), mild to moderate anxiety (OR=1.831, p=0.001), severe stress (OR=4.500, p=0.020), and mild to moderate stress (OR=2.508, p=0.009) were significantly associated with AIU and PIU. No significant association was observed between addictive, problematic Internet use and severe anxiety (p=0.087). Furthermore, the linear regression analysis (**Table 5**) shows female gender (β=-0.091, p<0.001), age (β=0.066, p=0.001), depression (β=0.257, p<0.001), and stress (β=0.323, p<0.001) were significantly correlated with the IAT total score (R=0.539, R^2 = 0.291, p<0.001).

DISCUSSION

Prevalence of AUI and PUI Among Chinese Children and Adolescents

To the best of our knowledge, there is limited information available on an overview study of IA and related risk factors of children and adolescents in a particular situation of stress and isolated condition. In the present study, 2.68% and 33.37% of the participants were classified as addicted and possibly addicted to the Internet. Several studies using the same criteria by the IAT reported with 1.2%~6.2% addicted Internet users and 12.5% ~46.0% problematic Internet users in China mainland, Hong Kong, Japan, South Korea, Malaysia, Philippines, Nigeria, and Greece (7, 20, 21). We found the prevalence was relatively higher than those reported earlier in China (2.2%/17.1%). The possible reason is that more students spent time in Internet use during this investigation. The fear resulting from the COVID-19 disease, and the consequences of lockdown, depression, and stress have been mounting affecting individuals' behaviors. It may also be explained by methodological differences (cross-sectional versus longitudinal study designs), and different statistical approaches (correlations versus time-lag models).

Age and IA

The results also showed that IA grew with age. Our research had a young group aged from 6 to 9 years old, and the incidence of

TABLE 2 | Recreational use of electronic devices among subsamples of addictive, problematic, and normal Internet use during and before the epidemic of COVID-19.

Recreational use of electronic devices	Addictive Internet Users (n = 55)		Problematic Internet Users (n = 684)		Normal Internet Users (n = 1311)		Comparison
	During the epidemic	Before theepidemic	During the epidemic	Before the epidemic	During the epidemic	Before the epidemic	
Frequency, *n (%)*							A*** P*** N***
several times per day	48 (87.27)	27 (49.09)	468 (68.42)	236 (34.50)	473 (36.08)	215 (16.40)	
once per day	2 (3.64)	10 (18.18)	146 (21.35)	178 (26.02)	493 (37.60)	400 (30.51)	
4-6 times per week	3 (5.45)	4 (7.27)	27 (3.95)	53 (7.75)	92 (7.02)	100 (7.63)	
1-3 times per week	1 (1.82)	7 (12.73)	34 (4.97)	160 (23.39)	159 (12.13)	358 (27.31)	
no use	1 (1.82)	7 (12.73)	9 (1.32)	57 (8.33)	94 (7.40)	238 (18.15)	
Duration per day, *n (%)*							A** P*** N***
>6 hours	28 (50.91)	18 (32.73)	129 (18.86)	66 (9.65)	87 (6.64)	49 (3.74)	
4-6 hours (including 6 hours)	9 (16.36)	3 (5.45)	140 (20.47)	90 (13.16)	134 (10.22)	84 (6.41)	
2-4 hours (including 4 hours)	11 (20.00)	11 (20.00)	222 (32.46)	138 (20.18)	317 (24.18)	173 (13.20)	
<2 hours	5 (9.09)	15 (27.27)	184 (26.90)	321 (46.93)	676 (51.56)	707 (53.93)	
no use	2 (3.64)	8 (14.55)	9 (1.32)	69 (10.09)	97 (7.40)	298 (22.73)	
Frequency of use after 00:00, *n (%)*							A** P** N**
>4 times per week	23 (41.82)	12 (21.82)	85 (12.43)	50 (7.31)	40 (3.05)	26 (1.98)	
3 times per week	8 (14.55)	2 (3.64)	43 (6.29)	23 (3.36)	28 (2.14)	18 (1.37)	
twice per week	3 (5.45)	5 (9.09)	76 (11.11)	54 (7.89)	62 (4.73)	37 (2.82)	
once per week	2 (3.64)	11 (20.00)	89 (13.01)	83 (12.13)	122 (9.31)	98 (7.48)	
no	19 (34.55)	25 (45.45)	391 (57.16)	474 (69.30)	1059 (80.78)	1132 (86.35)	
Frequency of use overnight, *n (%)*							N*
>4 times per week	12 (21.82)	4 (7.27)	35 (5.12)	26 (3.80)	27 (2.06)	12 (0.92)	
3 times per week	2 (3.64)	1 (1.82)	31 (4.53)	15 (2.19)	17 (1.30)	10 (0.76)	
twice per week	2 (3.64)	2 (3.64)	30 (4.39)	26 (3.80)	23 (1.75)	20 (1.53)	
once per week	4 (7.27)	7 (12.73)	36 (5.26)	45 (6.58)	72 (5.49)	60 (4.57)	
no	35 (63.64)	41 (74.55)	552 (80.70)	572 (83.63)	1172 (89.40)	1209 (92.22)	
Degree of addiction to electronic devices/mean *(SE)*	55.92 (3.21)	41.97 (2.90)	31.54 (3.27)	23.44 (2.95)	24.38 (1.10)	18.53 (0.99)	A*** P*** N***

A: Difference in addictive Internet users during and before the epidemic of COVID-19.
P: Difference in problematic Internet users during and before the epidemic of COVID-19.
N: Difference in average Internet users during and before the epidemic of COVID-19.
P < 0.05,P < 0.01, ***P < 0.001.*

AUI and PUI was 1.67% and 28.10%, respectively. Until now, most studies focused on the population of teenagers and young adults on Internet use (22). However, the onset use of the Internet in China has become earlier. The research report on the situation of Internet use among children and adolescents in China pointed out that the daily electronic devices (followed by mobile phones and tablets, followed by computers and televisions) exposure rate of Chinese children over 3 years old reached 92% (4). The average days of Internet use every week in children aged 3-8 years old in China are 3.7 (4). IA, just as Internet gaming disorder and gambling disorder, is a kind of addictive behavior without psychoactive substances. Behavioral addiction is generally developed incrementally and characterized by a change from fun, through losing control, to obsession (22). Internet use has its popularity and accessibility, so we should start to prevent IA in children according to the findings of this study.

Gender and IA

Gender difference is an essential issue in terms of IA. The proportion of males in AUI was much higher than that of females, which is consistent with previous studies (21, 23, 24). Boys who recreationally use the Internet prefer massive multiplayer online role-playing games (MMORPGs) (25) and violent games (26). In contrast, girls' online activities are mostly focused on playing time-killing games, socializing, texting, and online shopping, but less violent games (27–30). Girls present with shorter durations of online gaming and shorter online

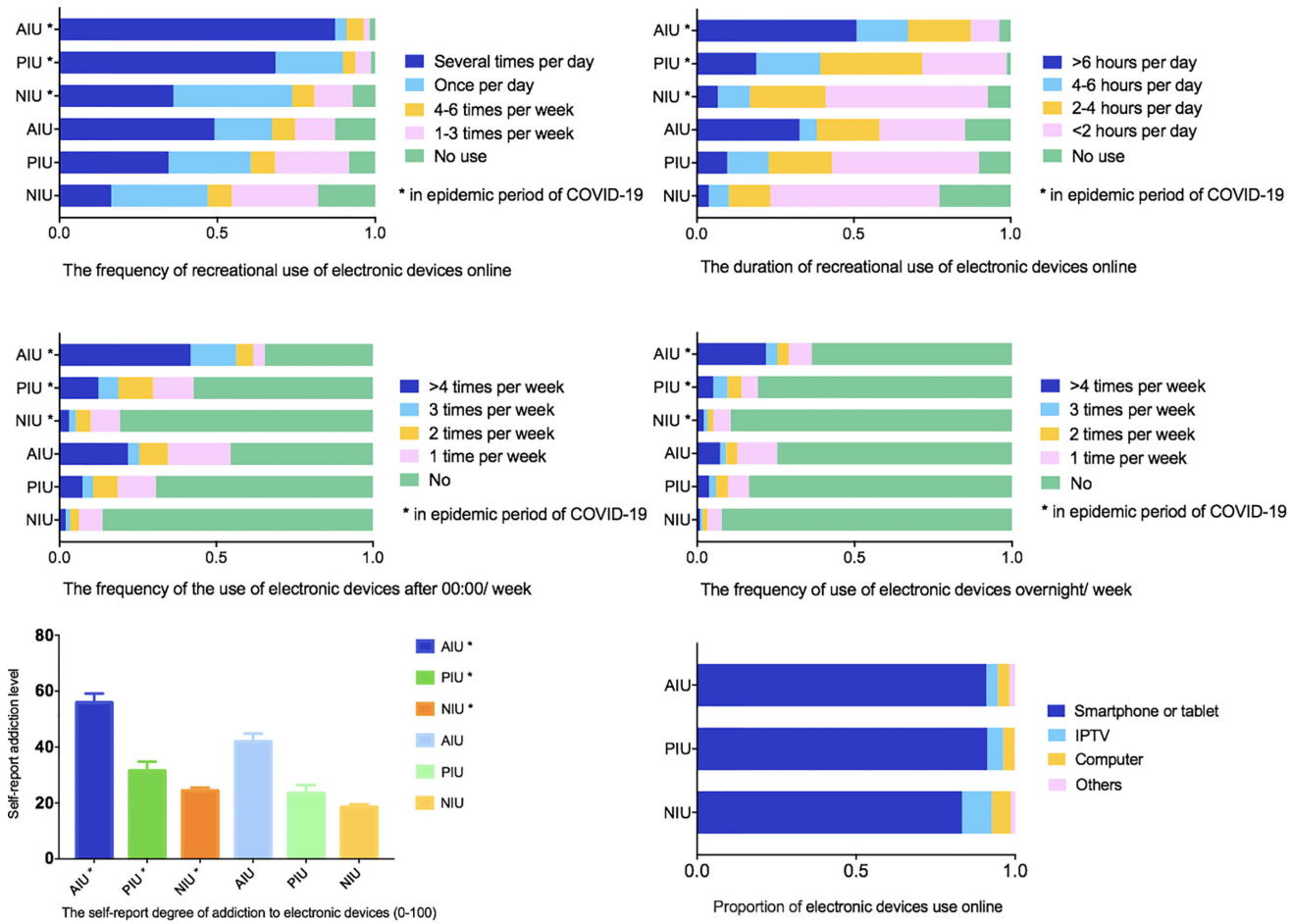

FIGURE 1 | Characteristic of recreational use of electronic devices online during and before the epidemic of COVID-19.

TABLE 3 | Prevalence of depression, anxiety, and stress among subsamples of addictive, problematic, and normal Internet users.

	Addictive Internet Users (n = 55)	Problematic Internet Users (n = 684)	NormalInternet Users (n = 1311)	$\chi 2$	*p*	Paired comparisons
Depression, *n (%)*				331.00	<0.001	1*;2*;3*
Severe	15 (27.27)	49 (7.16)	7 (0.53)			
Mild to moderate	17 (30.91)	182 (26.61)	92 (7.02.)			
No	23 (41.82)	453 (66.23)	1212 (92.45)			
Anxiety, n (%)				267.66	<0.001	1*;2*;3*
Severe	15 (27.27)	52 (7.60)	6 (0.46)			
Mild to moderate	9 (16.36)	138 (20.18)	78 (5.95)			
No	31 (56.36)	494 (72.22)	1227 (93.59)			
Stress, *n (%)*				207.58	<0.001	1*;2*;3*
Severe	11 (20.00)	32 (4.68)	3 (0.23)			
Mild to moderate	9 (16.36)	67 (9.80)	23 (1.75)			
No	35 (63.64)	585 (85.53)	1285 (98.02)			

$\alpha'=(2*\alpha)/[3*(3-1)+1]=0.014$
1: Addictive Internet Users vs Problematic Internet Users
2: Addictive Internet Users vs Normal Internet Users
3: Problematic Internet Users vs Normal Internet Users
**p < 0.014.*

TABLE 4 | Logistic regression analysis of risk factors of addictive, problematic Internet use.

Variables	OR (95% CI)	*P*
Male	1.491 (1.220~1.822)	<0.001
Age	1.073 (1.037~1.111)	<0.001
Depression		
Severe	3.672 (1.579~8.543)	0.003
Mild to moderate	2.881 (2.093~3.965)	<0.001
Anxiety		
Severe	2.095 (0.899~4.884)	0.087
Mild to moderate	1.831 (1.285~2.609)	0.001
Stress		
Severe	4.500 (1.266~15.995)	0.020
Mild to moderate	2.058 (1.198~3.534)	0.009

OR, odds ratio; CI, confidence interval.
**Analyzed by multiple logistic regression model.*

TABLE 5 | Linear regression of the relationships between research variables and IAT total score.

Variables	Standardized coefficient β	*p*
Female	-0.091	<0.001
Age	0.066	0.001
Depression	0.257	<0.001
Anxiety	-0.048	0.168
Stress	0.323	<0.001

screen times (31). The possible reason is girls are better in self-control and emotional regulation and physically and psychologically mature earlier, which can reduce pathological Internet use, especially when negative events occur (32, 33).

Internet Use and Mental State During the Epidemic of COVID-19

This study discovered that the frequency and duration of recreational electronic devices use, the frequency of electronic devices use after 00:00, and the self-score of addiction to electronic products were all significantly higher than those before the epidemic in all the groups. It is worth noting that the frequency of use overnight/week in NIU was higher than that before the epidemic, which might indicate risks in developing IA. Considerable attention should be paid to all children and adolescents, including those who have not yet reached the standard of IA.

Our data showed that a number of participants experienced significant depression, anxiety, and stress during the outbreak. In this particular period, due to the suspension of schools, the closure of living environments, the reduction of outdoor activities, and the increase of epidemic pressure, the mental health of school-age children and adolescents were threatened. The uncertainty and potentially adverse effect of a loss of academic progression could have a harmful impact. Furthermore, children and adolescents were not psychologically independent and still in the stage of psychological development, and also faced the challenge of massive online courses. Although guidance and handbooks for mental health care for Chinese people were posted and free online or telephone psychological counseling was widely promoted during the COVID-19 outbreak, there was little relative professional psychological assistance for children and adolescents. However, worth noting that we only assessed the psychological status once in the early phase of the outbreak. Given the ongoing pandemic, further studies on mental health over time and confirmation of psychological status development are needed.

Our study confirmed the role of depression and stress in IA in response to this outbreak, and these findings were inconsistent with the previous studies (8, 34–36). It is well-known that adverse experiences are associated with higher depression and stress levels (37). Internet is entertaining and easy to access, which may be a common way for children and adolescents to release emotions and stress and escape from reality (38). The Internet, especially online games, can stimulate individuals to have a sense of energy and autonomy and enhance self-esteem. Excessive users will be more focused on Internet and less interested in real life (39). Future studies are needed to assess whether expanding online consulting, particularly during the outbreak, effectively reduces depression and stress among children and adolescents, and indirectly prevents the development of IA.

Limitations

Several limitations should be noted in the present study. Firstly, the cross-sectional survey does not allow a conclusion to be drawn from the risk factors to IA among children and adolescents, so the results should be interpreted carefully. Secondly, IA lacks definite diagnostic criteria and is not determined by psychiatrists internationally. Young's Internet Addiction Test reflects mainly the DSM-IV's criteria of addiction, and the results may differ if other assessments were used. Thirdly, this study was based on the subjects' self-report, and pre-epidemic assessment may lead to memory bias. At last, as a response to the COVID-19 pandemic, the Chinese government has introduced steps of spatial distancing and "staying at home" to curb its spread and impact. So the sample was taken by a convenient sampling method to investigate mental health status and Internet use characteristics by online questionnaires. Despite these limitations, our findings still have important implications for research and intervention.

Conclusion

This study is a timely and large sample size investigation of Internet use in the representative areas of China in the COVID-19 outbreak. The findings provide substantial evidence of excessive Internet use among Chinese children and adolescents during this outbreak. Internet use is mainly influenced by the COVID-19 epidemic in various ways, including frequency and duration of recreational Internet use, and the rate of stay-up use. This study also represents a preliminary step towards understanding the relationship between IA and potentially related factors (male gender, age, depression, and stress). Therefore, it is necessary to provide preventive measures and strengthen education on IA for children and adolescents in countries experiencing or recovering from the epidemic. Further investigation is justified into whether reducing depression and stress can prevent IA among children and

adolescents. Nevertheless, because of the cross-sectional design of this study, large-scale prospective studies are warranted to confirm these associations.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The research protocol and consent procedures were approved by the Human Ethics Committee of the Xiangya Hospital of Central South University (the ethical approval number: 202003034). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

HD: writing of article, acquisition of data, and analysis/interpretation. FY: contributed to the conception and design, analysis/interpretation, and final approval for publication. XL: contributed to acquisition of data. WH: contributed to the analysis/interpretation, and final approval for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2020.00751/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Alterations of Metabolites in the Frontal Cortex and Amygdala Are Associated With Cognitive Impairment in Alcohol Dependent Patients With Aggressive Behavior

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Background: Alcohol dependence (AD) patients have a high prevalence of aggressive behavior (AB). The frontal cortex and amygdala contains various neurotransmitter systems and plays an important role in AB, which is also associated with cognitive deficits. However, to date, no study has addressed the association of metabolites in the frontal cortex and amygdala with cognitive deficits in Chinese aggressive behavior-alcohol dependent patients(AB-ADs).

Methods: We recruited 80 male AD and 40 male healthy controls (HCs), who completed the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), the Modified Overt Aggression Scale (MOAS), and the proton magnetic resonance spectroscopy (^{1}H MRS) scan using 3.0T Siemens. The ^{1}H MRS data were automatically fitted with a linear combination model for quantification of metabolite levels of n-acetyl-aspartate (NAA), glutamate (Glu), Choline (Cho) and creatine (Cr). Metabolite levels were reported as ratios to Cr.

Results: The AB-ADs group scored significantly lower than the non-aggression-alcohol dependent patients (NA-ADs) on these two RBANS subscales (immediate memory and attention function indices). The AB-ADs group showed a significant reduction in NAA/CR ratio in the left frontal cortex and Cho/Cr ratio in the left amygdala, and elevation in Glu/Cr ratio in the bilateral amygdala, compared with the NA-ADs group. The NAA/Cr ratio in the left frontal cortex was positively associated with immediate memory ($r=0.60$, $P<0.05$), and the Glu/Cr ratio in the right amygdala was negatively associated with delayed memory ($r=-0.44$, $P<0.05$) in AB-ADs group.

Conclusions: Metabolite alterations in the frontal cortex and amygdala may be involved in the pathophysiology of AB in AD and its associated cognitive impairment, especially immediate memory and delayed memory.

Keywords: alcohol dependence patients, frontal cortex, amygdala, cognitive impairment,^{1}H MRS

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INTRODUCTION

Alcohol dependence patients (AD) have a higher rate of aggressive behavior (AB) than patients with other psychiatric disorders, and the general population (1). The frequency of violent behavior in alcohol dependent patients has been reported to range from 20% to 50% (2). Moreover, the AB of AD patients can lead to economic burdens and social problems (3, 4) such as crime, and it predicts poor clinical outcome in AD patients (5). However, not all AD patients exhibit such aggressive behaviour (6) and it is unknown whether specific pathophysiological mechanisms cause AB in AD patients.

Alcohol-related AB is associated with cognitive deficits, these variables include the following: alcohol myopia (narrowed attention), hostile attribution bias (ambiguous social interactions being perceived as hostile (7), and disinhibition (executive functional abnormality) (8). This evidence has suggested that multi-faceted impairment in the cognition process is an important feature of the neuropsychology of AB in AD patients (9)

Many morphometric and functional neuroimaging studies have shown that aggressive individuals have structure and function abnormalities in regions related to the control of emotions, such as the frontal cortex, amygdala, and nucleus accumbens (10, 11). Studies that have used functional magnetic resonance imaging (MRI) have demonstrated that aggressive individuals showed deficient activation of the medial prefrontal cortex and the increased amygdala reactivity (12, 13). Importantly, several prefrontal cortex (PFC) subregions (anterior cingulate cortex) provide an inhibitory signal to the limbic areas (amygdala) (14). Considering the PFC-amygdala as a circuit, it helps to regulate emotional behavior (14). These pieces of evidence suggest that PFC and the amygdala play an important part in the regulation of AB (15, 16). Many neuroimaging studies have also found that long-term exposure to alcohol leads to serious regional brain changes (17), such as in the frontal cortex (18), amygdala (19) and anterior cingulate cortex (ACC) (20), including gray matter volume (GMV) integrity, regional activity, and cerebral glucose metabolism. These studies suggested that AD patients may share alterations in common brain regions with aggressive individuals, however, there is little known about the specific neurological mechanisms underlying AB and whether these cause AD patients to exhibit aggressive behavior.

Taken together, alcohol-related AB may be associated with specific neuroadaptive changes in the frontal cortex and amygdala by impairing various cognitive functions, including prefrontal control of emotional behavior (9, 21). However, no study has explored the association between activation in brain regions and cognitive functions (with imaging or electrophysiological techniques) in AD patients with AB. ^{1}H magnetic resonance spectroscopy (^{1}H MRS) is an invasive method, which can help to examine the metabolites in the brain regions (22). In this present study, we performed this method to explore the quantification of metabolite levels of n-acetyl-aspartate (NAA), glutamate (Glu), Choline (Cho), and creatine (Cr) in alcohol dependent patients with and without AB. We aimed to investigate whether AD patients with AB have specifically altered metabolites in the frontal cortex and amygdala associated with relevant cognitive functions. It was hypothesized that firstly, AB-AD patients would be associated with impaired cognitive functions and altered metabolite levels in the frontal cortex and amygdala; and secondly, that these impaired cognitive functions would be significantly correlated with altered metabolite levels in AB-AD patients.

METHODS

Participants

Eighty male AD inpatients and forty HCs were recruited from Hunan brain hospital, ChangSha city, Hunan, China. All subjects met the following inclusion criteria: 1) age 18-60 years, Han Chinese; 2) no contraindications for MRI. Participants were excluded if they (i) had any general medical conditions or neurological disorders, including infectious, hepatic, or endocrine disease; (ii) had a history of severe head injury with skull fracture or loss of consciousness of more than 10min; (iii) had any current or previous psychiatric disorder; (iv) had a family history of psychiatric disorder; (v) had contraindications for MRI. Alcohol dependence patients confirmed DSM-IV diagnosis of alcohol dependence and abstained from alcohol for at least 7 days before scanning. AD inpatients were excluded if they met criteria for other substance dependence (excluding nicotine dependence) at any time.

Written informed consent was given by all subjects. This study was approved by the Ethics Committee of the Brain Hospital, Traditional Chinese Medicine University.

Clinical Characters and Aggressive Behavior Measures

Each subject filled out a detailed questionnaire that recorded general information, sociodemographic characteristics, medical and psychological conditions, and smoking behavior. In addition, we administered an alcohol use questionnaire to record alcohol history and family history of alcohol from each subject. Additional information was collected from available medical records and collateral data (from family and/or treating clinician). The patient's degree of AB was assessed by the Chinese translation of the standardized Modified Overt Aggression Scale (MOAS). We divided the AD patients into groups based on their AB history and MOAS scores. AD patients with AB were defined as persons who had shown aggressive behavior on more than two instances within half a year, and rated with a score of≥8 in the MOAS. AD patients without AB were defined as individuals who had no AB history and rated with a score of < 8 in the MOAS.

Cognitive Tests

Cognitive functioning was assessed by the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS, Form A) (23). The RBANS consists of 12 subtests that are used to calculate 5 age-adjusted index scores and a total score. Test indices are Immediate Memory (comprised of List Learning and Story Memory tasks); Visuospatial/Constructional (comprised of Figure Copy and Line Orientation tasks); Language (comprised of Picture Naming and Semantic Fluency tasks); Attention

(comprised of Digit Span and Coding tasks); and Delayed Memory (comprised of List Recall, Story Recall, Figure Recall, and List Recognition tasks). The Chinese version of RBANS has good clinical validity and test-retest reliability (24).

Magnetic Resonance Data Acquisition

The MRS scan was performed using a Siemens Magnetom Trio Tim 3.0 MR scanner (Siemens, Erlangen, Germany) at the Magnetic Resonance Center of Hunan brain Hospital, China. T1-weighted three-dimensional images were acquired using a gradient echo sequence (repetition time=2,000ms, echo time=2.26ms, field of view=256×256mm, flip angle=8°, matrix size=256×256, number of slices=176, slice thickness=1mm). Using these images, a single ^{1} H MRS voxel was placed on the corpus callosum and centered on the intrahemispheric fissure, including frontal cortex (see **Figures 1A, B**), and amygdala (see **Figures 2A, B**).^{1} H MRS was performed using a short-echo point resolved spectroscopy sequence (PRESS;repetition time=1,000ms;echo time=144ms; voxel size 10×10×10mm; number of scans=328). Water suppression was achieved using a chemical shift selective (CHESS) sequence. The area under each peak was measured and the ratio of NAA/Cr to Cho/Cr was calculated using software provided by General Electric Company (GE Funtool2). The position of NAA is determined at the mass fraction of 2.0×10^{-6} on the spectral line, and the chemical shifts of other substances were determined by using it as a reference. The mass fraction of Cho is 3.2×10^{-6} and the mass fraction of Cr is 3.02×10^{-6}. In the calculation, the under-peak area of each peak is measured respectively. According to the usual calculation method, taking Cr as the internal standard, the ratio of NAA and Cho to Cr was calculated. All scans were performed by the same radiologist, and all images were recorded on disk for measurement and analysis at the same time.

Statistical Analysis

Demographic and clinical variables of the AD inpatients with AB and without AB groups and HCs were compared using t-test, analysis of variance (ANOVA) for continuous variables and chi-squared for categorical variables. We compared RBANS scores and metabolite ratios among the three groups using analysis of variance (ANOVA). Fisher's least significant difference (LSD) test was used to perform post-hoc pair-wise between-group comparisons. The relationship between the RBANS scores and metabolite ratios was examined by Pearson's or Spearman's correlation analysis, followed by the Bonferroni test. In these analyses, all variables were initially entered simultaneously to determine the overall influence, and then backward stepwise procedures were employed to determine the significant associations. SPSS version 19.0 was used to do all statistical analysis. Data were presented as mean and standard deviation (mean ± SD). All p values were 2 tailed with a significance level set at 0.05.

RESULTS

Participants Characteristics

The three groups, AB-AD patients (MOAS≥8, n=40), non-aggression-alcohol dependent (NA-AD) patients (MOAS< 8, n=40)and HCs were matched in terms of age, levels of education and the daily number of cigarettes smoked. The AB-AD patients and NA-AD patients were matched in terms of the duration of alcohol use, age of first alcohol use, and the dosage of alcohol used per day. Details of all self-report and behavioral measures are given in **Table 1**.

Group Differences in Cognitive Performance

RBANS index scores of 40 AB-AD patients, 40 NA-AD patients, and 40 HCs are shown in **Table 2**. The AB-AD patient group scored significantly lower than the NA-AD patients on these two RBANS subscales (immediate memory and attention function indices) (p<0.001).

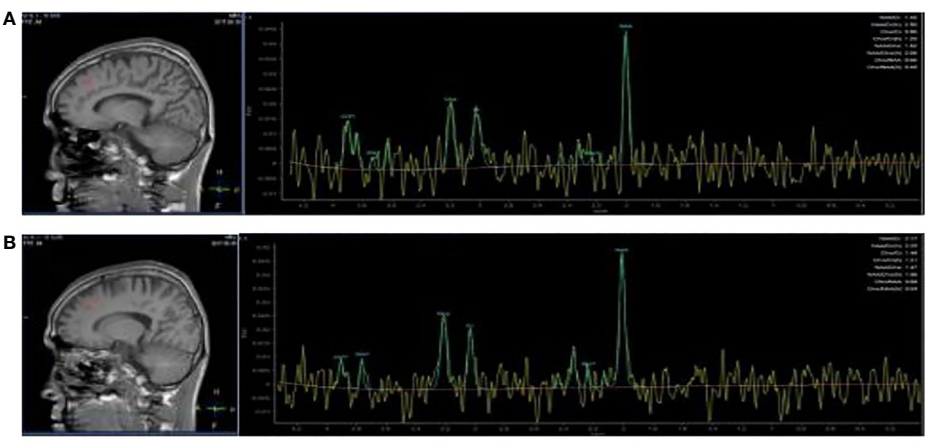

FIGURE 1 | Region of interest in the frontal cortex in views, and spectra of the unfiltered data superimposed with the LCModel fit. **(A)** Region of interest in the left frontal cortex in views, and spectra of the unfiltered data superimposed with the LCModel fit. **(B)** Region of interest in the right frontal cortex in views, and spectra of the unfiltered data superimposed with the LCModel fit.

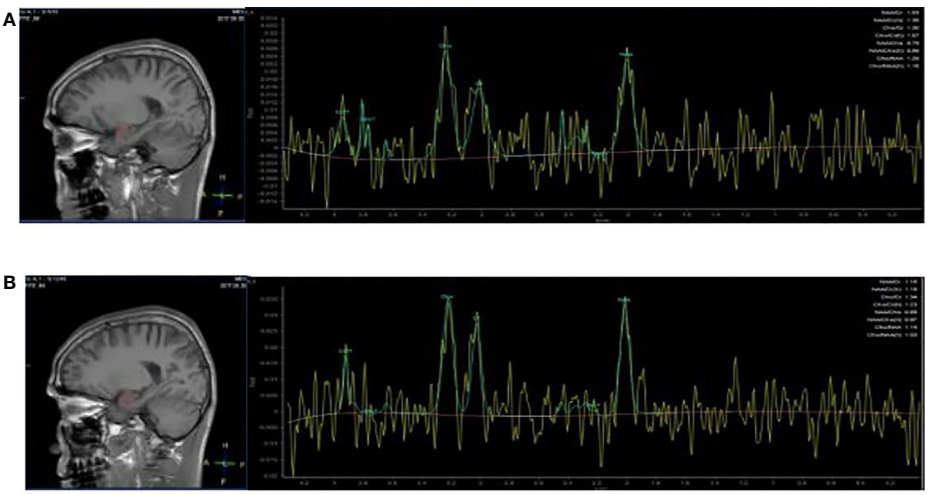

FIGURE 2 | Region of interest in the amygdala in views and spectra of the unfiltered data superimposed with the LCModel fit. **(A)** Region of interest in the left amygdala in views, and spectra of the unfiltered data superimposed with the LCModel fit. **(B)** Region of interest in the right amygdala in views, and spectra of the unfiltered data superimposed with the LCModel fit.

TABLE 1 | Demographic and alcohol use characteristics of alcohol dependent patients with Modified Overt Aggression Scale (MOAS) and health controls.

Characteristic	AB-ADs(n = 40)	NA-ADs(n = 40)	HCs(n = 40)	P
Age (years)	44.35 ± 5.52	41.28 ± 5.23	40.46 ± 6.24	0.10
Education (years)	9.30 ± 2.15	9.45 ± 1.74	9.64 ± 1.94	0.14
Age of first alcohol use (years)	16.55 ± 1.88	16.90 ± 1.93	17.65 ± 1.54	0.11
Alcohol used per day	233.28 ± 24.65	212.56 ± 31.41	–	0.10
Duration of alcohol use (years)	12.25 ± 8.24	10.52 ± 7.51	–	0.20
Number of abstinence	1.23 ± 1.17	0.88 ± 0.95	–	0.08
Cigarette smoked per day	28.50 ± 6.25	30.65 ± 4.55	27.45 ± 6.75	0.12

AB-ADs, aggressive behavior-alcohol dependent patients; HCs, healthy controls; NA-Ads, non-aggression-alcohol dependent patients.

^{1} H MRS Metabolite Ratios

The AB-AD patient group showed a significant reduction in NAA/CR ratio in the left frontal cortex and Cho/Cr ratio in the left amygdala, and elevation in Glu/Cr ratio in the bilateral amygdala, compared with the NA-AD patients group (**Table 3**).

Correlation Between Metabolite Ratios and Cognitive Performance

For the AB-AD patients, correlation analyses showed a significant positive association between NAA/Cr ratio in the left frontal cortex and the score of immediate memory index

TABLE 2 | Index scores on the RBANS in AB-ADs, NA-Ads, and health controls.

Index	AB-ADs(n=40)	NA-ADs(n=40)	HCs(n=40)	p^a	p^1	p^2	p^3
Immediate memory	30.50 ± 6.37	36.98 ± 6.07	49.43 ± 4.89	0.03	0.02	0.04	0.01
Visuospatial/ constructional	14.98 ± 4.76	16.43 ± 5.14	18.20 ± 4.31	0.12	0.13	0.11	0.12
Language	29.68 ± 5.55	28.68 ± 5.98	32.00 ± 5.51	0.06	0.08	0.07	0.08
Attention	63.03 ± 11.26	70.43 ± 10.62	75.85 ± 11.12	0.13	0.01	0.12	0.02
Delayed memory	55.56 ± 19.30	58.05 ± 12.56	67.23 ± 12.30	0.21	0.04	0.03	0.23

AB-ADs, aggressive behavior-alcohol dependent patients; HCs, healthy controls; NA-ADs, non-aggression-alcohol dependent patients.
p^a ANOVA,
p^1 aggressive behavior-alcohol dependent patients vs healthy controls,
p^2 non-aggression-alcohol dependent patients vs healthy controls,
p^3 aggressive behavior-alcohol dependent patients vs non-aggression-alcohol dependent patients vs healthy controls.

TABLE 3 | Metabolite concentrations in the region of interest in AB-ADs, NA-ADs, and health controls.

	AB-ADs(n=40)	NA-ADs(n=40)	HCs(n=40)	p^a	p^1	p^2	p^3
NAA/CR	2.27 ± 0.54	3.26 ± 0.78	3.87 ± 0.77	0.038	0.027	0.046	0.04
left frontal cortex							
right frontal cortex	3.18 ± 0.64	3.42 ± 0.56	3.74 ± 0.46	0.12	0.047	0.13	0.11
left amygdala	1.92 ± 0.72	1.96 ± 0.61	2.48 ± 0.74	0.044	0.042	0.043	0.12
right amygdala	1.87 ± 0.55	1.96 ± 0.51	2.03 ± 0.65	0.22	0.18	0.21	0.09
Cho/Cr	1.24 ± 0.33	1.32 ± 0.45	1.78 ± 0.32	0.045	0.03	0.032	0.10
left frontal cortex							
right frontal cortex	1.15 ± 0.34	1.08 ± 0.56	1.56 ± 0.65	0.03	0.043	0.037	0.08
left amygdala	3.25 ± 1.24	4.56 ± 1.03	4.58 ± 1.14	0.04	0.027	0.035	0.03
right amygdala	3.65 ± 0.55	3.97 ± 0.51	5.82 ± 0.45	0.044	0.038	0.043	0.034
Glu/Cr	5.21 ± 1.21	5.56 ± 1.32	5.34 ± 1.03	0.14	0.09	0.1	0.11
left frontal cortex							
right frontal cortex	4.85 ± 1.66	4.97 ± 1.56	5.01 ± 1.34	0.11	0.13	0.15	0.14
left amygdala	8.75 ± 3.65	5.06 ± 2.78	4.98 ± 2.65	0.03	0.01	0.07	0.02
right amygdala	7.82 ± 3.25	5.36 ± 2.02	4.54 ± 2.33	0.043	0.03	0.11	0.04

AB-ADs, aggressive behavior-alcohol dependent patients; HCs, healthy controls; NA-Ads, non-aggression-alcohol dependent patients; NAA, n-acetyl-aspartate; Cho, choline; Glu, glutamate; Cr, creatine.

p^a ANOVA,

p^1 aggressive behavior-alcohol dependent patients vs healthy controls,

p^2 non-aggression-alcohol dependent patients vs healthy controls,

p^3 aggressive behavior-alcohol dependent patients vs non-aggression-alcohol dependent patients vs healthy controls.

(r=0.60,P<0.05); and a significant negative association between Glu/Cr ratio in the right amygdala and the score of delayed memory (r=-0.44,P<0.05) (**Figure 3**).

DISCUSSION

The main findings of the present study were: (1) significantly lower scores on the two RBANS subscales (immediate memory and attention function indices) were found in AB-AD patients than NA-AD patients; (2) a reduction in NAA/CR ratio in the left frontal cortex and Cho/Cr ratio in the left amygdala, and an elevation in Glu/Cr ratio in the bilateral amygdala of AB-ADs, compared with NA-ADs group; (3) the NAA/Cr ratio in left frontal cortex was positively associated with immediate memory and the Glu/Cr ratio in the right amygdala was negatively associated with delayed memory in AB-AD patients group.

In this study, we found that significantly lower cognitive performance on the two RBANS subscales (immediate memory and attention function indices) were found in AB-AD patients than NA-AD patients. The impairment of attention and memory is associated with an increased manifestation of alcohol-related aggression and has been hypothesized to be an important factor in the onset of alcohol-related aggression (25, 26). Taken

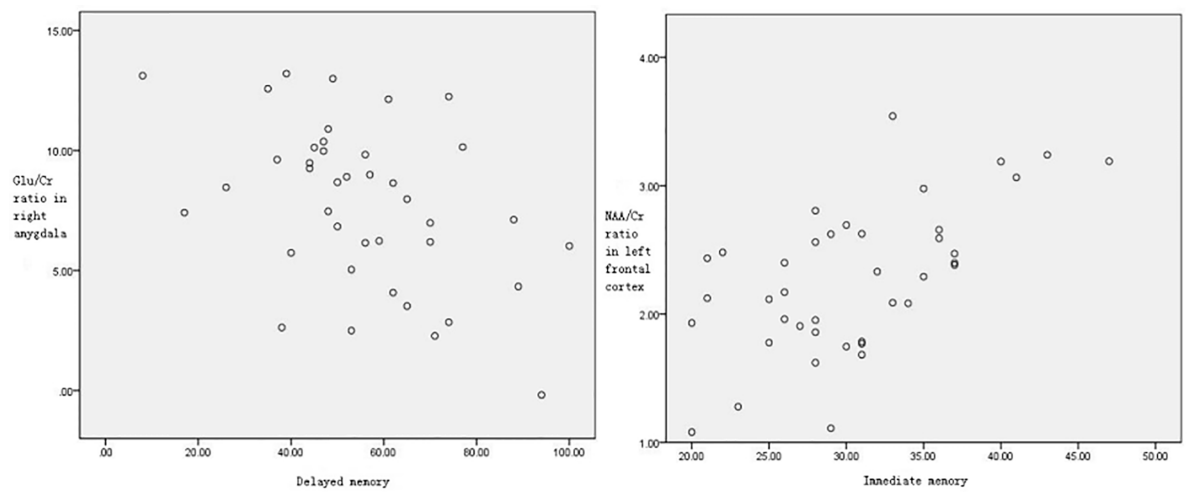

FIGURE 3 | NAA/Cr ratio in the left frontal cortex was significantly correlated with the score of immediate memory index (r=0.60,P<0.05). Glu/Cr ratio in the right amygdala was significantly correlated with the score of delayed memory (r=-0.44, P < 0.05).

together, these results suggest that AB-AD patients had more neuropsychological impairments than NA-AD patients.

Finding the reduced ratio of NAA/CR in the AB-AD patient group is consistent with the theory that substance abuse leads to long-lasting impairment of the frontal cortex and as well as the AD patients (27, 28). Some studies suggested that AD patients have structural and functional abnormalities in the frontal cortex, which is associated with decision-making and inhibition control of AB (9). In addition, NAA reflects neuronal integrity, viability, and number (29). The changes in NAA may reflect adverse neuron function disorder (30). This present finding suggests that there is decreased neuronal integrity, viability, number, or dysfunction in the frontal cortex in AD patients, which may be a neurobiological marker of alcohol-related aggressive behavior.

We also found that the AB-AD patient group showed a significant reduction in the Cho/Cr ratio in the left amygdala. The decreased ratio of Cho/Cr in the left amygdala for the AB-ADs group is in line with previous studies on AD patients (31, 32). Some studies have suggested that Cho is taken as a marker of glial density and represents the status of glial cells in the brain (33, 34). Reduction of Cho/Cr may suggest the proliferation of glial cells due to the damage of neurons induced by alcohol dependence patients (33), which is a marker of AD patients induced neurons damage after Cho decreased. We also found that the AB-AD patient group showed an elevation in Glu/Cr ratio in the bilateral amygdala, compared with the NA-AD patient group, previous studies on alcohol dependent patients have found such findings (35). Glu is the major excitatory neurotransmitter, which is the main precursor and involved in the regulation of brain amino acid metabolism (36). The glutamateric signal system plays an important role in inhibition and behavior control (37). The abnormal function of the glutamate energy system is related to the pathophysiology of mood disorder (38). Thus, our findings, which observed the increased Glu/Cr ratio in the bilateral amygdala, may be associated with an increased manifestation of alcohol-related aggression behavior.

The current findings indicate that metabolite alterations in the frontal cortex and amygdala were associated with worse cognitive function in aggressive behavior-alcohol dependent patients. This finding is consistent with two studies showing that metabolite alterations in the frontal lobes and limbic regions were associated with worse neuropsychological function in AD patients (39, 40). Studies that used as an animal model have discussed that frontal lobe lesion delays memory task completion time (41). Most importantly, frontal lobe lesions have lead to reduced working memory performance in human models (42). These results show that metabolites alterations may play a role in the cognitive process of AB-AD patients, suggesting that alcohol-related aggressive behavior is not just associated with cognitive function impairment but is also related to metabolite alterations in frontal cortex and amygdala.

Limitations

Several limitations of this study should be noted. First, all subjects in our study were male, making it impossible to explore the influence of gender confidently. Second, our study is cross-sectional, and it is unclear whether alterations in cognition performance and metabolites would persist or be reversed after a continued period of abstinence from alcohol. Finally, this study only focused on the metabolic changes in some brain regions (frontal lobe, amygdala) related to aggressive behavior. In the future, studies using whole-brain analysis may help to provide further insight into the relationship between brain regions and aggressive behavior in alcohol dependent patients. Future studies should also include more women AB-AD patients, and measure alterations longitudinally, examining AB-AD patients for a longer duration, starting from the beginning of abstinence, and covering a longer period of abstinence.

CONCLUSION

Our findings suggest that alterations in the ratios of NAA/CR, Cho/Cr, and Glu/Cr in the frontal cortex or amygdala may underline the pathophysiology of neurological impairment in AB-AD patients. These AD patients with AB also displayed a range of cognitive impairments, and metabolite alterations were associated with worse cognitive function in these AD patients. This study indicates that neurotransmitter alteration constitutes a risk factor for cognitive deficits in AB-AD patients. The reduction of NAA/CR in the frontal cortex and Cho/Cr ratio in the amygdala or elevation of the Glu/Cr ratio in the amygdala may be of great clinical significance in treatment.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the brain Hospital, Traditional Chinese Medicine University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CL, XZ, and XT designed the study. CL, YL, and JX managed the data collection. CL and YL undertook the statistical analysis. CL wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SYSTEMATIC REVIEW
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The Effects of Benzodiazepine Use and Abuse on Cognition in the Elders: A Systematic Review and Meta-Analysis of Comparative Studies

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Objective: Benzodiazepines (BZD) are one of the most frequently prescribed drugs worldwide. However, the cognitive effects of benzodiazepines in the elderly are highly debated. This systematic review and meta-analysis aims to explore the following two questions in the elderly population: (i) Do BZD lead to any impairments in cognitive functions in elderly users? and (ii) Which specific cognitive domains are most affected by BZD use and abuse?

Methods: First, we performed a literature search following the PRISMA guidelines. Electronic databases, including PubMed, PsycINFO, EMBASE, Cochrane Library, and Web of Science were searched until May 14th, 2020. After selecting the relevant articles, we integrated the results of the selected studies with a standardized cognitive classification method. Next, we performed meta-analyses with the random-effects model on the cognitive results. Finally, we specifically examined the cognitive impairments of BZD in the abuse subgroup.

Results: Of the included studies, eight of the thirteen had meta-analyzable data. Compared to the controls, elderly BZD users had significantly lower digital symbol test scores (n=253; SMD: -0.61, 95% CI: -0.91 to 0.31, I^2 = 0%, $p < 0.0001$). There was no significant difference in Mini-Mental State Examination, Auditory Verbal Learning Test, and Stroop Color and Word Test scores between BZD users and controls. According to the subgroup analyses, BZD abusers performed significantly worse than controls in Mini-Mental State Examination (n=7726; SMD: -0.23, 95% CI: -0.44 to -0.03, I^2 = 86%, p = 0.02), while there was no significant difference between the regular BZD users and the controls (n=1536; SMD: -0.05, 95% CI: -0.59 to 0.48, I^2 = 92%, p =0.85).

Conclusion: In the elderly population, the processing speed (digital symbol test scores) was significantly impaired in BZD users; global cognition (Mini-Mental State Examination scores) was significantly impaired in BZD abusers but not in BZD regular users. This study

provides insight into the factors that interact with BZD cognitive effects, such as aging, testing tools, and abuse. Clinicians should be cautious when prescribing BZD for the elderly.

Systematic Review Registration: PROSPERO, identifier CRD42019124711.

Keywords: benzodiazepines, cognitive function, substance addiction, aged, meta-analysis, cognitive dysfuction

INTRODUCTION

Benzodiazepines (BZD) are two-ring heterocyclic compounds consisting of a benzene ring fused with a diazepine ring. Since its discovery in the 1950s, BZD's sedative, hypnotic, anti-anxiety, and anti-convulsive effects have been increasingly accepted, making BZD use highly prevalent among adults (1) and especially in the elders (2). The prevalence of BZD use in elders varies between 10% and 42% worldwide (3). For example, BZD and related drugs are the third most abused prescription drug in America, with roughly 1-3% of the world population being subject to abuse (4). However, inappropriate BZD prescriptions can promote BZD misuse, facilitate the development of BZD addiction, and significantly affect the users' overall quality of life (5, 6). Therefore, it is critical for pharmacists, clinicians, and patients to be informed on the latest research regarding the adverse effects of BZD use and abuse.

Since the 1970s, research has found negative effects of BZD on recipients' cognitive functions (7). A meta-analysis in 2017 investigated the long-term cognitive impacts of BZD among adults. This analysis reported impairments in working memory, language, and processing speed, but not in executive function (reasoning and planning) (8). The participants in the meta-analysis, however, included adults of all ages. Compared to young adults, elderly populations require more cautiousness when undertaking BZD therapy. The elderly are more susceptible to cognitive impairment than young adults (9). During the progress of aging, cognitive function continues to decline with structural and functional neurological changes (10, 11). The pharmacokinetics of BZD in the elderly are different than in young adults, so the effects of BZD in older adults may have unique characteristics than in any other age group (12).

In recent years, several systematic reviews have found that BZD use was significantly associated with a higher risk of dementia and mild cognitive impairments (MCI). Dementia and MCI introduced a significant growth in mortality and financial burdens worldwide (13, 14). The most prominent symptom of these neurological disorders is a decline in cognitive function, measured by tests such as the Mini-Mental State Examination (MMSE). Sufficient evidence has shown that the risk of dementia correlates with cumulative dose, treatment duration, and long-acting effects of BZD molecules (15–18). However, previous literature failed to provide a causal account for the link between BZD use and the risk of dementia (18). To understand the mechanism behind the adverse effects of BZD, it is crucial to investigate the specific areas of cognitive functions that are impacted by BZD use and abuse.

Literature in the past 50 years presents contradicting evidence on whether BZD impairs cognitive functioning in the elderly (19–24). Among the studies that suggest an association between BZD use and cognitive impairment in elders, the type and degree of cognitive impairment reported are inconsistent across studies (19, 20, 22). Moreover, the neurological alterations due to the long-term effects of BZD use remains unclear (9). In 2018, Picton and colleagues summarized the cognitive effects of BZD in the elderly from published evidence. They found mixed findings of the association between BZD therapy and cognitive decline in elderly users (25). Given the lack of consensus in the current literature, a meta-analysis study may help reveal the critical effects of BZD use in the elderly and identify areas that require further research. To our knowledge, there are no systematic reviews with a meta-analysis that summarizes the current status of the cognitive effect of BZD use and abuse in the elderly population.

This systematic review and meta-analysis aims to explore the following two questions in the elderly: (i) Is BZD use associated with impairment in cognitive functions in the elderly? and (ii) Which cognitive domains have declined functionality associated with BZD use and abuse? The answers could help characterize the specific cognition impairments associated with BZD use, and identify individuals vulnerable to the negative effects of BZD. This meta-analysis may also help identify and monitor the cognitive effects associated with BZD use and abuse to prevent BZD addiction. With the high prevalence of BZD being prescribed to older populations worldwide, it is essential to inform clinicians and patients about the possible cognitive impairments associated with BZD use and abuse. Given the refractory rate and adverse effects of dementia and MCI (26), the results may also help reduce inappropriate BZD prescriptions to attenuate dementia risk in the elderly population.

METHODS

Systematic Review Protocol

The process of this systematic review follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, 2009 (27).

Databases and Search Strategy

We searched relevant articles in electronic databases, PubMed, PsycINFO, EMBASE, Cochrane Library, and Web of Science, from their inception to May 14, 2020. Specific search keywords included "benzodiazepines", "cognition", and "aged". The query used for PubMed is ((Benzodiazepines[mh]) OR (Benzodiazepines

Compounds[Title/Abstract]) OR (Benzodiazep*[Title/Abstract])) AND (cognit *' OR memory OR attention OR visual-spatial OR visuospatial OR recall OR recognition OR problem solving OR reaction time OR vigilance OR executive function*' OR reasoning OR psychomotor OR motor OR processing OR planning OR verbal fluency OR inhibit *') AND ((Aged[mh]) OR (Elder*[Title/Abstract]) OR (older adults[Title/Abstract])).

Additionally, we searched in Google Scholar and searched the reference list for relevant articles to ensure that no studies were missed.

Inclusion Criteria and the Process to Identify Studies

We developed the inclusion criteria according to the PICOS guideline. Appropriate papers met the following criteria: 1) Participants were human adults, older than 60 years, mean sample age over 65 years; 2) the treatment groups were BZD users; 3) the studies had placebo or non-users of BZD as controls; 4) the outcomes included performances of cognitive functions measured using any standardized neuropsychological instruments; and 5) any type of published clinical studies except for case reports or conference abstracts were used. We only included articles reported in English.

Endnote was used to delete the duplicated articles. Two reviewers (LL and JL) independently scanned the references by title/abstract to exclude irrelevant articles, then read the full text to identify the appropriate studies based on the above inclusion criteria. Finally, the debated studies were determined through discussions with a third reviewer (YT).

Risk of Bias and Quality Assessment

Two reviewers assessed the quality of each included study independently with the modified Newcastle-Ottawa Scale (mNOS) (28). The mNOS examines the quality of non-randomized studies by four bias reduction items: (1) selection bias, (2) performance bias, (3) detection bias, and (4) reporting bias. Each item is graded from 0 (low quality) to 3 (high quality) according to the example given for each risk level. Disagreements were resolved by discussions with a third reviewer (Y.T.). Publication bias was tested by using the funnel plot and Begg's tests on the outcome which synthesizes more than five studies.

Data Extraction

After generating a list of the included articles, two reviewers made the data extraction form collectively. The two reviewers then extracted the data independently. The data extraction form contained the following information: (1) author and publication year, (2) study design, (3) setting (country), (4) study design, (5) mean age, (6) gender distribution, (7) education, (8) participants sources, (9) details of BZD use (BZD types, using time, dosage, and the definition of BZD use), and (10) outcomes (cognitive task, cognitive domain, and main findings). The authors were contacted in order to obtain any missing data.

Data Synthesis and Statistical Analyses

The included studies utilized a variety of psychometric measurements, rendering it challenging to produce generalizable and informative conclusions. In this paper, we organize cognitive domains based on a commonly used framework (29, 30). According to this framework, we summarized the outcomes of the cognitive task from each included study to include a balanced and comprehensive view. Due to the discrepancy in psychometric screening tools between studies, the meta-analysis would only synthesis the data from the same neuropsychological instruments, such as the MMSE and the Auditory Verbal Learning Test (AVLT).

The RevMan software (31) was used to perform the meta-analysis. We adopted the random-effects model in our meta-analysis because it is more conservative than the fixed-effects model. Standardized mean difference (SMD) with 95% confidence intervals (CIs) was used for continuous outcomes. The study's heterogeneity was measured using I^2. I^2>50% indicated significant heterogeneity (32). The meta-analytic outcomes were two-tailed, with a significance level of 0.05. Based on BZD use information reported in each study, we divided all the BZD users into two categories: regular BZD users and BZD abusers. BZD regular use is an appropriate pattern of BZD use that follows the prescription instruction. BZD abuse behaviors include meeting sedative, hypnotic, and anxiolytic use disorder criteria according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, and using a higher frequency or dose, or longer use time than prescribed, or without a prescription (1, 4, 33). We then conducted a subgroup analysis to explore the cognitive changes in BZD abusers and regular BZD users.

RESULTS

Search Results and Studies Included

The study selection process of this systematic review is summarized in **Figure 1**. A total of 5072 references were returned by the initial search and scan, with 44 from relevant reviews and Google Scholar. After removing duplicated and irrelevant articles by title and abstract, the full texts of the remaining 79 were screened and 15 articles finally met the inclusion criteria. Two articles (34, 35) were from the same cohort study named *The Canadian Study of Health and Aging* (36). Another two articles had repeated participants (22, 37). Overall, 13 studies were included in the literature review. In the 13 studies, eight had meta-analyzable data.

Risk of Bias and Quality Assessment

The risk bias of the included studies was assessed by the mNOS and detailed in **Table S1** in the **Supplementary Materials**. The scores ranged from 13-19 out of 21. Nine studies (34, 38–45) had a sample size of more than one thousand participants. Nine studies (34, 38, 40–42, 44–47) used instruments that measured multi cognition domains. Eleven studies matched or adjusted education level as covariates and 12 studies matched or adjusted age as covariates. Therefore, the overall risk bias of the included studies is reasonably low. We tested the publication bias of the meta-analysis results of MMSE scores; the funnel plot (**Figure S1** in **Supplementary Materials**) and Begg's tests (p=0.673) did not find publication bias.

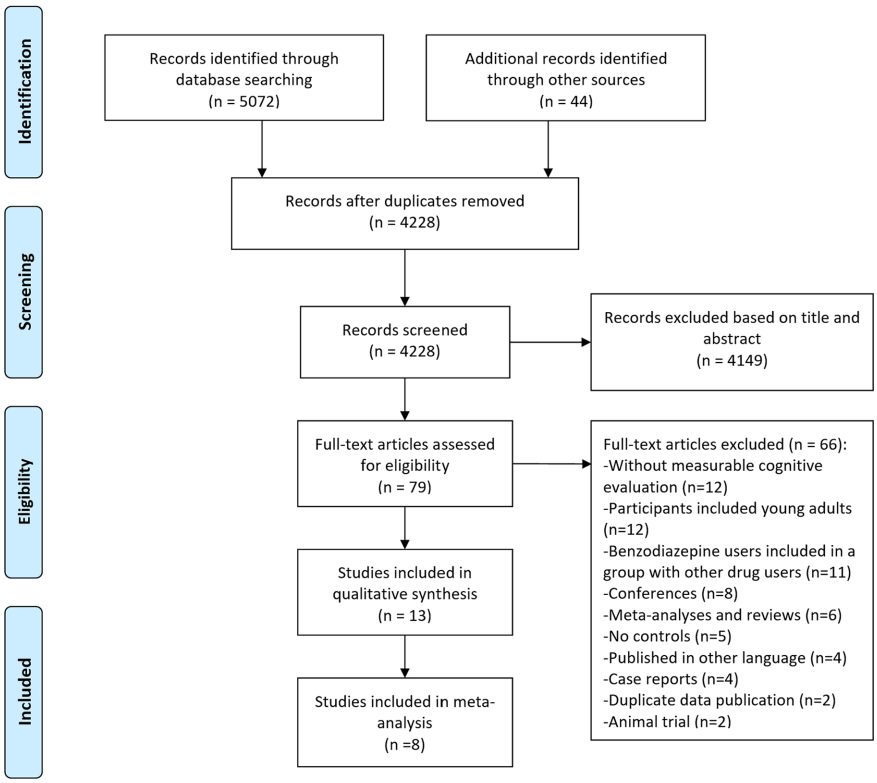

FIGURE 1 | Flow diagram of the search and study identification process.

Studies Characteristics

There were 26033 participants (6374 BZD users) included in this systematic review and 10666 (2318 BZD users) included in the meta-analysis. As shown in **Table 1**, all studies were carried out in America or Europe. Six out of thirteen were published in the last five years. Eight were cohort studies; the remaining five were cross-sectional studies. The mean age of the participants is 72.5 in the cohort studies at baseline, and 83.8 in the cross-sectional studies. Therefore, both users and controls included in this review were elderly participants. In the 13 studies, a total of 36 tasks were used to measure cognitive functions; each study utilized one to nine cognitive tasks.

Synthesized Findings

Benzodiazepine Use and Cognitive Decline in the Elders

Table 2 provides an overview of the effects on cognitive function from BZD use consolidated from all the included studies. The classification cognitive function division was adapted from a commonly used method of understanding and measuring cognitive domains (29). Eight of the thirteen studies with cognitive performance data had meta-analyzable data. They used the same cognitive measurements and reported the quantitative results required for meta-analysis (**Table 1**). Our meta-analysis synthesized data obtained with the same cognitive tasks across the included studies. Overall, the meta-analysis included the following cognitive functions: global cognition measured with the MMSE, processing speed measured with the digital symbol test, memory (recognition) measured with the AVLT, and executive functions (inhibitory control) measured with the Stroop Color and Word Test (SCWT) (**Figures 2A–D**).

Eleven out of 14 experiments on global cognition in the 13 studies showed no relationship between BZD use and decreased performance in global cognition. However, two experimental results (42, 47) showed that BZD users with higher socioeconomic status and BZD abusers performed worse in the MMSE than non-using controls. The remaining study by Bierman et al. (38) provided a third alternative result. The population-based 9-year cohort study concluded that, although there is a significant negative correlation between the MMSE scores and the accumulated BZD, the decrease in the MMSE scores with BZD use was relatively small. Moreover, the authors reported no correlation between dosage of BZD and MMSE scores. Taken together, the conclusions drawn from the literature are consistent with our meta-analysis results (**Figure 2A**); BZD users did not show significantly worse performance in the MMSE compared to the controls (n=9262; SMD: -0.18, 95% CI: -0.36 to 0.00, I^2 = 87%, p=0.05).

Seven studies tested the processing speed of BZD users. Four studies concluded that BZD use in the elderly population may result in significant impairment (41, 42, 47). They tested this

TABLE 1 | Summary of included articles.

Study	Study design	Country	Samples (BZD users)	Mean Age (BZD users)	Male percentage (BZD users)	Participants sources	Cognitive task	Cognitive domain
Gray et al. (39)	cohort study	U.S.	3434(1018)	74.4(74.5)	40.4(33.1)	population based	CASI	General Cognition
Hanlon et al. (40)	cohort study	U.S.	2765(400)	range(65-105)	33.5	community	SPMSQ; OMC	General Cognition
Paterniti et al. (42)[a]	cohort study	France	1176(159)	65(65.3)	41.6(23.7)	population based	TMT-B; DSS; RAVLT; FTT	General Cognition; Processing speed; Immediate recall: verbal/visual; Gross motor speed
Bierman et al. (38)[b]	cohort study	Netherlands	2105(1189)	69.2	47.5	population based	MMSE; Coding Task; RAVLT; RCPM	General Cognition; Processing speed; Immediate recall: verbal/visual; Reasoning/planning
Mura et al. (41)[a,b]	cohort study	France	5195(969)	73.5(74.6)	40.1(23.1)	community	MMSE; TMT-B; TMT-A; BVRT; IST	General Cognition; Processing speed; Immediate recall: verbal/visual; Verbal fluency
Zhang et al. (43)[b]	cohort study	U.S.	5423(405)	73.0(73.6)	34.1(30.6)	Alzheimer's disease center	MMSE; CDR-SB	General Cognition
Ros-Cucurull et al. (47)[a,b]	cohort study	Spain	64(33)	73.2(73.5)	28.13(21.9)	BZD users from hospital; controls are volunteers	MMSE; CPT-II; SDMT; RCFT; CVLT; COWAT FAS; IGT; Tower of London Test; N-Back; SCWT	General Cognition; Vigilance/focus; Processing speed; Immediate recall: verbal/visual; Delayed recall: verbal/visual; Recognition: verbal/visual; Verbal fluency; Reasoning/planning; Working memory; Inhibitory control
Del Ser et al. (44)	cohort study	Spain	1087(810)	74.7	36.1	community	MMSE; FCSRT; semantic verbal fluency (animals in one minute); CDT; DST	General Cognition; Immediate recall: verbal/visual; Delayed recall: verbal/visual; Verbal fluency; Reasoning/planning; Processing speed
Hoiseth et al. (46)[a]	cross-sectional study	Norway	241(168)	78.6(78.1)	27.8(25.0)	hospital	MMSE; HVLT; SCWT; DVT	General Cognition; Vigilance/focus; Immediate recall: verbal/visual; Delayed recall: verbal/visual; Recognition: verbal/visual; Inhibitory control
Helmes and Ostbye (34)[a]	cross-sectional study	Canada	1754(408)	79.7(79.6)	38.7	community	WAIS Block Design; Buschke Free Recall; AVLT Trial 6; Verbal Fluency; Token Test; WAIS Information; WAIS Similarities; WAIS Comprehension	Processing speed; Immediate recall: verbal/visual; Recognition: verbal/visual; Fine motor speed; Verbal Fluency; Semantic processing; Reasoning/planning
van Vliet et al. (45)[a,b]	cross-sectional study	Netherlands	2275(702)	range(85-90)	28.0(20.0)	community	MMSE; LDT; PLT-I; PLT-d; SCWT the Third Chart	General Cognition; Processing speed; Immediate recall: verbal/visual; Delayed recall: verbal/visual; Inhibitory control
Puustinen et al. (37)[a]	cross-sectional study	Finland	119(64)	81.6(82.1)	23.53(15.6)	hospital	MMSE	General Cognition
Hessmann et al. (48)[a,b]	cross-sectional study	Germany	395(49)	78.8(84.0)	31.9(28.6)	hospital	MMSE	General Cognition

CASI, Cognitive Abilities Screening Instrument; SPMSQ, Short Portable Mental Status Questionnaire; OMC, Orientation-Memory-Concentration Test; MMSE, Mini Mental State Examination; TMT-B, The Trail Making Test, part B; DSS, The Digit Symbol Substitution Test; RAVLT, Rey Auditory Verbal Learning Test; FTT, Finger Tapping Test; RCPM, Raven's Coloured Progressive Matrices; TMT-A, The Trail Making Test, part A; BVRT, The Benton Visual Retention Test; IST, Isaacs Set Test; CDR-SB, Clinical Dementia Rating Sum of Boxes; CPT-II, Conners Continuous Performance Test II-Omissions; SDMT, Symbol Digits Modalities Test; RCFT, Rey Complex Figure Test; CVLT, California Verbal Learning Test; COWAT FAS, Controlled Oral Word Association Test; IGT, Iowa Gambling Task; SCWT, Stroop Color and Word Test; FCSRT, total immediate and delayed recall in the Free and Cued Selective Reminding Test; CDT, clock drawing test; DST, digit symbol test; HVLT, Hopkins Verbal Learning Test; DVT, Digit Vigilance Test; LDT, Letter Digit Coding Test; PLT-I, Picture Learning Test-immediately; PLT-d, Picture Learning Test-delay.

Participants' characteristics were collected according to baseline information.

[a]*The authors report meta-analyzable data.*

[b]*The BZD abuse studies.*

TABLE 2 | Overview of tasks used to assess cognitive functioning in benzodiazepine users across different cognitive domains.

Cognitive domain	Task	Studies	Sensitivity[a]
Attention and processing speed			
Vigilance/focus	Conners Continuous Performance Test II-Omissions (CPT-II)	Ros-Cucurull et al. (47)*	1/1
	Digit Vigilance Test (DVT)	Hoiseth et al. (46)	0/1
Processing speed	TMT-B	Paterniti et al. (42); Mura et al. (41)*	2/2
	TMT-A	Mura et al. (41)*	1/1
	The Digit Symbol Substitution (DSS) test	Paterniti et al. (42)	1/1
	Coding task	Bierman et al. (38)*	0/1
	Symbol Digits Modalities Test (SDMT)	Ros-Cucurull et al. (47)*	1/1
	WAIS Block Design	Helmes and Ostbye (34)	0/1
	Letter Digit Coding Test (LDT)	van Vliet et al. (45)*	0/1
	Digit Symbol Test (DST)	Del Ser et al. (44)	1/1
			7/11
Memory and learning			
Immediate recall: verbal/ visual	Rey Auditory Verbal Learning Test (RAVLT)	Paterniti et al. (42); Bierman et al. (38)	0/2
	The Benton Visual Retention Test (BVRT)	Mura et al. (41)*	1/1
	Rey Complex Figure Test (RCFT) Immediate recall	Ros-Cucurull et al. (47)*	1/1
	California Verbal Learning Test (CVLT)	Ros-Cucurull et al. (47)*	1/1
	Hopkins verbal learning test (HVLT)	Hoiseth et al. (46)	0/1
	Buschke free recall	Helmes and Ostbye (34)	1/1
	Picture Learning Test (PLT-i)	van Vliet et al. (45)*	0/1
	Free and Cued Selective Reminding Test (FCSRT)-immediate recall	Del Ser et al. (44)	1/1
			5/9
Delayed recall: verbal/ visual	Rey Auditory Verbal Learning Test (RAVLT)	Paterniti et al. (42); Bierman et al. (38)*	1/2
	Rey Complex Figure Test (RCFT) Delayed recall	Ros-Cucurull et al. (47)*	1/1
	California Verbal Learning Test (CVLT)	Ros-Cucurull et al. (47)*	0/1
	Hopkins verbal learning test (HVLT)	Hoiseth et al. (46)	0/1
	Picture Learning Test (PLT-d)	van Vliet et al. (45)*	0/1
	the Orientation- Memory-Concentration Test (OMC)	hanlon1998* (40)	1/1
	Buschke free recall	Helmes and Ostbye (34)	1/1
	Free and Cued Selective Reminding Test (FCSRT)-delayed recall	Del Ser et al. (44)	0/1
			4/9
Recognition: verbal/visual	Rey Auditory Verbal Learning Test (RAVLT)	Paterniti et al. (42); Bierman et al. (38)*; Helmes and Ostbye (34)	1/3
	Rey Complex Figure Test (RCFT) recognition	Ros-Cucurull et al. (47)*	0/1
	California Verbal Learning Test (CVLT)	Ros-Cucurull et al. (47)*	0/1
	Hopkins Verbal Learning test (HVLT)	Hoiseth et al. (46)	0/1
			1/6
			9/23
Motor			
Gross motor speed	the Finger Tapping Test (FTT)	Paterniti et al. (42)	1/1
Fine motor speed	WAIS Block Design	Helmes and Ostbye (34)	0/1
			1/2
Language			
Verbal fluency	The Isaacs Set Test (IST)	Mura et al. (41)*	1/1
	Controlled Oral Word Association Test (COWAT FAS)	Ros-Cucurull et al. (47)*	1/1
	verbal fluency	Helmes and Ostbye (34); Del Ser et al. (44)	0/2
Semantic processing	Token Test	Helmes and Ostbye (34)	1/1
	WAIS Information	Helmes and Ostbye (34)	0/1
			3/6
Executive functions			
Reasoning/planning	Raven's Colored Progressive Matrices (RCPM)	Bierman et al. (38)*	1/1
	Iowa Gambling Task(IGT)	Ros-Cucurull et al. (47)*	0/1
	Tower of London Test	Ros-Cucurull et al. (47)*	1/1
	WAIS Similarities	Helmes and Ostbye (34)	0/1
	WAIS Comprehension	Helmes and Ostbye (34)	1/1
	clock drawing test	Del Ser et al. (44)	0/1
Working memory	N-Back	Ros-Cucurull et al. (47)*	1/1
Inhibitory control	SCWT	Ros-Cucurull et al. (47)*; Hoiseth et al. (46)	1/2
	the third chart of the 40-item SCWT	van Vliet et al. (45)*	0/1
			5/10

** for BZD abuse studies.*

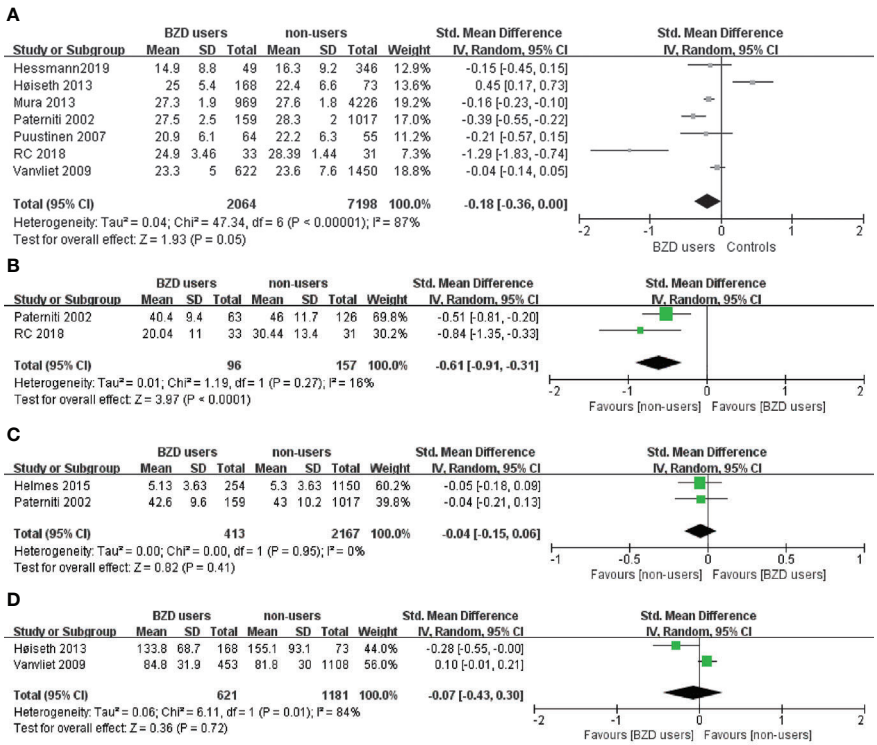

FIGURE 2 | **(A)** Effect of BZD use on Mini Mental State Examination in the elderly: forest plot. **(B)** Effect of BZD use on Digital Symbol test in the elderly: forest plot. **(C)** Effect of BZD use on Auditory Verbal Learning Test in the elderly: forest plot. **(D)** Effect of BZD use on Stroop Color and Word Test in the elderly: forest plot.

domain mainly with the Trail Making Test (TMT) or the digit symbol test. The results showed that BZD users performed significantly worse across all the abovementioned tests than the controls. A meta-analysis (**Figure 2B**) was performed using the two studies that utilized the TMT. The results showed that in the elderly, the BZD users performed significantly worse than controls in digit symbol tests (n=253; SMD: -0.61, 95% CI: -0.91 to 0.31, $I^2 = 0\%$, $p < 0.0001$). However, three other studies (34, 38, 45) suggested that the BZD users' processing speed was not significantly impaired during a coding task and block design task.

An overview of the eight studies that tested memory and learning ability is presented in **Table 2**. Five out of nine tasks showed impairment in verbal/visual immediate recall among BZD users. More specifically, three out of five verbal immediate recall tasks and two out of four visual immediate recall tasks showed worse performances in BZD users than controls. When measuring with verbal/visual delayed recall tasks, four out of nine studies showed less accurate responses among BZD users. In these tasks, three out of seven verbal delayed recall tasks and one out of two visual delayed recall tasks showed BZD users performed worse than controls. The meta-analysis of the AVLT results (**Figure 2C**) is high in heterogeneity and non-significant (n=2580; SMD: -0.04, 95% CI: -0.15 to 0.06, $I^2 = 84\%$, p=0.41).

Results of the tasks for motor, language ability, and executive functions are controversial (**Table 2**). In the domains of language ability and reasoning/planning function, three (34, 38, 47) out of four studies (44) that tested the respective domain showed a significantly worse performance in BZD users. Only one paper tested working memory (47) and showed that BZD users had a significantly worse performance than controls. In the domain of inhibitory control, the SCWT data sourced from two studies can be used for meta-analysis (**Figure 2D**). The results were non-significant and had high heterogeneity (n=1802; SMD: -0.07, 95% CI: -0.43 to 0.30, $I^2 = 84\%$, p =0.72).

Benzodiazepine Abuse and Cognitive Decline in the Elders

We conducted subgroup analyses of BZD abusers (**Figure 3**). Seven studies included participants with BZD abuse (38, 40, 41, 43, 45, 47, 48) (**Table 1**). In the BZD abuse subgroup, the abusers received significantly lower MMSE scores compared to the controls (n=7726; SMD: -0.23, 95% CI: -0.44 to -0.03, $I^2 = 86\%$, p =0.02). Meanwhile, in the BZD regular use subgroup, the MMSE scores of BZD users versus controls were not significantly different (n=1536; SMD: -0.05, 95% CI: -0.59 to 0.48, $I^2 = 92\%$, p =0.85). Considering $I^2 > 50\%$, the heterogeneity between the studies cannot be ignored.

Due to the discrepancy in the type of cognitive tasks used in the included studies, we could not obtain meta-analyzable data to examine the effects of BZD abuse on specific cognitive functions.

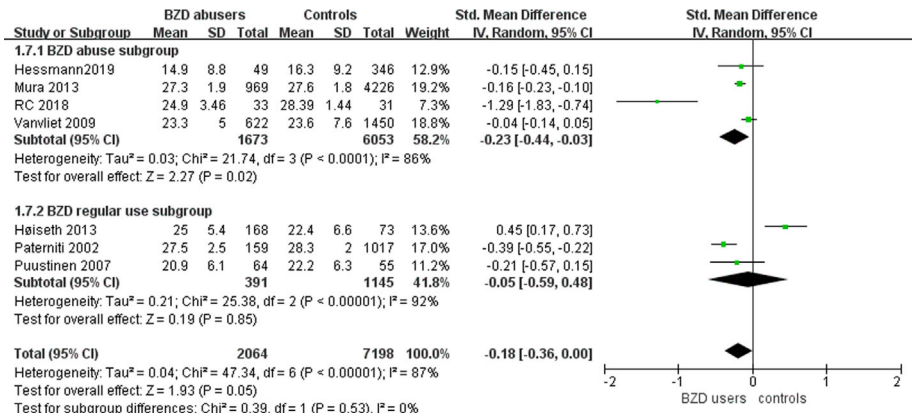

FIGURE 3 | Subgroup analysis of the effect of BZD abuse and regular use on mini mental state examination in the elderly.

Nevertheless, the results of the experiments in these studies were summarized in **Table 2**. Notably, for language ability, Mura et al. used the Isaacs Set Test to explore the effect of BZD abuse (41), and Ros-Cucurull used the Controlled Oral Word Association Test (47). Both results showed that BZD abusers performed worse than the control groups. In the domain of recognition, BZD abusers performed worse than controls in three different tasks in two studies (38, 47). Experiments testing performance on other cognitive divisions showed mixed results.

DISCUSSION

Main Findings of BZD Use

This meta-analysis and systematic review included 6374 BZD users and 19,659 controls to comprehensively investigate the affected cognition domains by BZD use and abuse in elders. A total of 13 papers met the inclusion criteria and were included in the literature review. Eight out of the 13 papers had appropriate tests and sufficient information for the meta-analysis.

Consistent with previous systematic reviews, our meta-analysis suggests no impairment in global cognition among elderly BZD users (49, 50). Interestingly, studies with young adults showed opposing results (51, 52), suggesting BZD use significantly impairs participants' global cognitive functioning. One reason for these results may be the fact that the negative effect of various risk factors on global cognitive decline decreases with age (53).

The results consistently showed impairment in elderly BZD users' processing speed, but not inhibitory control. Processing speed, defined by the time it takes for a person to complete a mental task, has been found to be associated with caudate activity in neuroimaging studies (54). Meanwhile, a higher BZD dose is associated with volume reductions in the caudate nucleus (55). This imaging evidence supports our results of decreased processing speed among BZD users. Our result in memory functions, however, is inconsistent with previous studies. While the meta-analysis (8) with adults of all ages found that BZD users had a significantly impaired working memory and immediate memory ability, our systematic review and meta-analysis did not find sufficient support for the effect. However, the interaction between age and BZD's effect on memory requires more robust validation.

Main Findings of BZD Abuse

The BZD abuse subgroup in this review included 1673 BZD abusers and 6053 controls. The subgroup analysis showed that BZD abusers received significantly lower MMSE scores than the controls, while the BZD regular users' scores were not significantly different from controls. These results demonstrated that impairment in global cognition occurs after the BZD user develops into abuse. However, the high heterogeneity in the results cannot be ignored. According to the results of the subgroup analysis, the confidence intervals of the two subgroups overlapped, and the difference between the two groups was not statistically significant (56). However, the subgroup analyses results, especially when not pre-specified at the beginning of the trial, cannot reflect the differences between the effect of interventions in subgroups (57). Future studies that directly contrast the cognition of BZD users and abusers are necessary. Only two studies tested language ability in BZD abusers; both found significantly worse language performance in BZD abusers compared to controls. Meanwhile, only two studies tested recognition ability; neither found a significant difference in the performance between BZD abusers and controls. These findings, however, are not conclusive because of the heterogeneity in the statistical results, and the small number of studies in the analyses. Therefore, more experiments are needed to reach more reliable conclusions.

After searching the databases, we did not find a meta-analysis investigating the cognitive effects of BZD abuse. The most relevant studies are two systematic reviews and meta-analyses papers on dementia risk in the elderly with long-duration and high dosage BZD users (58, 59). In 2015, Zhong and colleagues summarized six nested case-control or prospective cohort studies and concluded

that higher dosage BZD users had an increased risk of dementia (59). In 2019, He et al. found that the risk of dementia was higher in patients taking BZD for a longer duration (>3 years) among six case-control and four cohort studies (58). Although long-term or high dosage use is not equivalent to dependence, BZD addiction is more likely to occur in long-term and high-dose users (60). These studies support the results of our subgroup analysis to some extent, but the mechanism and effects behind BZD addiction and abuse needs further investigation.

Strengths and Limitations of This Review

This study is, to our knowledge, the first systematic review and meta-analysis of the cognitive effects of BZD use and abuse in elders. We attempted to consolidate results from studies testing different cognitive functions and data from a variety of cognitive tasks by utilizing a mature cognitive domain classification catalogue. The subgroup analysis of BZD abusers allowed us to preliminarily compare the effects of BZD use and abuse. This analysis encourages further studies to examine the qualitative difference of BZD use and abuse. These results can help identify and monitor the cognitive effects of BZD use and abuse, shedding light on awareness and prevention of BZD addiction at early stages, providing evidence for clinical decision-making, and improving the life quality of the elderly.

There are some limitations to this meta-analysis and systematic review. First, the number of studies and cognitive domains examined in this meta-analysis was limited. Five of the thirteen studies did not report cognitive tests data, and the domains of motor and language did not have meta-analysis results. Second, although the n is quite large, the meta-analysis results of the digital symbol test, AVLT, and SCWT were drawn from two small sample size studies, which is difficult to justify or interpret, considering the biased nature of the original studies. In the subgroup analysis of MMSE, the sample size of studies in the BZD regular users' subgroup varied from hundreds to 2500, which are much smaller than the sample size of the abusers' subgroup (>7000). Although the random effects model was used to reduce the impact caused by the difference between the sample size of the two subgroups, there was no analyzable data for further analysis. Third, due to the limitation of meta-analysis methodology, we could not directly compare the cognitive differences between BZD users and abusers. Fourth, other variabilities existed in the 13 studies in terms of the participants' source (hospital or community), sample size, sex ratio, and cognitive measurements. These factors could also bias the findings to an uncertain extent. Fifth, users and controls were not well matched in the analysis. Participants in the experimental groups of the studies had different preexisting conditions such as anxiety, depression, insomnia, and APOE e4 status. One study did not age match the intervention group with the control group or as a covariable, which means the study could not completely distinguish the cognition impaired by BZD and by natural aging. Other factors related to cognition, such as the use of other psychotropic medications, certain physical diseases, and educational level, were not examined in most studies. Finally, due to the limited number of studies, sources of heterogeneity were not examined.

Implications and Future Directions

Our meta-analysis confirmed the negative effects of BZD on elderly users' processing speed. Therefore, doctors should be cautious when prescribing BZD drugs to elderly patients, especially those with family histories of dementia, Alzheimer's disease, and other aging-related cognitive deficits. Additionally, although global cognition was not impaired in BZD regular users, BZD abusers had significantly worse performance in global cognition. This research can inform more individualized prescription decisions. For example, elderly patients whose daily activities require higher cognitive processing should be informed of BZD's potential side effects on their cognitive processing speed. Patients with a history of addiction should prioritize alternative treatments to BZD therapy to prevent BZD dependence and abuse.

Another important finding in this study is that the results of cognitive performance are highly dependent on the type of cognitive measurements in the study. For example, as previously reported, BZD users had significantly lower processing speeds when tested with the TMT. However, studies measuring processing speed with the coding task or block design task did not reveal any significant findings. Therefore, clinical practitioners should be mindful when selecting cognitive tests. It might be reasonable to use tests with higher sensitivity to reduce missed diagnoses.

In addition, through our exploration in the literature on the cognitive effects of BZD use, few studies paid attention to BZD abuse and addiction in participants (47, 61, 62). BZD use and abuse can be qualitatively different from BZD use. A survey conducted in 2015-2016 showed that BZD abusers accounted for about 17% of BZD users (6). Moreover, approximately half of the patients who used BZD for longer than 1 month are subject to BZD abuse or addiction (60). The neglect of the BZD abuse subgroup may be accountable for the mixed conclusions from studies on the cognitive effects of BZD use. Therefore, we encourage future researchers to separate BZD regular users and BZD abusers to achieve more precise and rigid conclusions.

CONCLUSIONS

In conclusion, this meta-analysis indicated no significant global cognition deficit (MMSE scores) in BZD users, but did reveal deficits in elders with BZD abuse behaviors. BZD users performed significantly worse in the cognition domain of processing speed (digit symbol test scores) than the controls, but not in memory and learning (AVLT scores) or inhibitory control (SCWT scores). Studies that tested the other cognitive domains, however, showed conflicting results. Unfortunately, these cognitive domains' measurements varied across studies, rendering it unavailable to be merged into meta-analysis. Clinicians should be cautious when prescribing BZD for the elderly, especially to patients with a family history of age-related cognitive deficits. Moreover, the majority of the included studies did not clearly distinguish between the use and abuse of BZD, making it challenging to evaluate the effects of BZD abuse. Future well-designed studies are needed in order to verify the cognitive effects of BZD use and abuse.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

LL and YT: conceptualization. LL and LJ: data curation and analysis. FW and YT: project administration. LL, YZ, JZ, and PJ: supervision, writing—review, and editing. LL and PJ: writing—original draft.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2020.00755/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Dysfunction of the Prefrontal Cortex in Betel-Quid–Dependent Chewers

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Betel quid is the fourth most popular psychoactive agent worldwide. Imaging studies have found altered brain structure in prefrontal cortex (PFC) in betel-quid dependent (BQD) chewers. However, the brain function in PFC associated with BQ use still remains unclear. The present study aimed to examine brain functional activity in PFC in individuals with BQD. This study recruited 48 participants with BQD and 22 normal controls (NCs). Both BQ-specific cue reactivity and Go/NoGo tasks were administered with functional magnetic resonance imaging (fMRI). Behavioral results showed a deficit in the choice reaction time task in BQD group. The fMRI results of the cue reactivity task suggested that, individuals with BQD exhibited responses in right ventromedial PFC, left posterior cingulate cortex (PCC), left lateral parietal lobe (LPL), left middle temporal gyrus and left visual cortex, when seeing BQ images compared with control images. In the Go/NoGo task, relative to NCs group, individuals with BQD showed higher activity in right dorsolateral PFC, right PCC and bilateral LPL between NoGo and Go trials. Across these two tasks, we consistently found disrupted function in PFC in individuals with BQD, which might lead to impaired craving and response inhibition in BQ addiction. Results of current study might shed light on the neural mechanisms involved in BQ use, which could be used as potential guidelines for diagnosis and treatment of BQ dependence.

Keywords: betel quid dependence, brain function, prefrontal cortex, cue reactivity task, go/nogo task

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INTRODUCTION

Areca nut, the fruit of the areca palm, contains the principal active agent Arecoline (1), which is demonstrated to have a chemical structure analogous to that of nicotine (2). Thus, betel quid (BQ, the product of areca nut) is recognized as a group 1 carcinogen by International Agency for Cancer Research (1), and categorized as "addiction" when heavy use according to DSM-IV and ICD-10 (3, 4). BQ is now the fourth most commonly consumed psychoactive substance in the world, following only alcohol, nicotine, and caffeine (5, 6). A recent study with a large sample size in 6 Asian populations revealed a vast prevalence of BQ use in these counties (typically over 10%) (7). BQ use is reported to be highly associated with oral sub-mucous fibrosis and oral cancer (8–11). However, the limited understanding of BQ use makes it difficult for the development of effective treatment to those patients (12).

Several lines of researches have suggested that intensive BQ use shows similar symptoms of other addictive disorders. First, in the Asian Betel-quid Consortium study of 8922 participants from 6 countries, betel-quid use disorder was reported to meet DSM-V criteria for substance use disorder and had a high prevalence among users of betel-quid (7). Another study suggested that the 12-month prevalence of BQ use disorder was 18.0%, which exceeded that reported for DSM-V defined drug use disorder (3.9%) (13) and was comparable to the results of alcohol (13.9%) and nicotine (20.0%) use disorders (14, 15). Second, animal studies suggested that high dose of BQ use could induce cocaine-like physiological states, such as anxiety, dilated pupils, tachycardia, and elevated blood pressure (16). In addition, BQ use was reported to be associated with altered cognitive ability, including spatial short-term memory (17), attention and inhibition control (18), executive function (19) and attention bias to BQ cues (20).

Functional neuroimaging studies have implicated that structural and functional alterations related to BQ chewing and dependence. Recently, a review systematically assessed previous functional magnetic resonance imaging (fMRI) studies on BQ addiction, and suggested that a key involvement of the prefrontal cortex (PFC) in BQ dependency (21). For example, studies from our group suggested that BQ use was associated with decreased gray matter volumes in bilateral dorsolateral PFC and ventral medial PFC (22), altered white matter integrity in anterior thalamic radiation highly associated with PFC (23), and less cortical thickness in PFC (19). These neuroimaging studies proposed that the role of prefrontal cortical structures in BQ addiction, which plays an important role in cognitive behavioral and emotional changes in various kinds of addiction (24–27). However, all the evidence in BQ addiction mainly came from studies of brain structure in BQ users. Little is known about functional role of PFC in individuals with betel-quid dependence (BQD).

Two common tasks employed in addiction neuroimaging studies are the cue reactivity and Go/NoGo tasks, in which neural activation is measured while the subject is viewing neutral versus addiction-related images and conducting the addiction-related Go and NoGo stimulus. Both of them can evoke the response from PFC. The present study aimed to investigate neural activity in PFC in individuals with BQD compared to normal controls (NCs). To do so, 48 men with BQD and 22 NCs were recruited to perform the BQ-related cue reactivity and Go/NoGo tasks under fMRI investigation. Consistent with previous findings that BQ use may be associated in part with structural deficits in PFC, we hypothesized that abnormal activation in PFC in individuals with BQD in both BQ-related cue reactivity and Go/NoGo tasks. We hoped that current study could improve our understanding of the roles of the PFC in BQ use.

MATERIALS AND METHODS

Participants

Seventy male volunteers were recruited to participant in this study including 48 subjects with BQD and 22 NCs. Individuals with BQD met the following inclusion criteria: (1) 18 to 50 years of age; (2) Han nationality; (3) right-handed; (4) nine or more years of education; (5) meeting the diagnostic criteria for BQD according to DSM-V criteria for substance use disorders; (6) with no attempt to quit or experience of BQ abstinence in the past 6 months. The BQ use behaviors of BQD participants were recorded, including duration of BQ use (years), age of first BQ use, daily weight of BQ use (g), the number of BQ use days in a week and the brands of BQ products. Body mass index (BMI) was also recorded. The severity of BQ dependence was measured by Betel Quid Dependence Scale (BQDS) (28). The BQD group was additionally screened for alcohol and nicotine addiction with Alcohol Use Disorders Identification Test (AUDIT) and Fagerstrom Test for Nicotine Dependence (FTND).

Healthy subjects with matched age without BQ use were defined as the NCs group, which were recruited through a combination of targeted site sampling, advertisement and snowball sampling referrals. Subjects were excluded both as BQD chewers and healthy controls if they: (1) met the DSM-V criteria for other substance dependency, such as alcohol and nicotine; (2) had medical history of any neurological or psychiatric disorder; (3) had systemic illnesses (e.g. diabetes mellitus, cardiovascular disease, thyroid disorders, renal disorders, and epilepsy); (4) had contraindication to MRI examination; (5) had current use of psychotropic medications; (6) were not able to read and write Chinese or left–handed. This study was carried out in accordance with the recommendations of the research ethical committee of Xiangya Hospital of Central South University of Hunan Province, Changsha, China. All subjects were given written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Institutional Review Board at Xiangya Hospital of Central South University of Hunan Province, Changsha, China.

Behavior Interviews and Cognitive Tests

Participants came to Xiangya Hospital to finish behavior interview and MRI scans in the same day. The behavior interview included several parts. The first part was the basic demographic information collection, including age, years of education, BMI, BQ use behaviors and so on. Then, they were asked to complete three simple cognitive tasks: choice reaction time task, working memory task, as well as the classic color-word Stroop task.

The choice reaction time task was adapted with default settings from Deary, Liewald and Nissan (29). The time required to finish this task was used as the index of general reaction time measure. The two-back working memory task with numbers was adapted from Xue and colleagues (30) and the number of correct response in each block was served as the index of working memory. The Chinese adaptation of Stroop task was used to assess the inhibition ability. The number of correct responses in 45s in the Color-Word condition was recorded as the index for this task.

MRI Protocol

In order to reduce the influence of addictive substances, all participants were required not to use caffeine, nicotine, alcohol and other addictive substance on the day of scanning. All MRI images were acquired using a Siemens 3.0T Prisma scanner at

Xiangya Hospital. Standard settings were used to perform the scan. For example, foam pads were used to minimize head motion. Participants were instructed to have a rest, kept their head very still during the structural scan and responded to the instructions when doing functional scans. Stimulus presentation and timing of all stimuli and response events were achieved using Matlab (Mathworks) and Psychtoolbox (www.psychtoolbox.org) on an IBM-compatible PC. Participants' responses were collected online using an MRI-compatible button box. The structural scan was performed using T1 MPRAGE sequence, covering whole brain with the following scanning parameters: TR/TE = 1900/3.18 ms, matrix = 320 × 320, number of slices = 256 and voxel size = 0.73 × 0.73 × 0.73 mm^3, sagittal slice position. Functional scans were performed using EPI sequence with the following parameters: TR/TE = 2000 ms/30 ms, Matrix = 94 × 94, number of axial slices = 75, voxel size = 2.34 × 2.34 × 2.00 mm^3.

fMRI Tasks

Participants performed one session of cue reactivity task and two sessions of Go/NoGo task inside the scanner. During the session of cue reactivity task, two types of cues were presented: the BQ image and control images. There were 20 images for each category, and each image were presented for three times. In order to keep the participants awake during this passive view task, 10 animal images were presented twice. These images were presented with a random order with each image was presented for 3 s. Participants were instructed to press a button whenever they saw an animal. There were two sessions of Go/NoGo tasks: (1) a control Go/BQ NoGo task (CGo task) in which they were asked to press a button when they saw a control image, and refrained from pressing the button when they saw a BQ-related image, and (2) a BQ Go/control NoGo task (BGo task) in which they were asked to press a button when they saw a BQ-related image, and refrained from pressing the button when they saw a control image. This Go/NoGo paradigm was adapted from previous studies (31), and allowed an examination of brain responses to BQ stimuli and the inhibition of pre-potent responses to BQ stimuli. Consistent with previous studies (31), each session consisted of 120 Go trials (75%) and 40 NoGo trials (25%). The NoGo trials were presented in pseudo-randomized order so that NoGo trial appeared with equal probability after one to five consecutive Go trials, and two NoGo trials appeared consecutively. Each stimulus was presented for 500 ms, followed by a fixation cross for 1.5 to 4 s with a mean of 2.5 s. The order of two versions of GO-NoGO tasks was counterbalanced across participants.

The correct ratio and reaction in the cue task were served as the behavior data of the task. The correct ratio for Go and NoGo trials as well as the reaction time for Go trials for each session were calculated as indexes for habitual-impulsive responding to the stimuli and inhibition of NoGo stimuli. Then, these behavior data were compared across groups.

fMRI Data Analysis

Image preprocessing and statistical analysis were carried out using FSL (www.fmrib.ox.ac.uk/fsl). Images were realigned to compensate for small residual head movements (32). Translational movement parameters never exceeded one voxel in any direction for any participant. Data was spatially smoothed using a 5-mm full-width-half-maximum (FWHM) Gaussian kernel and filtered using a nonlinear high pass filter with a 100-s cutoff.

A two-step registration procedure was used where EPI images were first registered to the MPRAGE structural image, and then into standard MNI space, using affine transformations (32). Registration from MPRAGE structural image to standard space was further refined using FNIRT nonlinear registration (33, 34). Statistical analyses were performed in the native image space with statistical maps normalized to the standard space prior to higher-level analyses. The data was modeled at the first-level using a general linear model within FSL's FILM module.

For the cue task, brain activations were modeled for BQ images, control images, and animal images separately. For the Go/NoGo task, brain activation in every trial was modeled for BQ Go, BQ Nogo, control Go, and control NoGo trials respectively. Error-related trials (misses and false alarms) were modeled together as a nuisance variable. The event onsets were convolved with canonical hemodynamic response function (HRF, double-gamma function) to generate regressors. Temporal derivatives were included as covariates of no interest in order to improve statistical sensitivity. Null events were not explicitly modeled, and therefore constituted an implicit baseline. The six movement parameters were also included as covariates in the model.

Higher-level analysis was created to model the activity difference across two groups. For cue reactivity task, the activation difference of BQ images vs. control images was compared between two groups to reveal the interaction between stimuli and group. For the Go/NoGo tasks in both sessions, the difference of NoGo trials vs. Go trials was compared to reveal the interaction between task and group. Higher-level random-effect models were tested for group analyses using FMRIB's Local Analysis of Mixed Effect stage 1 only (35, 36) with automatic outlier detection (37). The brain activation difference of BQ and control images, as well as NoGo and Go trials was also examined for BQD and NCs group independently using one-sample t-tests. Result images were thresholded with a height threshold of $Z > 3.1$ and a cluster probability of $p < 0.05$, corrected for whole-brain multiple comparisons based on Gaussian random field theory. The years of education was included as a covariate for all fMRI analysis.

RESULTS

Behavioral Results

Table 1 showed the demographic and cognitive characteristics for all participants. These two groups were matched on age and BMI, but they showed differences in years of education. In BQD group, they showed an average of 15.23 years of using BQ, average age 17.13 to try their first BQ and 40.19 grams of BQ per day. In terms of cognitive tests, these two groups showed no difference regarding the Stroop task as well as the working memory task. Compared with NCs group, BQD group showed deficits in the choice reaction task (**Table 1**). Correlation analysis showed that BQDS score was significantly correlated with the following variables in BQD group: (1) years of education, $r(48) =$

−0.338, $p = 0.019$; (2) estimated daily use in gram, $r(48) = 0.461$, $p < 0.001$. These results suggested that participants with higher BQDS scores were those with less education and higher daily consumption.

Cue Reactivity Task

The cue reactivity task was a very simple task and both groups performed very well in this task (over 97% of correct ratio of detecting animals) (**Table 2**). There was no difference in terms of either correct ratio or reaction time between groups. To investigate the activity difference between BQ and control images, contrasts of BQ-control images were tested with one sample t-test for each group and two sample t-test for group difference. Results suggested that in BQD group, BQ images activated a large set of brain areas than control images did, including right ventromedial PFC, left posterior cingulate cortex (PCC), left lateral parietal lobe (LPL), left middle temporal gyrus and left visual cortex (**Table 3** and **Figure 1**). However, brain activity in response to these two categories of images showed no significant difference in NCs group as well as in comparison between BQD and NCs group.

Go/Nogo Task

Table 2 also summarized major behavioral measures for both fMRI Go/NoGo tasks, including the correct ratio for Go and NoGo trials as well as the reaction time for Go trials. There was no difference if we compared these variables across groups. The 2 (Task: Go vs. NoGo) *2 (Stimuli: BQ vs. Control images) *2 (Groups: BQD vs. NC) ANOVA was performed on the correct ratio. The results suggested that there was a significant main effect of task ($F(1,67) = 4.87$, $p = 0.03$; NoGo trials showed lower correct ratio than Go trials) and a significant main effect of stimuli ($F(1,67) = 22.20$, $p < 0.001$; BQ images showed higher correct ratio than control images). But there was no significant main effect of group or other interaction effects (all $p > 0.05$).

We first compared brain activity difference between BQ and control images in each group. Results suggested there was significant difference in BQD group between NoGo and Go trials in the following regions: bilateral LPL, left lateral occipital cortex, and bilateral middle temporal gyrus (**Table 4**). However, there was no difference in NCs group when participants performed the Go/NoGo task. Group comparison showed some brain regions with higher activity between NoGo than Go trials in BQD than NCs group, including right dorsolateral PFC, right PCC and bilateral LPL (**Table 4** and **Figure 2**). No regions showed higher activity in NCs group compared with BQD group.

DISCUSSION

The present study aimed to investigate brain functional activity in PFC among subjects with BQD involved in the BQ-related cue reactivity task and Go/NoGo task. Behavioral results showed the

TABLE 1 | Demographic and clinical/cognitive characteristics of participants (M ± SD).

	BQD	NCs	Statistics
N	48	22	-
Age (years)	34.85 ± 8.10	32.05 ± 6.25	$t(68) = 1.44$, $p = 0.15$
Education (years)	11.63 ± 2.83	17.82 ± 2.82	$t(68) = -6.19$, $p < 0.001$
BMI (kg/m^2)	25.07 ± 3.74	23.32 ± 3.84	$t(68) = 1.81$, $p = 0.08$
BQDS	59.63 ± 14.55	–	–
AUDIT	4.35 ± 3.68	–	–
FTND	3.73 ± 2.79	–	–
Duration of BQ use (years)	15.23 ± 7.10	–	–
Age of first BQ use	17.13 ± 6.67	–	–
Daily weight of BQ use (g)	40.19 ± 33.11	–	–
Choice Reaction Time (s)	35.21 ± 9.77	41.50 ± 10.29	$t(68) = -2.46$, $p = 0.02$
Stroop	78.50 ± 32.49	69.72 ± 24.72	$t(68) = 1.13$, $p = 0.26$
Working Memory	9.19 ± 1.14	9.50 ± 1.01	$t(68) = -1.10$, $p = 0.28$

TABLE 2 | fMRI task behavioral data of different groups (M ± SD).

	BQD	NCs	Statistics
Cue Reactivity Task			
CR	0.99 ± 0.03	0.97 ± 0.05	$t(68) = 1.77$, $p = 0.16$
RT (ms)	609.5 ± 138.1	618.8 ± 144.3	$t(68) = -0.25$, $p = 0.80$
Go/Nogo Task			
BQ Go trial CR	0.92 ± 0.11	0.92 ± 0.12	$t(68) = 0.74$, $p = 0.998$
CN Go trial CR	0.85 ± 0.17	0.84 ± 0.13	$t(68) = 0.30$, $p = 0.77$
BQ Go trial RT (ms)	484.2 ± 36.6	486.8 ± 68.0	$t(68) = -0.21$, $p = 0.84$
CN Go trial RT (ms)	541.8 ± 62.5	551.7 ± 62.2	$t(68) = -0.60$, $p = 0.55$
BQ NoGo trial CR	0.85 ± 0.14	0.89 ± 0.07	$t(68) = -1.25$, $p = 0.22$
CN NoGo trial CR	0.84 ± 0.11	0.79 ± 0.15	$t(68) = 1.41$, $p = 0.16$

CR, correct ratio; RT, reaction time.

TABLE 3 | fMRI activity of the cue reactivity task.

L/R	Brain Region	Voxels	MNI x	MNI y	MNI z	Z
BQ-control contrast in BQD group						
R	Ventromedial prefrontal cortex	535	0	58	–2	4.38
L	Posterior cingulate cortex	1793	–2	–52	30	6.87
L	Lateral parietal lobe	809	–50	–70	32	6.04
L	Middle temporal gyrus	164	–60	–8	–16	4.34
L	Visual cortex	210	–6	–98	2	5.00
BQ-control contrast in NCs group						
None						
BQ-control contrast (BQD-NCs)						
None						

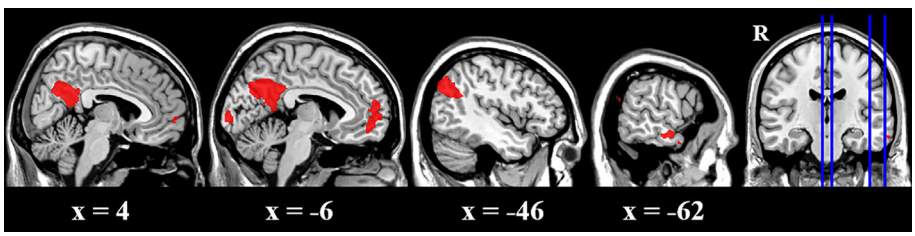

FIGURE 1 | Brain activity difference between BQ images and control images in BQD group in the cue reactivity task. BQ images activated a large areas of brain than control images, including right ventromedial PFC, left posterior cingulate cortex (PCC), left lateral parietal lobe (LPL), left middle temporal gyrus and left visual cortex. However, in NCs group, these two categories of images showed no difference.

TABLE 4 | fMRI activity of the Go/NoGo task.

L/R	Brain Region	Voxels	MNI x	MNI y	MNI z	Z
NoGo-Go trials in BQD group						
R	Lateral parietal lobe	2567	54	–50	36	6.27
L	Lateral parietal lobe	1058	–58	–54	44	5.20
L	Lateral occipital cortex	851	–16	–96	–4	6.21
R	Middle temporal gyrus	803	64	–22	–8	5.46
L	Middle temporal gyrus	718	–64	–18	–6	5.37
NoGo-Go trials in NCs group						
None						
NoGo-Go trials (BQD>NCs)						
R	Dorsolateral prefrontal cortex	1205	32	32	52	4.40
R	Posterior cingulate cortex	1429	16	–36	28	4.95
R	Lateral parietal lobe	1606	52	–66	36	5.56
L	Lateral parietal lobe	1190	–36	–78	38	4.35
NoGo-Go trials (NC>BQD)						
None						

BQD group showed deficits in the choice reaction time task but not in either the Stroop task or the working memory task. fMRI results of the cue task suggested that BQ images activated a large set of brain areas than control images did, including right ventromedial PFC, left PCC, left LPL, left middle temporal gyrus and left visual cortex when seeing BQ images compared with control images. In the Go/NoGo task, relative to NCs group, individuals with BQD showed higher activity in right dorsolateral PFC, right PCC and bilateral LPL between NoGo and Go trials. As hypothesized, individuals with BQD showed abnormal activation in PFC in both of these two tasks, which implies that functional abnormality of prefrontal area in BQ addiction.

These results highlighted functional alternation of PFC in BQD participants. Structural alteration in PFC has been widely reported in addiction, including cocaine (38–44), heroin (45–48), opiates (49), cannabis (50), nicotine (51, 52), alcohol (53, 54), ketamine (55), MDMA (56), methamphetamine (57), internet (58), and

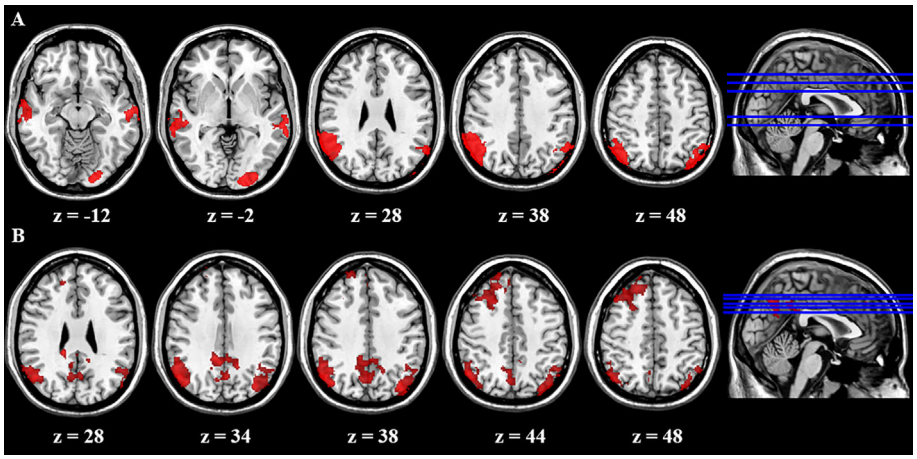

FIGURE 2 | Brain activity difference between Nogo and Go trials in the Go/Nogo task. **(A)** Results suggested in BQD group, there was significant difference between NoGo and Go trials in the following regions: bilateral LPL, left lateral occipital cortex, and bilateral middle temporal gyrus. However, in NCs group, there was no difference when participants performed the Go/Nogo task. **(B)** Group comparison showed some brain regions showed higher activity in BQD than NCs group in right dorsolateral PFC, right PCC and bilateral LPL between NoGo and Go trials. However, there was no brain region with higher activity in the NCs than BQD group. The right hemisphere is displayed at the left side of the paper.

online games (59, 60). In the field of BQ addiction, our group study investigated the relationship between cortical thickness and executive function in chronic chewers with BQD, suggesting that BQD group had thinner cortex in bilateral PFC, and cortical thickness of the bilateral dorsolateral PFC mediated the correlation of betel-quid chewing and executive function (19). Consistent with decreased gray matter volumes in bilateral dorsolateral PFC and ventral medial PFC (22), results suggested that structural alteration in PFC in BQ users with BQD. Recently, several studies using functional connectivity approach to investigate resting-state brain feature in BQD users consistently suggested that impaired resting-state functional connectivity in BQ users occurred in prefrontal regions (61, 62). Our current study extends these prior findings by providing new evidence with task fMRI for abnormal functional activity in prefrontal regions in individuals with BQD.

It is worth noting that we observed abnormal activity in ventromedial PFC in cue reactivity task and dorsolateral PFC in Go/NoGo task. Prefrontal cortex is one of the most frequently areas implicated in drug addiction, which was involved in addicted subjects during intoxication, craving, bingeing and withdrawal (24). Altered prefrontal cortex activity has been reported in nicotine and cocaine addiction (63–65). Although activity among prefrontal cortex is highly integrated and flexible, a meta-analysis study suggests different region of prefrontal cortex is involved in different function (66). The dorsal PFC has been predominantly implicated in top-down control and meta-cognitive functions, the ventromedial PFC in emotion regulation (including conditioning and assigning incentive salience to drugs and drug-related cues), and the ventrolateral PFC and lateral OFC in automatic response tendencies and impulsivity (66). Consistent with previous studies in addition, our study provides novel evidence for the notion that abnormalities of ventromedial PFC in drugs and drug-related cues and dorsolateral PFC in top-down control in BQ dependence and further suggests that the dysfunction of functional activity in the ventromedial and dorsolateral PFC may contribute to impaired cue-induced craving and response inhibition in BQ dependence.

It should be noted that there were three limitations in current study. First, the study used an imbalanced sample. There were more individuals with BQD than normal participants. Second, this study only recruited male participants, so it should be cautious in generalizing the findings of this study to the females. Third, this study was done with outpatients, so there were inaccuracies regarding exact amount and histories of BQ use. Finally, although we performed a careful physical examination and obtained routine laboratory tests, all potential disease and confounding by other substances could not be totally excluded.

CONCLUSION

Our study used neuroimaging approaches to assess the characteristic of brain activation with BQ specific cue reactivity and Go/NoGo tasks. The findings showed that individuals with BQD exhibited elevated responses in right ventromedial PFC, left PCC, left LPL, left middle temporal gyrus and left visual cortex when seeing BQ images compared with control images in the cue reactivity task. In the Go/NoGo task, relative to NCs group, individuals with BQD showed higher activity in right dorsolateral PFC, right PCC and bilateral LPL between NoGo and Go trials. Disrupted prefrontal function was consistently revealed in BQ cue induced craving and inhibitory control in individuals with BQD, which implies that treatments that target this region may help alleviate some symptoms of addictive BQ use.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board at Xiangya Hospital of Central South University of Changsha, Hunan Province, China. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XZ conceived and designed the experiments. FY, SL, DW, and CJ conducted the experiments and collected data. ZZ and ZQ analyzed the results. LK and CZ wrote and revised the main manuscript text. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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Ketamine Administration Leads to Learning-Memory Dysfunction and Decreases Serum Brain-Derived Neurotrophic Factor in Rats

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Objective: This study investigated the effects of acute or chronic ketamine administration on learning and memory function as well as levels of brain-derived neurotrophic factor (BDNF) in the hippocampus and blood in order to explore the potential correlation between learning-memory dysfunction and ketamine.

Methods: Rats were treated with 25 mg/kg ketamine for 3 d (n = 20) or 14 d (n = 20). Saline-treated rats were used as controls. The Morris water maze test was used to evaluate spatial learning and memory after 10 d of withdrawal. The level of BDNF in serum and the hippocampus were measured by ELISA.

Results: The number of platform crossings and residence time in the target platform quadrant were significantly reduced in ketamine 3 d and 14 d groups than in the saline controls (both $p < 0.05$). In addition, the average escape latency of ketamine 3 d and 14 d groups were significantly longer than that of the saline 3 d and 14 d groups ($p < 0.0001$), respectively. Further examination found that only serum samples from ketamine 14 d group showed significantly decreased BDNF level compared to that from saline 14 d groups ($p < 0.05$). However, no differences were detected in hippocampus samples.

Conclusion: Chronic ketamine exposure (25 mg/kg) causes spatial learning and memory deficits in SD rats, which may be associated with decreased serum BDNF levels.

Keywords: ketamine administration, spatial learning, memory, Morris water maze, brain-derived neurotrophic factor

INTRODUCTION

Ketamine is a commonly studied psychoactive substance and known drug of abuse. Recently, it has been widely abused as a new type of drug (also known as K powder) due to its addictive properties (1, 2). Ketamine abuse can lead to cognitive impairment (3, 4). Ketamine is an antagonist of the N-methyl-D-aspartate (NMDA) receptor, and can lead to similar symptoms of addiction (5). Recent studies have shown that low-dose ketamine does not have a significant effect on cognition function (6), but sub-anesthetic dose and anesthetic-dose ketamine could cause cognitive impairment by affecting executive function, procedural function, episodic memory, semantic memory, and working memory (2, 6, 7). Importantly, the effects of ketamine vary greatly with duration. In humans, acute ketamine treatment is known to impair episodic and working memory, recognition memory, and procedural learning (7). This has been further confirmed in mice, as chronic ketamine treatment also leads to memory impairment (8). Ketamine can induce behavioral changes in users and this change is related to the frequency and dose of use (9).

Brain-derived neurotrophic factor (BDNF), abundant in the central nervous system, is known to regulate synaptic plasticity as well as participate in learning and memory processes. BDNF is also likely to participate in the pathophysiology of drug addiction, including the cognitive impairment (10–12). In a study by Ke et al. serum BDNF concentrations were significantly lower in chronic ketamine users than in health controls (2). Importantly, it has been found that the level of BDNF in blood can be used to approximate protein concentration changes in brain tissues (12). It is therefore likely that BDNF concentrations are similarly lower in the brain of chronic ketamine users, particularly within the reward system of the brain. This network of brain structures, namely the ventral tegmental area of the midbrain and its connections to the nucleus accumbens and prefrontal cortex, governs reward, and is well established to be involved in drug addiction (13). In addition to reward circuity, exposure to drugs of abuse, including ketamine, can alter plasticity in cognition as well as learning and memory-related brain areas. Importantly, these changes can persist long after drug exposure has ceased (14).

Therefore, in order to further elucidate the potential relationship between BDNF and ketamine-induced learning-memory dysfunction, we investigated the effects of acute and chronic ketamine (25 mg/kg) on cognition as well as BDNF levels in the blood and hippocampus of Sprague-Dawley (SD) rats.

MATERIALS AND METHODS

Animals

A total of 75 adult healthy male SD rats were randomly divided into four groups: ketamine 3 d group (n = 20), saline 3 d group (n = 18), ketamine 14 d group (n = 20), saline 14 d group (n = 17). The experiment was carried out after a week of adaptive feeding.

Ketamine Exposure

All rats were intraperitoneally injected with ketamine, 25mg/kg for either 3 d or 14 d, and the saline group was injected with 0.9% saline for 3 d or 14 d as controls. Injections were performed daily at 4:30 p.m. In order to avoid the effect of withdrawal reaction on behavioral experiments, the rats were given a 10-day withdrawal period.

Morris Water Maze (MWM) Test

MWM test was conducted in a round stainless steel water tank with a diameter of 1.2 m and a height of 0.5 m, with an attached tracking and recording camera using SMART V2.5.21 program software (Panlab, Spain). A submerged transparent platform was placed 30 cm from the pool's edge and submerged 2 cm beneath the water surface. The water temperature was controlled at 22–24°C. The platform remained in the same position throughout trials.

The platform was removed, and the rats were free to swim for 2 min to screen out the insensitive rats before the start of the experiment. As a result, only 75 healthy SD rats were selected for further experiments. The selected healthy rats were tested for the navigation experiment and the space exploration experiment.

Positioning navigation experiment: Rats were trained for 4 consecutive days to determine their learning ability. At the beginning of the training, starting points were randomly selected in the southeast and the northwest quadrants, and the rats were placed gently against the pool wall. A video system tracked and recorded the rats swimming in the maze. The time taken to find the platform was recorded as escape latency. If rats failed to reach the platform within 60 s, the escape latency was recorded as 60 s, and the rats were guided to the platform and left for 10 s. Each rat was trained 4 times a day, 20-30 min for each interval, and the average latency across these trials was used as the latency value for each day. On the 5^{th} day, each animal was tested in four, single 60 s sessions from each quadrant.

Space exploration experiment: the main purpose was to measure the spatial memory ability of rats. After the 5th day of the water maze experiment, the platform was removed allowing the rats to swim freely for 60 s. The duration spent in the target quadrant and the number of crossing times in the quadrant were recorded

Material and Tissue Processing

Blood samples (4 ml) were collected from the inferior vena cava after abdominal anesthesia with 10% chloral hydrate (0.3 ml/100 g). All serum samples were separated, and stored in a -70°C ultra low temperature freezer for later use.

All rats were then sacrificed to harvest hippocampus. Pre-chilled physiological saline was added to obtain 10% hippocampal homogenate, and proteins extracted using RIPA lysates. 1 ml RIPA lysate (containing 1mmol PMSF) was mixed with 100 μg of sample and sonicated for 1 min (sonicated 2 s and interval 1 s) in a 1.5 ml EP tube on the ice. All samples were then centrifuged at 2,500 r/min for 10 min at 4°C to harvest supernatant, and stored in a -70°C ultra low temperature freezer before use.

Detection of BDNF Concentration by ELISA Method

Levels of BDNF were determined using Thermo Scientific's Rat BDNF ELISA kit per the manufacturer's instructions. Briefly, the sample (100 μl) was added to its appropriate well, and incubated

for 2.5 h at room temperature. The solution was discarded, and the wells were washed 4 times with 1× Wash Buffer. Next, 100 µl of 1× prepared biotinylated antibody was added to each well and incubated for 1 h at room temperature with gentle shaking. 100 µl of prepared Streptavidin-HRP solution was then added to each well and incubated for 45 min at room temperature with gentle shaking. The solution was than discarded and the wells were washed 4 times with 1× Wash Buffer. Finally, 100 µl of TMB Substrate was added to each well and incubated for 30 min at room temperature in the dark with gentle shaking. Each plate was evaluated within 30 min of stopping the reaction. Optical density OD values were measured at the wavelength of 450 nm, and the OD values of supernatant and serum in hippocampal homogenate were measured using a multi-function enzyme labelling analyzer.

Ethics Statement

All experiments were conducted in accordance with the animal experiment regulations of the Second Xiangya Hospital Ethics Committee of Central South University. The protocol was approved by the Second Xiangya Hospital Ethics Committee of Central South University.

Statistical Analysis

The statistical methods were analyzed by SPSS 22.0 software. One way ANOVA was used to analyze the data in accordance with normal distribution and homogeneity of population variance. Two way ANOVA and Bonferroni's multiple comparisons test were used to analyze the data of escape latency in water maze. LSD method was used for comparison between groups. The measurement data were expressed as mean ± standard deviation (X ± SD) or means ± sem. For data not in accordance with normal distribution, Mann Whitney U method in rank sum test was used for inter group comparison, and the results were expressed by relevant parameters. P represents the significant level of the test, $P < 0.05$ means the difference is statistically significant.

RESULTS

Quantitative Analysis of Experimental Animals

All 75 rats (ketamine 3 d group n = 20, saline 3 d group n = 18, ketamine 14 d group n = 20, saline 14 d group n = 17) were included in the statistical analysis of the experimental results. There were no accidental deaths in the experiment. A total of 75 rats were screened by water maze.

Experimental Results of Morris Water Maze

The results from the Morris water maze are shown in **Figure 1** and **Table 1**. The average escape latency of ketamine 3 d and 14 d groups were significantly longer than that of the saline 3 d and 14 d groups ($p < 0.0001$), respectively. The significant difference showed in day3 comparing saline 3 days group with ketamine 3 days group by multiple comparison ($p<0.05$).

On day six, in the space exploration experiment, a significant reduction in the number of platform crossings as well as residence time was observed in the ketamine 3 d group compared to the saline 3 d group ($p < 0.05$). Compared to the saline 14 d group, the number of platform crossings and the

TABLE 1 | Statistical results of the number of crossing platforms and residence time in each group of rats in space exploration experiments (X ± SD).

Group	Number	The number of crossing platform	The residence time in the target platform quadrant(s)
ketamine 3 d	20	6.5 ± 2.0^{bd}	6.7 ± 3.5^{bd}
saline 3 d	18	8.3 ± 3.5^{a}	9.6 ± 4.0^{a}
ketamine14 d	20	8.0 ± 2.8^{d}	8.5 ± 3.7^{d}
saline 14 d	17	10.0 ± 2.7^{ac}	11.0 ± 3.7^{ac}

[a]P value comparison with the ketamine 3 d group, P < 0.05, [b]P value comparison with the saline 3 d group, P < 0.05, [c]P value comparison with the ketamine 14 d group, P < 0.05, [d]P value comparison with the saline 14 d group.

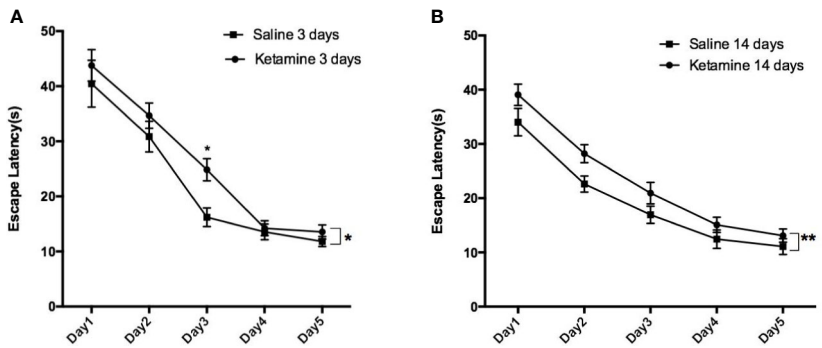

FIGURE 1 | The escape latencies of rats in water maze positioning navigation experiment. Data shown as means ± sem. Rats were grouped by different treatment, Two-way ANOVA with Bonferroni correction were used to determine whether difference existed based on experience level. Significantly difference were seen between saline 3 days group vs. ketamine 3 days group as shown in **(A)**, saline 14 days vs. ketamine 14 days group as shown in **(B)**. Further analysis each group at different time point by Bonferroni's multiple comparisons test in Prism6, the significant difference showed in day3 comparing saline 3 days group with ketamine 3 days group. *p<0.05, **p<0.0001.

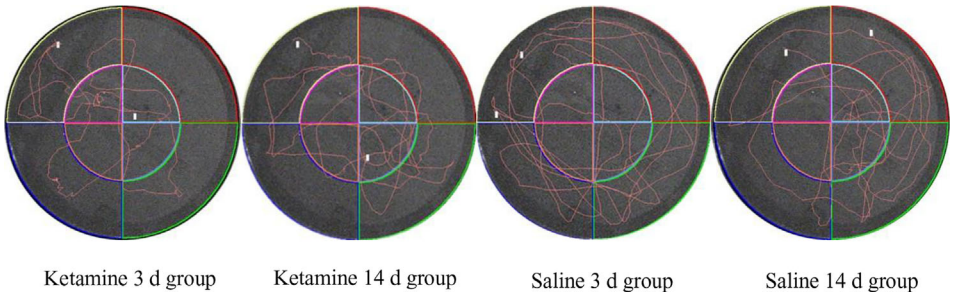

FIGURE 2 | Typical trajectory map of four groups of rats in space exploration.

residence time was also significantly reduced in the ketamine 14 d group ($p < 0.05$). However, there was no significant differences observed between the ketamine 3 d and ketamine 14 d groups.

As showed in **Figure 2**, the track map of the target platform quadrant in the 3 d ketamine group is thinner than in the 3 d saline group. Similar results were also observed between the ketamine 14 d group and the saline group. Therefore, as observed in both the 3 d and 14 d group, there appears to be a memory deficit in the ketamine group when compared to saline controls.

Results of BDNF Concentration in Serum and the Hippocampus

The serum concentration of BDNF in the ketamine 14 d group was significantly lower than that of the saline 14 d group (p <0.05). No significant difference was observed in serum BDNF concentration between the ketamine 3 d and saline 3 d group (**Table 2**).

BDNF concentrations in the hippocampus are showed in **Table 3**. No significant differences were found in hippocampal BDNF concentrations between any of the ketamine and saline groups (ketamine 3 d vs. saline 3 d: ketamine 14 d vs. saline 14 d).

TABLE 2 | Comparison of serum brain-derived neurotrophic factor (BDNF) concentration of each group.

Group	Total number	U	W	Z	P
A vs B	38	171.000	342.000	-0.263	0.792
C vs D	37	88.000	299.000	-2.469	0.014*
A vs C	40	183.000	393.000	-0.460	0.646

*Group A represents ketamine 3 d group, Group B represents saline 3 d group, Group C represents ketamine14 d group, Group D represents saline 14 d group, *indicates ketamine 14 d group compared with saline14 d group, P < 0.05. U, W, and Z value represent statistics. P represents significant difference.*

TABLE 3 | Comparison of hippocampus brain-derived neurotrophic factor (BDNF) concentration of each group.

Group	Total Number	U	W	Z	P
A vs B	38	175.000	385.000	-0.146	0.884
C vs D	37	124.000	334.000	-1.402	0.161
A vs C	40	199.000	409.000	-0.027	0.987

Group A represents ketamine 3 d group, Group B represents saline 3 d group, Group C represents ketamine14 d group, Group D represents saline 14 d group. U, W, and Z value represent statistics. P represents significant difference.

DISCUSSION

This experiment was designed to explore the neural mechanism related to ketamine addiction. Studies have found that ketamine abuse can lead to increased apoptosis of neurons in the frontal lobe, temporal lobe, hippocampus, among other regions, affecting cognitive function (1). Given that ketamine addiction can produce behaviors similar to schizophrenia, it has been suggested that ketamine addiction can be used as an experimental model of schizophrenia. Therefore, we assessed for differences in hippocampal and serum BDNF levels simultaneously to further elucidate the potential relationship between BDNF and ketamine-induced learning-memory dysfunction.

We first determined the effect of the acute and chronic ketamine (25mg/kg) exposure on learning ability and memory in rats. Our results showed that the continuous use of ketamine for 3 d or 14 d both lead to learning and memory dysfunction. The concentration of BDNF in serum and hippocampal homogenate was also measured by ELISA. Interestingly, we only observed a significant difference in serum BDNF concentrations between the ketamine 14 d group and the saline 14 d group. Based on this, we further assessed the effect of ketamine on learning-memory dysfunction in rats and its relationship with BDNF.

A recent study showed that the effect of ketamine on cognition was directly related to the dosage (6, 15, 16). However, there is no specific unity in the classification of low dose, subanesthetic dose and anesthetic dose among the present experimental literature. Low dose (2.5–10 mg/kg) ketamine can cause cognitive impairment, while lower doses (< 2.5 mg/kg) and a slightly higher dose (10–20 mg/kg) do not significantly affect cognition after 10 consecutive days of exposure (16). Some researchers have even used higher doses of ketamine (25 mg/kg) to study the effects on rats and found that they lead to positive and negative symptoms similar to schizophrenia as well as affect cognitive function (17). However, Imre et al. (9), suggest that 4–16 mg/kg of ketamine is a subanesthetic dose for rodents, and 20–50 mg/kg is a high dose (18). Meanwhile, Newcomer et al. have shown that for rats, the minimum response threshold is 40 mg/kg (19). Previous studies

have investigated the effect of ketamine on memory consolidation, and found that subanesthetic doses (5–50 mg/kg) did not affect the reconsolidation phase, whereas higher, anesthetic doses (125–150 mg/kg) did affect memory reconsolidation (15). Given the above findings, we chose the 25 mg/kg dose to study the effects of ketamine on learning and memory in rats. Consistent with previous studies, our results show that in the water maze experiment, the average latency of the ketamine-14 d group was longer than that of the saline-14 d group. The number of platform crossings and the residence time of the rats in the 3 d and the 14 d ketamine groups were lower than that of the control groups. These results suggest that repeated exposure to 25 mg/kg ketamine negatively affects learning and memory in SD rats.

It has also been reported that the effect of ketamine on cognition is related to the duration of exposure as well as the time since last exposure. Single ketamine exposure and repeated ketamine exposure both cause cognitive impairments (10, 20). For example, when ketamine (30 mg/kg) was injected continuously for one month, the spatial memory ability of rats was significantly reduced (21).

In animal studies, a single exposure as well as 3–5 d of exposure have all been defined as acute ketamine exposure (8, 10, 16, 22). Likewise, the definition of chronic ketamine exposure varies between experimental studies. In fact, some have defined chronic ketamine exposure in rodents as 5 d (2), 7 d (17), 10 d (16), 14 d (23), and 30 d (21). Therefore, based on the literature, we defined 3 d ketamine exposure as "acute" and 14 d as a "chronic" in order to study the difference in cognition between acute and chronic ketamine exposure in rats.

The half-life of ketamine is about 1 to 2 h, and the withdrawal reaction usually takes place between 0.5 d to 3 d (2). Most previous studies conducted the behavioral test 24 h after the last drug injection, thus not accounting for the effects of the withdrawal response. In order to avoid the effect of acute withdrawal in this experiment, the rats were given ketamine for 10 d after the last exposure. This study showed that acute and chronic ketamine (25 mg/kg) exposure both lead to learning and memory dysfunction in rats.

It had been reported that no matter acute or chronic exposure to ketamine, the damage to learning and memory of rats is persistent and irreversible (16, 23). In this experiment, the learning and memory abilities of rats were measured 10 days after ketamine withdrawal. Both the acute or chronic ketamine exposure led to significant impairment in cognitive dysfunction, comparing to control group.

n human and primates in general, cognition is mainly controlled by the prefrontal cortex and hippocampus, while rodents mainly depend on the function of the hippocampus (24). Importantly, deficiencies in BDNF is associated with hippocampal neuronal apoptosis. To verify whether learning-memory dysfunction in rats caused by ketamine is associated with BDNF deficiency in the hippocampus, we determined the levels of BDNF in the hippocampus of ketamine-treated rats. Interestingly, we found that there was no significant change in hippocampal BDNF concentrations after ketamine withdrawal. Intriguingly, we found that levels of BDNF were reduced in serum after chronic ketamine withdrawal. This suggests that serum BDNF levels are a better indicator of learning-memory dysfunction produced by ketamine exposure than hippocampal BDNF levels.

In conclusion, our study demonstrated that chronic ketamine (25 mg/kg) administration causes spatial learning and memory impairment in SD rats, and that this deficit might be associated with decreased serum BDNF levels. Additionally, our results also suggest that there are other mechanisms regulating BDNF levels in the brain. Nevertheless, it is still unclear how deficiencies in serum BDNF levels lead to learning and memory deficits in rats, requiring further exploration.

The limitation of this study is that we did not study the effects of ketamine addiction on human cognitive function and serum BDNF concentration. In the future, cognitive impairment caused by different doses and time of ketamine addiction patients can be measured with cognitive scale, and serum BDNF concentration can be measured at the same time. In order to understand the changes of brain structure, functional imaging of brain can be completed at the same time to understand the changes of neurotransmitters in the brain.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the Second Xiangya Hospital Ethics Committee of Central South University.

AUTHOR CONTRIBUTIONS

HS, XZ, and ZX conceptualized the experiments. ML, YL, and QZ performed the experiments. XZ, SH, and QH undertook the statistical analysis. ML and AX wrote the first draft of the manuscript. XZ and YC reviewed and edited the manuscript. XZ, TS, XC, and ZL contributed to the final manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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GENERAL COMMENTARY
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Commentary: Effect of Low-Frequency Repetitive Transcranial Magnetic Stimulation on Impulse Inhibition Methamphetamine in Abstinent Methamphetamine Patients

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Keywords: transcranial magnetic stimulation, low-frequency, methamphetamine, commentary, prefrontal cortex (PFC)

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A Commentary on

Effect of Low-Frequency Repetitive Transcranial Magnetic Stimulation on Impulse Inhibition in Abstinent Patients With Methamphetamine Addiction: A Randomized Clinical Trial
by Yuan, J., Liu, W., Liang, Q., Cao, X., Lucas, M.V., and Yuan, T.F. (2020). JAMA Netw Open 3, e200910. doi: 10.1001/jamanetworkopen.2020.0910

Drug addiction and relapse are characterized by compulsive drug seeking behavior and high impulsivity throughout different stages of addiction (e.g., formation and relapse) (1). Methamphetamine (MA) dependents exhibited high impulsivity, which act as important risk of relapse (2). Neuroimaging studies reported dopaminergic transmission and cortical-striatal circuitry mechanisms underlying the changes (3). It is conceivable that targeting these aberrant circuits might facilitate the behavioral control and reduce behavioral impulsivity in MA dependents (4) and, finally, prevent the patients from relapse. A recent study published on *JAMA Netw Open* proved the clinical potential of brain stimulation approach for impulsivity control in MA patients (5).

The authors firstly developed a two-choice oddball task that allows for quantification of behavioral impulsivity with improved sensitivity, in compared to the classical Go/Nogo task (6). The task contains both standard (frequently) and deviant (infrequently) trials; the subject needs to habituate for standard trial responses and response for deviant trial by press the button for inhibition. The behavioral results therefore provided both accuracy responses and reaction time (RT) differences between the two conditions. The authors found that the MA dependents reported lower accuracy and shortened response time, both of which reflecting the increased impulsivity associated with MA dependence.

Considering the importance of prefrontal cortex (PFC) in behavioral inhibition and risk decision making processes (7), the authors therefore designed a randomized controlled trial to investigate the effects of 1 Hz non-invasive repetitive transcranial magnetic stimulation (rTMS) on impulsivity of MA dependents, which is found to be effective in reducing impulsivity in other types of patients (8).

Single session 1-Hz treatment at left PFC successfully improved accuracy and slowed down the response time, while repeated treatment with 10 sessions demonstrated lasting effects for at least 30 days. These findings suggest that 1 Hz rTMS could reduce behavioral impulsivity and improve the cautious decision making.

The study for the first time reported the effects of rTMS intervention on impulsivity of MA dependence. It is possible that this finding might be generalize to other substances of abuse as well and warrants future large trials investigating the effects of chronic rTMS on impulsive drug seeking behaviors and relapse. Recent studies emphasized the potency of rTMS treatment on drug intake behavior, improve sleep quality and cognition, and efficiency to reduced cue reactivity or craving in a variety of drug dependents (9–17). It should be noted that most of these results focused on high-frequency (e.g., 10 Hz) rTMS stimulation at left PFC region (18).

Previous studies reported that, for MA addicts, the craving rate decreased after high-frequency (10 Hz) rTMS at left PFC, and the rate increased after low-frequency (1 Hz) rTMS at the same cortex (19, 20). There is a theory that the excitation of unilateral cortical regions leads to suppression of the contralateral side, so the high-frequency stimulation on the left can achieve the same therapeutic effect in the right position by low frequency (21). It is consistent with the results observed by Yuan et al. that no matter high-frequency rTMS at left PFC or low-frequency rTMS at right PFC effectively reduced craving rate for MA abusers (22). Whether the patient requires two different and separate treatment protocols for drug seeking and impulsivity is yet to be understood.

The study is limited by a short period of treatment and follow-up time window. It is still necessary to understand the lasting effects of rTMS on impulsivity improvement; it is also important to examine if rTMS also improves risk decision making, which partly relies on behavioral inhibition ability. In addition, it will be interesting to understand if targeting motor cortical areas (e.g., SMA, M1) would also modulate the motor impulsivity and provide a full picture of impulsivity treatment in substances of abuse.

Taken together, the recent study by Yuan et al. opened novel possibility of impulsive behavior intervention for drug dependence and potentially imply on relapse behavior. Neuroimaging studies are required to elucidate the underlying neuroplasticity and circuitry changes following rTMS treatment.

AUTHOR CONTRIBUTIONS

HW, CY, and XL contributed equally to this work. HW and CY drafted the manuscript. XL revised the manuscript. WL and FH provided funds. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Quality of Life in Patients With Methamphetamine Use Disorder: Relationship to Impulsivity and Drug Use Characteristics

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Background: The quality of life (QOL) of patients with methamphetamine use disorder (MAUD) is increasingly recognized as an important outcome. Previous studies have found that impulsivity is negatively associated with QOL in mental disorders, but this relationship is rarely confirmed in patients with MAUD. We hypothesized that impulsivity is negatively correlated with QOL in patients with MAUD based on previous findings. In addition, a variety of drug use characteristics of patients that may potentially affect their QOL need to be further explored. Therefore, the purpose of this study was to explore the relationship between impulsivity, multiple drug use characteristics, and QOL in patients with MAUD.

Methods: A total of 379 patients with MAUD were recruited, and the majority of them were male (85.5%), with an average age of 33.93 ± 7.08 years. Two psychiatrists conducted semi-structured interviews with methamphetamine (MA) users in two compulsory drug rehabilitation centers to obtain their demographics and drug use characteristics. The Barratt Impulsiveness Scale-11 (BIS-11) and Brief WHO Quality of Life Assessment (WHOQOL-BREF) were used to assess patients' impulsivity and QOL, respectively. Correlation and univariate regression analysis were used to explore the relationships between impulsivity, a series of drug use characteristics and patients' QOL in different domains. Further multiple linear regression analysis was used to identify what extent the above clinical variables explained the variations in patients' QOL.

Results: Age, marital status, employment, and various drug use characteristics were significantly associated with at least one QOL domain. Among them, married and full-time job were positively correlated with QOL, while others were negatively correlated with QOL. The total score of BIS-11 was significantly negatively correlated with all four domains of QOL. Impulsivity, a range of drug use characteristics and certain

demographic characteristics collectively explained varying degrees of variation in different domains of QOL.

Conclusions: Impulsivity and various drug use characteristics can significantly predict QOL in all fields of MAUD patients. In addition, we have also found differences in the predictors of QOL in different domains. Overall, this study provides clinical guidance for the treatment of MAUD patients, that is, management of impulsivity in patients with MAUD may help improve their QOL and even sustain their drug rehabilitation.

Keywords: methamphetamine, quality of life, impulsivity, substance use disorder, drug abuse, drug use

INTRODUCTION

Quality of life (QOL) refers to individuals' subjective perception of their own life status, which involves the domains of physical health, psychological state, social relations and living conditions (1). In the past decade, QOL has been paid more and more attention by psychiatrists because of its importance for outcomes of patients with mental illness (2–4), especially for schizophrenia (5, 6), major depression (7, 8) and bipolar disorder (9, 10). In fact, QOL is not one of the traditional or formal measures of outcomes. Therefore, in contrast to these common psychiatric diseases, a more insidious condition named substance use disorders (SUDs), has received relatively little attention in terms of QOL from both clinicians and researchers (11). Despite the limited number of studies, it does not prevent evidence that QOL has increasingly become a determinant of the outcomes of patients with SUDs (12, 13). More specifically, it has been shown that improved QOL can effectively reduce the use of drugs during treatment seeking (14, 15). In addition, a high QOL contributes to the sustainable withdrawal of patients with SUDs after leaving treatment (16, 17). However, patients with SUDs often reported more impaired QOL than those without SUDs (18, 19), or even poorer than some other chronic disease (20, 21), which may greatly hinder their rehabilitation. Consequently, it is urgent for clinicians to understand the factors leading to the poor QOL in patients with SUDs, so as to improve it in a cost-effective manner.

Previous studies have consistently concluded that impulsivity was significantly associated with QOL, especially in a group of mental disorders characterized by impulsivity, such as bipolar disorder (9, 22), attention deficit hyperactivity disorder (23, 24) and emotional eating (25, 26). In fact, although impulsivity has been shown to be a non-core symptom, in popular diagnostic systems (such as DSM-5 and ICD-11), certain impulsive behaviors can still be used to diagnose SUDs, such as uncontrolled drug seeking (27, 28). Therefore, we hypothesized that the high impulsivity of patients with SUDs may have an impact on their QOL. Unfortunately, there are few studies on the relationship between impulsivity and QOL in patients with SUDs. Although rare, fortunately, we have drawn from this small number of studies that in the SUDs population, patients with higher impulsivity have worse QOL than those with lower impulsivity (29, 30). Furthermore, impulsivity may be a predictor of QOL in patients with SUDs. More specifically, high impulsivity is associated with lower QOL (31, 32). However, there are some shortcomings in the existing studies. On the one hand, they rarely pay attention to the relationships between impulsivity and QOL, and on the other hand, they did not determine whether there is a different relationships between the various domains of QOL (i.e., QOL is basically divided into four domains, namely physical, psychological, social, and environmental, see Method Section). In view of this, our research will focus on solving all these problems ignored by previous studies.

In addition to impulsivity, there are other factors that were consistently associated with the QOL of patients with SUDs, of which the most commonly studied were the characteristics related to drug use, including the frequency of drug use (33, 34), the severity of SUDs (35, 36), comorbidities with mental disorders (37, 38) and polysubstance use (39). In fact, in addition to the above-mentioned drug use characteristics, there are also the age of onset of SUDs, the number of relapse and comorbidities with other SUDs, which seem to have been overlooked in previous studies. Overall, the main purpose of this study was to identify the degree to which impulsivity and some previously less discussed drug use characteristics explain the variations in QOL, especially to clarify the relationships between these clinical variables and QOL in different domains. It is worth emphasizing that most previous studies have used the Addiction Severity Index (ASI) to assess the severity of SUDs (35, 36), which may produce some bias due to the self-reported data. In order to modify this, we obtained the severity of SUDs through the semi-structured interviews in this study, according to the diagnostic criteria of SUDs in DSM-5. In addition, the patients we recruited were recent users of methamphetamine (MA), as MA has increasingly become an illegal drug of abuse worldwide, especially in Asia (40). Currently, methamphetamine use disorder (MAUD) has become an global public health issue (41). Therefore, improving the QOL of MAUD patients is critical to prevent an outbreak.

METHOD

Participants and Procedures

A total of 379 MA users participated in this study, including of 324 men (85.5%) and 55 women (14.5%), aged from 20 to 54 years old (M =33.93 ± 7.08). They were recruited from two compulsory drug

rehabilitation centers in Changsha, Hunan Province. From March to September 2018, two psychiatrists who validated the raters' consistency conducted semi-structured interviews with MA users who entered drug rehabilitation centers. The inclusion criteria for this study were 1) the core drug used in the last three years was MA; 2) diagnosed as a stimulant use disorder (that is, meeting at least 2 criteria in DSM-5); 3) at least two weeks of detoxification at the time of enrollment. The exclusion criteria were 1) lifetime/current diagnosis of mental illness or personality disorder; 2) alcohol use disorder; 3) intellectual disability or cognitive impairment; 4) presence of any other serious diseases.

This study was approved by the Ethics Committee of The Second Xiangya Hospital of Central South University. The subjects understood the purpose and procedure of the study and signed an informed consent for. They could quit unconditionally and all information was confidential.

Measures

Demographics and Drug Use Characteristics

To ensure data quality, we administrated a semi-structured interview to collect demographics and drug use characteristics of the patients as follows: 1) demographic data included gender, age, education, marital status (whether married), whether they had children, who they lived with (family member or non-family member), employment (full-time or part-time job, unemployment); 2) drug use characteristics included the age of onset of MAUD, the number of relapse (i.e., reused drugs after at least three months of absolute withdrawal), comorbidities with other SUDs [i.e., the patients in this study were classified as simple MAUD, having comorbidities with opioid use disorder (OUD) and comorbid ketamine use disorder (KUD), depending on their diagnosis] and the severity of MAUD (i.e., patients who met six or more criteria were diagnosed as severe MAUD, and those with less than 6 criteria were diagnosed as mild to moderate according to the DSM-5).

Impulsivity

We used the Chinese version of Barratt Impulsiveness Scale-11 (BIS-11) to assess three aspects of impulsivity (42), namely no-planning impulsivity (i.e., the tendency to live an irregular life), motor impulsivity (i.e., act without considering the consequences) and cognitive impulsivity (i.e., defects in thought or difficulty in solving problems). BIS-11 is a self-reported scale of 30 items and is rated on a 5-point Likert scale, ranging from 1 "never" to 5 "always" (43). For the purpose of the current study, the total score was calculated, and a higher score indicates a higher level of trait impulsivity, which was validated in patients with MAUD in our previous studies (44, 45). In this study, the Cronbach's α of the total scale was 0.909.

Quality of Life

The brief WHO Quality of Life Assessment (WHOQOL-BREF) was used to assess patients' QOL (1). It has been validated in patients with SUDs (14, 46). WHOQOL-BREF is a self-reported scale composed of 26 items, in which item 3 to 26 constitutes the individual's four domains of QOL, namely physical QOL (PHYSQOL, seven items), psychological QOL (PSYCHQOL, six items), social QOL (SOCIALQOL, three items) and environmental QOL (ENVIRQOL, eight items). These items are scored with a Likert-5 point scale and all items are positively scored except for item 3, 4, and 26. The sum of each subscale was calculated, and the higher score indicates the higher QOL in corresponding filed. In addition, item 1 and 2 independently assess a person's overall QOL and physical condition. Since we aimed to examine how clinical variables affect different aspects of QOL, we did not analyze these two overall items. The Cronbach's α of the four subscales ranged from 0.615 to 0.808 in this study.

Statistical Analysis

Descriptive statistics were used to describe demographic and drug use characteristics. Pearson correlation and univariate regression analysis were used to initially identify the relationship between impulsivity, drug use characteristics and QOL. Multiple linear regression analysis was used to further examine the association of the aforementioned clinical variables. Considering the differences in the four aspects of QOL, we executed four regression models with PHYSQOL, PSYHQOL, SOCIALQOL, and ENVIRQOL as dependent variables, and those measures with p <0.1 in the previous univariate regression analysis together with gender and age as independent variables. All analysis was performed using SPSS software (version 23.0) with a bilateral significance level <0.05.

RESULTS

Description of Demographics and Clinical Variables

Demographic data is shown in **Table 1**. The majority (85.5%) of MAUD patients were male. Their average age and years of education were 33.93 (7.08) and 9.96 (2.70) respectively. Regarding their marital status, 175 (46.2%) were married, while others (53.8%) were not. When asked who they usually lived with, 250 (66.0%) reported their family members. In addition,

TABLE 1 | Demographics of the patients (N = 379).

Variables	M (SD)	N (%)
Gender		
male		324 (85.5)
female		55 (14.5)
Age	33.93 (7.08)	
Education (years)	9.96 (2.70)	
Married		
yes		175 (46.2)
no		204 (53.8)
Live with family member		
yes		250 (66.0)
no		129 (34.0)
Have children		
yes		243 (64.1)
no		136 (35.9)
Employment		
full-time job		229 (60.4)
part-time job/unemployment		150 (39.6)

243 (64.1%) had at least one child. In regard to their employment, 229 (60.4%) had a full-time job, while others were part-time or unemployed.

Table 2 shows the drug use characteristics and scores of BIS-11 and WHOQOL-BREF. First, patients reported that their age of onset of MAUD was 30.25 (7.26), with an average of relapse for 2.47 (1.01) times since the onset of MAUD. Regarding comorbidities with other SUDs, 227 cases (59.9%) were diagnosed without comorbidities (that is, simple MAUD), while 46 cases (12.1%) were diagnosed as comorbidities with OUD, and the remaining 106 cases (28.0%) were diagnosed with comorbidities with KUD. In terms of the severity of MAUD, the majority (73.9%) met more than six diagnostic criteria of DSM-5 and they were therefore diagnosed as severe. Then, as we can see in **Table 2**, the total score of BIS-11 was between 36 and 126, with an average score of 80.85 (15.95). Meanwhile, the sum of four domains of QOL was 14.91 (SD = 2.13, PHYSQOL), 13.35 (SD = 2.12, PSYCHQOL), 14.38 (SD = 2.59, SOCIALQOL), and 13.54 (SD = 2.30, ENVIRQOL), respectively.

Correlations of Drug Use Characteristics, Impulsivity With QOL

We conducted correlation and univariate regression analysis to initially explore the relationship between multiple clinical variables and QOL. First, Pearson's correlation results are shown in **Table 3**. We found that the total score of BIS-11 was negatively correlated with QOL in all four domains, with a correlation coefficient ranging from 0.308 (SOCIALQOL, $p < 0.01$) to 0.467 (PHYSQOL, $p < 0.01$). Except for PSYCHQOL, the number of relapses was negatively associated with QOL in other three domains, with correlation coefficients of 0.187 (PHYSQOL, $p < 0.01$), 0.175 (SOCIALQOL, $p < 0.01$), and 0.108 (ENVIRQOL, $p < 0.05$), respectively.

TABLE 2 | Drug use characteristics and scores of BIS-11 and WHOQOL-BREF (N = 379).

Variables	M (SD)	N (%)
Age of onset of MAUD	30.25 (7.26)	
Number of relapses	2.47 (1.01)	
Comorbidity with other SUDs		
without		227 (59.9)
comorbidity with OUD		46 (12.1)
comorbidity with KUD		106 (28.0)
Severity of MAUD		
mild to moderate		100 (26.4)
severe		279 (73.6)
Total score of BIS-11 (0.909)[a]	80.85 (15.95)	
WHOOQOL-BREF (0.886)[a]		
PHYSQOL (0.703)[a]	14.91 (2.13)	
PSYCHQOL (0.615)[a]	13.35 (2.12)	
SOCIALQOL (0.647)[a]	14.38 (2.59)	
ENVIRQOL (0.808)[a]	13.54 (2.30)	

MAUD, methamphetamine use disorder; SUDs, substance use disorders; OUD, opioid use disorder; KUD, ketamine use disorder; BIS-11, Barratt Impulsiveness Scale-11; WHOQOL-BREF, Brief WHO Quality of Life Assessment; PHYSQOL, physical quality of life; PSYCHQOL, psychological quality of life; SOCIALQOL, social quality of life; ENVIRQOL, environmental quality of life.
[a]*Cronbach's α.*

TABLE 3 | Correlations and univariate regression of impulsivity, drug use characteristics and QOL.

	PHYSQOL	PSYCHQOL	SOCIALQOL	ENVIRQOL
Correlations[a]				
age of onset of MAUD	0.021	0.045	-0.028	0.039
number of relapses	-0.187**	-0.079	-0.175**	-0.108*
total score of BIS-11	-0.467**	-0.459**	-0.308**	-0.438**
Univariate regression[b]				
severe MAUD	-0.157**	-0.235***	-0.190***	-0.205***
comorbidity with OUD[c]	-0.227***	-0.129*	-0.134*	-0.175**
comorbidity with KUD[c]	-0.041	-0.073	-0.037	-0.074

$^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.
QOL, quality of life; PHYSQOL, physical quality of life; PSYCHQOL, psychological quality of life; SOCIALQOL, social quality of life; ENVIRQOL, environmental quality of life; MAUD, methamphetamine use disorder; BIS-11, Barratt Impulsiveness Scale-11; OUD, opioid use disorder; KUD, ketamine use disorder.
[a]*Pearson correlation coefficient.*
[b]*Standardized Beta coefficient.*
[c]*The reference was simple MAUD.*

Table 3 also presented the results of univariate regression. We firstly found that severe MAUD was negatively associated with QOL in all four domains, which had the highest correlation with PSYCHQOL ($r = -0.235$, $p < 0.001$), and the lowest correlation with PHYSQOL ($r = -0.190$, $p < 0.001$). In addition, compared with the simple MAUD, patients comorbid with OUD had significantly lower QOL in all four domains, especially PHYSQOL ($\beta = -0.227$, $p < 0.001$).

Multiple Linear Regression of Drug Use Characteristics, Impulsivity, and QOL

We performed four separate multiple regression models by using Stepwise and Enter methods. First, PHYSQOL was set as the dependent variable, and then gender, age, education, marital status, who lived with, employment, comorbidity with OUD or KUD, number of relapses, severity of MAUD, and impulsivity were set as independent variables. As shown in **Table 4**, impulsivity ($\beta = -0.446$, $p < 0.001$), number of relapse ($\beta = -0.130$, $p = 0.004$), full-time job ($\beta = 0.093$, $p = 0.039$) and comorbidity with OUD ($\beta = -0.127$, $p = 0.008$) explained 28.1% of the variation in PHYSQOL ($F = 24.266$, $p < 0.001$, adjusted $R^2 = 0.270$).

Second, we designated PSYCHQOL as dependent variable, and gender, age, marital status, whether or not have children, employment, comorbidity with OUD or KUD, severity of MA and impulsivity as independent variables. The results showed that 26.4% ($F = 22.184$, $p < 0.001$, adjusted $R^2 = 0.252$) of the variation in PSYCHQOL were explained by impulsivity ($\beta = -0.417$, $p < 0.001$), married ($\beta = 0.177$, $p < 0.001$) and severe MAUD ($\beta = -0.140$, $p = 0.003$).

In the next analysis, SOCIALQOL was set as a dependent variable, and gender, age, marital status, having children, who lived with, number of relapses, comorbidity with OUD or KUD, severity of MAUD and impulsivity were included in the regression model. We found that impulsivity ($\beta = -0.276$, $p < 0.001$), number of relapse ($\beta = -0.138$, $p = 0.005$), severe MAUD ($\beta = -0.146$, $p = 0.004$), married ($\beta = 0.129$, $p = 0.009$) and age

TABLE 4 | Multiple linear regression of impulsivity and drug use characteristics to QOL.

QOL domains	Predictors	β	T	*p*
PHYSQOL				
	impulsivity	-0.446	-9.953	<0.001
	number of relapses	-0.130	-2.863	0.004
	full-time job	0.093	2.071	0.093
	comorbidity with OUD	-0.127	-2.671	0.008
PSYCHQOL				
	impulsivity	-0.417	-8.822	<0.001
	married	0.177	3.842	<0.001
	severe MAUD	-0.140	-2.961	0.003
SOCIALQOL				
	impulsivity	-0.276	-5.478	<0.001
	number of relapses	-0.138	-2.795	0.005
	severe MAUD	-0.146	-2.884	0.004
	married	0.129	2.608	0.009
	age	-0.116	-2.190	0.029
ENVIRQOL				
	impulsivity	-0.387	-8.144	<0.001
	married	0.162	3.500	0.001
	full-time job	0.128	2.831	0.005
	severe MAUD	-0.106	-2.232	0.026

QOL, quality of life; PHYSQOL, physical quality of life; PSYCHQOL, psychological quality of life; SOCIALQOL, social quality of life; ENVIRQOL, environmental quality of life; MAUD, methamphetamine use disorder; OUD, opioid use disorder.

(β = -0.116, p = 0.029) accounted for 16.3% of the variation in SOCIALQOL (F = 10.344, p < 0.001, adjusted R^2 = 0.148).

Finally, ENVIRQOL was set as a dependent variable, while gender, age, marital status, having children, who lived with, employment, number of relapses, comorbidity with or KUD, severity of MAUD, and impulsivity were independent variables. The results showed that 26.2% of variation in ENVIRQOL (F = 18.790, p < 0.001, adjusted R^2 = 0.248) was explained by impulsivity (β = -0.387, p < 0.001), married (β = 0.162, p = 0.001), full-time job (β = 0.128, p = 0.005) and severe MAUD (β = -0.106, p = 0.026).

It is worth mentioning that we conducted the Bonferroni test, which tested the deviation caused by multiple comparisons, and the results were still significant.

DISCUSSION

To the best of our knowledge, this is the first study to explore the relationship between impulsivity, drug use characteristics and QOL in the MAUD population. The main findings were as follows. First, certain demographic and a range of drug use characteristics, more specifically, age, marital status, employment, number of relapses, comorbidity with OUD as well as the severity of MAUD were correlated with different QOL. Second, impulsivity was negatively associated with QOL in all four domains among patients with MAUD. Finally, impulsivity, drug use characteristics and some demographics collectively explained the varying degrees of variation in the four domains of QOL.

Regarding demographics, age, married, and having a full-time job can predict one or more QOL domains. Specifically, married positively predicted three domains (ENVIRQOL, SOCIALQOL, and PSYCHQOL), while having a full-time job positively predicted PHYSQOL and ENVIRQOL. These were partially consistent with previous studies in patients with SUDs (39, 47, 48) and other mental disorders (49), indicating that stable social support is more constructive for positive perception of ENVIRQOL. In addition, age was negatively associated with SOCIALQOL which was consistent with one study in patients with depression (50). One of the possible reasons is that SOCIALQOL is involved in the sexual well-being of patients, which is generally reported as unsatisfactory in the elderly (51, 52). In terms of drug use characteristics, number of relapses, severe MAUD, and comorbidity with OUD negatively predicted at least one domain of QOL. First, more relapses predicted poorer PHYSQOL and SOCIALQOL, which was consistent with the findings of some recurrent diseases such as acne (53), depression (54) and SUDs (55). Our findings suggested that relapse was an important risk factor for QOL of SUDs patients, especially in their physical and social domains. Therefore, when treating patients with a higher relapse frequency, special attention should be paid to the recovery of their physical and social functions. Second, compared with simple MAUD, patients comorbid with OUD had significantly lower PHYSQOL. This indirectly supports previous studies that opioid abusers had a particularly poor physical QOL (56–58), even lower than other SUDs (59). In fact, previous studies have also found that comorbidities with alcohol use disorders are a risk factor for QOL (60). Collectively, clinicians should pay special attention to patients with multiple SUDs because their potentially low quality of life may affect the outcomes. Finally, most QOL domains in patients with severe MAUD were worse than those patients with mild to moderate MAUD, confirming the results of previous studies that severity is a stable predictor of poor QOL no matter what assessments was used (35, 61).

In addition to the above results, there were more important findings supporting the previous conclusion that impulsivity was closely related to QOL in patients with impulse-related disorders (10, 22–24, 62), including patients with SUDs in this study. Specifically, impulsivity was significantly negatively correlated with different domains of QOL in patients with MAUD, and even after controlling for demographics and multiple drug use characteristics, impulsivity can still predict each different domain of QOL. This was consistent with our previous hypothesis that impulsivity was a powerful predictor of QOL in patients with SUDs (32, 63), while in the current study, MAUD patients (31, 64). In other words, patients with higher impulsivity have lower QOL. More interestingly, we found that although impulsivity was associated with QOL in all domains, the degree was different. The results of correlation and multiple linear regression showed that impulsivity was most closely related to PHYSQOL and least related to SOCIALQOL. This completely replicated the results of patients with bipolar disorder (22), suggesting that impulsivity was even a stable predictor of

cross-domain QOL. Furthermore, impulsivity was the strongest predictor of PHYSQOL which refers to the patients' physical pain, fatigue, sleep, mobility, daily life, dependence on medical treatment, and ability to work (65). Previous studies have found that patients with SUDs tend to report physical discomfort, such as pain (66, 67) and poor quality of sleep (68, 69), which may interfere with their treatment. Our findings may provide advice to clinicians that reducing impulsivity may improve their perception of PHYSQOL, thereby promoting treatment.

However, this study was not without limitations. First, all patients were recruited from compulsory drug rehabilitation centers, excluding those from voluntary drug rehabilitation centers. In fact, there were some differences in a variety of clinical variables between the two groups, which may lead different results. Second, there were fewer female patients in our sample, resulting an imbalanced sex ratio. Therefore, we did not perform a gender difference analysis when exploring these relationships. We will continue to supply female samples to achieve a balanced sex ratio and explore potential gender differences in our findings. In addition, this study was cross-sectional, and we cannot determine whether impulsivity is a longitudinal predictor of QOL in patients with MAUD. Therefore, additional research is needed to examine whether controlling impulsivity will improve the QOL of patients.

Despite these limitations, this study still has obvious advantages. We have identified that impulsivity can significantly predict all domains of QOL in patients with MAUD, even after controlling for other clinical variables. Our results, combined with previous studies, confirm that higher impulsivity is harmful to QOL of patients with impulsivity-related symptoms. In addition, we have also found that there are some differences between the predictors of different QOL domains. For example, comorbidity with OUD can only predict PHYSQOL. It is worth mentioning that, as the most populous country worldwide, our results are worthy of reference for other regions, especially those with large numbers of drug abusers. Overall, this study provides clinical guideline for treatments, that is, regulating impulsivity in patients with SUDs may help improve their QOL, and even maintain their drug rehabilitation.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: The database can be obtained from the corresponding authors with any reasonable reasons. Requests to access these datasets should be directed to TL, liutieqiao123@csu.edu.cn.

ETHICS STATEMENT

This study was approved by the Ethics Committee of The Second Xiangya Hospital of Central South University. The subjects understood the purpose and procedure of the study and signed an informed consent.

AUTHOR CONTRIBUTIONS

TL and WH designed the study. HS and XJZ supervised the whole process. QD designed the structure of the interviews and measures. YW, MQL, ZZ and LZ performed the semi-structure interviews and data collection. YZ and MYL participated in data collation. JZ computed statistical analyses and interpreted the results. YW managed the literature search and wrote the manuscript. XYZ designed the framework of the manuscript and revised it. All authors contributed to the article and approved the submitted version.

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Genome-Wide DNA Methylation Analysis in Male Methamphetamine Users With Different Addiction Qualities

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This paper aimed to explore the genome-wide DNA methylation status of methamphetamine (MA) abusers with different qualities to addiction and to identify differentially methylated candidate genes. A total of 207 male MA abusers with an MA abuse frequency of ≥10 times and an MA abuse duration of ≥1 year were assigned to the high MA addiction quality group (HMAQ group; 168 subjects who met the diagnostic criteria for MA dependence according to the DSM-IV) or to the low MA addictive quality group (LMAQ group; 39 subjects who did not meet the criteria for MA dependence). In addition 105 healthy controls were recruited. Eight HMAQ subjects, eight LMAQ subjects, and eight healthy controls underwent genome-wide DNA methylation scans with an Infinium Human Methylation 450 array (Illumina). The differentially methylated region (DMR) data were entered into pathway analysis, and the differentially methylated position (DMP) data were screened for candidate genes and verified by MethyLight qPCR with all samples. Seven specific pathways with an abnormal methylation status were identified, including the circadian entrainment, cholinergic synapse, glutamatergic synapse, retrograde endocannabinoid signaling, GABAergic synapse, morphine addiction and PI3K-Akt signaling pathways. SLC1A6, BHLHB9, LYNX1, CAV2, and PCSK9 showed differences in their methylation levels in the three groups. Only the number of methylated copies of CAV2 was significantly higher in the LMAQ group than in the HMAQ group. Our findings suggest that the circadian entrainment pathway and the caveolin-2 gene may play key roles in MA addiction quality. Further studies on their functions and mechanisms will help us to better understand the pathogenesis of MA addiction and to explore new targets for drug intervention.

Keywords: methamphetamine, addiction quality, genome-wide DNA methylation analysis, circadian entrainment pathway, caveolin-2

INTRODUCTION

Methamphetamine (MA) is an amphetamine-type stimulant drug with a strong propensity for abuse, and it promotes the release of monoamine neurotransmitters in the central and peripheral systems (1). A low dose of MA can induce a series of short-term effects, including euphoria, an increase in energy, positive emotions, increases in heart rate and blood pressure, mydriasis, peripheral high fever, a loss of appetite and weight, improvements in mental acuity, and social and sexual disinhibition (2). A high dose or repeated abuse of MA can cause mental disorders with typical symptoms, including paranoid delusions, auditory hallucinations, increased activity, and stereotypic behavior (3).

To date, MA abuse remains a serious social problem worldwide. The report of the United Nations Office on Drugs and Crime (UNODC) shows that amphetamine-type stimulants, especially MA, rank as the second most widely used illegal drugs worldwide, and 0.7% of the world's population used amphetamine-type stimulants in 2016 (4). According to the 2017 Report on Drug Control in China, by the end of 2017, more than 1.54 million individuals abused synthetic drugs (especially MA), accounting for 60.2% of drug abusers, which did not include double hidden drug users (5). Numerous clinical observations have indicated that different individuals experiencing the same level of repeated MA exposure show different qualities to addiction. A large sample epidemiological survey also exhibited the same situation. In 2012, the Substance Abuse and Mental Health Services Administration (SAMHSA) estimated that 12 million individuals (∼4.7% of the population aged 12 years or older) had used MA once in their lifetime, 1.2 million individuals (∼0.4% of the population aged 12 years or older) had used MA in the past year, and 0.44 million individuals (∼0.2% of the population aged 12 years or older) had used MA in the past month (6). Moreover, 0.54 million individuals (also ∼0.2% of the population aged 12 years or older, roughly close to the proportion of the individuals using MA in the past month) in the United States in 2012 met the diagnostic criteria for stimulant drug abuse or dependence according to the DSM-IV (6).

However, the mechanisms for differences in quality to addiction remain unclear. Over the years, classic DNA polymorphism studies have not clearly resolved this issue. Most of the studies have focused on the potential susceptible genetic variations for addiction and have found that genetic polymorphisms of multiple candidate genes are significantly associated with susceptibility to addiction (7). It should be noted, however, that the currently identified risk locus associated with addiction susceptibility could account for only a moderate degree of the genetic variance (<5%) (8). Moreover, the results of molecular genetic studies are non-repeatable because of the small sample size and significant heterogeneity. In general, differences in quality to addiction among different individuals are currently thought to arise from genetic predisposition and the environment (e.g., acute drug exposure). At present, epigenetic changes are generally considered to be manifestations of genetic-environment interactions. Epigenetic refers to heritable but reversible regulation of various genetic functions, including gene expression mediated by modifications of DNA and histones (9). Various aspects of epigenetic changes are very stable, making them ideal vectors for addiction susceptibility research (10).

Disturbances in epigenetics, particularly DNA methylation, have been implicated in the pathophysiology of several psychiatric disorders, such as eating disorders (11), depression (12), schizophrenia (13), cocaine addiction (14), and alcohol dependence (15). Epigenetic research on MA addiction is at a relatively early stage, far behind that of cancer or other complex disorders, and far behind that of cocaine, heroin, and other substance abuse. A review discussed recent advances in epigenetic mechanisms underlying amphetamine- or MA-induced behavioral, transcriptional, and synaptic plasticity, and found drug exposure induces major epigenetic modifications in a very complex manner, such as histone acetylation and methylation, DNA methylation (16). DNA methylation is an important gene regulatory mechanism underlying MA-induced learning and memory alterations (17). The genome-wide epigenetic approaches will accelerate the speed of discovery in the field of addiction. However, there has only a few genome-wide DNA methylation study on MA. Desplats et al. (18) reported genome-wide profiling of DNA methylation in postmortem frontal cortex tissue of with HIV-1 infection and MA dependence patients showed differential methylation on genes related to neurodegeneration; dopamine metabolism and transport; and oxidative phosphorylation. Cadet et al. (19) reported genome-wide DNA hydroxymethylation analysis of rats with MA exposure indicated that changes in differentially hydroxymethylated regions and increased expression of specific potassium channels in the NAc may promote abstinence from drug-taking behaviors. González et al. (20) reported that the global DNA cytosine methylation (5-mC) levels in genomic DNA were significantly increased in the medial prefrontal cortex (mPFC) of mice treated with a 7-days repeated MA administration.

This study aimed to explore the genome-wide DNA methylation status of individuals with different qualities to MA abuse through a two-step experiment, including screening for candidate genes with genome-wide DNA methylation microarrays and verifying abnormally methylated genes from a large number of samples using MethyLight quantitative polymerase chain reaction, which were divided into samples from high MA addictive quality and low MA addictive quality groups.

MATERIALS AND METHODS

Participants

Two hundred and seven male MA abusers, with an MA abuse frequency of ≥10 times and an MA abuse duration of ≥1 year from 577 volunteer participants of Xinkaipu Compulsory Rehabilitation Institute (Changsha, Hunan) and White Mud Lake Compulsory Rehabilitation Institute (Xiangyin, Hunan), underwent a Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I/P) from March 2013 to January 2014. Compulsory rehabilitation is the primary form of treatment for illegal drug dependence (61.6% drug users received compulsory treatment by 2012) in China (21). The residential MA users

were admitted in the treatment facilities after confirmed MA use by urine or hair test. Among them, 168 individuals were classified into the high MA addictive quality group (HMAQ group; MA-dependent patients according to the DSM-IV), and 39 individuals were classified into the low MA addictive quality group (LMAQ group; did not meet the diagnostic criteria for MA dependence). In addition, 105 healthy controls who received physical examinations at the Second Xiangya Hospital were recruited.

A complete medical history, physical examination and laboratory tests were obtained from all subjects. All of them were male, Han Chinese, aged 18–50 years, and without severe acute or chronic medical illnesses. The demographic data and substance abuse characteristics of the three groups are shown in **Table 1**. Polydrug abuse in methamphetamine users is very common (22), and this paper wants to focus on methamphetamine users. So, the subjects with other illicit drugs use more than three times, including but not limited to heroin, marijuana, and Ketamine, were excluded from this study. There were only 207 male MA abusers met the requirements in 577 volunteer participants from two compulsory rehabilitation institutes. The co-use of alcohol and tobacco were recorded in **Table 1**.

This study was conducted in accordance with the principles expressed in the Declaration of Helsinki and was approved by the Ethics Committee of the Second Xiangya Hospital. Prior to the study, the procedure was fully explained, and written informed consent was obtained from each subject. All the subjects were free to participate in or abstain from this study, and free to withdraw from this study anytime without threat of punishment.

Sample Processing, DNA Extraction, and Bisulfite Conversion

Fasting blood samples were collected and stored at −80°C. Genomic DNA was isolated from blood samples using DNeasy blood & tissue kits (Qiagen, Valencia, CA, USA), and bisulfite modification was performed using the EZ DNA Methylation-Gold kit (Zymo, Irvine, CA) after all blood samples were collected. The bisulfite-converted DNA was resuspended in TE buffer and stored at −80°C until analysis.

Illumina Infinium® HumanMethylation450 BeadChip Microarray Analysis

We randomly selected eight subjects (dosage: 675.22 ± 93.26 mg/time) in the HMAQ group, eight subjects (dosage: 384.32 ± 87.75 mg/time) in the LMAQ group, and eight subjects in the healthy control group for genome-wide DNA methylation analysis. The bisulfite-converted DNA was then hybridized to the Infinium HumanMethylation450 BeadChip microarray (Illumina, San Diego, USA) in Shanghai Biostar Genechip, Inc. (Shanghai, China) using Illumina-supplied reagents and protocols. This method simultaneously profiles the methylation status for > 485,000 CpG sites at a single-nucleotide resolution. The methylation status of each individual CpG locus was calculated as the ratio of the fluorescent signals (β = Max (M,0)/[Max (M,0) + Max (U,0) + 100]), which ranged from 0 (no methylation) to 1 (complete methylation), using the average probe intensity of the methylated (M) and unmethylated (U) alleles. The quality control information is provided in the supplement as **Supplementary Figures 1–3**. The original data of the chip were preprocessed using the ChAMP package of R software with the BMIQ method for type 2 probe calibration, the ComBat method of the SVA package for the correction of multiple batch effects, the RefbaseEWAS for cell-type heterogeneity calibration, the minfi package for functional normalization function analysis, and the Lasso method for differentially methylated region (DMR) analysis. The screening of differentially methylated loci screening used the pooled *t*-test with $p < 0.05$ and |beta.difference|>0.14 as the threshold for significant differences.

Functional Annotation and Pathway Analysis and Potential Candidate Gene Selection

After the genome DNA methylation chip data were analyzed, the differentially methylated positions (DMPs) and differentially methylated regions (DMRs) were screened. DMRs were included in the pathway analysis of the Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.ad.jp/kegg/), and DMPs were used to screen for candidate genes to perform MethyLight qPCR verification with large samples. To screen for candidate genes from the complex DMP results and to eliminate the impact of confounding factors, the following screening strategies were performed: first, the abnormally methylated sites should correspond to the specific gene name or the UCSC_REFGENE_ACCESSION number; second, the abnormally methylated sites should be located in TSS200 and TSS1500 of the gene promoter region; third, Gene Ontology (GO) analysis (http://www.geneontology.org) should confirm the function of the abnormally methylated genes related to the nervous system; and fourth, the methylation statuses of the candidate gene should be different in all pairwise comparisons of the three groups.

After further literature review and gene functional analysis, solute carrier family 1 member 6 (SLC1A6), basic helix-loop-helix family member b9 (BHLHB9), Ly6/neurotoxin 1 (LYNX1), caveolin 2 (CAV2), and proprotein convertase subtilisin/kexin type 9 (PCSK9) were selected for MethyLight qPCR verification.

Verification of DNA Methylation With MethyLight qPCR

MethyLight qPCR was used to verify the methylation status of the candidate genes, and it is based on the principle of fluorescence-based real-time PCR with two probes. We prepared standard gene products of SLC1A6, BHLHB9, LYNX1, CAV2, and PCSK9 by the amplification of target DNA fragments and the purification of agarose gel DNA fragments using a Gel Extraction Kit and DNA Methylation Kit (CWBiotech, Beijing, China). Their primers and probes were designed by Beacon Designer 7.0, as shown in **Table 2**. MethyLight qPCR was performed on a PikoReal 96 Real-Time PCR System (Thermo Fisher Scientific Inc., US) using bisulfite-treated DNA and a GoldStar TaqMan Mixture (CWBiotech, Beijing, China). The MethyLight qPCR

TABLE 1 | Demographic data and substance abuse characteristics of three groups.

	HMAQ group ($n = 168$)	LMAQ group ($n = 39$)	Healthy controls ($n = 105$)
Age (years old)	30.30 ± 6.45	32.21 ± 7.61	30.05 ± 6.51
Usage mode	Heated suction	Heated suction	NA
Total duration of drug abuse (months)*	36.78 ± 21.34	26.52 ± 18.10	NA
Age of first drug abuse (years old)	25.31 ± 17.37	28.08 ± 7.52	NA
Withdrawal time (months)	9.75 ± 3.21	10.66 ± 4.48	NA
Relapse number*			
1 (first use)	91 (54.17%)	28 (71.79%)	NA
2–3	59 (35.12%)	11 (28.21%)	NA
≥4	18 (10.71%)	0	NA
Polydrug Abuse History			
Tobacco	163 (95.83)	36 (92.31)	NA
Alcohol	57 (33.93%)	14 (35.90%)	NA

*A total of 207 male MA abusers with MA abuse frequency ≥ 10 times per year and MA abuse time ≥ 1 year were recruited. One hundred and sixty-eight cases were classified to high MA addictive quality group (HMAQ group, MA dependent patients according to DSM-IV), and 39 cases to low MA addictive quality group (LMAQ group) who abused MA at least 10 times and more than 1 year, but did not meet the diagnostic criteria for MA dependence on DSM-IV. Significant differences were noted in the total duration of drug abuse ($t = 2.7781$, $P = 0.006$) and relapse numbers ($\chi^2 = 6.784$, $P = 0.032$) between the HMAQ and LMAQ groups. *$p < 0.05$.*

TABLE 2 | Primers and probes of the candidate genes for DNA methylation analyses.

Gene	Primers	Probes
SLC1A6	F: 5′-GGAAACAGAGAAGCCTGG-3′ R: 5′-CTCAGGAAGCGCTCATTA-3′	M: 5′-TTCGCAGCCTTCGCCATC-3′ U: 5′-TTCACAGCCTTCGCCATC-3′
BHLHB9	F: 5′- TTAGTGTGGTTTTTTTTAATTTT−3′ R: 5′- TAACATAACAACCACCAC−3′	M: 5′- AACAAACGAACAACTAAAAACCC-3′ U: 5′- AACAAACAAACAACTAAAAACCC−3′
LYNX1	F: 5′- TTAGTTTAGTTAGGTTGGAAA-3′ R: 5′- TTCACATTATCTACACTTCTC-3′	M: 5′- ACCTCGACCTAAACTCAAACCTCAC-3′ U: 5′- ACCTCAACCTAAACTCAAACCTCAC−3′
CAV2	F: 5′- TGAGTGGTTAGTAGGTTAA-3′ R:5′- ACAATCACATCTATAATTATCTTAC-3′	M: 5′- CACGAAAACAAAACCCTAACACAAAACC-3′ U: 5′- CACAAAAACAAAACCCTAACACAAAACC-3′
PCSK9	F: 5′- TTGTGTTTATTATAGAAT-3′ R: 5′- TTTATACTACAAAAATTC-3′	M: 5′- AACGTCATATAAATACATTCAA-3′ U: 5′- AACATCATATAAATACATTCAA-3′

F, forward; R, reverse; M, Methylated; U, Un-Methylated.

standard curves for methylated SLC1A6, BHLHB9, LYNX1, CAV2, and PCSK9 are shown in **Supplementary Figures 4–8**.

Statistical Analysis

The chip data and MethyLight qPCR data were processed by R version 3.2.4 (R Statistics), and raw-intensity files were preprocessed and normalized together in quantiles using the ChAMP package with the BMIQ method for type 2 probe calibration, the ComBat method of the SVA package for the correction of multiple batch effects, the RefbaseEWAS for cell-type heterogeneity calibration, the minfi package for functional normalization function analysis, and the Lasso method for differentially methylated region (DMR) analysis. The screening for differentially methylated loci used the pooled *t*-test with $p < 0.05$ and |beta.difference|>0.14 as the threshold for significant differences. The remaining data were analyzed using SPSS 19.0 software with a *t*-test, chi-squared test, Pearson correlation analysis, and covariance analysis with age as the covariant. The discrete trend of CAV2 methylation level in LMAQ group was very serious after the outliers were winsorized at level 1 and 99% (The sample size of LMAQ group is 39, only the maximum value was assigned as 99%). In covariance analysis, the CAV2 methylation level was adjusted with age as covariance from 1.05E6 ± 9.83E5 to 1.05E6 ± 8.51E4, and its discrete trend was relieved.

RESULTS

Analysis of Genome-Wide DNA Methylation Microarray Data in Patients With Different Addictive Qualities

After genomic DNA methylation chip data were analyzed, the genome wide methylation level in human chromosomes was shown in **Figure 1**. The distribution of differential methylation sites on chromosomes was shown in **Supplementary Figure 9**. The heatmaps and volcano plots of differential methylation sites in pairwise comparisons of three groups were shown in **Supplementary Figures 10**, **11**. All the chip data had

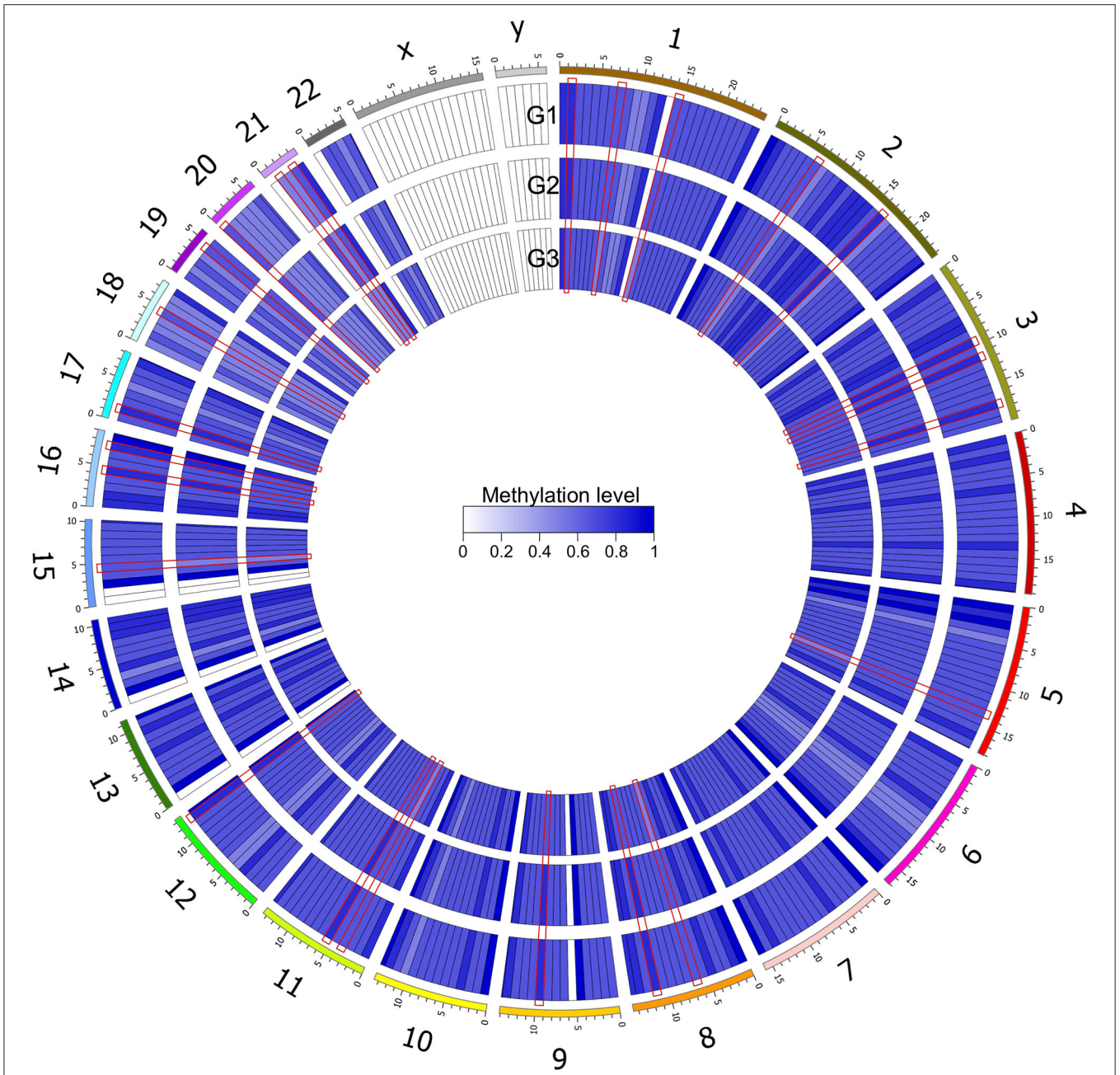

FIGURE 1 | Genome wide methylation level shown in human chromosomes. Taking 10 MB as the sliding window, the average methylation level of each window was calculated. The color depth indicated the average methylation level of the region. G1, HMAQ group; G2, LMAQ group; G3, Health control group.

been uploaded in GEO database (GSE154971). After all DMRs in the pairwise comparisons of the three groups were entered into the GO analysis and KEGG pathway analysis, the top 30 categories of the GO analysis and enrichment analysis results of methylation chip data are shown in **Figures 2**, **3**. The top 30 pathways of the KEGG pathway analysis results of methylation chip data are shown in **Figure 4**. After the non-specific pathways were removed, there were seven pathways associated with the nervous system or neuronal function in the KEGG pathway analysis results of the methylation chip data, as shown in **Table 3** and **Figure 5**.

After the DMP results were screened following the strategy described in the Methods section, 78 significantly differentially methylated genes remained. There were 32 significantly differentially methylated genes (15 genes with downregulated methylation levels and 17 genes with upregulated methylation levels) between HMAQ patients and healthy controls, 21

FIGURE 2 | The GO classification analysis results of methylation chip data. The top 30 categories of GO classification analysis results of Methylation chip data were showed. The neural function related classifications with interest of authors were highlighted in red.

significantly differentially methylated genes (12 genes with downregulated methylation levels and nine genes with upregulated methylation levels) between LMAQ patients and healthy controls, and 25 significantly differentially methylated genes (14 genes with downregulated methylation levels and 11 genes with upregulated methylation levels) between HMAQ patients and LMAQ patients, as shown in **Figure 6**.

After further literature review and gene functional analysis, SLC1A6, BHLHB9, LYNX1, CAV2, and PCSK9 were selected for MethyLight qPCR verification with a large sample, and they all had significant differences in the pairwise comparisons of methylation chip data among the HMAQ group, LMAQ group, and healthy control group.

Comparison of Methylation Levels of SLC1A6, BHLHB9, LYNX1, CAV2, and PCSK9 in the Three Groups

MethyLight qPCR verification of DNA methylation results of the five target genes indicated significant differences in the number of methylated copies of CAV2, BHLHB9, LYNX1, PCSK9, and SLC1A6 among the three groups (**Table 4** and **Figure 7**). Pairwise comparisons indicated that the number of methylated copies of CAV2 was significantly higher in the LMAQ group than in the HMAQ group, while it was significantly lower than that in the healthy control group. The number of methylated copies of BHLHB9, LYNX1, PCSK9, and SLC1A6 was not significantly different between the HMAQ group and the LMAQ group. However, the number of methylated copies of BHLHB9 and LYNX1 was significantly higher in both the HMAQ group and the LMAQ group than in healthy control group, while the number of methylated copies of PCSK9 and SLC1A6 was significantly lower in both the HMAQ group and the LMAQ group than in the healthy control group.

Correlation Analysis of Candidate Gene Methylation Levels With MA Abuse Characteristics in the Three Groups

The number of methylated copies of the CAV2 gene was significantly associated with age and the total duration of drug abuse in the LMAQ group. The number of methylated copies of the BHLHB9 gene was significantly correlated with the age of first drug abuse and the total duration of drug abuse in the LMAQ group. No other correlation was identified in the HMAQ group or the healthy control group (**Table 5**).

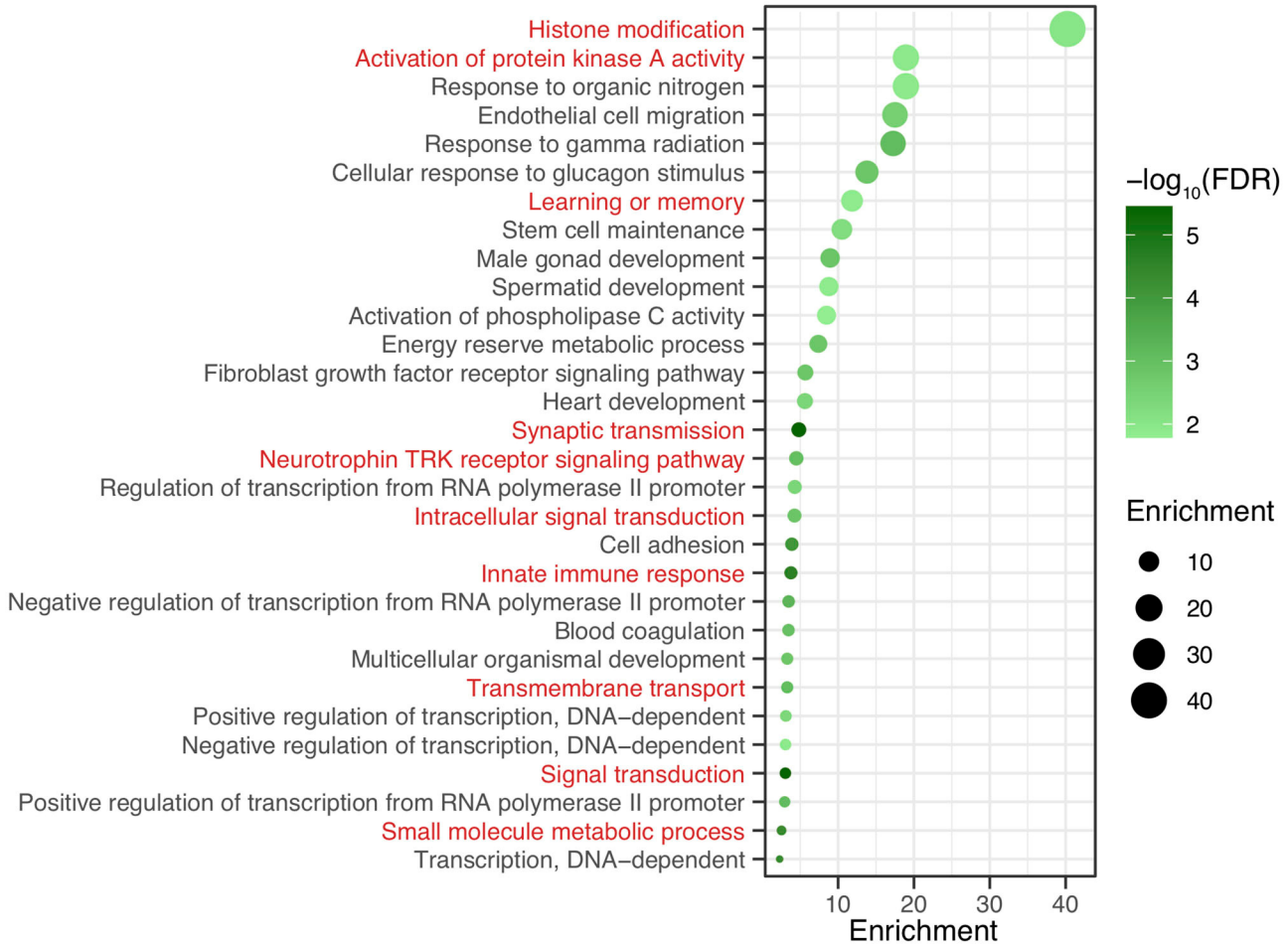

FIGURE 3 | The GO Enrichment analysis results of methylation chip data. The top 30 categories of GO Enrichment analysis results of Methylation chip data were showed. The neural function related enrichments with interest of authors were highlighted in red.

DISCUSSION

To the best of our knowledge, this is the first study to examine genome-wide DNA methylation in patients who abuse MA. The main findings of this study included (1) the important role of the circadian entrainment pathway in the pathogenesis of MA addiction inferred by the analysis of DMR data and (2) the important role of CAV2 in different qualities mechanisms of MA addiction revealed by DMP data.

Analysis of DMR Data Inferred the Role of the Circadian Entrainment Pathway in the Pathogenesis of MA Addiction

In this study, all DMRs of the genomic DNA methylation chip data in the pairwise comparisons of the three groups were included in the KEGG pathway analysis, which showed that seven specific pathways were statistically significant. Moreover, all seven pathways, including the Circadian entrainment (23), Cholinergic synapse (24), Glutamatergic synapse (25), Retrograde endocannabinoid signaling (26), GABAergic synapse (27), Morphine addiction (28), and PI3K-Akt signaling pathways (29), were identified to be associated with MA addiction or reward circuits.

The most significant finding of our current study was that the circadian entrainment pathway, which plays an important role in helping organisms cope with dynamic environmental changes and maintain survival with adaptive behavior, was involved in susceptibility to MA addiction (30). In mammals, the circadian entrainment pathway consists of two parts. One is a light signal mechanism that converts light signals into glutamic acid and pituitary adenylate cyclase activating peptide (PACAP) signals and stimulates the suprachiasmatic nucleus (SCN) of the hypothalamus to form a circadian rhythm. Then, the SCN connects the auxiliary oscillators of the whole brain and the body through peptide and neurotransmitter signals (including dopamine, glutamic acid, gamma-aminobutyric acid, etc.) and regulates the physiology and behavior of organisms in a 24-h cycle with molecular clocks throughout various cells throughout the body (31). Second, in the non-optical signaling mechanism,

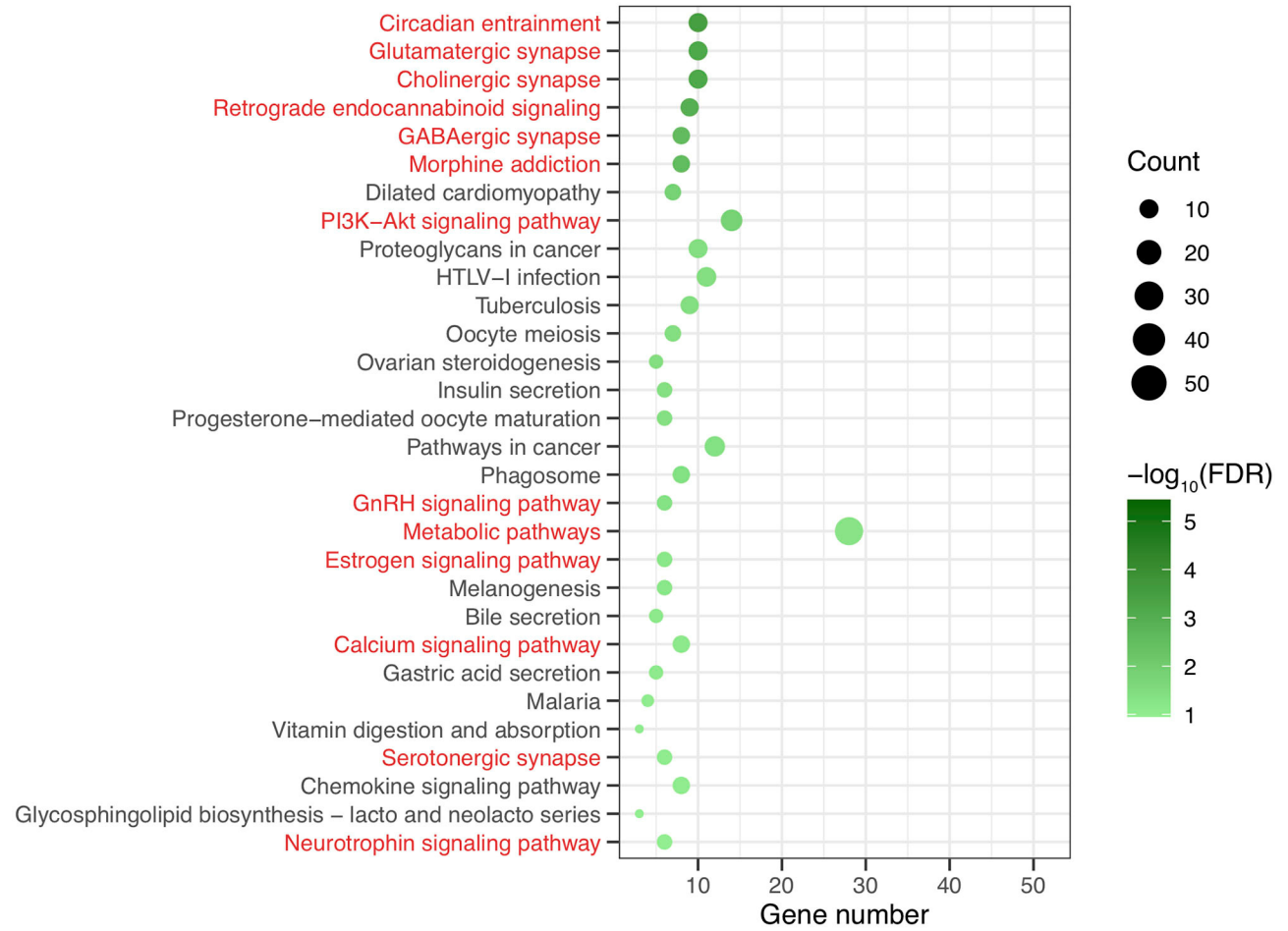

FIGURE 4 | The KEEG pathway analysis results of methylation chip data. The top 30 pathways of KEEG pathway analysis results of Methylation chip data were showed. The neural function related pathways with interest of authors were highlighted in red.

melatonin inhibits light-induced circadian rhythms by binding to melatonin receptors (MT1, MT2) to inhibit adenylate cyclase (AC) (32).

The circadian rhythm transcription factors are also deeply involved in the mechanism of the reward pathway. The SCN directly sends gamma-aminobutyric acid (GABA)-ergic projections to connect the lateral habenular nucleus (LHb), dorsomedial hypothalamic nucleus (DmH), medial anterior optic area (mPOA) and paraventricular thalamic nucleus (PVT), which can affect reward circuits through indirect neural connections (33). The crucial brain structural regions in the reward circuits, such as the ventral tegmental area (VTA), nucleus accumbens (NAc), prefrontal cortex (PFC), hippocampus (Hipp) and amygdala (Amy), have been shown to express proteins related to clock regulation (circadian transcription factors) (34) and non-clock regulation (melatonin receptors) (32) to maintain circadian rhythm stability and control emotion and reward behavior through neural circuits and molecular mechanisms (35).

Further, circadian rhythm transcription factors play an important role in the pathogenesis of substance addiction disorder. First, genome-wide association studies (GWASs) have shown close relationships between polymorphisms and other mutations in the circadian rhythm core genes and the pathogenesis of addiction disorder (35). Second, circadian rhythm disorders increase susceptibility to addiction. Repeated light/dark conversion can increase alcohol intake (36) and the preference for MA (37) in pre-exposure-treated rats. In humans, epidemiological studies reported that shift work or jet lag was associated with increased smoking and alcohol consumption (38, 39). Third, substance abuse can alter the functional state of the circadian entrainment pathway. Acute or chronic substance abuse in rodents has been found to alter the expression of circadian rhythm genes, including per1, per3, cry1, bmal1, npas2, and clock, in brain areas such as the NAc, dorsal striatum and hippocampus (40). Finally, manipulation of circadian rhythm genes can significantly alter dopaminergic signaling and reward-related behavior. ClockΔ19 mice showed a high dopaminergic state characterized by increased tyrosine hydroxylase expression in the VTA region and increased dopamine release in the NAc region, with strong sensitization to cocaine, increased preference

TABLE 3 | The neural specific pathways of genome wide DNA methylation microarray in patients with different addictive qualities.

Pathway name	Diffgene count	Gene count	Enrichment	*P*-value	FDR
Circadian entrainment	10	97	8.30	1.54E-06	3.21E-04
Cholinergic synapse	10	113	7.12	6.38E-06	6.57E-04
Glutamatergic synapse	10	118	6.82	9.48E-06	6.57E-04
Retrograde endocannabinoid signaling	9	103	7.03	2.35E-05	1.22E-03
GABAergic synapse	8	90	7.15	6.85E-05	2.85E-03
Morphine addiction	8	93	6.92	8.71E-05	3.02E-03
PI3K-Akt signaling pathway	14	347	3.25	5.31E-04	0.02

Diffgene count: differentially methylated gene count.
All the DMRs of genome wide DNA methylation microarray in the pairwise comparisons of the three groups were entered into the KEGG pathway analysis, which showed that seven pathways were statistically significant (FDR < 0.05 and p < 0.05) and associated with nervous system or neuronal function.

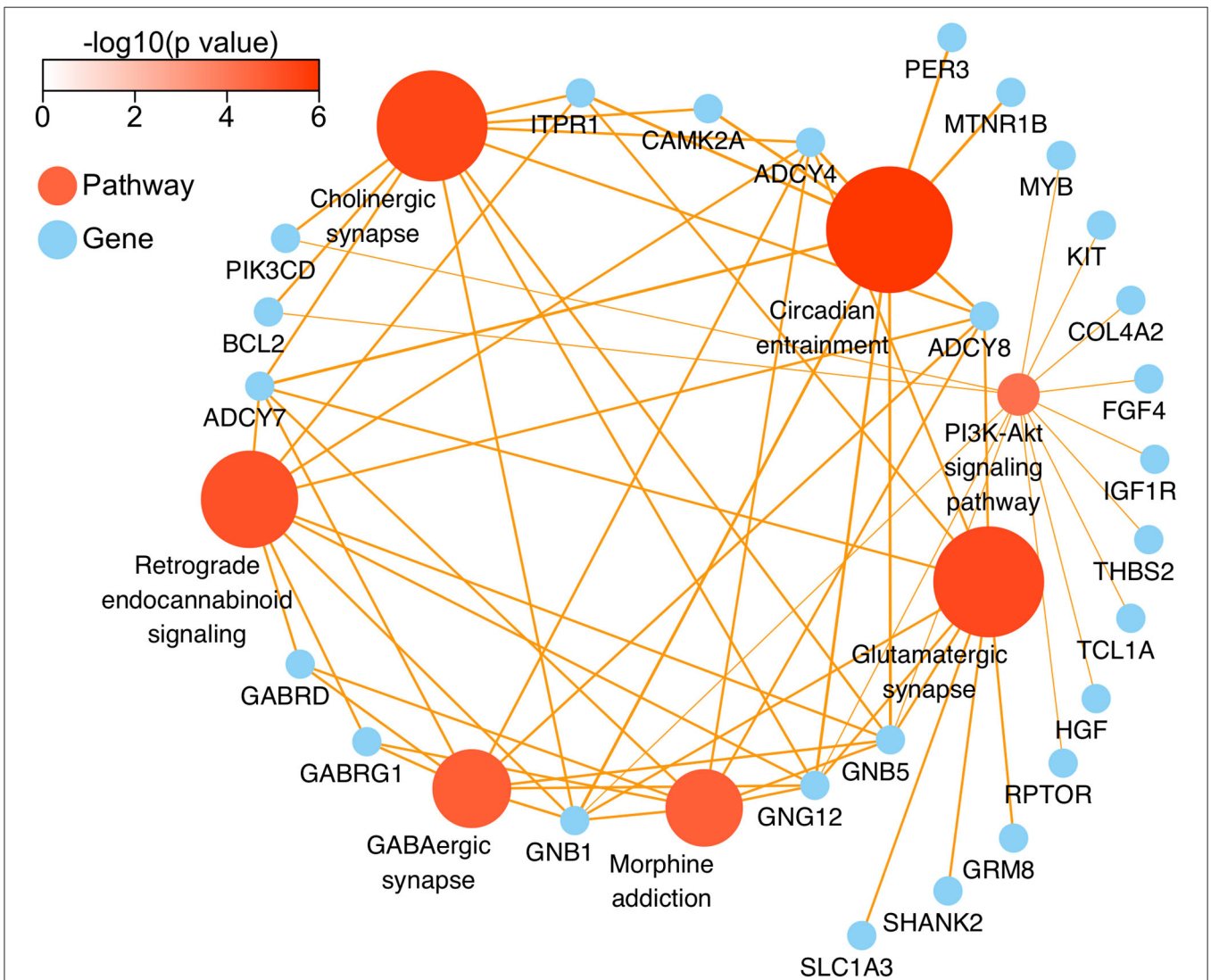

FIGURE 5 | The network diagram of top seven neural specific KEEG pathways of methylation chip data. The neural specific KEEG pathways with the top seven FDR values are shown as brown ball, which inconsistent size is related to their -$\log_{10}$(FDR), and the depth of brown is according to -$\log_{10}$(p). Their 26 enrichment genes are shown as sky-blue ball. With an orange color, the arrow pointing at the linked pathway, and the inconsistent thickness of the lines are also according to the Enrichment value.

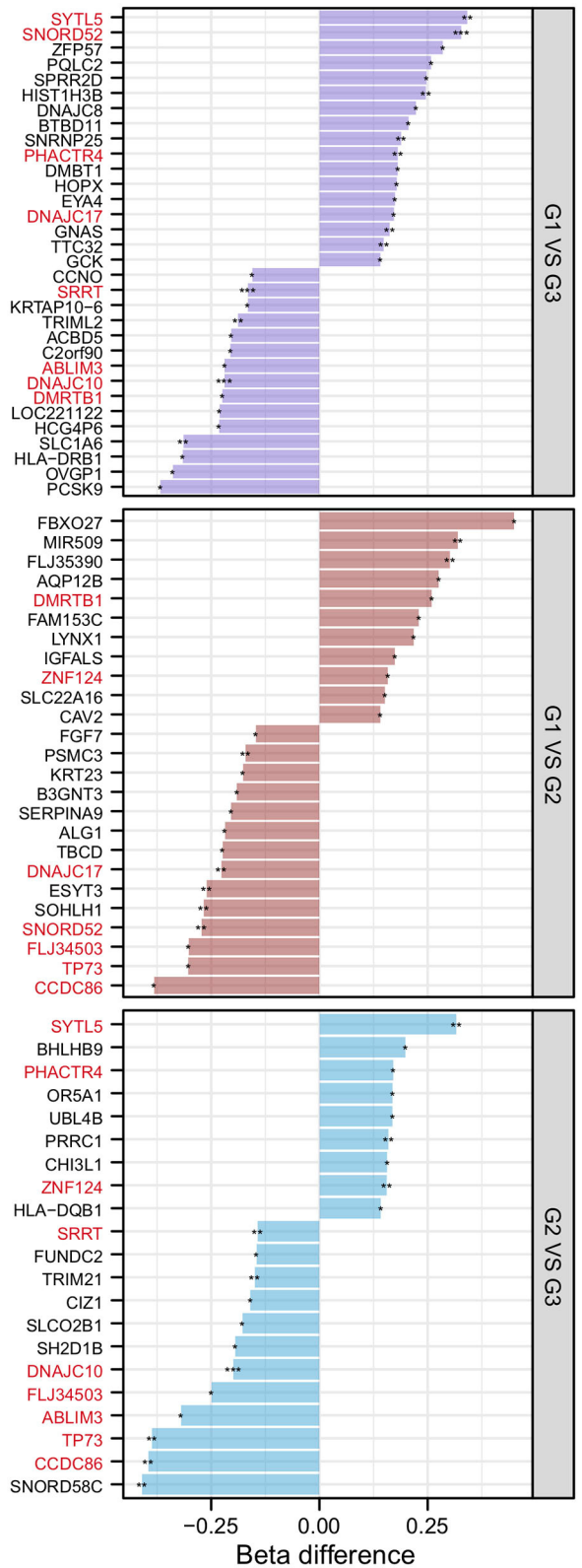

FIGURE 6 | The list of differential DNA methylation genes screened out from methylation chip data. After the DMP results were screened following the *(Continued)*

FIGURE 6 | strategy described in the Methods section, 78 significantly differentially methylated genes remained. Totaling 32 significantly differentially methylated genes (15 genes with downregulated methylation levels and 17 genes with upregulated methylation levels) between HMAQ patients and healthy controls, 21 significantly differentially methylated genes (12 genes with downregulated methylation levels and nine genes with upregulated methylation levels) between LMAQ patients and healthy controls, and 25 significantly differentially methylated genes (14 genes with downregulated methylation levels and 11 genes with upregulated methylation levels) between HMAQ patients and LMAQ patients.

for cocaine, increased goal-directed behavior, and increased motivation for the self-administration of cocaine (41).

Another interesting finding is that the results of KEGG pathway analysis inferred the role of the morphine addiction pathway, instead of amphetamine addiction pathway, in the pathogenesis of MA addiction. Georgiou et al. (28) reported chronic methamphetamine use and abstinence can induce brain-region specific neuroadaptations of the m-opioid receptor (MOR) in mice. Regional manipulation of MOR expression in NAc and VTA of mice may be a novel approach to differential modulate MA behavioral sensitization (42). Guo et al. (43) reported acute intragastrical administration of naltrexone, a non-selective opioid receptor antagonist, could significantly reduce the cue-induced drug-seeking behavior of rats induced by intraperitoneal injection of MA after extinction training. Also, we checked the pathway annotation and including genes, and found morphine addiction pathway related to GABAergic synapse pathway and dopaminergic synapse pathway, but amphetamine addiction pathway only related to dopaminergic synapse pathway. Our results also reported other related pathways included cholinergic synapse pathway, glutamatergic synapse pathway, and GABAergic synapse pathway. This situation implies that MA addiction is more complicated than only dopaminergic mechanism.

There are few studies on genome-wide methylation of addictive substances, especially methamphetamine, and they are scattered in brain tissue samples of animal models and human blood samples, so the results are difficult to be inconsistent. Only Desplats et al. (18) reported genome-wide profiling of DNA methylation in postmortem frontal cortex tissue of with HIV-1 infection and MA dependence patients showed differential methylation on genes related to neurodegeneration; dopamine metabolism and transport; and oxidative phosphorylation. Fonteneau et al. (14) reported a genome-wide DNA methylation study with cocaine self-administering rats treated with DNA methyltransferase inhibitor, and the Ingenuity pathway analysis (IPA) of differentially methylated genes from the PFCx showed Akt/PI3K pathway and Rho-GTPase family was involved in the plasticity of axonal growth, synaptogenesis, and spine remodeling, underlying the effect of DNA methyltransferase inhibitor on the observed behavioral changes. Akt/PI3K pathway also highlighted in our results.

Meanwhile, there are a few DNA methylation researches of candidate genes in people or animals addicted to MA. Yuka

TABLE 4 | Comparison of methylation copy numbers of the candidate genes in three groups (Unadjusted: ± *SD*; Adjusted: ± *SE*).

	HMAQ group ($n = 168$)		LMAQ group ($n = 39$)		Health control group ($n = 105$)		*F*	*p*
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted		
CAV2[a,b,c]	3.43E5 ± 5.08E5	3.45E5 ± 4.10E4	1.05E6 ± 9.83E5	1.05E6 ± 8.51E4	1.78E5 ± 2.65E5	1.77E5 ± 5.18E4	38.673	0.000
BHLHB9[b,c]	1.09E5 ± 6.42E4	1.09E5 ± 4.34E3	1.18E5 ± 7.39E4	1.18E5 ± 9.01E3	4.56E4 ± 2.69E4	4.56E4 ± 5.49E3	47.075	0.000
PCSK9[b,c]	2.39E6 ± 1.62E6	2.40E6 ± 1.41E5	1.85E6 ± 1.40E6	1.84E6 ± 2.93E5	5.89E6 ± 2.23E6	5.89E6 ± 1.78E5	136.373	0.000
SLC1A6[b,c]	3.11E4 ± 1.86E4	3.10E4 ± 2.01E3	3.03E4 ± 2.06E4	3.05E4 ± 4.18E3	7.05E4 ± 3.61E4	7.06E4 ± 2.55E3	80.477	0.000
LYNX1[b,c]	3.01E5 ± 1.59E5	3.01E5 ± 1.15E4	3.27E5 ± 2.33E5	3.26E5 ± 2.38E4	1.18E5 ± 6.85E4	1.18E5 ± 1.45E4	56.107	0.000

HMAQ group (high MA addictive quality group): MA abusers conformed to the diagnostic criteria of MA dependence according to the SCID-I/P with drug abuse frequency≥10 times and drug abuse time≥1 year; LMAQ group (low MA addictive quality group): MA abusers did not conform to the diagnostic criteria of MA dependence according to the SCID-I/P with drug abuse frequency≥10 times and drug abuse time≥1 year.

*No linear regression between the interaction of age * group and the methylation level of SLC1A6 ($F = 0.637$, $p = 0.530$), CAV2 ($F = 1.971$, $p = 0.141$), LYNX1 ($F = 0.004$, $p = 0.996$), PCSK9 ($F = 1.184$, $p = 0.308$), BHLHB9 ($F = 0.534$, $p = 0.587$), respectively were found. Covariance analysis with age as covariance was used to compare the methylation copy numbers of the candidate genes in three groups, and Bonferroni post-hoc test was used to do the pairwise comparisons.*

[a] *The pairwise comparison between HMAQ group and LMAQ group, $p < 0.05$.*

[b] *The pairwise comparison between HMAQ group and health control group, $p < 0.05$.*

[c] *The pairwise comparison between LMAQ group and health control group, $p < 0.05$.*

et al. (44) found that the rate of DNA methylation at six CpG islands of SHATI/NAT8L promoter sites is significantly higher in MA users when compared to healthy subjects. Jayanthi et al. (45) reported that a single prior injection of MA enhanced MA self-administration (SA) and blocked SA-induced changes in DNA methylation and mRNA expression of potassium channels in the rat nucleus accumbens. MA produces a variety of epigenetic effects in the brain, which are seminal to establish long-lasting alterations in neuronal activity. Exposure to high and/or prolonged doses of MA induced persistently demethylation within alpha-synuclein gene (SNCA) promoter, which matches the increase in alpha-synuclein protein. Demethylation was remarkable (10-fold of controls) and steady, even at prolonged time intervals being tested so far (up to 21 days of MA withdrawal) (46). MA-induced changes in long interspersed element-1 (LINE-1) partial methylation patterns are associated with MA-induced paranoia and could explain in part the pathophysiology of this type of psychosis. It is argued that MA-induced neuro-oxidative pathways may have altered LINE-1 partial methylation patterns, which in turn may regulate neuro-oxidative and immune pathways, which may increase risk to develop MA-induced paranoia (47). Veerasakul et al. (48) found that the methylation levels in the parvalbumin (PVALB) promoter were increased in MA-dependent patients using pyrosequencing, which might contribute to the GABAergic deficits associated with MA dependence. Hao et al. (49) reported that the methylation levels of the CHN2 gene, which encodes chimeric protein-2, which regulates axonal pruning, were increased in MA addiction patients. Nohesara et al. (29) demonstrated that DNA hypomethylation of the promoter regions of DRD3, DRD4, MB-COMT, and AKT1 was associated with increased expression of the corresponding genes in MA-dependent patients with psychosis and, to a lesser extent, in non-psychotic MA-dependent patients. Xu et al. (50) measured the methylation levels of five CpGs (CpG1–5) on the BDNF promoter using pyrosequencing, and only the CpG5 methylation level was found to be significantly lower in MA addicts, which played a key role in the regulation of BDNF gene expression. Moreover, significant associations were identified between CpG5 methylation and addiction phenotypes, including tension-anxiety, anger-hostility, fatigue-inertia, and depression-dejection.

Screening and the Validation of DMP Data Revealed the Role of CAV2 in Different Qualities Mechanisms of MA Addiction

After further literature review and gene functional analysis, SLC1A6, BHLHB9, LYNX1, CAV2, and PCSK9 were selected from the DMP results for MethyLight qPCR verification with a large sample. The results indicated that the number of methylated copies of CAV2 in the LMAQ group was significantly higher than that in the HMAQ group, while the number of methylated copies of CAV2 was significantly lower than that in the HMAQ group and the healthy control group.

The number of methylated copies of BHLHB9, LYNX1, PCSK9, and SLC1A6 was not significantly different between the HMAQ group and the LMAQ group. However, the number of methylated copies of BHLHB9 and LYNX1 was significantly higher in both the HMAQ group and the LMAQ group than in the healthy control group, while the number of methylated copies of PCSK9 and SLC1A6 was lower in both the HMAQ group and the LMAQ group than in the healthy control group.

In the LMAQ group, the number of methylated copies of the CAV2 gene was significantly correlated with age and the total duration of drug abuse. The number of methylated copies of the BHLHB9 gene was significantly correlated with the age of first drug abuse and the total duration of drug abuse in the LMAQ group. No other correlations were identified in the HMAQ group or the healthy control group. These five genes with abnormal methylation levels in MA abusers were all associated with neurological function and neurocognition.

It has been reported that only SLC1A6 and PCSK9 were associated with MA and/or ethanol abuse (51–54). SLC1A6 is

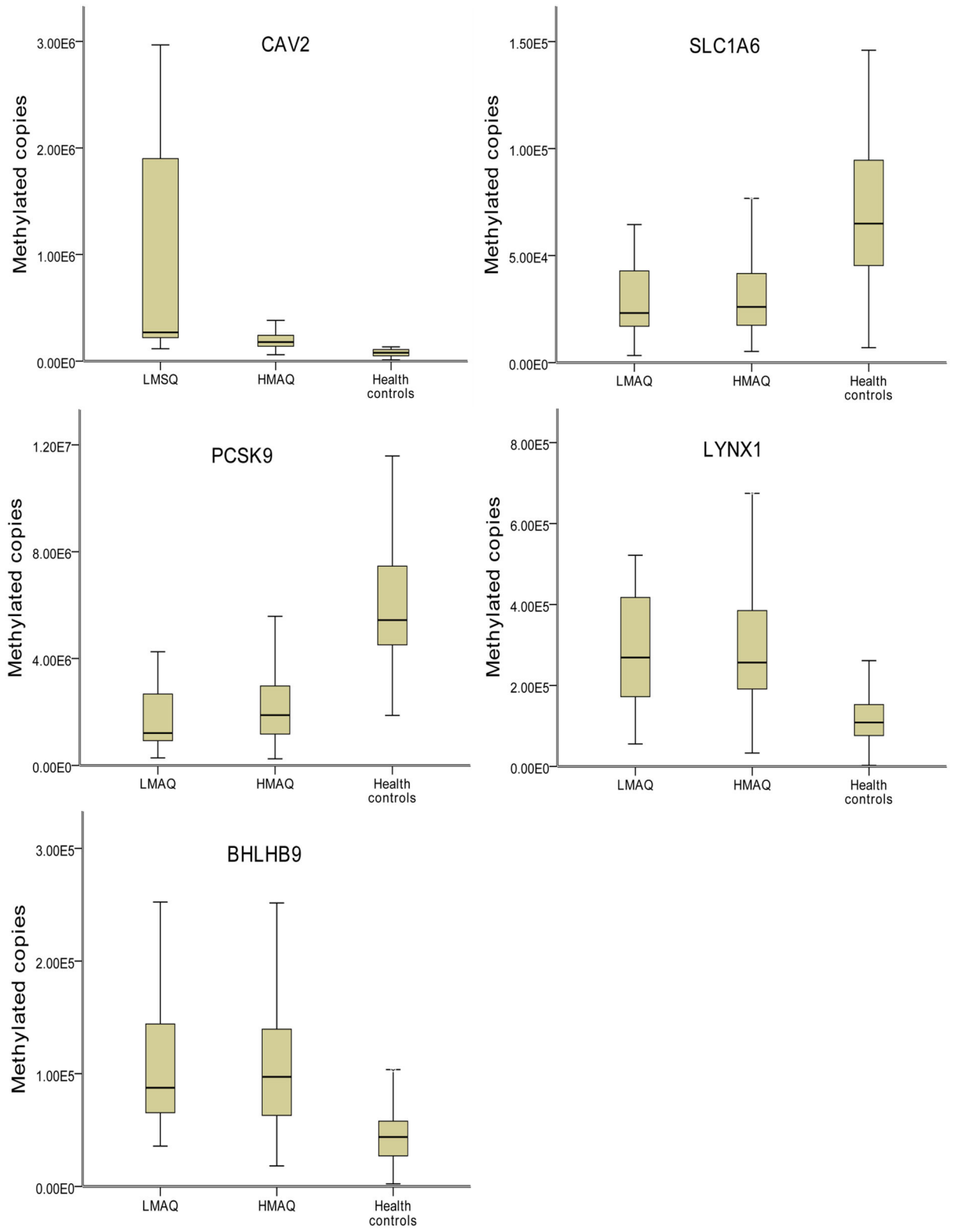

FIGURE 7 | Comparison of methylation levels of SLC1A6, BHLHB9, LYNX1, CAV2, and PCSK9 in three groups. Covariance analysis with age as covariance and Bonferroni *post-hoc* test as pairwise comparisons method showed that the significant differences in the methylated copies of CAV2, BHLHB9, LYNX1, PCSK9, and

(Continued)

FIGURE 7 | SLC1A6 in the three groups. The methylation copies of CAV2 were significantly higher in LMAQ than those in HMAQ groups, while the latter were significantly lower than those in healthy control groups. Methylation copies of BHLHB9, LYNX1, PCSK9, and SLC1A6 were not significantly different between the HMAQ and LMAQ groups. However, the methylation copies of BHLHB9 and LYNX1 were significantly higher in both HAMQ and LAMQ than those in healthy control groups, while the methylation copies of PCSK9 and SLC1A6 were significantly lower in both HAMQ and LAMQ than those in the healthy control groups.

also referred to as excitatory amino acid transporter 4 (EAAT4), a high-affinity glutamate transporter. The maintenance of low levels of extracellular glutamate and the avoidance of dose-dependent neurotoxicity can be achieved only by glutamate transporter-mediated cellular uptake, such as SLC1A6, because there is no extracellular glutamate metabolic system in humans (55). Szumlinski et al. (51) identified the hyperglutamatergic state within the nucleus accumbens as a biochemical trait that corresponds to both genetic and idiopathic vulnerability for high MA preferences and consumption. Alshehri et al. (52) found that sequential exposure to ethanol and MA produced an additive effect in the downregulation of glutamate transporter 1 (GLT-1, also EAAT2) in the striatum and hippocampus rather than the glutamate aspartate transporter (GLAST, EAAT1) and cysteine/glutamate antiporter (xCT). Pickering et al. (53) found significantly decreased correct alterations and increased time in the choice area at the Y-maze center, as well as decreased mRNA expression of SLC1A6 in the medial prefrontal cortex of male Wistar rats after 2.5 g/kg ethanol treatment and 5 days of intermittent treatment (53).

BHLHB9, also referred to as p60TRP (transcription regulator protein), is a member of the G-protein-coupled receptor (GPCR)-associated sorting protein (GPRASP) family. BHLHB9 can regulate the survival and differentiation of NGF-dependent neurons, and its expression level is decreased in the brains of Alzheimer's disease (AD) patients (56). BHLHB9, similar to GPRASP1, downregulates the expression of delta opioid receptor 1 (Oprd1) in neural stem cells (57). Mishra et al. demonstrated that BHLHB9 improved memory and learning abilities in BHLHB9 transgenic mice, which was attributed to increased synaptic connections and plasticity (57). Zhao et al. (58) reported that GPCRs interacted with β-site APP cleaving enzyme 1 (BACE1), a key secretase in AD pathogenesis. Moreover, the allosteric modulators and biased ligands of GPCRs displayed potential for the pharmaceutical treatment of AD patients, suggesting the possibility that GPCRs may be a therapeutic target for AD.

LYNX1 is a member of the Ly6/neurotoxin family associated with nicotinic acetylcholine receptors (nAChRs), which affect a wide array of biological processes, including learning and memory, attention, and addiction (59). Lynx1 modulates nAChR function *in vitro* by altering agonist sensitivity and desensitization kinetics. Dopaminergic cells of the substantia nigra pars compacta (SNc) and nearly all of the parvalbumin interneurons (GABAergic neurons) express lynx1 mRNA transcripts, whose dysfunctions are characteristics of psychiatric disorders (60). The titration of the lynx1 dosage can maintain neuronal health, while the lynx1 gene may participate in the trade-off between neuroprotection and augmented learning (61). Lynx1 prevents Aβ1-42-induced cytotoxicity in cortical neurons by competing with binding to nAChR subunits, and cortical Lynx1 levels are decreased in a transgenic mouse model with concomitant β-amyloid and tau pathology, which might have functional and pathophysiological implications in Alzheimer's disease (62).

PCSK9 is a member of the *Bacillus subtilis* protease (proprotein invertase) family, which is involved in the systemic control of blood cholesterol levels, neuronal differentiation, apoptosis, migration and nervous system development (63). Monoclonal antibodies that target PCSK9, a novel lipid-lowering approach, have recently been found to be accompanied by an increase in neurocognitive events (64). Picard et al. (65) found that PCSK9 expression levels in autopsy-confirmed AD brains were elevated in the frontal cortices compared to levels found in controls, both at the mRNA and protein levels. PCSK9 regulation seems to be under tight genetic control in females only, with specific variants that could predispose them to increased risk of AD. Hyperlipidemia regulates neuronal apoptosis in the hippocampal CA3 of mice by increasing PCSK9 and BACE1 expression, which may elucidate the role of lipid metabolism disorder in AD pathogenesis (66). A cross-tissue and cross-phenotypic analysis of genome-wide methylomic variation performed in alcohol use disorder (AUD) using samples from three discovery, four replication, and two translational cohorts indicated that replication in the AUD datasets confirmed PCSK9 hypomethylation. A translational mouse model of AUD showed that alcohol exposure caused a downregulation of PCSK9 (54).

CAV2, a membrane microdomain or "lipid raft," has emerged as an essential functional module of the cell that is critical for the regulation of nerve growth factor (NGF) signaling and subsequent cell differentiation (67). BDNF increased the level of CAV2 in the rafts of hippocampal neurons for synapse development (68). Single-cell gene expression profiles showed downregulation of CAV2, GNB4, and lipase A in AD Rac1b-positive/p75 (NTR)-labeled basal forebrain (CBF) nucleus basalis neurons (69). CAV2 is also involved in the binding of the dopamine D1 receptor and the positive regulation of the dopamine receptor signaling pathway. Only two studies have reported the relationship between CAV1 and addiction. CAV-1 plays an important role in morphine-induced changes in the structural plasticity of primary cultured mouse cortical neurons (70). The compulsive-like intake behavior of MA was associated with an enhanced expression of D1Rs, increased activity of ERK1/2, and a decrease in Cav-1 expression in the dorsal striatum (71).

There were several limitations in this study. First, the subjects who abused MA in this study were recruited from the

TABLE 5 | Correlation analysis of candidate gene methylation levels with methamphetamine abuse characteristics in three groups (*r*).

	HMAQ group (*n* = 168)			LMAQ group (*n* = 39)			Health control (*n* = 105)
	Age	Age of first drug abuse	Total drug abuse time (months)	Age	Age of first drug abuse	Total drug abuse time (months)	Age
CAV2	−0.101	0.105	−0.069	0.351*	0.308	0.405*	0.078
BHLHB9	0.103	0.129	0.145	0.288	0.368*	0.312*	−0.132
PCSK9	−0.014	0.004	0.156	−0.235	−0.191	0.134	0.046
SLC1A6	0.146	−0.098	0.009	0.117	0.129	−0.059	0.017
LYNX1	0.023	−0.044	0.104	−0.149	−0.057	−0.203	−0.129

*Methylation copies of the CAV2 gene were significantly associated with age and total duration of drug abuse in the LMAQ group. The methylated copies of the BHLHB9 gene were significantly correlated with the age of first drug abuse and the total duration of drug abuse in the LMAQ group. *$p < 0.05$.*

rehabilitation facilities in one province of China. They had not used MA for at least 3 months or even 2 years; hence, they were not acute users. Thus, there was a broad range of past frequencies and dosages of MA abuse. Second, in this study, we reported only the genome-wide DNA methylation status of MA abusers with different qualities to addiction and did not further explore its underlying mechanisms. Third, this is a DNA methylation research of peripheral blood cells of MA abusers. There are affirmative differences in the environment and differentiation between peripheral blood cells and neurons, but it is the only convenient method to study the DNA methylation alteration of alive MA abusers. Also, Uno et al. (72) reported that the ratios of Shati/Nat8l CpG island methylation were significantly decreased in both the nucleus accumbens and the peripheral blood of MA-induced murine models of schizophrenia-like phenotype compared with those of control mice. To our knowledge, this is the first study to show similar alterations of DNA methylation status in both the brain and peripheral blood of MA treated mice, which suggests the feasibility of this kind of research. For the next step, gene knockout or transgenic animals should be used to investigate the roles of circadian entrainment pathways and the caveolin-2 gene in the pathogenesis of MA addiction.

CONCLUSIONS

This study investigated the genome-wide DNA methylation status of different addiction qualities in MA abusers and identified seven specific pathways with abnormal methylation status, which were all reported to be associated with MA addiction or reward circuits, especially the circadian entrainment pathway, a relatively new pathogenesis. Moreover, CAV2 was the only gene among the five candidate genes screened from the genome methylation chip data that showed significant differences in methylation levels in all pairwise comparisons of the three groups. Further studies on their functions and mechanisms will help us to better understand the pathogenesis of MA addiction and to explore new targets for drug intervention.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in GEO database (GSE154971).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Second Xiangya Hospital. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from each individual for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

WH was responsible for the study design and supervision. LL, TLu, HD, CZ, and TLi had participated in the acquisition, analysis, and interpretation of the data. LL wrote a draft of the manuscript. XZ critically revised the manuscript for important intellectual content and provided the technical support. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2020.588229/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Is the Long-Term Use of Benzodiazepines Associated With Worse Cognition Performance in Highly Educated Older Adults?

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Background: Benzodiazepines (BZD) are common medications for sedative, hypnotic, and anxiolytic that are especially prevalent in older adults. Previous studies have shown that BZD use could impair users' cognition, significantly affecting their quality of life. Past research has shown that higher education might play a protective role in the process of cognitive decline. Very few studies had examined the cognitive effects of BZD on highly educated older adults. The study aimed to explore how cognitive functions would be affected by benzodiazepines among highly educated older adults.

Method: 140 older adults with an average education period of 14.8 years were included in this study. The subjects were divided into three separate groups, the long-term BZD users (≥180 days), short-term BZD users (<180 days), and non-users. Demographics and cognitive assessments for the three groups were analyzed using the analysis of variance (ANOVA), the chi-squared test, and the analysis of covariance (ANCOVA). To examine the association between BZD use and cognition a multiple linear aggression approach was used.

Result: All three groups were significantly different from each other when looking at executive functioning in the Trail Making Test B (TMT-B). Compared to the control group, short-term BZD users showed significant defects in TMT-B time ($p = 0.002$) and TMT-B errors ($p < 0.001$); long-term BZD users showed significant defect on TMT-B time ($p = 0.041$). Compared to short-term BZD users, long-term BZD users showed significant merit on TMT-B errors ($p = 0.001$). No significant differences were found in other cognitive tasks that reflected general cognition, verbal memory, language fluency, and visual memory. After adjusting for demographic, increased BZD use over time was positively associated with scores for the revised Brief Visuospatial Memory Test ($r = 0.377$, $p = 0.012$).

Conclusion: BZD use may be significantly associated with worse executive functioning in highly educated older adults. However, there is no association between the duration of BZD use and increased cognitive deficits in highly educated older adults. This study

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identified future experimental directions including potential longitudinal studies, within-subject studies comparing mood disorder patients' cognitive performance before and after onset of BZD use, and between-subject studies that directly compare BZD's effect on subjects with the same baseline of cognitive functioning.

Keywords: cognition, benzodiazepines, elderly, education, sedative-hypnotics

INTRODUCTION

Benzodiazepines (BZD) are a class of psychoactive drugs that can enhance the binding of the neurotransmitter gamma-aminobutyric acid (GABA) on the GABA-A receptor, resulting in sedative, hypnotic, and anxiolytic effects (1). BZDs have been used for the treatment of a wide range of clinical conditions such as anxiety, insomnia, and alcohol dependence. Treatment using BZD has increased over time, with a significant number of older adults being prescribed BZD as their main treatment option (2). Increased prescribing of BZD is due to the high prevalence of pain, anxiety, and insomnia in this age group (2, 3). Research has suggested that BZD use was positively correlated with age (4, 5), with approximately 9–12% of old adults in the United States using BZD (6). Developed countries have reported a higher prevalence of BZD use with roughly 7–43% of older adults using BZD (7). Globally, BZD use is approximately 10–42% older adults (8–10).

In recent years, several studies have shown that BZD use may impact the cognitive functioning of older adults, but studies varied in the cognitive domains observed and the participant's demographics. A cohort study by Paterniti et al. (11) using 1,389 old adults with an average education level of 10 years examined the relationship between BZD use and cognitive functioning. The results showed that long-term BZD users were at a higher risk for reduced performance in general cognition tasks for executive functioning than non-BZD users (11). A study by Bierman et al. (12) also suggested that general cognition, executive functioning, and memory in older adults was negatively affected by BZD use. Participants utilized in this study had an average age of 69 years with varied education levels (60.3% participants with low-level education, 27.3% participants with medium-level education, 14.4% participants with high-level education) (12). However, research has also suggested that there are no significant changes in cognitive functioning following BZD use in older adults. Research conducted by Hanlon et al. found that performance on cognition tasks was not associated with BZD use in individuals over 65 with different education levels (33.7% participants $<$8 year, 36.7% participants 8–12 year, 29.6% participants $>$12 year) (13). Zhang et al. found no evidence to suggest a relationship between BZD use and decline in general cognition in older adults with an average education level of 15 years (14). Consistently, Grossi et al. found no significant association between BZD use and dementia among older adults with 10 years of education (15). To sum up, research on the impact of BZD on the cognitive function in older adults were inconsistent with the large heterogeneity for demographics.

During the process of aging, structural, and functional neurological changes that occur renders older adults more vulnerable to risk factors such as psychotropic drugs, stress, and negative mental health states. However, higher education level is a protective factor of age-related cognitive decline. It has been widely accepted that higher education is consistently related to better cognitive performance in the older adults (16, 17). The protective effect of high education on cognition can be explained by the cognitive reserve hypothesis (18, 19), which suggests that people with higher cognitive reserves are more resilient to age-related neurological changes, through the maintenance of more intact cognitive functioning (20). Evidence suggests that a higher educational level is associated with more cognitive reserve (21). With the development of the economy, there was a worldwide expansion of higher education since the twentieth century (22), which led to an increased population of highly educated older adults. Based on the popularity of BZD prescription in the older adults, there would be more highly educated individuals taking BZD than ever. Research focusing on BZD's cognitive effects on highly educated older adults may provide not only evidence for the security of BZD, but also guidance on clinical application in medicine. Moreover, it may provide a new perspective to explore the protective effect of education on cognition, allowing researchers to further examine the cognitive reserve theory.

Unfortunately, very few studies have examined the effect of BZD on cognition, especially for specific cognitive domains, such as memory and executive function, of highly educated older adults. Moreover, the literature currently lacks information on the cognitive effects of BZD in highly educated older adults, and if the duration of BZD use plays a role in cognitive performance. The purpose of this study is to explore if the cognitive function of highly educated older adults is associated with BZD use and how the cognitive effect changes with the duration of BZD use. If higher education level has protective effects on cognitive performance, we expect to see a decrease in lower cognitive performance in the BZD use groups compared to the non-use group. As well, we expect that as BZD use duration increases, there will be no significant decline in cognitive scores as BZD.

METHOD

Subjects

Participants were recruited from inpatient, outpatient, and annual medical examination services at the First Affiliated Hospital of China Medical University, Shenyang, China from May 2019 to December 2019. This study was approved by the ethics committee in the First Affiliated Hospital of China Medical

TABLE 1 | Demographic information of the participants.

	Comparison group ($n = 47$)	Short-term using group ($n = 46$)	Long-term using group ($n = 47$)	*P-value*
Sex, male/female	30/17	31/15	28/19	0.735
Age, year, mean ± SD	71.40 ± 8.40	74.93 ± 10.78	75.06 ± 10.25	0.129
Education, year, mean ± SD	15.47 ± 2.67	14.11 ± 3.76	15.07 ± 2.92	0.103
Smoke, yes/no	5/42	5/41	7/40	0.778
Drink, yes/no	6/41	3/43	5/42	0.348
BMI, kg/m^2, mean ± SD	24.30 ± 2.73	24.15 ± 2.60	23.00 ± 3.06	0.036*
Cerebrovascular disease, yes/no	17/30	20/26	25/22	0.249
Hypertension, yes/no	29/18	31/15	29/18	0.806
Coronary heart disease, yes/no	17/30	22/24	22/25	0.232
Diabetes, yes/no	19/32	12/34	18/29	0.295

*BMI, Body Mass Index; *P < 0.05.*

University. All individuals used in this experiment signed the informed consent.

The inclusion criteria were (1) aged 60 or older, (2) with a more-than-or-equal-to a high school education level. Participants were excluded if (1) they met the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V) diagnostic criteria for Schizophrenia Spectrum and other Psychotic Disorders, and Neurocognitive Disorders, (2) were in a state of anxiety (the Self-Rating Anxiety Scale, SAS score≥20) (23) or depression (Geriatric Depression Scale, GDS scores≥15) (24), (3) were taking drugs that affect cognitive performance such as cholinesterase inhibitors and memantine, (4) were in other statuses of abnormal cognition such as head trauma, and (5) were unable to complete cognitive assessments such as severe visual or hearing impairment, illiteracy, or color blindness. These exclusion criteria were set to ensure that the participants were not subject to other conditions that might confound cognitive performance. After screening, 140 individuals aged more than 60 years (females $N = 51$) were included in the study.

Procedure

Screening

After signing the consent form, participants were evaluated for anxiety and depression symptoms using the SAS and GDS. BZD users are more likely to experience depression and anxiety than the non-users (25). However, depression and anxiety can significantly affect cognitive test performance, confounding the result of the study (26). To reduce the influence of depression and anxiety on the cognition results in this study, participants with moderate symptoms of anxiety or depression, reflected by scores of SAS score of ≥20 and GDS scores of ≥15, respectively, were excluded. Participants not in a state of anxiety or depression then participated in face-to-face interviews to collect demographic and clinical information including age, sex, education, height, weight, smoking status, drink status, medication information, and chronic disease history. Participants' heights and weights were collected to calculate the body mass index (BMI). The information on medication and chronic disease history was supplemented by the electronic medical record system of the First Affiliated Hospital of China Medical University and the physical examination system of the geriatrics department of the First Affiliated Hospital of China Medical University. A total of 140 participants remained after screening with the exclusion criteria. The demographic and clinical information of the included participants are presented in **Table 1**.

BZD Use Groups and Controls

This study included all classes of benzodiazepines: anxiolytic (N05BA), hypnotic and sedative (N05CD), antiepileptic (N03AE), and myorelaxant (M03BX07). Hypnotic drugs derived or connected with benzodiazepines (N05CF) were also included. Based on the self-report medication history obtained in the interviews and prescription record in the electronic medical record system, we converted the accumulated BZD dosage and average daily BZD dosage of each participant into diazepam equivalents (27). Based on the BZD use behavior provided in the participants medical information, the participants were divided into three BZD use groups: long-term BZD users ($N = 47$, BZD using time ≥180 day), and short-term BZD users ($N = 46$, BZD using time <180 day), and non-users ($N = 47$, never used BZD) (28).

Cognitive Performance Tests

The cognitive function evaluation process consisted of six cognitive tasks reflecting general cognition and four subdomains of cognition: memory (visual memory and verbal memory), attention, executive function, and verbal fluency. Cognitive tasks were selected based on high credibility among older adults in China (29).

The Mini-Mental State Examination test (MMSE) (30, 31) is a widely used cognition evaluating instrument. It includes the assessment of multiple cognitive dimensions. The test was used to assess the participants' general cognitive levels and excluded dementia patients.

The Rey Auditory Verbal Learning Test (AVLT) (32, 33) has multiple measures of memory and was easy to apply. This test was used to evaluate the participants' functions of verbal memory.

The Trail Making Test (TMT) (33, 34) is a commonly used executive function detection tool and plays an auxiliary role in the identification of dementia and early stage of

TABLE 2 | BZD use information of the participants.

	Short-term BZD users ($n = 46$)	Long-term BZD users ($n = 47$)
Accumulated BZD using dosage, diazepam equivalent, mean ± SD	0.50 ± 0.61	0.92 ± 1.53
Average BZD using dosage, diazepam equivalent, mean ± SD	31.27 ± 38.06	955.06 ± 1578.98
BZD using time, d, mean ± SD	65.72 ± 42.58	1221.23 ± 1556.04

cognitive impairment. TMT-A assesses attention and visuomotor processing speed, while the TMT-B assesses attention and visuomotor processing speed, visual-motor ability, and working memory (35, 36).

The Controlled Oral Word Association Test (COWAT) (37, 38) is a widely used verbal fluency test. The purpose of the test is to evaluate the spontaneous production of words within a limited amount of time. This test is sensitive to language function.

The Digit Span Test (DST) (33, 39) is often used to test short-term attention function and is widely used in geriatrics research.

The Brief Visuospatial Memory Test-Revised (BVMT-R) (40, 41) are widely used tests of visual memory in both clinical and research settings.

Data Analysis

IBM SPSS Statistics for Windows, Version 22 was used for all analyses. The analysis of variance (ANOVA) tests were used to compare the continuous demographic variables. Chi-square tests were used to compare the classified demographic variables and chronic conditions history of disease among the two BZD use groups and the control group. These statistical tests on the demographic information aimed to identify potential covariates among the three BZD use groups. BZD use information was determined with descriptive statistics. In order to evaluate the cognitive performance among the three BZD use groups, a comparison of cognitive task results was conducted using analysis of covariance (ANCOVA) was performed with BZD use group as a fixed effect adjusting for the demographic variables. Furthermore, to investigate whether BZD use duration can predict cognitive performance, we conducted a bivariate analysis and a multiple linear regression analysis within the long-term and short-term BZD use groups using participants' BZD use duration and cognitive tasks results. The covariates included in the model were age, gender, education, and BMI. A significance level of 0.05 was used for all tests of significance.

RESULT

The descriptive demographic information is shown in **Table 1** and the BZD use is shown in **Table 2**. A total of 140 subjects were included in this study: 51 females and 89 males. The average age of all subjects was 73.79 ± 9.94 years, and the average number of years in education was 14.88 ± 3.19 years. The three groups were matched for gender, age, education years, smoking, drink, and history of chronic diseases. There was a significant difference between groups in the BMI ($p = 0.036$). Compared to the non-users and short-term BZD users, the BMI of the long-term BZD use group is significantly lower. Among the 93 BZD users, the average accumulated dose of BZD users was 498.13 diazepam equivalent, the average daily dose of BZD users was 0.71 diazepam equivalent, and the average duration of medication was 649.69 days. Seventeen BZD users took BZD because of anxiety and the other 76 participants use it for insomnia.

The results of the ANCOVA for differences in cognitive tasks performance with BMI as a covariate is shown in **Table 3**. The three groups showed significant differences in the performance of TMT-B time and TMT-B errors, but no significant differences in the performance of other tests. The post-multiple comparisons results are shown in **Figure 1**. For the result of TMT-B time, the completion time of non-users was 187.28 ± 71.61 s, which was significantly faster than 249.36 ± 94.53 s of short-term users ($p = 0.002$) and 227.48 ± 113.52 s of long-term BZD users ($p = 0.041$). As for TMT-B errors, the number of errors of short-term BZD users was 1.85 ± 1.86, which was significant higher compared to both the non-users (number of errors = 0.64 ± 0.96, $p < 0.001$) and the long-term users (number of errors = 0.87 ± 1.24, $p = 0.001$).

Consistent with our prediction, multiple linear regression analyses showed no significant decline in the cognitive tests of MMSE, AVLT, TMT, COWAT, and DST with longer duration of BZD use. Interestingly, as shown in **Table 4**, the duration of BZD use was positively correlated to better performance in the BVMT-R N1 test ($r = 0.235$, $p = 0.023$). After adjusted for demographic variables, the BVMT-R N1 scores of BZD users were still correlated with BZD over time ($r = 0.377$, $p = 0.012$).

DISCUSSION

In this study, we explored the association between BZD use and the cognitive functioning of highly educated older adults by comparing performance in cognitive tasks for long-term BZD users, short-term BZD users, and non-users. Within our study, the two BZD use groups and the control group were matched in most demographic information, including age, gender, education, smoking status, and drinking status. There was, however, a significant difference in the BMI of the three groups, with BZD user groups having the lowest BMI scores. This is consistent with participants' demographic information in a previous study on BZD use (42). With the BMI value as a covariate, the main results of this study include: (1)There were significant differences in the executive function of TMT-B test among the three groups, while there were no significant differences in the scores of MMSE reflecting general cognition, AVLT reflecting verbal memory, COWAT reflecting language fluency, DST reflecting attention, and BVMT-R reflecting visual

TABLE 3 | Results of Analysis of Covariance (ANCOVA) of differences in the cognitive tasks results with BMI as covariate.

Test	Variable	Non-users	Short-term BZD users	Long-term BZD users	*F*-value	*P-value*
MMSE	Total scores	27.48 ± 2.64	25.90 ± 4.79	27.30 ± 2.60	1.71	0.186
AVLT	AVLT N1	3.98 ± 1.53	3.43 ± 1.80	3.51 ± 1.60	1.18	0.311
	AVLT N2	5.30 ± 1.60	5.11 ± 2.08	4.91 ± 1.91	0.49	0.612
	AVLT N3	6.40 ± 1.88	6.09 ± 2.50	5.77 ± 1.90	1.06	0.349
	AVLT N4	4.94 ± 2.65	4.00 ± 2.55	4.11 ± 2.43	1.53	0.221
	AVLT N5	19.80 ± 5.33	18.96 ± 5.57	19.64 ± 3.91	0.17	0.841
TMT	TMT A time	73.49 ± 51.85	91.08 ± 52.17	83.15 ± 57.77	0.62	0.542
	TMT A errors	0.62 ± 2.27	0.61 ± 0.99	0.64 ± 1.17	0.12	0.886
	TMT B time	187.28 ± 71.61	249.46 ± 94.53	227.48 ± 113.52	5.23	0.006*
	TMT B errors	0.64 ± 0.96	1.85 ± 1.86	0.87 ± 1.24	9.68	0.000*
COWAT	COWAT 15s	8.67 ± 2.76	9.48 ± 3.34	8.81 ± 2.43	1.24	0.293
	COWAT 60s	19.56 ± 4.98	19.23 ± 4.08	18.95 ± 5.31	0.18	0.836
DST	DST forwards	12.41 ± 2.23	11.39 ± 1.88	11.82 ± 2.37	2.88	0.059
	DST backwards	5.34 ± 1.15	4.89 ± 1.26	4.92 ± 1.42	1.80	0.169
BVMT-R	BVMT-R N1	5.73 ± 1.82	5.22 ± 1.57	5.15 ± 2.24	1.31	0.273
	BVMT-R N2	8.09 ± 2.48	7.83 ± 2.74	6.98 ± 2.55	1.88	0.158
	BVMT-R N3	9.21 ± 2.74	9.09 ± 2.86	7.95 ± 2.82	2.19	0.118

**Result statistically significant; MMSE, Mini-Mental State Examination; AVLT, the Rey Auditory Verbal Learning Test (AVLT N1-N4 = the number of words the participant can repeat correctly each time, AVLT N5 = the number of words the participant can repeat correctly 5 min after N4); TMT, the Trail Making Test; COWAT, the Controlled Oral Word Association Test (COWAT 15 s/60 s = the number of words the participant can speak in 15 s/60 s); DST, the Digit Span Test; BVMT-R, the Brief Visuospatial Memory Test-Revised (BVMT N1-N3 = the scores the participant can gain each time).*

memory; and (2) Within highly educated older adults using BZD, the duration of medication was significantly correlated with a higher BVMT-R N1 score.

Comparing to other studies on BZD use and cognitive functioning, some demographic data of our study were inconsistent. For example, some previous studies included more women in the BZD use group than the non-use group (5, 6). In our study, there was no significant difference in gender distribution between the BZD use and non-use groups. There are two possible reasons for this discrepancy. The small sample size of our study might not accurately reflect the demographic attributes of the entire patient population. Another possible explanation for the inconsistency may be related to the specific participants group in our study. It is likely that the population of highly educated adults has the same sex ratio in the subgroups of BZD short-term users, BZD long-term users, and non-users. Therefore, the restriction to high education in sample selection reduced the difference of demographic information between the BZD condition groups.

BZD Use and the Altered Cognition Domains in Highly Educated Older Adults

In the ANCOVA result for differences in cognitive tasks, the three groups only showed significant differences in the completion time and number of errors in the TMT-B test. Previous research has shown that although TMT-B time and TMT-B errors can both reflect participants' executive function, TMT-B time has a stronger correlation with visual movement, while the performance of TMT-B errors has a stronger correlation with working memory (35, 36). In the regression analysis, there was a significant correlation between BZD using time and BVMT-R N1 score reflecting immediate visual memory. These results suggest that the effects of BZD use on the cognitive function of highly educated old adults may be mainly manifested in the immediate visual memory function and executive function including visual motor and working memory.

Although there has been no previous research on the specific cognitive domains affected by BZD use in highly educated older adults, some research on general aged BZD users has reported that the cognitive effects of BZD exist in a variety of cognitive domains including executive function and visual memory (14, 43–48). A 7-year cohort study in France by Mura et al. (44) suggested that there was a significantly associated between BZD use and lower performance of immediate visual memory and executive function, but no significant association between BZD use and lower global cognitive function in older adults. In their study, 29.6% non-users and 33.8% BZD users had 9–12 years of education, and 40.9% non-users and 32.1% chronic users had at least 12 years or more of education (44). In addition, some studies' results were not entirely consistent with our findings. Ros-Cucurull et al. found that auditory memory function and visual-spatial ability of BZD users were also significantly impaired compared to non-users with 75% of participants' education level fewer than 10 years (45). In a study with participants with an average education level of <9 years, Helmes and Ostbye found that auditory memory functioning in BZD users was significantly impaired compared with that of non-users (47). These inconsistencies may be related to the high educational level of the participants in our study (14.88 ± 3.19 years). The inconsistency across studies of cognitive performance tests in highly educated older adults may be because of the protective effects of higher education

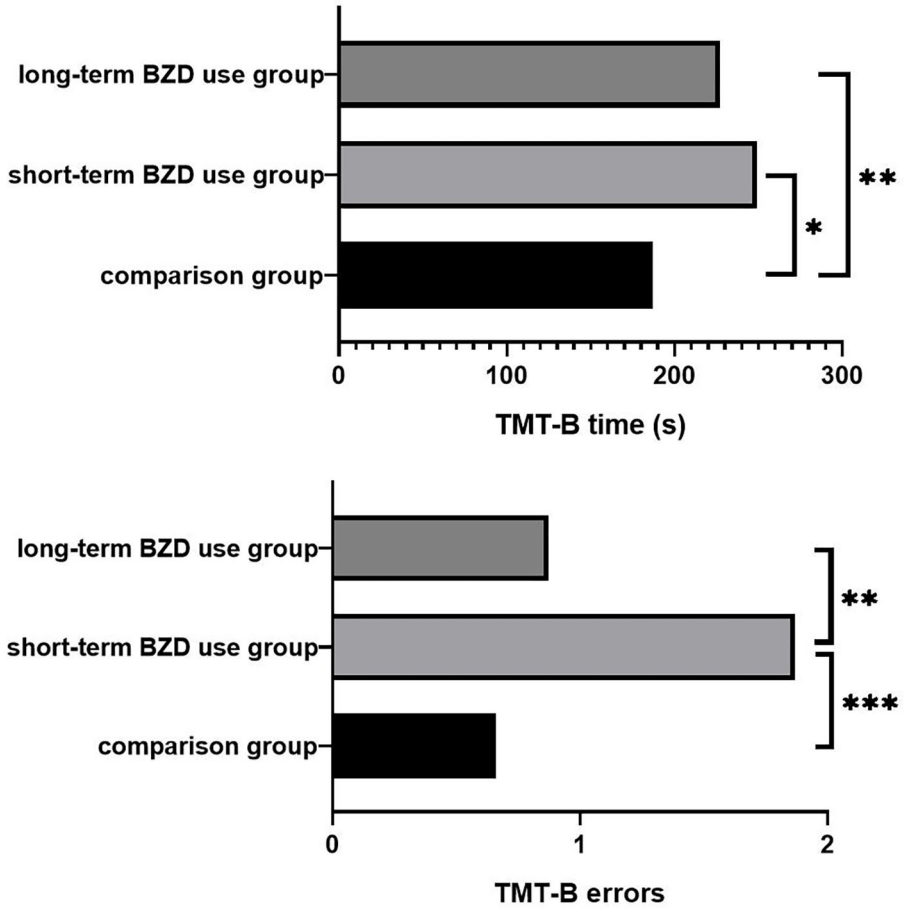

FIGURE 1 | Post-comparison of TMT-B results between groups. *$p < 0.05$; **$p < 0.005$; ***$p < 0.001$.

TABLE 4 | Multiple linear regression analysis for the factors determining BVMT-R N1 scores in BZD users.

Model	Beta	*t*	*P*	*R*	R^2	Adjusted R^2
Crude	0.235	2.306	0.023	0.235	0.055	0.045
Model 1	0.256	2.553	0.012	0.377	0.142	0.092

Model 1: adjusted for age, sex, education, and BMI.

on certain cognitive domains. Batterham's study showed that higher education affected cognition decline differently across the domains. They explored the protective effects of education in three cognition domains, including global cognition, processing speed, and memory, and found that higher education was associated with worse global cognition but not memory and processing speed (49). Therefore, the nature and mechanism of higher education's protective effects are still unclear.

BZD Using Time and the Cognition Decline in Highly Educated Older Adults

In this study, compared to non-users and long-term BZD users, short-term BZD users showed a significant increase in TMT-B errors, reflecting worse working memory function. At the same time, there was no significant difference between the non-users and the long-term BZD users. It was also found that the numbers of TMT-B errors in long-term BZD users were significantly fewer than that of the short-term BZD users, and there was no significant difference between the long-term BZD users and the non-users. In addition, although there was no significant difference in BVMT-R scores among the three groups, there was a significant correlation between the number of BVMT-R N1 scores and the BZD using time among all BZD users. These results suggest that the negative cognitive effects of BZD (at least in the domain of immediate visual memory and executive control) in the highly educated older adults did not increase with longer duration of BZD use. Previous studies have shown that long-term BZD use might result in physical dependence (2), and patients with BZD dependence could significantly impair cognitive functioning (25). Exploring the protective factors on the progress of cognition decline in dependent patients may help prevent severe cognitive impairment and improve an individual's prognosis. The results of our study may provide a new perspective to explore the protective effects of high education level on BZD dependence. However, since this study is a cross-sectional study and there is no follow-up data, it should be interpreted with caution.

Past research on general aged BZD users is consistent with our findings (14, 44, 46, 50). A prospective study of 3,434 BZD users over 10 years by Gray et al. showed that the risk of dementia in the older adults increased and then decreased with increased BZD use (46). The results of Zhang et al. 7-year cohort study of 5,423 BZD users with an average education level of 15 years found no faster decline of general cognition reflected by MMSE with long-term BZD use (14). A double-blind randomized controlled study conducted by Voshaar et al. (51) observed the long-term BZD users' resilience of the negative cognition effect by BZD. After taking 10 mg or 30 mg diazepam 2.5 h, long-term users can almost completely tolerate acute effects on cognition, but the control group still showed cognitive damage (50). A 7-year cohort study of 5,195 BZD users by Mura et al. showed no longitudinal association between long-term BZD use and accelerated cognitive decline (44). The phenomenon that the cognitive impairment of BZD users did not increase with the duration of medication may not be due to their high education level as previous research has shown (52, 53). One possible explanation was that after a period of the therapeutic use of BZD, the elevated mental health of the BZD users can promote the cognition (54). However, some research showed that aged BZD users suffered more cognitive impairment as their time on the drug increased (51, 55–57). These inconsistencies may be due to researchers not considering that BZD users had higher levels of anxiety than the control group, which results in a reduction in cognitive functioning (26). This explanation is supported by the research of Lucki et al., which reported that the cognitive performance of long-term BZD users did not differ significantly from that of non-users after matching for age, gender, education, and anxiety levels (58).

Strengths and Limitations

This study aims to assist with the clinical decision making of prescribing BZD to older adults. The therapeutic effects of BZDs are widely supported, but a major concern with BZD treatment is its potential negative effects on patients' cognitive functions, especially in older patients. With the increase in the accessibility of higher education, as well as the prevalence of BZD prescription, it is expected that there will be an increasing population of highly educated older adults using BZD. This research directly responds to these growing trends, attempting to address an expected clinical question: Are older patients less at risk for the negative cognitive effects BZD treatment's if the patients are highly educated? This forward-seeing perspective of this study provides unique strength and clinical meaning. This study focused on the population of highly educated old adults when investigating the cognitive effect of BZD and controlled for several demographic and clinical variates. Other than BMI, all other demographic information of the groups well-matched.

However, there are also some limitations. First, although BZD users were divided into long-term users and short-term users to explore the relationship between the BZD use duration and BZD's cognition effects, as a cross-sectional investigation, this study cannot directly reflect the causal relationship between BZD use and how did the BZD's cognition effects change with using time. Second, due to the outbreak of COVID-19 in China in December 2019, the study had to be discontinued, leaving the sample size of the study unsatisfactory. Third, we did not consider the role of sleep in the cognition effect of BZD. Past research has shown that sleep could influence cognitive performance in older adults (59) While many older adults use BZD as a treatment for insomnia (5), this study did not assess the participants' sleep quality. Therefore, the differences in cognitive outcomes might be due to the differences in sleep quality among the three groups. Further research is needed to exclude the influence of sleep. Additionally, some other factors related to older adults' cognition, such as occupation and daily activity, might also have influenced the results of the study.

This current study inspires multiple options for future research. The next step of this project could be to design an experimental study that assigns BZD non-users into BZD use and other anxiolytics groups to further reduce the curative effect of the drug on the cognition outcomes. A longitudinal study can also be informative in providing insight into the causality and long-term effects of BZD use. To eliminate the effects of depression and anxiety on cognitive performance, we excluded patients with those conditions. However, because BZD is widely prescribed to manage mood disorders, it is clinically relevant to include those populations in future studies. A preliminary proposal includes a within-subject design examining the cognitive performance of mood disorder patients before and after the onset of BZD treatment. This current study investigated the relationship between BZD use and cognitive performance. Other side-effects of BZD use such as addictive effects should also be evaluated in the context of highly educated adults.

CONCLUSION

In conclusion, we investigated the cognitive effects of BZD use on highly educated older adults and obtained valuable clinical findings. The results of this study found that in the population of highly educated older adults, short-term BZD use is significantly associated worse executive function (TMT), but functioning in general cognition (MMSE) and other cognitive domains such as verbal memory (AVLT), language fluency (COWAT), attention (DST), and visual memory (BVMT-R) was not associated with BZD use. Moreover, the cognition effect of BZD in highly educated old did not increase with the duration of BZD use. The result of this study can contribute to the discourse of the security of BZD use among older adults and provides guidance on clinical medicine in the future.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee in the First Affiliated Hospital of China Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LL and YT: conceptualization. LL and MT: data curation and analysis. RZ and YT: project administration. LL, YZ, LJ, and PJ: supervision, writing—review, and editing. LL and PJ: writing—original draft. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2020.595623/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Prevalence of Internet Gaming Disorder and Its Association With Personality Traits and Gaming Characteristics Among Chinese Adolescent Gamers

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Background: Internet gaming is extremely popular in China. However, some players overuse it, with negative outcomes. Knowing the prevalence rate and specific risk factors can provide a better understanding of the etiology of Internet Gaming Disorder (IGD). This study aimed to estimate the prevalence of IGD among Chinese adolescents and its association with their personality traits and Internet gaming characteristics.

Methods: A cross-sectional study design was utilized in this study. Participants were recruited from 34 provincial administrative districts in China and consisted of 6,379 adolescent game players aged 15–25 (males/females = 3,701/2,678, mean age: 19.35 ± 1.61). A self-report structured questionnaire containing questions regarding demographic information and Internet gaming use characteristics, the Video Gaming Dependency Scale, and the Chinese Big Five Inventory-brief version, was used in the study.

Results: The prevalence of IGD among Chinese adolescent game players was 17.0%. All participants were divided into the IGD group (males/females = 751/333, mean age: 19.74 ± 1.85) or the non-IGD group (males/females = 2,950/2,345, mean age: 19.27 ± 1.54). Specifically, twelve factors were significantly associated with IGD ($p < 0.001$), including neuroticism (β = 0.17), conscientiousness (β = −0.14), Internet gaming time per day (Hour) (β = 0.21), Internet gaming charge per month (Yuan) (β = 0.21), motive: escaping (β = 0.15), motive: sensation seeking (β = 0.13), motive: maintaining (β = 0.08), motive: coping (β = 0.06), having one or two long-term game partners (β = 0.06), male (β = 0.12), undergraduate and above (β = 0.04), and relationship status of couple (β = 0.04).

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Conclusion: Our findings offer evidence with respect to the prevalence of IGD and its relationships with specific personality traits and Internet gaming characteristics in China. It is necessary for Chinese parents, teachers, and social workers to pay more attention to adolescents' personality traits of high neuroticism and low conscientiousness, long time and high expense they spend on game, as well as their motives.

Keywords: internet gaming disorder (IGD), internet gaming use characteristics, personality traits (big five), big five model, Chinese adolescent gamers

INTRODUCTION

Given the characteristics of Internet games (free access, unlimited use, strong interactivity, and instant rewards), immersion in Internet gaming has become increasingly common and an important part of daily activities for adolescents and adults (1). As of June 2019, there were 494 million Internet game users in China, accounting for 57.8% of all Internet users (854 million) (2). Most people play games healthily, which can provide benefits for users (3). However, some players show overuse and symptoms similar to those of substance addictions (4), which may result in negative outcomes such as depression, anxiety, academic dysfunction, poor sleep quality, exacerbating relationships with family and peers, and even substance use (5, 6).

As a non-substance addiction (7), like gambling disorder (8), Internet Gaming disorder (IGD) was enrolled in the Fifth Edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) in 2013, warranting further research (9). In 2018, the International Classification of Diseases (ICD-11) also considered including it as a disease (10). Due to the differences in measurement tools and cultural backgrounds, different prevalence rates of IGD were reported among young people. For example, prevalence in Europe was between 1.2 and 1.6% (11, 12), while in Asia it ranged from 1.6 to 18.4% (13–15). Some studies reported that the rates of possible IGD among adolescents in China ranged between 2.0 (15) and 17.0% (16). However, more research on epidemiological investigation based on large samples should be conducted to verify those figures.

IGD is not only related to the stimulation of the game itself but also linked to the personality traits of the players. Personality traits can be considered as determinants of one's behaviors (17), which are enduring dispositions characterized by individuals' unique adaptation to others, themselves, objects, and even to the whole environment. According to the diathesis-stress model of behavior, addictive disorders may result from specific personality traits (1, 17). Strong empirical evidence has indicated that some personality traits were linked to antisocial behaviors, violence (18), substance use (19), and gambling (20). For example, heightened extraversion may be a predictor of smoking and alcohol consumption (21). Smokers scored higher on neuroticism and lower on conscientiousness and agreeableness than non-smokers (19).

Despite some controversies (22), the Five-Factor model is the most common classification of personality characteristics (23). The Big-Five model includes five personality dimensions: extraversion (people who are sociable and talkative), agreeableness (well-mannered and soft-hearted), neuroticism (unstable and short-tempered), conscientiousness (organized and hardworking), and openness to experience (curious and original) (24). A recent meta-analysis review revealed all personality dimensions had a significant effect on Internet addiction, based on 12 different studies (25). Easygoing, outgoing, responsible, and more neurotic individuals who were less open to experience showed higher levels of Internet addiction. Furthermore, individuals showing high levels of high conscientiousness (persistence, reliability, and an orientation toward achievement) may better predict general and mental health states (26). Studies of personality and health have shown that neuroticism in particular can be a common risk factor for poor health (27). To date, there has been limited research on the relationship between personality traits and IGD (1, 28–30), and these studies assessed personality traits using different assessment tools. Therefore, it is hard to compare or synthesize the results of their findings. Most research suggested a positive correlation between neuroticism and IGD; however, results for other personality traits have been inconsistent.

To better understand the risk factors of IGD, it is equally crucial to comprehend the association between IGD and Internet gaming use characteristics. Some researchers demonstrated that Internet gaming characteristics were significantly correlated with IGD. For example, Rho et al. (29) found that many significant behavioral factors can predict IGD, such as weekday game time, money spent on gaming, game club membership status, and offline game club attendance. Middle school students who played games more than 3 h a day were identified as having higher degrees of gaming addiction than others (30). Over 75% of gamers played with guild members; compared to playing solo, playing with others may be more stimulating (28).

This study is the first to investigate the association of IGD with the Big-Five factors and Internet gaming characteristics in a large sample of Chinese adolescent game players. It provides empirical evidence for understanding the etiology of IGD.

MATERIALS AND METHODS

Subjects

We conducted our study as an online survey in China in October and November 2019 via WeChat (a social app used by more than one billion people) based on the survey program Questionnaire Star (a free online questionnaire survey evaluation and voting platform). People could browse the questionaire page by scanning the quick response (QR) code. In the first page,

there was a brief introduction on the aim of this investigation, and its voluntary, anonymity and confidentiality, as well as the notes for filling in the questions. At the bottom of this page, persons could choose "reject" to refuse participating this survey, or "accept" to switch to the next page and continue filling the queastionaire. Anyone with access to the Internet who understand Chinese could participate. As an incentive for completing the questionnaire, participants could receive feedback on their results, including personality characteristics, and suggestions for their gaming behavior.

Online participants comprised adolescents and young adults, aged 15–25 years, from 34 provincial administrative districts in China. Among 8,592 respondents, 6,379 Internet game users were identified. Those who never played Internet games ($n = 1403$) or had severe physical or mental disorders ($n = 462$) were excluded. Additionally, we removed exaggerated responses (e.g., selected the same option for all items, $n = 319$) and multiple responses from the same IP addresses ($n = 29$).

The Institutional Review Board of Second Xiangya Hospital of Central South University approved this study protocol. All subjects were fully informed about the purpose of this investigation and signed online informed consent.

Procedures and Measures

We used self-administered composite questionnaires to obtain demographic information, gaming use characteristics, IGD, and personality traits. We conducted a preliminary study in a small sample of adolescents and finalized the questionnaire before starting the study.

Demographic information included age, gender, relationship status (dating/married vs. single), highest education, and family structure (having siblings or being an only child).

For gaming use characteristics, respondents reported the game device (mobile or computer) they most commonly used, the number of long-term game partners they had, their motives for gaming, and the amount of money (monthly) and time (daily) they spent gaming over the past 12 months.

IGD was assessed by the Chinese version of Video Gaming Dependency Scale (VGD-S), which was revised from the English version developed by Rehbein et al. (11), covering all nine criteria of the DSM-5. For each criterion, there were two items. Participants were asked to choose each of the 18 descriptive items they had experienced in the past 12 months on a four-point Likert scale ranging from 1 (*total disagreement*) to 4 (*full agreement*). A criterion was met if at least one of the two items was answered with 4 (*full agreement*). Participants who endorsed five or more of the nine criteria were classified with IGD. The VGD-S was evaluated in a sample of 11,003 German students, aged 13–18, demonstrating good reliability of Cronbach's α with 0.93 (11). The Chinese version of the VGD-S yielded an internal consistency of 0.94 in this research.

Personality traits were assessed by the 40-item brief version of the Chinese Big Five Inventory (CBF-PI-B) (31–33). Big-five personality traits were measured with eight items mapping each dimension: conscientiousness, extraversion, agreeableness, neuroticism, and openness. Responses were on a six-point Likert scale ranging from 1 (*strongly disagree*) to 6 (*strongly agree*). The CBF-PI-B demonstrates high construct validity and reliability, and the internal consistency coefficient was between 0.76 (agreeableness) and 0.81 (neuroticism), with an average of 0.79 (31). The retest coefficient of the 10-week interval ranged from 0.67 (agreeableness) to 0.81 (openness), with an average of 0.74 (31). In this study the CBF-PI-B yielded an internal consistency of 0.86.

Statistical Analysis

Prevalence of IGD in the whole sample was calculated. We used chi-square tests and *t*-tests to compare demographics, Internet gaming use characteristics, and personality traits between the IGD group and the non-IGD group. In univariate analysis, via a backward stepwise entry, multiple linear regression was performed to determine risk factors related to IGD. The statistical significance level was set at $p < 0.001$ (two-sided). We used SPSS software version 23.0 package for all analyses.

RESULTS

In this study, a total of 6,379 adolescent game players were recruited from 34 provincial administrative districts in China. The average age of the 6,379 participants was 19.4 years [standard deviation [SD]:1.6, range:15–25], and 57.1% were male adolescents (males/females = 3,701/2,678). The average time spent on gaming was 1.8 h per day (SD:1.3) and the average amount of money spent on gaming was 63.9 yuan per month (SD:145.2). **Table 1** displayed the detailed sociodemographic and Internet gaming characteristics of the participants.

According to the positive criteria, endorsement of five or more of the nine criteria, a total of 1,084 adolescents (17%) were classified with IGD. All participants were divided into the IGD group (males/females = 751/333, mean age: 19.74 ± 1.85) and non-IGD group (males/females = 2,950/2,345, mean age: 19.27 ± 1.54). Results from the Chi-square tests and *T*-test (**Table 1**) show that IGD groups were more likely to be male ($p < 0.001$), single ($p < 0.001$), undergraduate or above ($p < 0.001$), older ($p < 0.001$), use computers to play ($p < 0.001$), have one or two long-term game players ($p < 0.001$), play games for seeking ($p < 0.001$) and escaping ($p < 0.001$), and spend more time and money on gaming ($p < 0.001$). To control the effect of socio-demographic variables, sex, education and relationship status, personality traits and Internet gaming characteristics were included in a multiple linear regression. All variables included in the linear regression model did not show multicollinearity (VIF: 1.021–2.207), and the Durbin–Watson was 1.906, so assumptions of collinearity were not violated. The association between IGD and predictive variables were strongly relative. Together, all the predictive variables accounted for 38.7% of the variance in IGD scores and the explained variability was also significant, $F_{(18,6378)} = 224.276$, $p < 0.001$.

Multiple linear regression analysis showed that twelve factors were significantly associated with IGD, including neuroticism ($\beta = 0.17$, $p < 0.001$), conscientiousness ($\beta = -0.14$, $p < 0.001$), Internet gaming time per day (Hour) ($\beta = 0.21$, $p < 0.001$), Internet gaming charge per month (Yuan) ($\beta = 0.21$, $p < 0.001$), motive: escaping ($\beta = 0.15$, $p < 0.001$), motive: sensation seeking

TABLE 1 | Socio-demographic and Internet gaming characteristics of participants and comparison between IGD group and Non-IGD group by variables.

Variables		Total (N = 6,379)	IGD group (n = 1,084)	Non-IGD group (n = 5,295)	c^2 or t	p
Gender	Male	3,701	751	2,950	68.001	<0.001
	Female	2,678	333	2,345		
Age (years)		19.35 ± 1.61	19.74 ± 1.85	19.27 ± 1.54	−8.743	<0.001
Relationship status	Couple	135	83	52	48.483	<0.001
	Single	6,244	1001	5,243		
Highest education	Below	2,641	353	2,288	42.035	<0.001
	Above	3,738	731	3,007		
Family structure	having siblings	3,166	530	2,636	0.285	0.773
	being an only child	3,213	554	2,659		
Gaming use characteristics						
Device	PC	2,008	685	1,323	608.93	<0.001
	Mobile	4,371	399	3,972		
Long-term game partners	none	2,485	192	2,293	739.886	<0.001
	3>N≥1	2,027	479	1,548		
	6>N≥3	1,144	261	883		
	N≥6	723	152	571		
Daily gaming time(hours)		1.83 ± 1.30	2.86 ± 1.81	1.62 ± 1.05	−30.664	<0.001
Money spent on gaming/month(yuan)		63.92 ± 145.19	179.35 ± 241.44	40.29 ± 100.92	−30.791	<0.001
Motives						
Seeking	Yes	1,915	589	1,326	367.529	<0.001
	No	4,464	495	3,969		
Escaping	Yes	694	345	349	590.99	<0.001
	No	5,685	739	4,946		
Coping	Yes	2,656	593	2,063	20.942	<0.001
	No	3,723	491	3,232		
Passing time	Yes	4,275	657	3,618	24.259	<0.001
	No	2,104	427	1,677		
Making friends	Yes	1,163	267	896	55.259	<0.001
	No	5,216	817	4,399		
Maintaining	Yes	1,350	355	995	116.45	<0.001
	No	5,029	729	4,300		
Personality traits						
Neuroticism		25.68 ± 7.93	29.63 ± 6.38	24.87 ± 7.98	−18.492	<0.001
Conscientiousness		33.16 ± 6.34	30.52 ± 5.40	33.70 ± 6.39	15.286	<0.001
Openness		33.27 ± 6.96	32.35 ± 6.17	33.45 ± 7.10	4.781	<0.001
Agreeableness		35.10 ± 6.11	32.88 ± 5.85	35.56 ± 6.06	13.332	<0.001
Extraversion		29.65 ± 7.14	28.58 ± 6.57	29.87 ± 7.24	5.445	<0.001

Below, Below the undergraduate; Above, Undergraduate or above; Seeking, Sensation seeking; Escaping, Escaping reality; Coping, Coping negative emotion; Friends, Making friends; Maintaining, Maintaining status.

($\beta = 0.13$, $p < 0.001$), motive: Maintaining($\beta = 0.08$, $p < 0.001$), motive: coping ($\beta = 0.06$, $p < 0.001$), having one or two long-term game partners ($\beta = 0.06$, $p < 0.001$), male ($\beta = 0.12$, $p < 0.001$), education of undergraduate or above ($\beta = 0.04$, $p < 0.001$), and relationship status of couple ($\beta = 0.04$, $p < 0.001$) (see **Table 2**).

DISCUSSION

Our study is one of few to determine the relationships between IGD, Internet gaming use characteristics, and Big-Five personality traits. This is the first investigation of the prevalence of IGD in a large sample (>6,000) of adolescent game players in China. We found that nearly 17.0% of Chinese adolescent game users reported IGD, which was significantly associated with gender, highest education, relationship status, Internet gaming use characteristics (money spent per month, time spent per day, and long-term game partners), motivation (escape, sensation seeking, maintaining, and coping) and personality traits (neuroticism, conscientiousness).

In this study, we found a 17% prevalence of IGD among Chinese adolescent gaming users, the exact same percentage

TABLE 2 | Multivariable linear regression of factors associated with IGD.

	B	SE B	β	*P*	VIF
Neuroticism	0.053	0.003	0.172	<0.001	1.206
Conscientiousness	−0.050	0.005	−0.140	<0.001	1.812
Agreeableness	0.011	0.005	0.028	0.016	1.438
Openness	0.006	0.005	0.017	0.237	2.207
Extraversion	−0.011	0.004	−0.027	0.027	1.523
Device	−0.03	0.059	−0.005	0.639	1.173
Long-term game partner	0.329	0.052	0.062	<0.001	1.021
Money spent on gaming/month	0.003	0.000	0.206	<0.001	1.354
Gaming time/day	0.395	0.021	0.209	<0.001	1.298
Sensation seeking	0.675	0.056	0.126	<0.001	1.141
Escaping	1.179	0.084	0.149	<0.001	1.180
Coping negative emotion	0.321	0.051	0.064	<0.001	1.075
Passing time	0.026	0.052	0.005	0.623	1.042
Making friends	0.062	0.064	0.010	0.327	1.039
Maintaining	0.481	0.060	0.080	<0.001	1.049
Gender	0.580	0.054	0.116	<0.001	1.217
Highest education	0.191	0.050	0.038	<0.001	1.023
Relationship states	0.719	0.176	0.042	<0.001	1.098

This analysis was based on the IGD group and the Non-IGD groups. B, Regression coefficient; SE, standard error; β, standardized β coefficient. Model: $F(18) = 224.28$, $p < 0.001$, $R^2 = 0.39$.

as the Experts Consensus of Gaming Disorder in China (2019 Edition) (16), which is much higher than many other countries. Using the same tools as our study, Rehbein et al. (11), the authors of the VGD-S English version, conducted a state-representative survey consisting of 11,003 middle school students and reported a 1.2% prevalence of GD. Müller et al. (12) found an IGD prevalence of 1.6% in 12,938 adolescents from seven European countries. Other studies reported significant figures: between 0.3 and 1.0% in four international groups of 18,932 people (34); 1.3% in a national sample of 902 Dutch gamers (35); and 1.8% of 1,287 Australian adolescents (36). The higher rate of IGD in this study might be related to differences in sample characteristics (based on the general population or gamers), assessments of IGD, or cultural factors. These studies, conducted in different socio-cultural populations, suggest that China's IGD prevalence is more concerning. This may be a cultural difference. Western countries have more individualistic cultures than Asian countries (37, 38), where higher levels of individualism were significantly associated with lower levels of Internet addiction, manifested in an increased need for achievement, dominance, and autonomy, and a reduced need for affiliation (39). Considering the negative health consequences of IGD and the vast number of Chinese adolescents, a 17% prevalence suggests that adolescent IGD in China deserves preventive and clinical attention.

Certain sociodemographic variables were more significantly associated with IGD. Consistent with previous studies (40, 41), we found that being male was significantly related with IGD, possibly due to gender differences in online use. To illustrate, females mostly use online networking for chatting, sharing information, tightening communication, and visiting personal websites, whereas males mostly use the Internet to play games (42). Furthermore, certain temperamental characteristics of males such as high comorbidity of Attention Deficit Hyperactivity Disorder (ADHD), high levels of novelty seeking, and high levels of impulsivity may be responsible for higher rates of IGD. Research also indicated that roughly four in five Massively Multiplayer Online Roleplaying Game (MMORPG) players were males (28). Many games on the market are invented by males for males (43); gaming companies strategically target boys and men to increase their sales (44, 45), partly explaining the high rate of male gaming usage.

Unlike previous studies (29, 46, 47), we found that dating or married and highly educated young adults were more likely to have IGD. Rho et al. (29) found there was no significant differece on marital status and education between the IGD and concrol group. A clinical research showed that patients with IGD were more likely single (46). Being single was positively associated to addictive video gaming, which may be related with their loneliness (47). The opposite results in this study may due to the unbalanced relationship status (135 couple vs. 6244 single) of these participants, which influenced the outcomes. The results of *t*-test showed that the IGD group was older than the non-IGD group. It can be speculated that young adults and college students tend to have more autonomy and opportunities to use smart devices than younger students do. Therefore, due to their technological proficiency and lack of external supervision, they may be more prone to suffer from gaming disorders (14). More research is needed to verify these results.

In the current study, IGD was significantly associated with the personality traits of neuroticism and conscientiousness, which is consistent with previous reports (48–50). Our study indicated

a significant relationship between neuroticism and IGD, which correlates to a previous finding that when highly neurotic individuals consider the real world threatening, they often turn to digital worlds, where they feel in control and safe (1). Since previous research has reported that high level of neuroticism is a predictor for smoking (51) and correlated with other mental disorders (52), it may represent a general health risk factor. Our results also suggested a significant relationship between low conscientiousness and IGD. Low conscientiousness indicates one has less commitment to personal goals, a tendency to be disorganized, and trouble organizing and following a schedule (53); therefore, the digital environment may be more attractive (1). Consequently, paying particular attention to adolescents with high neuroticism or low conscientiousness may help prevent IGD.

We found that personality traits like openness, agreeableness, and extraversion were not associated with IGD. However, several studies reported a slightly significant correlation between low openness and IGD (54), low agreeableness associated with Internet addiction (55), and IGD patients displaying lower extraversion than control groups and groups of pathological gamblers (1). For example, game players with low levels of openness are prone to stick to their gaming behavior rather than exploring new activities (1). Further studies are needed to provide more evidence.

Certain Internet gaming characteristics, like having one or two long-term game partners, significantly predicted IGD. For example, MMORPGs encourage gamers to team up with other players to progress (28), inspiring teamwork and interaction (56, 57). Generally, gamers with IGD were considered to be isolated and have not many social relationships in the real world, while they developed long-term relationships with their game partners, such as attending offline game clubs and having game club memberships (29).

Consistent with findings on risk factors of IGD in the general population, the motives of escaping reality, sensation seeking, and coping with negative emotions were significantly associated with IGD. Sensation seeking may provide a coping strategy for boredom (58); this is a positive reinforcement for IGD. Furthermore, the diversity of online games is a preferable alternative to dealing with negative emotions (59) that are often associated with high neuroticism. Players who enjoy role-playing games engage in gaming to relax and temporarily escape real life (60). Alternatively, coping with negative emotions such as fear of failure can be seen as negative reinforcement for IGD (44, 61, 62). Many studies have shown that the feeling-of-escape motive is most strongly associated with and most predictive of IGD (28, 44).

Gaming costs and daily gaming time were additional risk factors of IGD. Users with IGD spent more time (2.86 vs. 1.62 h per day) and more money (179.35 vs. 40.29 yuan per month) than the non-IGD group. Those scoring low in conscientiousness had difficulty controlling the amount of time and money they spent on gaming. Previous studies reported that spending large amounts of time and money was a predictor of IGD (29). Many gamers report difficulty in controlling gaming time. Rho et al. (63) also showed that money and time spent on gaming are risk factors for IGD. Zaheer Hussain found that if a player spends more time playing games online, the risk of being addicted increases (28); therefore, corresponding measures should be taken to prevent and manage IGD, such as strictly controlling gaming time and money spent.

This study had some limitations. First, using self-reported questionnaires instead of face-to-face structured interviews requires that more caution is taken when generalizing the findings. However, self-reported investigations completed online are thought to improve honesty levels (64). Second, this study was a cross-sectional survey, so findings regarding relationships may not be causal. However, personality traits are relatively stable in life, so interpreting differences in personality traits is reasonable (1). Third, we used short versions of the Big-Five scale; although the complete versions may be more reliable and detailed, the short version appears to be psychometrical and more helpful for a large-scale survey. Fourth, the sociodemographic characteristics of the non-IGD group did not exactly match those of the IGD group, possibly leading to confounding results.

CONCLUSIONS

This study found significant associations between IGD and personality factors, Internet gaming characteristics in China. Given that China appears to have a higher IGD prevalence rate than other regions, it is necessary for Chinese parents, teachers, and social workers to pay more attention to the mental needs of adolescents in terms of personality traits and gaming use. A three-level prevention system is worth adopting to assist with general prevention, targeted prevention, and early detection and treatment (16). Future research should be conducted to explore how these factors influence IGD and examine the development of IGD.

DATA AVAILABILITY STATEMENT

All relevant data is contained within the article: The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Institutional Review Board of Second Xiangya Hospital of Central South University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to this study. ZL, QH, and HS conceptualized and designed the research, wrote the first draft of the manuscript, and contributed to the final manuscript. TS, TF, and XC prepared the assessment tools. LT, SL, and JQ performed the experiments. SH and YC undertook the statistical

analysis. All authors critically reviewed content and approved final version for publication.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Association Between Brain-Derived Neurotrophic Factor Val66Met Polymorphism and Methamphetamine Use Disorder: A Meta-Analysis

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Background: Several studies had examined the association between brain-derived neurotrophic factor (BDNF) Val66Met polymorphism and methamphetamine (METH) use disorder, whereas the results were conflicting. The aim of this study was to conduct a meta-analysis to achieve a pooled effect size of the association between BDNF Val66Met polymorphism and METH use disorder.

Methods: Literature searches were conducted in PubMed, EMBASE, and Cochrane Library up to July, 2020. All relevant studies on the relationship of BDNF Val66Met polymorphism and METH addiction were retrieved. Pooled odds ratios (ORs) with 95% confidence intervals (95% CIs) were calculated in the dominant, recessive, co-dominant, and allele model to appraise the association.

Results: Seven case–control studies with a total of 2,204 subjects (956 METH-dependent cases and 1,248 healthy controls) were included in this meta-analysis. The results showed a significant correlation between BDNF Val66Met polymorphism and METH dependence in overall population under different genetic models. However, subgroup analysis indicated that the association only existed in Han Chinese but not in other Asian populations.

Conclusion: Although the current data indicate that BDNF Val66Met polymorphism might be a potential genetic factor for METH use disorder, more researches are needed to prove its role in different populations.

Keywords: BDNF Val66Met, polymorphism, methamphetamine, addiction, meta-analysis

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INTRODUCTION

Methamphetamine (METH) is highly abused throughout the world, which remains an extremely serious public health issue. Although efforts to investigate the biological processes of METH use disorder (i.e., abuse, dependence, and psychosis) have uncovered several potential pathogenic factors, there is still a lack of putative biomarkers to predict its susceptibility. Former family and twins studies indicated that the development of substance use disorders was closely connected

with genetic factor (1–3). Kendler et al. (4) found a heritability estimate of 57% for stimulants abuse/dependence in males, while Mcgue et al. (5) found that the heritability of use and abuse of amphetamines was 16% in adolescents. Besides, many genetic animal models supported the effects of genetic factors on METH addiction (6, 7). However, to date, the key genetic variations that increase susceptibility to METH addiction have not been identified. Brain-derived neurotrophic factor (BDNF) belongs to the neurotrophin family, which is widely expressed in the adult brain and plays a key role in the facilitation of development, differentiation, and survival of midbrain dopaminergic neurons as well as the modulation of synaptic transmission. Many reports showed that BDNF was involved in the development of psychiatric and substance use disorders and had a potential role in the treatment of these disorders (8–11). Experiments on animal models showed that increasing BDNF expression could promote the survival and protection of dopaminergic neurons following METH administration (12, 13).

BDNF Val66Met (rs6265; G196A), an early found functional single-nucleotide polymorphism (SNP) in BDNF gene, resulted in a valine (Val)-to-methionine (Met) substitution in the prodomain. BDNF Val66Met not only affected the secretion of BDNF but also changed the structure of BDNF and affected its interaction with HAP1 and Sortilin1 (14–16). BDNF Val66Met was involved in the modulation of neuronal morphology and synaptic plasticity as well as the development of neuronal, psychiatric, and drug use disorders (17–23). Researches suggested that BDNF Val66Met polymorphism was a potential predisposing genetic factor for different substance use disorders, such as cigarette, alcohol, cocaine, heroin, and METH (24–28). Significant differences in BDNF Val66Met genotype distribution were found between METH addicts and controls, and higher 66Val allele frequencies were found in both Asian and Caucasian drug addiction cases (28, 29). Excessive use of METH can induce psychotic episodes. Greening et al. (30) found that METH differentially altered dopamine signaling markers between mice with different BDNF Val66Met genotypes (Val/Val vs. Met/Met), implicating involvement of BDNF in Meth-induced reprogramming of the mesolimbic proteome, while the research by Sim et al. (31) suggested that the BDNF Val66Met polymorphism might contribute to METH dependence and psychosis in the Chinese population but not in other Malaysian ethnicities.

As mentioned above, though more and more studies have found that METH addiction is associated with BDNF, there are still many contradictions about the association between Val66Met polymorphism and METH addiction. The reason for the conflicting results might be ethnic specificity, limited samples, participant heterogeneity, and insufficient statistical ability. Therefore, the meta-analysis aimed at synthetizing current studies on the association of BDNF Val66Met polymorphism and susceptibility to METH use disorder.

METHODS

Literature Search

Any literature concerning the association between BDNF polymorphisms and METH addiction was sought by using the Medical Subject Headings terms "methamphetamine," "polymorphism," "variant," "BDNF," "brain-derived neurotropic factor," "rs6265," "rs6265 G>A," "G rs6265A," and "Val66Met" in databases (PubMed, EMBASE, and Cochrane Library) up to July, 2020. To find more appropriate studies, the references of the key studies were hand-searched. When detailed information for calculating effect size was lacking, we contacted the authors directly. The study was registered at PROSPERO (CRD42020193010).

Inclusion and Exclusion Criteria

Case–control and cross-sectional studies were included irrespective of publication status or language. In this meta-analysis, all included studies met the following criteria: (a) studies focused on the association between BDNF Val66Met polymorphism and METH use disorder susceptibility, (b) patients were diagnosed using standard diagnostic criteria by a psychiatrist, (c) genotypes distribution of the control group should be in Hardy–Weinberg Equilibrium (HWE), and (d) there were sufficient data of the genotypes in the case and control group to compute the odds ratios (ORs) and 95% confidence intervals (CIs). The exclusion criteria were as follows: (a) METH-dependent subjects involving other substance use disorders, (b) publications with patients with other neuropsychiatric disorders, and (c) no genotypic data available.

Quality Assessment

The methodological quality assessment of inclusion studies was performed with the Newcastle-Ottawa scale (NOS) (32). A "star system" has been used to assess data quality based on three aspects: the selection, comparability, and exposure or outcome of interest. Two independent researchers identified the literatures, and divergences between reviewers were resolved through discussion or with the assistance of a third researcher. The power of meta-analysis was estimated by STATA 12.0 software (Stata Corporation, College Station, Texas, USA). When the power of test was higher than 0.8, the result was considered acceptable.

Data Extraction

Data extraction from an individual study included the following: first author, publication year, site of study, ethnicity, sample size, age, gender distribution, diagnosis criteria, genotyping method, HWE of cases and controls, and distribution of genotypes. Two researchers extracted the data independently, and disagreements were settled through discussion or with the assistance of a third researcher.

Statistical Analysis

Statistical analysis was performed by STATA 12.0 software (Stata Corporation, College Station, Texas, USA). The relationship between BDNF Val66Met polymorphism and METH use disorder was appraised by pooled ORs and 95% CIs in dominant model (Met/Met + Val/Met vs. Val/Val), recessive model (Met/Met vs. Val/Val + Val/Met), co-dominant model (Met/Met vs. Val/Val; Val/Met vs. Val/Val), and allele model (Met vs. Val). Summary OR results and publication bias were evaluated by Z test and Egger's test, respectively. $P < 0.05$ was considered statistically significant. I^2 and Q test statistics were

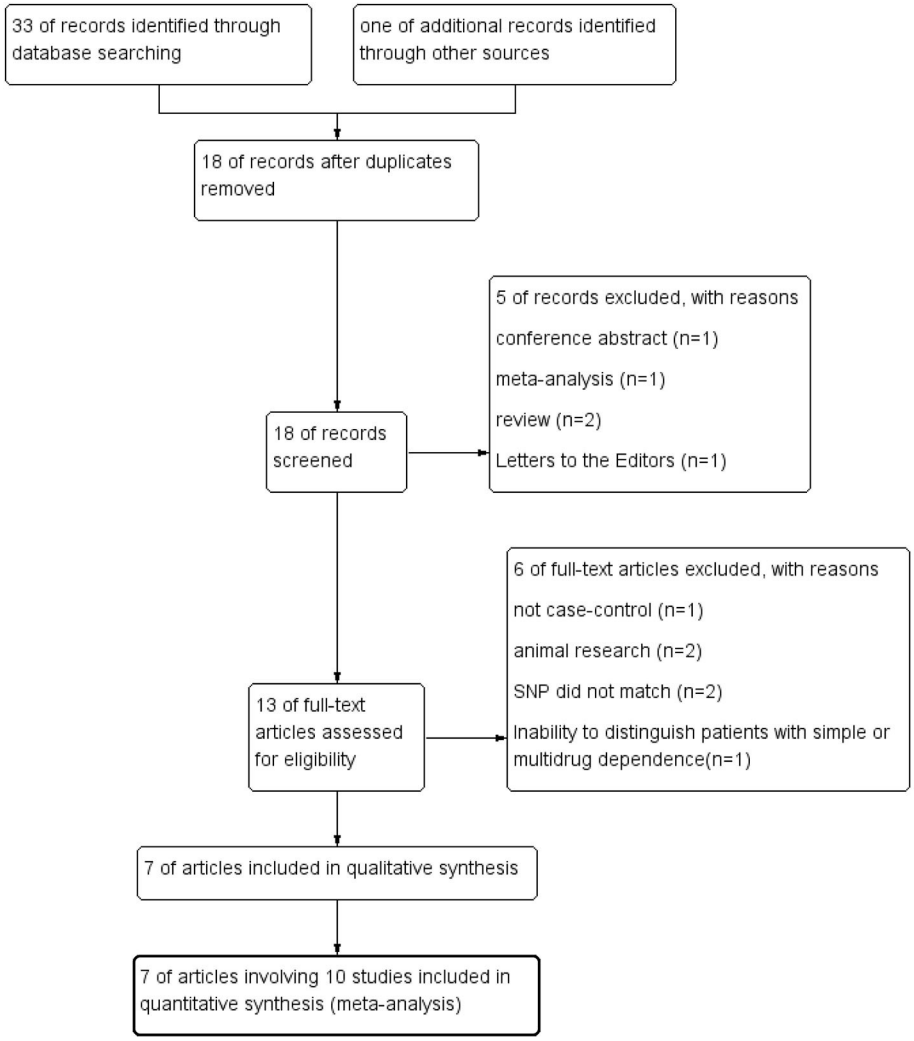

FIGURE 1 | Flow diagram of literature retrieval and selection.

employed to ascertain the heterogeneity among studies. When the heterogeneity was high ($I^2 > 50\%$ or $P < 0.1$), a random-effect model was selected. Otherwise, the fixed effects model was chosen. Subgroup analyses were conducted according to ethnicity. Sensitivity analysis was performed with leave-one-out approach to examine the stability of analysis.

RESULTS

Literature Search

The process of systematic literature search and selection was displayed in **Figure 1**. We identified 33 articles through database searching and 1 additional article through reference lists searching. After excluding duplicates, 18 articles remained. Screening the titles and abstracts of these 18 articles resulted in 13 relevant studies. After the full-text review of the remaining 13 articles, 7 studies were qualified for final data analysis.

Study Characteristics

Seven case–control studies (28, 29, 31, 33–36) with a total of 2,204 subjects (956 METH-dependent cases and 1,248 healthy controls) were included in this meta-analysis. **Table 1** summarized the general characteristic of inclusion studies. All included studies were in accordance with HWE. The NOS scores of all studies were >6, indicating that they had good methodological quality (**Supplementary Table 1**). Except for one (33) employing ICD-10 criteria, all studies used DSM-IV criteria to identify METH-dependent subjects. One study from Malaysia were carried out in four different ethnic groups (31). After classification of this study into four subgroups, the number of studies was raised to 10.

Of the 10 different cohorts included in our study, 9 were from Asia (28, 31, 33–36). Two studies from China, one study from Taiwan, and one study from Malaysia were carried out in the Han Chinese population (28, 31, 34, 36). Cheng et al. (28) studied BDNF Val66Met polymorphism in both METH- and heroin-dependent patients, and only the former was included in the

TABLE 1 | General characteristics of the included studies in the meta-analysis.

References	Origin	Population	Sample size (case/control)	Sex Male (case/control)	Age (case/control) (mean ± SD)	Diagnosis	Genotyping	Genotypes distribution (case/control)			Allelic distribution (case/control)		p_{HWE} (case/control)
								Met/Met	Met/Val	Val/Val	Met	Val	
Cheng et al. (28)	Taiwan	Han Chinese (Asian)	103/122	103/122	27.8 ± 6.8/29.8 ± 8.5	DSM-IV	PCR-RFLP	15/31	57/68	31/23	87/130	119/114	0.17/0.19
Itoh et al. (33)	Japan	Japanese (Asian)	189/202[a]	150/158	36.6 ± 11.9/37.2 ± 10.6	ICD-10	PCR-RFLP	23(8[a])/25	96(27[a])/107	70(21[a])/70	43/157	69/247	0.25/0.10
Bousman et al. (29)	USA.	American (Caucasian)	117/76	117/76	36.6 ± 11.9/37.2 ± 10.6	DSM-IV	MassArray	2/2	29/29	86/45	33/33	201/119	0.80/0.29
Sim et al. (Malay) (31)	Malaysia	Malay (Asian)	59/51	59/51	31 ± 7.8/36 ± 10.6	DSM-IV	PCR-RFLP	6/10	33/25	20/16	45/45	73/57	0.15/0.97
Sim et al. (Chinese) (31)	Malaysia	Han Chinese (Asian)	24/45	24/45	40 ± 9.6/30 ± 9.4	DSM-IV	PCR-RFLP	2/12	12/27	10/6	16/51	32/39	0.54/0.14
Sim et al. (Kadazan-Dusun) (31)	Malaysia	Kadazan-Dusun (Asian)	50/30	50/30	29 ± 6.6/33 ± 13.1	DSM-IV	PCR-RFLP	9/6	28/18	13/6	46/30	54/30	0.37/0.27
Sim et al. (Bajau) (31)	Malaysia	Bajau (Asian)	53/28	53/28	28 ± 6.4/31 ± 16.7	DSM-IV	PCR-RFLP	14/6	26/14	13/8	54/26	52/30	0.89/0.98
Su et al. (34)	China	Han Chinese (Asian)	200/219	167/173	30.79 ± 7.99/33.68 ± 9.87	DSM-IV	MassArray	40/50	96/118	64/51	176/218	224/220	0.71/0.25
Iamjan et al. (35)	Thailand	Thai (Asian)	100/102	100/102	NA	DSM-IV	Real-time PCR	20/25	42/54	38/23	82/104	118/100	0.19/0.55
Su et al. (36)	China	Han Chinese (Asian)	194/378	160/149	31.46 ± 8.40/45.99 ± 12.96	DSM-IV	MassArray	39/90	93/189	62/94	171/369	217/377	0.70/0.79

[a]*Fifty six patients abuse methamphetamine only in their lifetime and 122 patients abuse some other drugs besides methamphetamine in the present or past;* p_{HWE}*, p-value for Hardy–Weinberg equilibrium; NA, not available.*

TABLE 2 | The results of the meta-analyses under different genetic models for all studies.

Genetic model	Comparison	Association			Heterogeneity		Publication bias[a]	Power of test
		OR	95% CI	*P*	I^2 (%)	*P*		
Dominant model	Met/Met + Val/Met vs. Val/Val	0.65	0.53–0.79	<0.01	0.00	0.49	0.70	0.999
Recessive model	Met/Met vs. Val/Val + Val/Met	0.76	0.60–0.95	0.02	0.00	0.70	0.48	0.728
Co-dominant model	Met/Met vs. Val/Val	0.59	0.46–0.77	<0.01	1.40	0.43	0.55	0.990
	Val/Met vs. Val/Val	0.67	0.55–0.82	<0.01	0.00	0.66	0.80	0.991
Allele model	Met vs. Val	0.76	0.67–0.86	<0.01	1.30	0.43	0.59	0.998

[a]*P-value for Egger's test.*

study. In the study by Itoh et al. (33), 56 patients abused MTEH only, while 122 patients abused other drugs besides METH. According to the predetermined inclusion criteria, the 56 subjects with METH dependence only were included in the data analysis. Four of the included (28, 29, 31, 35) studies were carried out in male only. Itoh et al. (33) analyzed the association of two BDNF gene SNPs with METH abuse in Japan. Bousman et al. (29) investigated association of six putative SNPs in six genes (AKT1, ARRB2, BDNF, COMT, GSTP1, and OPRM1) with METH abuse in Caucasian.

Meta-Analysis

The summary data of different genetic models indicated a significant association between BDNF Val66Met polymorphism and METH addiction in overall population, and no publication bias and significant heterogeneity were identified (**Table 2** and **Figure 2**). Except for the recessive model, the power of meta-analyses was acceptable (**Table 2**). **Figure 3** displayed the results of sensitivity analysis for different genetic models in the overall population. When the study by Cheng et al. was removed, the pooled OR changed a lot (from OR = 0.76, 95% CI: 0.60–0.95 to OR = 0.80, 95% CI: 0.63–1.02, **Figure 3E**) under the recessive model. While for the dominant, co-dominant, and allele model, the overall effect was not reversed after the deletion of study by Cheng et al. (**Figures 3A–D**). Therefore, this study was retained.

Considering the conflicting results between different populations, the further subgroup analysis by ethnicity was employed. Four studies of Han Chinese origin and five studies of East and Southeast Asian origin (except for Han Chinese) were enrolled in the subgroup analysis (**Table 3** and **Figure 2**). Due to insufficient data, we were unable to perform a subgroup analysis of Caucasian population, but we listed a study of Caucasian origin as a reference in **Table 3** and **Figure 2**. In the subgroup analyses, we found a statistically significant association between BDNF Val66Met polymorphism and METH addiction in Han Chinese, but not in other Asian populations. Heterogeneities were increased in the Han Chinese group under different genetic models.

DISCUSSION

BDNF Val66Met, a non-synonymous SNP of BDNF gene, is related to a variety of neuropsychiatric diseases, such as Parkinson's disease, Alzheimer's disease, schizophrenia, and posttraumatic stress disorder (19–21, 37). Egan et al. (14) firstly examined the effects of BDNF Val66Met polymorphism and demonstrated a role for the variants in memory and hippocampal function. Subsequently, Greening et al. (30) found that BDNF Val66Met involved in METH-induced reprogramming of the mesolimbic proteome. The reward system of the midbrain and memory function of the hippocampus plays an important role in the formation of METH addiction. Therefore, due to the role of BDNF Val66Met in these processes, its relationship with METH addiction has attracted more and more attention. Many studies have examined the association between BDNF Val66Met polymorphism and METH abuse, but the results of these studies were not entirely consistent with each other. In this meta-analysis, we found that there was an association between BDNF Val66Met polymorphism and METH addiction in the overall population, suggesting that BDNF Val66Met polymorphism might be a potential marker for METH-dependent susceptibility. METH is a psychostimulant and has obvious neurotoxicity to dopamine neurons. In the animal METH-treated model, increased BDNF resulted in preservation of corpus striatal dopamine levels, indicating that the BDNF expression had a certain effect on antagonizing the nerve damage induced by METH (38). BDNF expression was increased in METH-dependent patients, and the BDNF Val66Met genotypes can affect the secretion of BDNF (36, 39). Therefore, the effects of BDNF Val66Met polymorphism on BDNF expression might be relevant to METH addiction.

As the use of METH penetrated the geographic regions of different ethnicities in the world, racial differences among METH abusers were observed (40). In the subgroup meta-analysis by ethnicity, we found a significant association between BDNF Val66Met polymorphism and METH addiction in Han Chinese, but not in other Asian populations. This result was different from that of Haerian et al., who found that the BDNF Val66Met polymorphism may be a risk factor for addiction to METH in a South Asian population (17). The difference might result from the differences in the included trials and subgroup analysis. In the study by Haerian et al., five inclusion studies investigated the association between BDNF Val66Met polymorphism and METH addiction, and one of them recruited patients with simple or multidrug dependence.

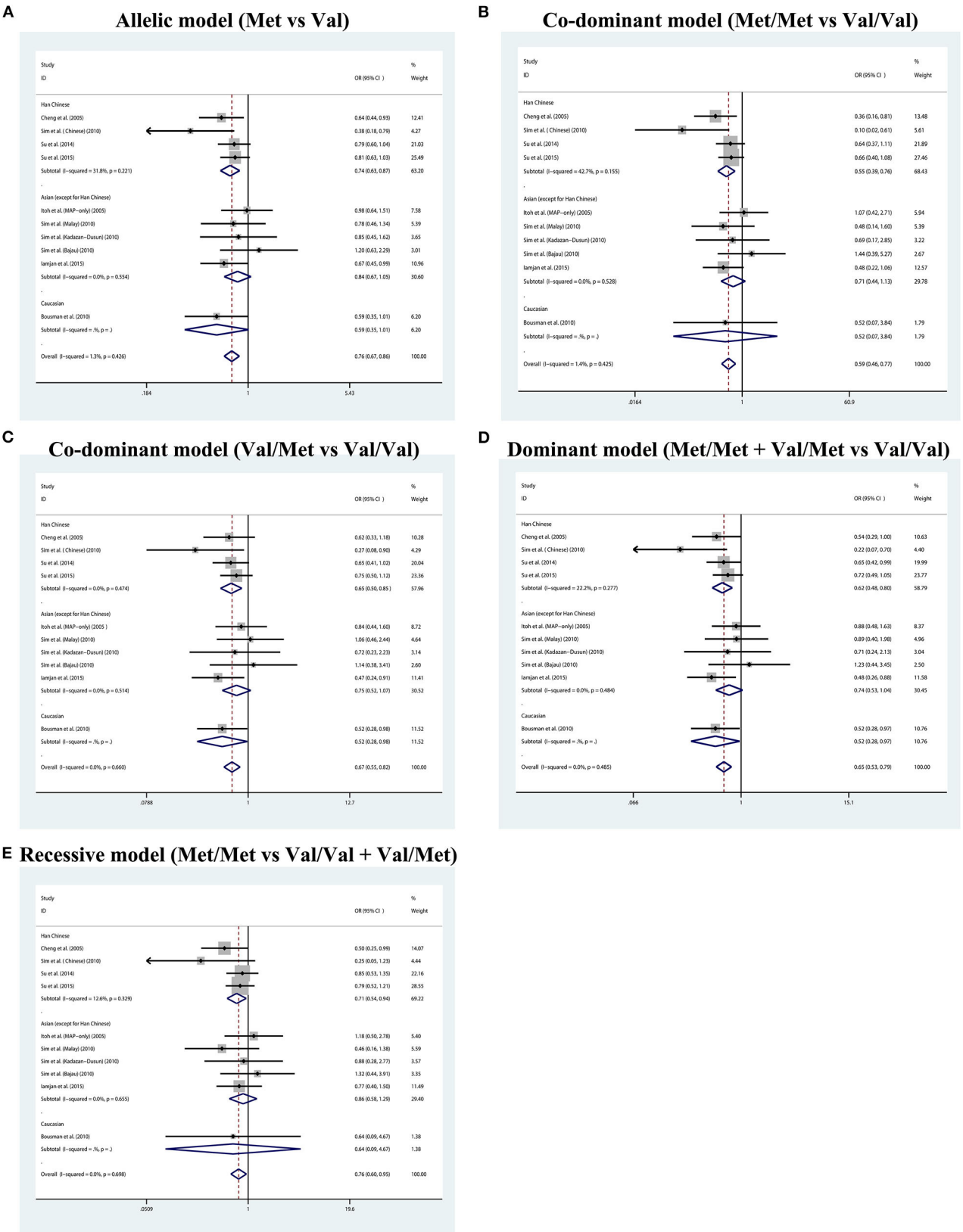

FIGURE 2 | Forest plot for the association between BDNF Val66Met polymorphism and methamphetamine dependence susceptibility in five genetic models. **(A)** Allelic model (Met vs. Val). **(B)** Co-dominant model (Met/Met vs. Val/Val). **(C)** Co-dominant model (Val/Met vs. Val/Val). **(D)** Dominant model (Met/Met + Val/Met vs. Val/Val). **(E)** Recessive model (Met/Met vs. Val/Val + Val/Met).

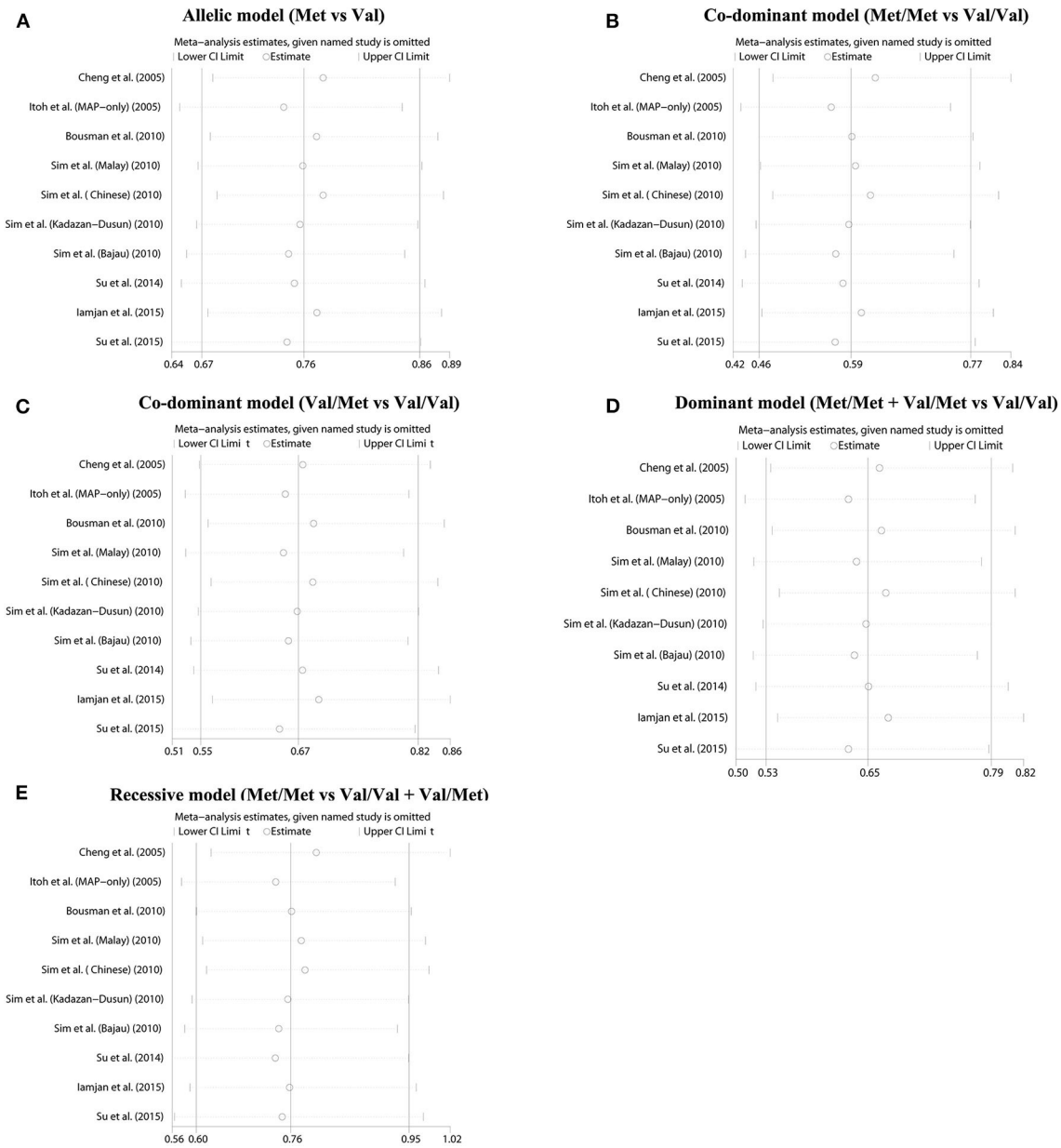

FIGURE 3 | Sensitivity analysis results. **(A)** Allelic model (Met vs. Val). **(B)** Co-dominant model (Met/Met vs. Val/Val). **(C)** Co-dominant model (Val/Met vs. Val/Val). **(D)** Dominant model (Met/Met + Val/Met vs. Val/Val). **(E)** Recessive model (Met/Met vs. Val/Val + Val/Met).

In this meta-analysis, we only included studies that explored the relationship between simple METH addiction and BDNF Val66Met polymorphism. Therefore, seven trials were included in this meta-analysis, including four trials the same as the inclusion studies by Haerian et al. and three additional trials. Haerian et al. conducted subgroup analysis only according to Asian and Caucasian ethnicity. Our study further separated Han Chinese from Asian ethnicity; for many studies, it was found that the 66Val allele frequency in Han Chinese was significantly different from the controls and the weight of Han Chinese in the overall analysis was relatively high. This meta-analysis found that BDNF Val66Met might be a genetic factor for METH use disorder among the Han Chinese population. The allele frequency of BDNF Val66Met varied between different geographical areas. The frequency of 66Val allele in East Asians is 0.510, while that in South Asians is 0.807 (41). The distribution of BDNF Val66Met genotypes was also different among the inclusion studies, implying that there might be differences in susceptibility to METH among people with diverse genetic backgrounds. In the subgroup analysis, heterogeneities were increased in the Han Chinese group. This heterogeneity might result from the participants' heterogeneity among the inclusion

TABLE 3 | The results of subgroup meta-analyses under different genetic models.

Subgroup	Genetic model	Comparison	Association			Heterogeneity	
			OR	95% CI	*P*	I^2 (%)	*P*
Asian (Han Chinese)	Dominant model	Met/Met + Val/Met vs. Val/Val	0.62	0.48–0.80	**<0.01**	22.20	0.28
	Recessive model	Met/Met vs. Val/Val + Val/Met	0.72	0.54–0.95	**0.02**	12.60	0.33
	Co-dominant model	Met/Met vs. Val/Val	0.55	0.39–0.76	**<0.01**	42.70	0.16
		Val/Met vs. Val/Val	0.66	0.50–0.86	**<0.01**	0.00	0.47
	Allele model	Met vs. Val	0.74	0.63–0.87	**<0.01**	31.80	0.22
Asian (except for Han Chinese)	Dominant model	Met/Met + Val/Met vs. Val/Val	0.74	0.53–1.04	0.08	0.00	0.48
	Recessive model	Met/Met vs. Val/Val + Val/Met	0.86	0.58–1.29	0.47	0.00	0.66
	Co-dominant model	Met/Met vs. Val/Val	0.71	0.44–1.13	0.15	0.00	0.53
		Val/Met vs. Val/Val	0.75	0.52–1.07	0.11	0.00	0.51
	Allele model	Met vs. Val	0.84	0.67–1.05	0.12	0.00	0.55
Caucasian[a]	Dominant model	Met/Met + Val/Met vs. Val/Val	0.52	0.28–0.97	**0.04**	/	/
	Recessive model	Met/Met vs. Val/Val + Val/Met	0.64	0.09–4.67	0.66	/	/
	Co-dominant model	Met/Met vs. Val/Val	0.52	0.07–3.84	0.52	/	/
		Val/Met vs. Val/Val	0.52	0.28–0.98	**0.04**	/	/
	Allele model	Met vs. Val	0.59	0.35–1.01	0.05	/	/

[a]*Only one study was included.*

The bold values indicate that the p-value is less than 0.05.

studies. Of the four studies carried out in Han Chinese, half (28, 31) only included METH-dependent male as research subjects. Research has reported the gender differences in socio-demographic and clinical characteristics of METH abusers (42). Furthermore, Heinzerling et al. (43) found that there was a significant interaction between sex and BDNF Val66Met among METH-dependent patients. Although the biological mechanism contributing to these differences is unclear, additional researches on the different biological basis of response to METH among male and female are warranted.

In summary, considering the race specificity, limited samples, participant heterogeneity, and insufficient statistical ability of the current studies on the association between BDNF Val66Met polymorphism and simple METH use disorder, we applied a meta-analysis to get a pooled effect size, illustrating the potential effect of BDNF Val66Met on METH addiction. Besides, in the subgroup analysis according to the ethnicity, we found that the effect of BDNF Val66Met on METH addiction had ethnically specific differences. However, there were still several limitations in this meta-analysis. First of all, the majority of the inclusion studies were conducted on men, so the relationship between BDNF Val66Met polymorphism and gender cannot be observed among METH abusers. Second, the limitation of sample size made a relative low power of the recessive model. Besides, too few Caucasian studies were carried out, and a stratified analysis by Caucasians could not be done under different genetic models because of the insufficient frequency data. Finally, this study only focuses on the association between BDNF Val66Met polymorphism and METH-dependent susceptibility without considering gene–gene or gene–environment interactions. Therefore, this conclusion still needs to be supported by more researches carried out in different populations, and more researches are needed to be conducted.

CONCLUSIONS

This is the first meta-analysis to study the relationship between simple METH addiction and BDNF Val66Met polymorphism. In this meta-analysis, we found that the expression of BDNF 66Met in METH-dependent patients was lower than that in the controls, indicating that BDNF 66Met might be a protective factor for METH addiction. Besides, in the subgroup analysis, we found that BDNF 66Met was differentially expressed only in the Han population, suggesting that the protective effect of BDNF 66Met might be ethnic-specific. The mechanism of BDNF Val66Met gene polymorphism on METH addiction in the Han population remains to be further studied. The current studies on association between METH addiction and BDNF Val66Met polymorphism are mainly from Asia, while few are from other districts. Therefore, the current results might be modified with

the increase of studies in other regions. Considering these limitations, large-sample studies with different ethnicities should be conducted to determine the role of the BDNF Val66Met polymorphism in different populations.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

LH and TL: study design. LH, QW, and YL data collection, analysis, and interpretation. LH: drafting of the manuscript. TL and YL: critical revision of the manuscript. All authors approval of the final version for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2020.585852/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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A Community-Based Investigation of Stigma Toward Individuals Receiving Methadone Maintenance Treatment in China: A Randomized Case Vignette Study

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Background: Even though methadone maintenance treatment (MMT) is effective and widely recommended, it is widely misunderstood and stigmatized. This study aimed to explore people's attitudes and beliefs toward MMT, and stigmatization of MMT patients in China.

Methods: This randomized, vignette-based study enrolled 1,066 household respondents (552 males and 514 females, response rate is 88.83%, 86.00% in vignette 1 and 91.66% in vignette 2) from two communities in China. Respondents initially completed questionnaires on sociodemographic characteristics and their knowledge about methadone and MMT. They were then randomly assigned to vignette 1 (described a patient receiving MMT) or vignette 2 (described a patient receiving amlodipine treatment). The labeling, stereotyping, and social distance toward the individual described in the vignettes were evaluated.

Results: In these two vignettes, respondents showed a significantly higher level of stigma and discrimination toward the patient receiving MMT than the patient receiving amlodipine treatment. Approximately 60% of respondents believed that methadone is a type of addictive drug and that participating in MMT is a way to get high. Over 60% of respondents labeled the heroin-dependent patient who is receiving MMT as an addict even though the patient has not used heroin for several years; about 80% of them believed that the patient has undesirable characteristics and expressed a strong desire for social distance.

Conclusions: People's misunderstanding of MMT, and stigmatization of MMT patients were very common among populations in the communities in China. To maximize MMT patients benefiting from MMT programs, more efforts are needed to minimize the impact of MMT-related stigma.

Keywords: stigma, methadone maintenance treatment, MMT, China, public attitudes

INTRODUCTION

Methadone Maintenance Treatment in China

In China, opioids remain one of the most widely used illegal drugs (1), and illicit opioid use continues to drive the human immunodeficiency virus (HIV) epidemics and increase crime (2, 3). To address the severe social and medical problems associated with illicit opioid use, China initiated the methadone maintenance treatment (MMT) program with just eight sites in 2004. By the end of 2016, the program has been extended to 789 sites, with 162,000 heroin users under treatment, as stated by the annual report on drug control in China in 2017 (from the Office of China National Narcotics Control Commission, http://jhak.com/grwz/20170125.pdf). MMT has a long-established efficacy in preventing illicit opioid use, reducing crime, and decreasing the risk of HIV and hepatitis C virus (HCV) transmission (4–6).

Even though MMT is effective and widely recommended, it is still largely misunderstood and stigmatized (7–10). Negative attitudes and beliefs toward MMT are widespread, such as taking methadone is a way to get high, and patients receiving MMT are characterized as incompetent, untrustworthy, irresponsible, and has a weak willpower (11). Due to these prejudice, stereotypes, and discrimination, MMT patients have been stigmatized widely in the community (12). Research indicates that stigma against MMT gives rise to many negative consequences, including increased involvement in risky behavior, non-participation, treatment withdrawal, delayed recovery, and reintegration process (13–16).

Stigmatization Toward MMT Patients

A previous study reported that MMT service providers had a high-level stigma toward MMT patients (17). Community-based social support may play a more crucial role in improving and continuing the treatment of individuals under MMT (18). Exploring the community population's attitudes and beliefs toward MMT and stigmatization of MMT patients in China will help us develop better MMT. Thus, the present study aimed to assess public stigmatizing attitudes toward the patients receiving MMT in China, based on Link and Phelan's conception of the stigma process. According to Link and Phelan's (19) view of stigma [stigma occurs when the following interrelated components converge: "(1) labeling: people distinguish and label human differences; (2) stereotyping: the labeled is linked to undesirable characteristics; (3) separating: the labeled is separated as an out-group; (4) the labeled experience status loss and discrimination; and (5) a power situation is necessary to allow these process to unfold]", we hypothesize that misunderstanding of MMT and stigmatization of MMT patients would also be very common among populations in the communities in China.

METHODS

Research Procedure and Respondents

The data was collected between December 2018 and March 2019. Participants were enrolled from an urban community in the Tianxin district of Changsha city and a rural community in the Junshan district of Yueyang city, Hunan province. Participants were randomly selected from the household-based sample in these communities by Excel worksheet. In total, 800 households were selected from the urban community with 3,363 households, and 400 households were selected from the rural community with 1,046 households. One person at least 16 years old was randomly chosen in each family. A total of 1,200 individuals were included in this study. A local organizer in each community brought interviewers into each house and gave a brief account of the survey. Respondents were provided with 10 CNY to compensate for their participation. Ethical approval was obtained from the Ethics Committee of Jiangxi Mental Hospital (No. 20180204).

Research Instruments

In this community-based, randomized case vignette study, we developed a survey booklet, including the respondents' demography and knowledge about MMT, as well as two vignettes to assess stigmatization of MMT. Respondents in the study group received vignette 1, describing a patient receiving MMT, while respondents in the control group received vignette 2, illustrating a patient receiving amlodipine treatment. Each participant was randomly given one of the vignettes.

Public Knowledge About MMT

A set of questions were asked to examine the participants' knowledge about MMT, such as "In your opinion, methadone is a—(1) medicine, (2) addictive drug, (3) do not know? In your opinion, participating in MMT is a—(1) therapeutic method, (2) means of drug addiction, (3) do not know?"

Labeling

Participants were asked to define the person depicted in the vignette (1) normal person, (2) patient, and (3) addict with the question "In your opinion, Zhangsan is a _____."

Stereotyping

The semantic differential scale was used to assess the measure of stereotyping (20). The scale comprises seven pairs of polar adjectives on seven 7-point rating scales. Scores ranged from 1 to 7, lower values indicating a more negative stereotype. Participants were categorized as having a negative stereotype about the person depicted in the vignette if they scored below 4 points.

Social Distance

The social distance scale (21–23) was used to assess participants' desire for social distance. Participants were asked about their willingness to engage in five forms of social contact with the person depicted in the vignette including (1) "become a neighbor," (2) "spend an evening socializing," (3) "make friends," (4) "start working closely," and (5) "become related as a family." These items were rated on a 4-point scale from 1 (definitely) to 4 (definitely not). The mean score ranged from 1 to 4 points, with a higher score reflecting a stronger desire for social distance. Participants were viewed as unwilling to engage in these forms of interaction if they scored above the point of 2.5.

TABLE 1 | Demographic characteristics.

Variable	Total ($n = 1,066$)	Participants respond to the vignette of the patient receiving AT ($n = 516$)	Participants respond to the vignette of the patient receiving MMT ($n = 550$)	χ^2/t	*P*
	M (SD)	M (SD)	M (SD)		
Age (years)	37.38 (15.88)	37.52 (16.71)	37.25 (15.06)	*0.28*	*0.78*
	n (%)	*n* (%)	*n* (%)		
Gender				1.76	0.19
Male	552 (51.8)	278 (53.9)	274 (49.8)		
Female	514 (48.2)	238 (46.1)	276 (50.2)		
Marital status				3.87	0.05
Currently married	648 (60.8)	218 (42.2)	200 (36.4)		
Not married	418 (39.2)	298 (57.8)	350 (63.4)		
Education (years)				4.91	0.19
≤6	44 (4.1)	14 (2.7)	21 (3.8)		
7–12	682 (63.9)	224 (43.4)	258 (46.9)		
13–17	322 (30.2)	268 (51.9)	254 (46.2)		
>18	18 (1.7)	10 (1.9)	17 (3.1)		
Income per month				4.8	0.10
≤¥3,000	652 (61.2)	316 (61.24)	336 (61.09)		
>¥3,000	414 (38.8)	200 (38.76)	214 (38.91)		
Residence				0.07	0.79
Urban	690 (64.7)	180 (34.9)	196 (35.6)		
Rural	376 (35.3)	336 (65.1)	354 (64.4)		

AT, amlodipine treatment; MMT, methadone maintenance treatment. χ^2 represents chi square value of chi-squared test for categorical variables; t represents t-value of t-tests (for continuous variables).

Vignettes

We constructed one vignette depicting a patient receiving MMT and the other describing a patient receiving amlodipine treatment. Patients in the two vignettes met ICD-10 criteria for opioids dependent, currently abstinent, but receiving treatment with methadone, and hypertension, respectively.

Vignette 1: Patient Receiving MMT

Zhang San is a 38-year-old male. His first ingestion of heroin was at his friend's house approximately 10 years ago. In the subsequent months, the intake dosage gradually increased. He gradually lost interest in other activities in life. Because of heroin use, he lost his job. Three years ago, he initiated an attempt to stop heroin use and went to an MMT clinic to receive MMT. In the past 3 years, he has taken his medication on time and has not used heroin. He has found a job and has been working continuously since then.

Vignette 2: Patient Receiving Amlodipine Treatment

Zhang San is a 38-year-old male. Three years ago, he received an examination at the hospital due to occasional dizziness and lack of strength. His blood pressure was repeatedly measured over 170/100 mmHg. Zhang San was prescribed with amlodipine. In the past 3 years, he has taken his medication on time and keep his blood pressure in the normal range. He has been working continuously.

Statistical Analysis

All statistical analyses were conducted in SPSS version 20.0 software package. The *t*-tests (for continuous variables) and χ^2 analysis (for categorical variables) were performed to compare the differences in public stigma toward the MMT patient and the hypertension patient. Moreover, correlation analysis was performed to examine the correlation between stigmatization and the age, sex, marital status, education, resident, and monthly income of the community residents.

RESULTS

Of the 1,200 individuals identified in the survey, 134 (11.17%) lost contact. Thus, 1,066 (88.83%) participants were (86.00% in vignette 1 and 91.66% in vignette 2) included in this study. The demographic information is shown in **Table 1**. There are no differences in the sociodemographic characteristics between participants who responded to the vignette of the patient receiving amlodipine treatment and those who responded to the vignette of the patient receiving MMT.

Community Residents' Understanding of MMT

Approximately 60% of respondents believed that methadone is a type of addictive drug and that participating in MMT is a way to get high. Over 30% of the respondents expressed that they did not know what methadone is or what MMT entails. Only 10.13% of the respondents acknowledged methadone as medicine and perceived participating in MMT as a treatment method (**Table 2**).

TABLE 2 | Community residents' understanding of methadone and MMT.

	N	%
In your opinion, methadone is a		
Medicine	108	10.13
Addictive drug	608	57.04
Do not know	350	32.83
In your opinion, participating in methadone maintenance treatment is a		
Therapeutic method	108	10.13
Way to get high	600	56.28
Do not know	358	33.58

MMT, methadone maintenance treatment.

Community Residents' Stigmatization of MMT Patients

Labeling

More than half (64.00%) of the respondents labeled the patient receiving MMT as addicts, 18.18% identified a patient's label. Only 17.82% identified a normal label for the MMT patient, whereas 67.83% labeled the hypertension patient receiving amlodipine treatment as normal (**Table 3**).

Stereotyping

The stereotypes about the patient receiving MMT were generally negative. Compared with the hypertension patient (5.35 ± 1.12), the heroin-dependent patient receiving MMT (3.47 ± 1.75) was rated far more negatively. The majority (76.46%) of respondents had a negative stereotype about the MMT patient, whereas only 10.47% had a negative stereotype about the patient receiving amlodipine treatment (**Table 3**).

Social Distance

Most of the respondents expressed a high-level desire for social distance from the patient receiving MMT. Approximately 90% of respondents were unwilling to make friends with the heroin-dependent patient receiving MMT and have the person marry into the family. Most (78.54%) were unwilling to start working closely with the person; more than half of them were unwilling to move next door to and spend an evening socializing with the person. Respondents showed a higher level of desire for social distance from the patient receiving MMT (3.33 ± 0.79) than the patient receiving amlodipine treatment (1.72 ± 0.31). More details are shown in **Table 3**.

Correlation analysis did not find any associations between sociodemographic with the views about MMT, negative stereotype, or social intolerance toward the patient receiving MMT (as shown in the **Supplementary Material**).

DISCUSSION

As far as we know, this is the first study to evaluate the misunderstanding of MMT and stigmatization toward MMT patients in China. Findings of this study reveal that misunderstanding of MMT is widely spread in the community in Hunan, China. Although MMT effectively reduces opioids use, criminality, and adverse health effects, only 10.13% of the respondents acknowledged methadone as medicine and perceived MMT as a treatment method. Consistent with reports that MMT continues to be largely misunderstood (7, 11, 12), many respondents perceived methadone as a type of addictive drug and that participating in MMT is a way to get high.

High Level of Stigma and Discrimination Toward MMT Patients

In these two vignettes, respondents showed a significantly higher level of stigma and discrimination toward patients receiving MMT than patients receiving amlodipine treatment. Approximately 60% of respondents believed that methadone is a type of addictive drug and that individuals participate in MMT to get high. Over 60% of respondents labeled the heroin-dependent patient who is receiving MMT as an addict. About 80% believed the patient has undesirable characteristics and expressed a strong desire for social distance.

These findings also show stigmatization of MMT patients compared with patients with other chronic conditions that require treatment such as hypertension. The majority of the community respondents in the community labeled the methadone patient as an addict, believing the patient receiving MMT is bad, insincere, dangerous, unpredictable, unreliable, weak, and selfish. Since negative stereotype could evoke community rejection (24), it is no surprise that the respondents expressed a strong desire for social distance across different domains of social interaction with patients receiving MMT. Participants expressed that the more intimate the interaction, the stronger the desire to keep a distance. Thus, the vast majority were unwilling to make friends with the patient receiving MMT or have the person marry into the family.

These findings in our study suggest that widespread misunderstanding of MMT and stigmatization of the patients in the community should be taken into account in the community-based MMT program. China has established the largest MMT network in the world to address the serious social and medical problems associated with illicit opioid use. However, the present study indicates that community residents are far from being well-prepared to accept methadone patients. The continuing stigmatization of methadone patients may undermine the government's efforts to help them tackle their condition. Interventions to resolve misunderstandings and reduce stigmatization are necessary for all sections of the community.

Many studies have demonstrated that the MMT stigma is an extension of substance use stigma (8, 11, 25–28). Therefore, interventions that reduce MMT stigma may need to co-address substance use stigma (27). Keeping a reasonable balance between raising public awareness of drugs while avoiding discrimination and rejection against drug users is an important issue in public media communication. Previous evidence demonstrated that communicating with the public using sympathetic stories or

TABLE 3 | Community residents' stigmatization toward the patient receiving AT and the patient receiving MMT.

Stigmatization	Vignettes				*t*	χ^2
	The patient receiving AT ($n = 516$)		The patient receiving MMT ($n = 550$)			
	Mean (SD)	*n* (%)[#]	Mean (SD)	*n* (%)[#]		
Labeling						
Normal person		350 (67.8)		98 (17.8)		256.36*
Patient		166 (30.6)		100 (18.2)		
Addict		0 (0.0)		352 (64.0)		
Stereotyping						
Good–bad	5.57 (1.21)	56 (10.9)	3.53 (1.79)	410 (74.6)		
Sincere–insincere	5.39 (1.20)	78 (15.1)	3.42 (1.74)	426 (77.5)		
Safe–dangerous	5.42 (1.22)	84 (16.3)	3.46 (1.71)	422 (76.7)		
Predictable–unpredictable	5.40 (1.20)	80 (15.5)	3.43 (1.78)	424 (77.1)		
Reliable–unreliable	5.35 (1.26)	94 (18.2)	3.56 (1.58)	426 (77.5)		
Strong–weak	5.12 (1.28)	158 (30.6)	3.57 (1.86)	398 (72.4)		
Unselfish–selfish	5.22 (1.24)	160 (20.9)	3.34 (1.78)	426 (77.5)		
Mean score	5.35 (1.12)	54 (10.5)	3.47 (1.75)	420 (76.5)	86.92*	210.14*
Social distance						
Become neighbor	1.71 (0.65)	56 (10.9)	2.99 (0.72)	352 (64.0)		
Become acquaintance	1.73 (0.68)	58 (11.2)	3.01 (0.73)	373 (67.7)		
Make friends	1.74 (0.65)	64 (12.4)	3.57 (0.74)	481 (87.5)		
Become co-worker	1.73 (0.65)	64 (12.4)	3.12 (0.74)	432 (78.5)		
Become family	1.74 (0.67)	64 (12.4)	3.53 (0.76)	485 (88.2)		
Mean score	1.72 (0.31)	64 (12.4)	3.33 (0.79)	450 (81.8)	81.71*	232.06*

AT, amlodipine treatment; MMT, methadone maintenance treatment.

[#]Percentages in the part of stereotyping represent proportion of respondents who scored below than the 4 midpoint of the Semantic Differential Scale. Percentages in the part of social distance represent proportion of respondents who scored above than the 2.5 midpoint.

**P < 0.001.*

narratives that humanize the experiences of individuals with substance use disorder is a promising technique for reducing stigma (29–31). In contrast, messages blaming individuals with substance use disorder will increase public negative emotions and desire for social distance toward them (32, 33). Furthermore, information about the therapeutic quality of MMT is especially needed to address stigma. Stories or narratives portraying individuals with successful treatment recovery would reduce MMT stigma (34–36).

Limitations

There are several limitations to the study. First, this study is based on self-report data which may induce self-report bias. Second, information was obtained through closed-ended questions, which may guide participants to provide socially desirable responses. Third, other factors other than MMT depicted in the vignette, such as heroin dependence, may impact responses, thus leading to overestimated stigma levels. Fourth, participants were recruited from two communities in Hunan, which may not fully represent all community members in this province or in China. Despite this, participants were randomly selected from these two communities, which reduced bias and increase the representation of findings. Fifth, as 11.17% of the identified individuals could not be contacted, it is unknown if their views and attitudes toward MMT patients are different from the respondents. Sixth, the vignette response rate described that patient receiving MMT (86.00%) was much lower than the patient receiving amlodipine treatment (91.66%). These non-responders who received MMT vignette may have more negative attitudes toward patients receiving MMT, which may lead to underestimation of the stigmatization toward MMT. Yet, the present result still reflects a high level of stigmatization attitudes toward MMT and patients receiving MMT in community residents. Last but not least, we did not evaluate participants' personal experience, such as having encountered such patients in the past, which may influence results and could provide some information about prevention and intervention.

CONCLUSIONS

Our findings demonstrate that misunderstanding of MMT and stigmatization of MMT patients are very common among populations in the communities in China. Educating community residents regarding MMT's therapeutic quality and the patient identity of the person receiving MMT is recommended.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethic Committee of Jiangxi Mental Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

TL and QW performed the statistical analysis. QD, QW, and TL prepared the first draft of the manuscript. QD, MH, FY, and QL assisted in the collection of data and implementation. QD, TL, and QW have contributed to the interpretation of data and writing. WH commented on the manuscript. All authors participated in the research, study design, manuscript preparation, and have approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2020.601266/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Alcohol Consumption in China Before and During COVID-19: Preliminary Results From an Online Retrospective Survey

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Background: Alcohol is an important aspect of Chinese culture, and alcohol use has been traditionally accepted in China. People with stress, anxiety, and depression may use more alcohol. More people reported symptoms of anxiety and depression during the outbreak of COVID-19. Thus, people may drink more alcohol during the outbreak of COVID-19 than before COVID-19.

Methods: An online retrospective survey was conducted on a total sample of 2,229 participants. Drinking behaviors before and during COVID-19, current risky drinking and hazardous drinking, and the association between high-risk drinking and mental health problems (depression, anxiety, and stress) were assessed *via* self-reported measures on the Alcohol Use Disorders Identification Test (AUDIT) and the 21-item Depression Anxiety Stress Scales (DASS−21).

Results: This study found that, compared with before COVID-19, alcohol consumption was slightly decreased during COVID-19 (from 3.5 drinks to 3.4 drinks, $p = 0.035$) in the overall sample. Most (78.7%) alcohol drinkers were males. Before and during COVID-19, males consumed more drinks per week (4.2 and 4.0 vs. 1.3 and 1.2 drinks), had a higher percentage of heavy drinking (8.1 and 7.7% vs. 4.4 and 2.7%), and more drinking days per week (2.1 and 2.1 vs. 1.0 and 0.9 days). Males also had more risky drinking (43.2 vs. 9.3%) and hazardous drinking (70.2 vs. 46.6%) than female counterparts. This study also found that high-risk drinking predicted anxiety in females.

Conclusions: This study suggests a slight reduction in alcohol consumption during COVID-19. However, hazardous drinking is common, especially among male alcohol

drinkers. Males consumed more alcohol, had more risky and hazardous drinking than female counterparts both before and during COVID-19. Public health policy makers should pay more attention to developing effective, population-based strategies to prevent harmful alcohol consumption.

Keywords: alcohol consumption, heavy drinking, risky drinking, before and during COVID-19, retrospective survey

INTRODUCTION

A 2018 global status report on alcohol and health by the World Health Organization (WHO) shows that the numbers of current drinkers (those who have consumed alcoholic beverages in the previous 12-month period) aged 15 and over reached more than two billion (accounting for 43% of the population) (1). There are only three WHO regions in which alcohol is consumed by more than half of the population, including the European region (EUR) (59.9% of current drinkers), the region of the Americas (AMR) (54.1%), and the Western Pacific region (WPR) (53.8%), and the increase in current drinkers in WPR is dominated by China (1). In 2016, alcohol use led to a large burden of disease and injury, causing 132.6 million disability-adjusted life years (DALYs, the total loss of healthy life years from onset to death), which represented 5.1% of all DALYs (1). The harmful use of alcohol is associated with more than 200 diseases and unintentional injury deaths (e.g., in 2016, approximately three million drinkers lost their lives due to harmful use of alcohol globally) (1, 2). The total alcohol consumption per capita remained at the level of 6.4 L (equivalent to 13.9 g of pure alcohol per day) globally over the period 2010–2016, but that was increased from 7.1 to 7.2 L in China (1). Consequently, the number of alcohol-attributable deaths also increased from 484,000 to 670,000 over the past decade (from 2007 to 2017) (3). The burden caused by alcohol use has become the third highest behavioral risk factor after inappropriate diet and smoking in China (4).

Alcohol use disorder (AUD), including alcohol abuse and dependence, is a chronic and relapsing disease, which is characterized by compulsive, heavy alcohol use and loss of control over alcohol intake (5). Globally, nearly 300 million adults (mainly males) suffer from AUD, and 2.6% of people aged 15 and over suffer from alcohol dependence (AD, the most severe form of AUD) (1). The current prevalence estimate of AD in China is about 2.2% (6). AD is characterized by craving, tolerance, and withdrawal (7) and is associated with physical and mental disease, causing impairments in overall health-related quality of life (HRQOL) (8). Data from 302 hospitals in Beijing, China, found that the number of hospitalized patients with alcoholic liver disease (ALD) increased from 1.68% in 2002 to 4.59% in 2013 (9).

There are several factors that can impact the levels and patterns of alcohol consumption, including biological, individual, and social factors (10). A meta-analysis of twin and adoption studies showed that about 50% of AUDs are heritable (11). The prevalence tends to be higher if alcohol is readily available, and people adopt a more permissive attitude toward heavy drinking (12, 13). Individuals with low socioeconomic status are at least twice as likely to die from their disorders and prolonged heavy alcohol use than their counterparts with high socioeconomic status (14). Negative emotions, such as stress, anxiety, and depression, are associated with alcohol use and may increase an individual's vulnerability to AUD (15–17). Alcohol is an important aspect of Chinese culture. Alcohol use has been traditionally accepted in China, especially during major social events (such as the spring festival and parties) (18). A survey of the general population in China found that males used alcohol more heavily and frequently than females (19).

An online survey of mental health problems caused by the COVID-19 outbreak and mass isolation found that, during the COVID-19 outbreak (n = 1,074), Chinese residents (mainly Hubei Province) reported a rate of 9.5% of harmful drinking and 1.6% of AD, and drinking problems were higher among people from Hubei (which had the most COVID-19 cases) than other provinces (20). Assessing the changes in Chinese people's drinking behavior before and during the outbreak of COVID-19 may provide evidence for public health policy making on preventing harmful alcohol consumption.

The primary objective of this online retrospective survey is to assess the impact of COVID-19 on alcohol drinking patterns among people from the general population of China by comparing alcohol drinking before and during the outbreak of COVID-19. Considering the association between alcohol use and anxiety and depression (21) and more people reporting symptoms of anxiety and depression during the outbreak of COVID-19 (20, 22), we hypothesize that, compared with before the outbreak of COVID-19, Chinese people may drink more alcohol during the outbreak of COVID-19. We also hypothesize that high-risk drinking would be associated with depression, anxiety, and stress.

METHODS

Participants and Inclusion and Exclusion Criteria

In this cross-sectional, retrospective, anonymous online survey, participants were 18 years of age or older, Chinese literate, alcohol drinkers, able to access online services, had interest, and were

Abbreviations: WHO, World Health Organization; WPR, Western Pacific Region; DALYs, disability-adjusted life years; AUD, alcohol use disorder; AD, alcohol dependence; ALD, alcoholic liver disease; TLFB, Timeline Followback; AUDIT, Alcohol Use Disorders Identification Test; AUDIT-C, Alcohol Use Disorders Identification Test for Consumption; DASS−21, the 21-item Depression Anxiety Stress Scales.

TABLE 1 | Inclusion and exclusion criteria of participants.

Inclusion criteria
1. 18 years of age or older
2. Alcohol drinker
3. Being able to access online services
4. Being able to read and write in Chinese
5. Expressing an interest in participant this study
6. Willing to provide informed consent to participate in the study
Exclusion criteria
1. Under 18 years of age
2. Unable to access online services
3. Unable to read and write in Chinese

willing to provide informed consent to participate in the study. An overview of participant eligibility criteria is given in **Table 1**.

Recruitment

An Internet-based recruitment method was used in this cross-sectional survey study. We used Internet-based advertisements (such as websites and social media) to invite any adults to participate in an approximately 10- to 20-min online survey between May 7, 2020, and August 3, 2020. Advertisements contained a hyperlink that directed potential participants to the study's ethics approved consent form. After obtaining electronic informed consent, eligible participants were asked to complete a demographic questionnaire and measures of addictive behaviors (smoking behavior and the behavior of using social media and Internet gaming, reported elsewhere). Participants were required to answer all questions before submitting the survey but could quit the survey at any time. Thus, this survey had no missing data.

Measures

Socio-Demographics

Socio-demographic information of all respondents included gender, age, years of completed education, employment status (employed, unemployed, student), marital status, and region (rural, urban).

Alcohol Drinking Behavior and Possible Alcohol Dependence

Alcohol consumption (number of alcohol units) before and during the outbreak of COVID-19 was measured by a Timeline Followback (TLFB) questionnaire (23), including questions about (1) average amount (mL) of alcohol intake per day, (2) average amount (mL) of alcohol intake per week, and (3) average alcohol drinking days per week (0–7).

The Alcohol Use Disorders Identification Test (AUDIT) (24, 25) is a widely used tool to assess hazardous drinking and AUDs. AUDIT is a comprehensive, 10-question screening tool. The total score ranges from 0 to 40, from low risk (0–7), increasing risk (8–15), higher risk (16–19), to possible dependence (20 or more). The AUDIT alcohol consumption questions AUDIT-C (Alcohol Use Disorders Identification Test for Consumption) (26, 27) is a modified version of the AUDIT and includes three items: (1) How often do you have a drink containing alcohol? (scores: never = 0, monthly or less = 1, 2-4 times per month = 2, 2-3 times per week = 3, 4+ times per week = 4); (2) How many units of alcohol do you drink on a typical day when you are drinking? (1–2 = 0, 3–4 = 1, 5–6 = 2, 7–9 = 3, 10+ = 4); (3). How often have you had 6 or more units if female or 8 or more if male on a single occasion in the last year? (Never = 0, Less than monthly = 1, Monthly = 2, Weekly = 3, Daily or almost daily = 4). The AUDIT-C is scored on a scale of 0–12. In male individuals, a score of $\geq$4 is considered positive, optimal for identifying hazardous drinking or active AUDs. In female individuals, a score of $\geq$3 is considered positive. Generally, the higher the score, the more likely it is that an individual's drinking is affecting his or her safety. The severity of risk drinking in AUDIT-C points are ranked as severe (8–12 points), high (6–7 points), moderate (4–5 for males and 3–5 for females), and low (0–3 for males and 0–2 for females).

Mental Health (Depression, Anxiety, and Stress)

The 21-item Depression Anxiety Stress Scale (DASS−21) (28) was used to identify signs of anxiety, depression, and stress. The DASS-21 has been translated and validated in Chinese (27, 29). It is a self-report instrument consisting of three subscales, and each of the three DASS-21 scales contains 7 items to assess depression, anxiety, and stress over the last week. The responses are given on a 4-point Likert scale, ranging from "I strongly disagree" (0) to "I totally agree" (3). Overall scores for the three constructs are calculated as the sum of scores for the relevant seven items multiplied by two. Ranges of scores correspond to levels of symptoms, ranging from "normal" to "extremely serious." Scores for depression, anxiety, and stress are calculated by summing the scores for the relevant items, ranging from 0 to 21 scores. Recommended cutoff scores for conventional severity labels (normal, mild, moderate, severe, extremely severe) are as follows (multiplied by 2): depression: 0–9, 10–13, 14–20, 21–27, 28+; anxiety: 0–7, 8–9, 10–14, 15–19, 20+; stress: 0–14, 15–18, 19–25, 26–33, 34+.

Definition

Alcohol Drinker

Defined as a person who drinks no <30 g alcohol (equal to 900 mL beer) per week.

Standard Drink

A standard drink is equal to 14.0 g (0.6 ounces, equal to 18 mL) of pure alcohol. Generally, this amount of pure alcohol is found in: 12 ounces (equal to 355 mL) of beer (5% alcohol content); 8 ounces (equal to 237 mL) of malt liquor (7% alcohol content); 5 ounces (equal to 148 mL) of wine (12% alcohol content); 1.5 ounces (equal to 44 mL) or a "shot" of 80-proof (40% alcohol content) distilled spirits or liquor (e.g., gin, rum, vodka, whiskey).

Heavy Drinker

If a man drinks more than 14 drinks per week or a woman drinks more than 7 drinks per week (30).

Hazardous Drinking or Active AUDs

Women who score 3 or higher in AUDIT-C recommended limits; men who score 4 or higher in AUDIT-C recommended limits (26).

Investigation Methods/Data Collection

This online survey study was performed using a professional online survey service, Questionnaire Star (https://www.wjx.cn), released nationwide through social media (such as WeChat, Weibo, QQ, etc.).

Quality Control

The computer IP address can be tracked to determine whether multiple entries were made from the same computer. However, considering that respondents may share the same Internet and same IP address with their families, this study did not limit the number of questionnaires from the same IP address. However, the survey allowed only one response per phone or computer.

Data Analysis

All data was automatically collected by Questionnaire Star. A user-specified Excel file was downloaded from the database. Statistical analysis was performed using SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Descriptive statistics, chi-square (χ^2) tests, and independent sample *t*-tests were applied to measure differences in alcohol drinking behaviors between before and during the outbreak of COVID-19. Regression analysis was applied to explore whether high-risk drinking predicted depression, anxiety, and stress in males and females. Statistical significance was set for two-sided $p < 0.05$.

RESULTS

Demographic Information

Demographic characteristics for the overall sample, males, and females are shown in **Table 2**. The total sample is 2,229 participants (78.7% males). Male participants were older, more employed, more married, and younger for first use of alcohol than their female counterparts. There were no differences between male and female participants in the rates of mental health problems with more than 30% individuals reporting depression, about 40% of people reporting anxiety, and more than 20% of them reporting stress during the last week of the survey.

TABLE 2 | Demographic characteristics, overall and for males and females.

Variables	Overall (*N* = 2,229)	Male (*n* = 1,755, 78.7%)	Female (*n* = 474, 21.3%)	*p*-value*
Age (mean ± SD)	36.6 ± 9.92	37.5 ± 9.71	33.4 ± 10.03	<0.001
Education (%)				
High school or lower	11.2	11.0	11.8	0.616
College or higher	88.8	89.0	88.2	
Employment (%)				
Unemployed	3.3	2.8	5.1, 24	0.014
Employed	96.7	97.2	94.9	
Marriage (%)				
Married	71.7	75.6	57.4	<0.001
Unmarried	28.3	24.4	42.6	
Living place (%)				
Urban	96.6	96.4	97.3	0.367
Rural	3.4	3.6	2.7	
Mental health problems (%)#				
Depression	34.0	33.7	34.8	0.660
Anxiety	39.5	38.6	42.8	0.097
Stress	22.3	22.2	22.6	0.849
Age of first drinking	17.6 ± 4.98	17.3 ± 4.85	18.6 ± 5.31	<0.001

Data are n (%) or mean (SD).

**Significant difference, $p < 0.05$ (two sample t-tests for continuous variables and χ^2 tests for categorical variables).*

#Mental health problems ranged from mild, moderate, severe, to extremely severe.

Drinking Behaviors Before and During COVID-19

Drinking behaviors before and during COVID-19 for the overall sample, males, and females are shown in **Table 3**. Compared to the time before COVID-19 and during COVID-19 in the overall sample, the average drinks (a standard drink is equal to 14.0 g or 0.6 ounces, = 18 mL, of pure alcohol) per week decreased from 3.5 drinks to 3.4 drinks, $p = 0.035$; the percentage of heavy drinking also insignificantly decreased from 7.4 to 6.7%, and the average of drinking days per week reduced from 1.9 to 1.8 days.

Gender Differences in Drinking Behaviors Before and During COVID-19

Gender differences in drinking behaviors before and during COVID-19 are shown in **Table 4**. Males reported about 4 drinks per week both before and during COVID-19, which was almost four times more than females; males also reported higher rates of heavy drinking (almost twice before and more than twice during COVID-19) and more drinking days per week (more than twice) than females both before and during COVID-19.

Risky and Hazardous Drinking

Severity of drinking risks by AUDIT-C overall and for males and females are shown in **Table 5**. The overall high-risk drinking was 36% with 43.2% of males and 9.3% of females. Among high-risk drinkers, half of males and one-third of females may have AD. More than 70% of males and almost half of females (46.6%) reported hazardous drinking.

High-Risk Drinking Predicting Depression, Anxiety, and Stress

A regression model of high-risk drinking predicting depression, anxiety, and stress is shown in **Table 6**. Regression analysis found no association between high-risk drinking and mental health problems of depression, anxiety, and stress in males. However, high-risk drinking predicted anxiety in females.

DISCUSSION

This online retrospective survey found that, compared with before COVID-19, alcohol consumption was slightly decreased during COVID-19. Most (78.7%) alcohol drinkers were males. They consumed more alcohol and had more risky and hazardous drinking than female counterparts both before and during

TABLE 3 | Drinking behaviors before and during COVID-19, overall and for males and females.

	Before COVID-19	During COVID-19	*p*-value*
Drinks per week (mean ± SD)			
Overall	3.5 ±6.29	3.4 ± 6.18	0.035
Male	4.2 ± 6.83	4.0 ± 6.71	0.070
Female	1.3 ± 2.59	1.2 ± 2.65	0.190
Heavy drinker[#] (%)			
Overall	7.4, (164)	6.7, (149)	0.379
Male	8.1, (143)	7.7, (136)	0.662
Female	4.4, (21)	2.7, (13)	0.162
Drinking days per week (mean ± SD)			
Overall	1.9 ± 1.82	1.8 ± 1.90	0.030
Male	2.1 ± 1.83	2.1 ± 1.92	0.104
Female	1.0 ± 1.49	0.9 ± 1.55	0.104

[#]Heavy drinker: if a man drinks more than 14 drinks per week or a woman drinks more than 7 drinks per week. A standard drink is equal to 14.0 g (0.6 ounces, equal to 18 mL) of pure alcohol.
**Significant difference, $p < 0.05$.*

TABLE 4 | Gender differences in drinking behaviors before and during COVID-19.

	Males (*n* = 1,755)	Females (*n* = 474)	*p*-value*
Drinks per week (mean ± SD)			
Before COVID-19	4.2 ± 6.83	1.3 ± 2.59	<0.001
During COVID-19	4.0 ± 6.71	1.19 ± 2.650	<0.001
Heavy drinker[#] (%)			
Before COVID-19	8.1	4.4	<0.001
During COVID-19	7.7	2.7	<0.001
Drinking days per week (mean ± SD)			
Before COVID-19	2.1 ± 1.83	1.0 ± 1.49	<0.001
During COVID-19	2.1 ± 1.92	0.9 ± 1.55	<0.001

[#]Heavy drinker: if a man drinks more than 14 drinks per week or a woman drinks more than 7 drinks per week.
**Significant difference, $p < 0.05$.*

TABLE 5 | Severity of drinking risks by AUDIT-C, overall and for males and females.

Drinking risks	Overall (*n* = 2,229) % (*n*)	Males (*n* = 1,755) % (*n*)	Females (*n* = 474) % (*n*)	*p*-value*
High-risk drinking (AUDIT-C >5)	36.0 (802)	43.2 (758)	9.3 (44)	<0.001
Severe, possible dependence	19.3 (428)	23.4 (411)	3.6 (17)	
High	16.8 (374)	19.8 (347)	5.7 (27)	
Low-risk drinking (AUDIT-C 0-5)	64.0 (1,427)	56.8 (997)	90.7 (430)	<0.001
Moderate	–	27 (474)	37.3 (177)	
Low	–	29.8 (523)	53.4 (253)	
Hazardous drinking		70.2 (1,232)	46.6 (221)	<0.001

AUDIT-C, alcohol use disorders identification test for consumption.
Hazardous drinking: women who score 3 or higher in AUDIT-C recommended limits; men who score 4 or higher in AUDIT-C recommended limits.
**Significant difference, $p < 0.05$.*

TABLE 6 | Regression model of high-risk drinking predicting depression, anxiety, and stress.

Variable[#]	OR	CI	*p*-value*
Male			
Depression	0.99	0.73–1.34	0.962
Anxiety	1.09	0.82–1.45	0.566
Stress	0.90	0.68–1.20	0.479
Female			
Depression	1.01	0.43–2.40	0.976
Anxiety	2.62	1.14–6.01	0.023
Stress	0.56	0.24–1.35	0.198

[#]Normal as reference; OR, odds ratios; CI, 95% confidence intervals.
**Significant difference, $p < 0.05$.*

COVID-19. This study also found that high-risk drinking predicted anxiety in females.

Compared with the time of before COVID-19, the average drinks per week, percentage of heavy drinking, and average drinking days per week all showed a reduction during COVID-19. A similar online survey of mental health problems caused by the COVID-19 outbreak and mass isolation during the earlier COVID-19 outbreak reported that Chinese residents from Hubei Province reported a higher rate of hazardous (33.5%) and harmful alcohol use (11.1%) than those from other provinces (21.5 and 1.9%, respectively); both rates are lower than the rates in the current study (20). The high rate of problematic drinking among Hubei residents may be due to distress experienced as a result of the pandemic. The result in our current study of lowered levels of consumption may be due to the decreased physical and financial availability of alcohol as well as the reduction of social interactions, such as celebrations and birthday parties (31). Our result shows that, in China, overall, alcohol consumption during the epidemic was lower than that before the epidemic. Although the Chinese government did not release a stricter alcohol control policy, due to strict national isolation and traffic control measures during the epidemic (32), the decline in alcohol availability is likely to be the main reason for the reduction of alcohol use. Interestingly, a study in Italy showed that, during the COVID-19 lockdown period, the craving level of inpatients was lower than real-life samples of outpatients with substance use disorders, and this may be related to the lack of availability of the substance and has relevant therapeutic implications for addicted patients (33). Further monitoring of alcohol drinking patterns during the COVID-19 pandemic would be necessary to better understand the effects of COVID-19 on alcohol consumption and to develop better comprehensive alcohol control policies.

The current study found gender differences in drinking behaviors before and during COVID-19. Males reported about 4 drinks per week both before and during COVID-19, which was

almost four times more than females; males also reported higher rates of heavy drinking (almost twice before and more than twice during COVID-19) and more drinking days per week (more than twice) than females both before and during COVID-19. These differences are consistent with the results of previous studies in China and abroad, i.e., men drink more than women (19, 34, 35).

We also found more than one-third (36%) of high-risk drinking with 43.2% in males and 9.3% in females. Among high-risk drinkers, half of males and one-third of females may have AD. In addition, more than 70% of males and almost half of females (46.6%) reported hazardous drinking. These gender differences may partly be due to the different roles of men and women in society and family (36). Men reported drinking more when exposed to pleasant emotions and social pressure and drinking more, more frequently, and with a greater risk of harmful drinking than women (1, 37, 38). Women reported drinking more when experiencing family interpersonal problems, the death of someone close, and negative emotions (36, 39). This study also found high-risk drinking predicted anxiety in females but not in males. There is increasing concern about the increase in alcohol consumption and alcohol-related harms during the COVID-19 pandemic, such as AUD, intimate partner violence, harm to children, suicide, and non-communicable diseases (40). For example, interpersonal violence (especially intimate partner violence) may cause physical and mental harm to women (or men) during quarantine, which may be related to harmful alcohol consumption (41).

Limitations

This study has some limitations that need to be considered. First, this online survey study only recruited participants online (many through social media, such as WeChat, QQ). As a result, more than 90% of the participants were from urban areas, and almost 90% of the participants had a college or higher degree; thus, the sample is not representative of the general adult population in China. Another limitation is that the measurements were all self-reported, which may result in bias of reported alcohol consumption; individuals who had drinking problems may be underestimated in this study. Furthermore, this is a retrospective survey, and alcohol consumption reported before COVID-19 may not be accurate. Further prospective cohort studies are needed to explore how social events affect alcohol drinking patterns and hazardous drinking as well as measures to prevent harmful alcohol use.

CONCLUSIONS

The present study provides an insight into the impact of the COVID-19 pandemic on alcohol use patterns. This study suggests a slight reduction in alcohol consumption during COVID-19. However, hazardous drinking is common, especially among male alcohol drinkers. Males consumed more alcohol and had more risky and hazardous drinking than female counterparts both before and during COVID-19. Public health policy makers should pay more attention to developing effective, population-based strategies to prevent harmful alcohol consumption.

DATA AVAILABILITY STATEMENT

All data in the current study was stored in the PI's affiliation, and is available from the corresponding authors on reasonable request and with completion of data user agreement.

ETHICS STATEMENT

The study protocol has been approved by The Ethics Committee of Sir Run Run Shaw hospital, an affiliate of Zhejiang University, Medical College (No. 20200505-33). Informed consent was communicated and obtained from each participant prior to participation. Participants were informed with the purpose, assessments, potential risks and benefits of the study before survey. All of the obtained data have no personal information, none information from the data can be linked back to the participants.

AUTHOR CONTRIBUTIONS

YW, QD, and YLiao conceived the study. YW, QD, and YLiao did the literature review, statistical analyses, and drafted the report. YW, QD, HL MH, SW, JC, LW, TL, ZW, and YLiu collected the data. YLiao and YW took the lead in writing the manuscript. JT and WC interpreted the data and commented on the manuscript. All authors contributed to the article and approved the submitted version.

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Insomnia in Chinese College Students With Internet Addiction: Prevalence and Associated Clinical Correlates

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Background: Internet addiction (IA) has gained more and more attention for its negative impact on the subjects' study and daily life. However, in a large sample, there is little research on the association between IA and insomnia in Chinese college students. This study aimed to investigate the prevalence of insomnia and its related risk factors among Chinese college students with IA.

Methods: A cross-sectional design was used to investigate 627 Chinese college students with IA. Each student completed a survey on demographic data, Internet addiction (Revised Chinese Internet Addiction Scale), depression (Self-Rating Depression Scale), insomnia (Athens Insomnia Scale), anxiety (Self-Rating Anxiety Scale), and suicidal behavior. Binary logistic regression analysis was employed to adjust for confounding factors.

Results: The prevalence of insomnia among students with IA was 54.86%. Compared with IA students without insomnia, IA students with insomnia were more likely to be younger, smoking, drinking, have anxiety, depression, suicidal ideations, suicide plans, and suicide attempts (all $p < 0.05$). Moreover, drinking [OR, 1.664; 95% confidence interval (CI), 1.139–2.431; $p = 0.008$], anxiety (OR, 2.321; 95% CI, 1.116–4.826; $p = 0.024$), and suicidal ideation (OR, 1.942; 95% CI:1.295–2.911; $p = 0.001$) were independently associated with insomnia in IA students.

Conclusions: Insomnia is very common in Chinese IA students. Drinking, anxiety, and suicidal ideation are independently correlated with insomnia. This study provides valuable evidence for school counselors and clinical professionals to assess Internet addiction, insomnia, and suicide risk.

Keywords: Chinese college students, risk factors, prevalence, internet addiction, IA, insomnia

INTRODUCTION

Internet addiction (IA) refers to the excessive use of the Internet, which leads to damage to personal psychological states (1). Participants with IA are often unable to control their desire to surf the Internet, escape from a dysphoric mood, and lose interest in other things (2, 3). With the development of the Internet and multimedia, the use of the Internet is becoming an essential part of people's daily life. It is reported that by 2010, the amount of global Internet users has reached 4.39 billion (4). Internet addiction has attracted more and more attention. A meta-analysis conducted in 31 countries and seven world regions showed that the pooled prevalence of IA was 6.0% (5). The Internet has brought enormous convenience to people's life. It is estimated that Chinese Internet users had reached 854 million by June 2019 (6). Millions of people use the Internet to send messages (825 million), browse news (686 million), make online purchase (639 million), order take-outs (421 million), or watch videos (759 million) (6). Despite the benefits of the Internet, spending too much time on the Internet can also have a negative impact on subjects' study, work, and daily life. Studies have shown that people with IA usually have poorer academic performance (7), lower happiness (8), and are more likely to feel lonely (9). In addition, adolescents with IA are characterized by anger, distress, social withdrawal, familial conflict, and loss of control, and they are more likely to have personality disorders (10). Studies have also indicated that IA is associated with mental health problems, such as insomnia (11, 12), anxiety (13), depression (13), bipolar disorders (14), self-harm, and suicidal behavior (13). IA has become the main concern of the world society today.

Insomnia refers to the difficulty of initiating and/or maintaining sleep, or early morning awakening, which can cause damage to the individual's daily life, such as fatigue or daytime sleepiness (15). Previous studies have revealed the link between IA and insomnia. For example, Li et al. observed a higher prevalence of insomnia among middle school students with IA (16). A meta-analysis indicated that compared with those subjects without IA, subjects with IA were 2.2 times more likely to suffer from insomnia [overall pooled odds ratio (OR) = 2.20] and present with shorter sleep duration [overall pooled standardized mean difference (SMD) = −0.24 h] (17). Further, studies have shown that individuals with IA show a higher proportion of sleep disorders, such as shorter sleep duration (18, 19), longer sleep duration required (18), poor sleep quality (18–20), excessive daytime sleepiness (21), and sleep–wake behavior problems (21).

Compared with younger students, college students have more free time after studying. In addition, courses and homework in universities often need to be finished with the help of the Internet. Therefore, college students have more opportunities to surf the Internet and are susceptible to Internet addiction. Sleep disorders such as insomnia for college students with IA are also issues of great concern to us. Previous studies have reported that college students with IA have significantly reduced sleep quality and quantity (22, 23). On the other hand, Kitazawa et al. reported that Japanese college students with poor sleep quality were 1.52 times more likely to have IA (22). Generally speaking, although previous studies have revealed the relationship between IA and insomnia, most of the studies have mainly focused on young people, particularly the teenagers in foreign countries; however, there is little research on Chinese college students. As far as we know, our study was the first of its kind to investigate the relationship between IA and insomnia in a large sample of Chinese college students. The main purpose of this study was to investigate the prevalence of insomnia and its related risk factors among IA college students in China.

METHODS

Participants

A cross-sectional design was adopted in this study. The Chinese Internet addiction scale (CIAS-R) was utilized to screen for students with IA with convenience sampling method. The CIAS-R was delivered online anonymously to students from the Technology and Changsha Medical University and Changsha University of Science. Students completed the scale and present with a CIAS-R total score reached, or more than 53 were enrolled in this study. Therefore, this study enrolled 627 college students with IA. The inclusion criteria for participation were 1) participants were between 17–25 years old; 2) their score on the Chinese Internet addiction scale (CIAS-R) reached or exceeded 53; 3) they were in good health; and 4) they agreed to sign informed consent to participate in this study. All participants were informed of their right to decide to take part in or quit. They were also informed of their right to refuse to participate, and they can withdraw from this study at any time. The survey was provided online through social media app (WeChat). In addition, the survey was delivered anonymously to protect the privacy of the students. College counselors were responsible to guide the students to fill the survey online, and they were trained on how to collect the data before providing guidance. This study was approved by the Ethics Committee of the Second Xiangya Hospital. Each college students provided informed consent before taking part in this study.

Assessment

Participants' sociodemographic data, including sex, age, community, nationality, one-child family, parents' education level, good relationship with mother (yes or no), good relationship with father (yes or no), family income, smoking, drinking habits, right handedness, history of mental disorders, history of physical disorders, family history of mental disorders (FHMD), and suicidal behaviors (suicidal ideation, suicide plans, and attempts) were collected by trained college counselors.

Internet addiction was assessed by the revised Chinese Internet addiction scale (CIAS-R); CIAS-R is a 19-item self-report questionnaire designed to measure participants' addiction to the Internet. Each item of the scale was rated from 1 (1 for complete inconformity) to 4 (complete conformity). The total score of CIAS-R ranges from 0 to 76. When the total score of CIAS-R reached or exceeded 53, the students were divided into Internet addiction group. According to reports, CIAS-R has good psychometric properties (24). When their score in the CIAS-R reached or exceeded 53, they were regarded as having IA.

Participants' anxiety and depression were measured by the Self-rating Depression Scale (SDS) and Self-rating Anxiety Scale (SAS) (25, 26). The cutoff score for anxiety was 50 on SAS, and the cutoff score for depression was 53 on SDS (27). SAS and SDS were widely used and show good psychometric properties among the Chinese population (28, 29).

The Athens Insomnia Scale (AIS) was utilized to measure participants' insomnia symptoms. AIS is an eight-item self-report scale, and each item has a four-point Likert scale from 0 to 3 (0 for no problem or equivalent, and 3 for very severe problem or equivalent). The total score of AIS is between 0 and 24, the cutoff score of insomnia is 6 (30). A higher AIS score indicates a higher level of insomnia symptoms. It is reported that AIS has good psychometric properties in the Chinese population (30).

Statistical Analyses

In this study, chi-squared test was employed to compare the group differences for categorical variables, and t-test was utilized for continuous data (continuous data conformed to a normal distribution). The odds ratio (OR) was calculated through the univariate logistic analysis. Binary logistic regression analysis was employed to adjust for confounding factors (adjusted OR); all of the variables in **Table 1** were put into the binary logistic model. The significance level of this study was set at two-tailed p value of 0.05. IBM SPSS 22 was utilized to perform all the statistical analyses.

RESULTS

Table 1 shows that the prevalence of insomnia among students with IA was 54.86% (344/627). IA students with insomnia were younger, and more likely to smoke, drink alcohol, suffer from depression or anxiety, used to have suicidal thoughts, make suicide plans, and with a history of committing suicide (all $p < 0.05$). In addition, no significant group difference was observed in sex, nationality, one-child family, good relationship with parents, family income, parents' education level, right-handedness, physical disorder history, mental disorder history, and family history of mental disorder (FHMD). The following variables remained to be significant after controlling for covariate variables in binary logistic analysis: drinking [OR, 1.664; 95% confidence interval (CI), 1.139–2.431; $p = 0.008$], anxiety (OR, 2.321; 95% CI, 1.116–4.826; $p = 0.024$), and suicidal ideation (OR, 1.942; 95% CI: 1.295–2.911; $p = 0.001$) (see **Table 2**).

DISCUSSION

To the best of our knowledge, this study was the first of its kind to investigate the association between insomnia and IA in Chinese college students with a large sample size. We found that insomnia was very common among Chinese college students with IA, with a prevalence rate of 54.86%. We also revealed that insomnia was associated with younger age, smoking, drinking alcohol, anxiety, depression, suicidal thoughts, suicide plans, and suicide attempts. Moreover, we found that after controlling for confounding variables, drinking alcohol, anxiety, and suicidal ideation were still independent risk factors for insomnia in IA college students.

This study revealed that IA and insomnia coexisted at a high level (54.86%), which was similar to the prevalence rate reported in Hong Kong (52.7%) (19), but lower than that in Taiwan (63.4%) (12). This difference may be due to different measurement methods utilized in different studies. Another possible reason may be that each study recruited a different population. Their study recruited participants of all ages, while the subjects in our study were college students. In addition, previous studies conducted among college students have also revealed a significant association between IA and insomnia (11, 23, 31). There are several reasons that may have contributed to this association. Participants with IA are frequently reported to have poor time management skills, and excessive use of the Internet may lead to reduced sleep time (32). Furthermore, studies have shown that electronic devices such as computers and smartphones, can emit light, especially blue light, thereby suppressing individual's melatonin levels (33). Melatonin is secreted by the pinealocytes in the pineal gland and plays a very important role in human sleep (34).

We demonstrated that anxiety was independently associated with insomnia in IA students. The association between IA and anxiety was consistent with previous studies (11, 12, 22, 35, 36). For example, Evren et al. found that Turkish college students with a high probability of insomnia had higher levels of IA and anxiety (35). Younes et al. and Kitazawa et al. found that college students in Lebanon and Japan with potential IA or problematic Internet use had significantly higher anxiety levels than normal Internet users. Moreover, having anxiety tendencies contributed to increased IA risk (11, 22). A meta-analysis demonstrated that IA was positively associated with anxiety, especially among young adults (19–39 years old). Moreover, people with anxiety were 2.7 times more likely to suffer from IA than those without anxiety (pooled OR = 2.70) (36). As for the relationship between anxiety and insomnia, Taylor et al. proposed that people with insomnia were 17.35 times more likely to suffer from anxiety than those without insomnia (37). The possible causes for the associations between insomnia and anxiety may include psychological factors, neural correlates of the brain, and genetic factors.

Psychologically, patients with insomnia usually exhibit higher levels of "neuroticism," "internalization," anxiety, and perfectionism-related traits (38), which may contribute to the susceptibility and permanence to insomnia. Insomnia patients often "keep their emotions to themselves," which may lead to increased physiological activation (39) "Perfectionism" tends to set excessively high performance standards, which may lead to failure to fall asleep. Meanwhile, patients with insomnia are often characterized by excessive attention to sleep (40), decreased ability to concentrate (41), and daytime irritability (41), which may increase the likelihood of anxiety. In addition, treatment for insomnia can improve anxiety symptoms and vice versa (42). As for the neural substrates, previous studies have shown that the anterior cingulate cortex (ACC) is part of the pre-frontal network responsible for controlling amygdala activity from top to bottom (43). In patients with primary insomnia, the average connectivity between the left amygdala and right anterior cingulate cortex

TABLE 1 | Demographics and clinical characteristics between participants with and without Internet addiction (IA) and between IA students with and without insomnia.

Variables	IA with insomnia ($n = 344$)	IA without insomnia ($n = 283$)	p	
Age (years), mean (SD)	20.12 (1.525)	20.25 (1.50)	0.047*	
Sex				
Males, *n* (%)	144 (41.9%)	115 (40.6%)	0.757	
Females, *n* (%)	200 (58.1%)	168 (59.4%)		
Community urban				
Urban	135 (39.2%)	119 (42.0%)	0.476	
Rural	209 (60.8%)	164 (58.0%)		
Nationality				
Han	305 (88.7%)	249 (88.0%)	0.793	
Others	39 (11.3%)	34 (12.0%)		
One-child family	122 (35.5%)	109 (38.5%)	0.431	
Good relationship with mother	323 (93.9%)	269 (95.1%)	0.530	
Good relationship with father	301 (97.5%)	261 (92.2%)	0.053	
Smoking	54 (15.7%)	18 (6.4%)	0.000***	2.741 (1.568–4.794)
Alcohol	158 (45.9%)	92 (32.5%)	0.001**	1.764 (1.272–2.445)
Family income/year (yuan)				
Less than 30,000	81 (23.5%)	74 (26.1%)	0.612	
30,000∼70,000	160 (46.5%)	121 (42.8%)		
More than 70,000	103 (29.9%)	88 (31.1%)		
Father's education level				
JMSB	172 (50.0%)	143 (50.5%)	0.976	
HSTS	125 (36.3%)	103 (36.4%)		
CUA	47 (13.7%)	37 (13.1%)		
Mother's education level				
JMSB	204 (59.3%)	170 (60.1%)	0.938	
HSTS	110 (32.0%)	87 (30.7%)		
CUA	30 (8.7%)	26 (9.2%)		
Right-handedness	300 (87.2%)	252 (89.0%)	0.481	
Physical disorder history	24 (7.0%)	14 (4.9%)	0.289	
Mental disorder history	13 (3.8%)	4 (1.4%)	0.070	
FHMD	14 (4.1%)	5 (1.8%)	0.094	
Anxiety	197 (57.3%)	77 (27.2%)	0.000***	3.585 (2.557–5.027)
Depression	172 (50.0%)	63 (22.3%)	0.000***	3.492 (2.459–4.960)
Suicidal ideation	192 (55.8%)	102 (36.0%)	0.000***	2.241 (1.623–3.095)
Suicide plans	52 (15.1%)	22 (7.8%)	0.005**	2.113 (1.249–3.574)
Suicide attempts	96 (27.9%)	38 (13.4%)	0.000***	2.496 (1.648–3.780)

*IA, Internet addiction; JMSB, junior middle school and below; HSTS, high school or technical school; CUA, college or university and above; FHMD, family history of mental disorder. *p < 0.05; **p < 0.01; ***p < 0.001.*

(rACC) decreases with increasing state anxiety, which means that decreased top–down control of the amygdala may increase the risk of developing anxiety disorder accompanied by preexisting primary insomnia (44). Moreover, the lower overall functional connectivity density values of the left anterior cingulate cortex (lACC) and right insula are associated with their anxiety level (45), but another study shows that the enhanced connectivity between the left insular lobe and the right anterior cingulate cortex (rACC) was negatively correlated with anxiety level (46). Therefore, more research is needed to explore the potential neural basis of the association between insomnia and anxiety. As for heredity, a twin study found that the genetic factors related to the etiology of insomnia overlap with those related to anxiety (42), suggesting that there is a biological genetic basis underlying insomnia and anxiety.

We also found that suicidal ideation was independently associated with insomnia in IA college students, which was consistent with previous studies (12, 20). For example, previous studies have found that poor sleep quality was associated with lifelong suicidal ideation (12) and lifelong suicide attempts (20) among people with IA. Many studies have identified insomnia as an independent, modifiable risk factor for suicide (47, 48). Studies

TABLE 2 | Multivariate analysis for variables associated with Internet addiction participants with insomnia.

Variable	B	S.E.	Wald	df	Sig.	Exp(B)	95%CI for EXP(B)	
							Lower	Upper
Age	0.061	0.059	1.075	1	0.300	1.063	0.947	1.193
Sex	0.107	0.194	0.304	1	0.582	1.113	0.761	1.626
Community	0.199	0.227	0.771	1	0.380	1.221	0.782	1.905
Nationality	−0.251	0.274	0.837	1	0.360	0.778	0.455	1.332
One-child family	−0.048	0.209	0.053	1	0.818	0.953	0.633	1.435
Good relationship with mother	0.827	0.450	3.377	1	0.066	2.286	0.947	5.519
Good relationship with father	−0.488	0.337	2.097	1	0.148	0.614	0.317	1.188
Smoking	0.601	0.330	3.315	1	0.069	1.825	0.955	3.486
Alcohol	0.509	0.193	6.934	1	0.008**	1.664	1.139	2.431
Family income			1.679	2	0.432			
Family income 1	0.290	0.224	1.678	1	0.195	1.336	0.862	2.073
Family income 2	0.193	0.260	0.550	1	0.458	1.213	0.728	2.019
Mother's education level			1.070	2	0.586			
Mother's education level 1	−0.251	0.243	1.069	1	0.301	0.778	0.483	1.252
Mother's education level 2	−0.216	0.449	0.231	1	0.631	0.806	0.334	1.942
Father's education level			1.212	2	0.546			
Father's education level 1	0.246	0.225	1.196	1	0.274	1.279	0.823	1.989
Father's education level 2	0.245	0.399	0.377	1	0.539	1.278	0.584	2.793
Right-handedness	0.122	0.288	0.181	1	0.671	1.130	0.643	1.986
Physical disorder history	−0.343	0.435	0.621	1	0.431	0.710	0.303	1.665
Mental disorder history	0.553	0.732	0.571	1	0.450	1.739	0.414	7.298
FHMD	0.877	0.644	1.855	1	0.173	2.404	0.680	8.490
Anxiety	0.842	0.374	5.081	1	0.024*	2.321	1.116	4.826
Depression	0.391	0.390	1.006	1	0.316	1.479	0.689	3.175
Suicidal ideation	0.664	0.207	10.317	1	0.001**	1.942	1.295	2.911
Suicide plans	−0.273	0.354	0.594	1	0.441	0.761	0.380	1.524
Suicide attempts	0.180	0.289	0.390	1	0.532	1.198	0.680	2.109

*CI, confidence interval; FHMD, family history of mental disorder; family income 1, 30,000–70,000; family income 2, more than 70,000; mother's education level 1, high school or technical school; mother's education level 2, college or university and above; father's education level 1, high school or technical school; father's education level 2, college or university and above. *$p < 0.05$; **$p < 0.01$.*

have shown that treatments for insomnia, such as cognitive behavioral therapy for insomnia (CBT-I) (49) and controlled-release zolpidem (50) (a hypnotic drug) can reduce the risk of suicide (51). There are several reasons that may contribute to this association. Perlis et al. proposed a hypothesis that insomnia usually results in "hypofrontality" and diminished executive function, and then executive dysfunction can lead to difficulties in regulating moods, thoughts, or actions tend to think about things in a negative way and fail to inhibit inappropriate actions, such as suicidal ideation and suicide attempts (52). Similarly, McCall et al. found that false beliefs and attitudes toward sleep may be a specific mediator, making insomnia as a risk factor for suicidal ideation (53). Patients with insomnia are often mentally overactive and do not know how to handle it, and may have false ideas such as committing suicide to escape the disease.

This study should be interpreted with caution due to the following limitations. First, our research was a cross-sectional study, so we cannot draw any conclusions about causality. Future studies should employ a longitudinal research design to better explore the casual relationship between these variables. Second, despite the advantage of a large sample size, the participants in this study were from two universities in Hunan province, and more students from different places should be recruited in further studies to increase the representativeness. Third, this study employed self-report scales to measure the demographic and clinical features of participants, which may be biased compared to studies conducted utilizing diagnostic interview tools.

In conclusion, we have demonstrated that insomnia is very common among Chinese college students with IA. Further, this study revealed the association between insomnia and drinking alcohol, anxiety, and suicide ideation. These findings underscore the importance of addressing insomnia symptoms and related factors such as anxiety and suicide ideation among IA college students. Overall, this study provides valuable evidence for us to deepen our understanding of the pathological mechanisms underlying insomnia in the subjects with IA, and also provides a reference for school counselors and clinical professionals when assessing IA, insomnia and the risk of attempted suicide.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Second Xiangya Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XL, XZ, and YS were responsible for the study design. YS, XJ, and YZ were responsible for recruiting the participants. CH and JL were involved in statistical analysis. YS, YZ, and XJ were involved in manuscript preparation and drafting the paper. CH and JL were involved in editing and revising the manuscript. XL and XZ were responsible for the critical revision of the manuscript. All authors have contributed to and have approved the final manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Interaction Between Smoking and Internet Gaming Disorder on Spontaneous Brain Activity

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Converging lines of evidence indicates that smoking and internet gaming disorder (IGD) affect spontaneous brain activity, respectively. However, little is known about whether these two factors work together on the human brain. In this study, we investigated the interaction between smoking and IGD on local spontaneous brain activity using amplitude of low-frequency fluctuation (ALFF) based on resting-state fMRI (rs-fMRI). Forty-six cigarette smokers, 38 IGD individuals, 34 participants with both IGD and cigarette smoking (IGD-Smoking), and 60 healthy individuals involved in the study. Voxel-wise analysis of covariance of ALFF revealed that there were significant interactions between IGD by smoking in the right medial pre-frontal cortex (MPFC)/ventral striatum, bilateral cerebellar, and visual-related regions as well as the left temporal gyrus. In the right MPFC/ventral striatum and left temporal gyrus, ALFF in smoking group was significantly higher than healthy group while there were no significant ALFF differences between IGD-Smoking group and IGD group. While in the bilateral cerebellar and visual-related regions, ALFF in the smoking group was significantly lower than healthy group while ALFF in IGD-Smoking group did not show significant difference with IGD group. In addition, in the smoking group, ALFF of the right MPFC/ventral striatum was associated positively with anxiety and depression scores while the ALFF value in the smoking group had a trend toward negative correlation with SDS scores in the bilateral cerebellar and visual-related regions. The ALFF value in the smoking group was associated positively with anxiety score in the left temporal gyrus. These findings indicate that smoking and IGD interacted with each other in the human brain. Our results, in terms of spontaneous brain activity, may imply the fact that IGD people are more tended to get smoking. Moreover, it is possible to predict that smokers may be more easily to get internet addiction than healthy people.

Keywords: interaction, internet gaming disorder, smoking, spontaneous brain activity, ALFF

INTRODUCTION

Addiction is a complex phenomenon involving psychological and social consequences such as dependence, tolerance, sensitization, and craving (1, 2). Internet gaming disorder (IGD) is classified as an addictive disorder in the Diagnostic and Statistical Manual of Mental Disorders (5th Edition, DSM-5), characterized by the use of online games in a manner that leads to significant distress and functional impairments of general life (3). Smoking is one of the largest single causes of preventable morbidity and natural mortality, which may also cause cognitive decline and dementia (4). IGD and smoking, the typical representative of behavioral and substance addiction, respectively, have common core clinical features: diminished control and a hedonic quality over the problematic behavior, unsuccessful trying to stop, and impairment of major life functioning (5), and may share similar neural circuits including the reward circuits, memory and learning circuits, cognitive control loops (6, 7).

Studies based on questionnaires suggest that the youthful initiation of internet addiction in teenage students could be predicted by smoking (8–10). Subjects with both nicotine dependence and IGD demonstrated a greater degree of urge activities in the anterior cingulate cortex (ACC) and parahippocampus in comparison to the controls (11). Another study showed that compared with the non-smokers with IGD, smokers with IGD had motivation and executive function changes (12). Additionally, compared with healthy controls, both IGD and smoking groups showed significantly different resting-state functional connectivity (rsFC). Significant rsFC differences were also found between IGD and smoking groups (13).

Resting-state fMRI (rs-fMRI) has been shown to study spontaneous brain activity by assessing the baseline brain activity based on the low-frequency (<0.1 Hz) fluctuations of blood oxygenation level-dependent signals. Amplitude of low-frequency fluctuation (ALFF) has been applied to detect abnormalities of brain activity in IGD individuals and smokers, respectively, by measuring the local spontaneous brain activity (14). Compared with healthy controls, IGD individuals had increased ALFF in brain areas of the bilateral middle cingulate cortex, right parahippocampal gyrus, left precuneus, supplementary motor area, and medial orbitofrontal cortex (OFC) (15). Abnormal OFC and ACC activation might be associated with cue-induced gaming urge (16). The OFC connects extensively with the striatum and limbic regions and it is involved in cognitive and impulse control and reward processing by assessing the motivational significance of stimuli and selecting behavior to achieve desired outcomes (15). So abnormal OFC activity in addiction might be related to dysfunction of cognitive control ability (15). Activation was also found in ACC in IGD group when compared to the control group in resting state (17). ACC involved in modulating emotion, motivation and attention and monitoring conflicts to trigger desired execution and control outputs (18). Increased fractional ALFF (fALFF) was found in the superior temporal gyrus. The temporal gyrus serves as regulating sense perception including visual and auditory and its nerve fibers project to frontal lobe (19). Compared to non-smokers, smokers showed decreased fALFF in the precuneus and cerebellum anterior lobe (20, 21). Diminished regional homogeneity of bilateral cuneus and lingual gyrus was found in heroin-dependent individuals (22) and regional cerebral blood flow of bilateral cuneus was also decreased in opioid addiction (23). The major function of bilateral cuneus is visual processing and inhibitory control. This abnormity may suggest the impaired inhibitory control ability in addicted subjects. All these reports have studied the effects of IGD or smoking on ALFF in brain areas, respectively. However, less is known about the changes on spontaneous brain activity when subjects have both IGD and smoking disorders and whether they work on human brain independently or interactively in alterations of brain functions.

The primary goals of this study were to explore the effects of smoking and IGD on spontaneous brain activity. Given the high IGD risk associated with smoking and high smoking risk associated with IGD mentioned in many studies (9, 24), we hypothesized that IGD and smoking were not independently working on human brain. Therefore, in this study we investigate the interaction between smoking and IGD on the spontaneous brain activity. Four groups of healthy controls, smokers, IGD and IGD-Smoking individuals were under resting-state fMRI scanning. ALFF was calculated and the voxel-wise two-way analysis of covariance (ANCOVA) was performed to investigate the interaction between smoking and IGD.

MATERIALS AND METHODS

Subjects

One hundred and eighty-five right-handed subjects, including 62 healthy controls (age range: 16–28 years), 50 cigarette smokers (age range: 18–29 years), 39 IGD addicts (age range: 16–27 years), and 34 participants with both IGD and cigarette smoking (IGD-Smoking) (age range: 16–28 years) were involved in this study. Psychiatric medical disorders were screened by the Mini International Neuropsychiatric Interview for (25). All subjects did not have any drug abuse or dependence, psychiatric or neurological diseases history (other than nicotine and internet dependence for smokers and IGD individuals, respectively), or intellectual disability. All participants were cleared of substance addiction by a urine drug screening. Smokers were defined as those who smoked at least 10 cigarettes a day during the last year. Fagerström test for nicotine dependence (FTND) was used to evaluate the severity of smoking addiction (26). None of the smokers had been abstinent for more than 3 months in the previous year. Non-smokers smoked no more than five cigarettes during their lifetime. The IGD participants were enrolled from the psychological outpatient clinic at the Shanghai Mental Health Center and were interviewed by two experienced psychiatrists with the criteria of modified Young's Diagnostic Questionnaire for Internet Addiction (YDQ) (27). Ko et al. Internet Addiction Scale (CIAS) was performed to access the severity of internet addiction (28). Before MRI scanning, all participants were assessed by questionnaires of the Self-rating Anxiety Scale (SAS) (29), Self-rating Depression Scale (SDS) (30) and Barratt Impulsiveness Scale (BIS) (31).

Image Acquisition

All subjects were scanned by a 3.0-Tesla GE Signa HDx (Milwaukee, WI, United States) with a standard 8-channel head coil and the head motion and scanner noise were minimized by foam pads. The parameters of the echo-planar imaging sequence for the rs-fMRI data collection were as follows: repetition time/echo time: 2,000/24 ms; matrix: 64 × 64; flip angle: 90°; field of view: 230 × 230 mm^2; 4 mm with no gap; 34 slices. The rs-fMRI scanning lasted for 440 s and 220 volumes were obtained. All participants were instructed to lie still, keep their eyes closed but not to fall asleep during MRI scanning. All the resulted images were visually accessed by two experienced neuroradiologists to exclude pathological findings.

Data Pre-Processing

Images were pre-processed by the Data Processing Assistant for Resting-State fMRI (32). The first 10 time points of each subject were excluded and the remaining 210 volumes were corrected for the acquisition time delay. Participants with a maximum head motion >2 mm in the x, y, or z direction or a head rotation >2° or mean framewise displacement larger than 0.2 mm were excluded. Using this criterion, 2 healthy controls, 4 smokers and 1 IGD addict were discarded, and a total of one hundred and seventy-eight subjects (60 healthy controls, 46 cigarette smokers, 38 IGD addicts, and 34 IGD-Smoking participants) were ultimately used in the ALFF analysis. The regression of head motion effects was carried out by Friston 24-parameter model (33). After that, the images normalization to the Montreal Neurological Institute (MNI) space and resampling to a 3-mm isotropic voxel and the smoothing procedure with a 6 mm full width at half maximum Gaussian kernel to reduce noises was performed. Linear and quadratic trends as well as white matter and corticospinal fluid signals were then removed. Finally, individual ALFF map was calculated within a mask without non-brain tissue, divided by its mean ALFF for further statistical analysis.

Statistical Analysis

The voxel-wise two-way (IGD and smoking) ANCOVA controlling for age, gender, years of education and mean frame-wise displacement was performed to investigate the main effects as well as the interaction between smoking and IGD on ALFF. The multiple comparisons correction of statistical F-maps was performed with family-wise error (FWE) cluster-corrected ($p < 0.05$) when using a primary voxel determining threshold of $p < 0.001$ to protect against false-positive findings. Then ALFF values from significant clusters showing interaction effects were extracted to perform the *post-hoc* pairwise comparison controlling for age, gender, educational level and head motion and a Bonferroni correction level of 0.0083 (0.05/6) was applied to protect against false-positive findings. The correlation analysis with ALFF among groups as dependent variable and variables related to smoking such as duration of smoking, age at first smoking, and FTND and IGD related variables (CIAS), questionnaire scores of SAS, SDS, BIS and its three dimensions controlling for age, gender, educational level, and head motion was performed to investigate whether the altered ALFF was related to the smoking and IGD related features and questionnaire scores. Results with $p < 0.05$ (uncorrected) were considered significant.

RESULTS

Demographic and Clinical Measures

The four groups showed significant sex differences according to the Chi-square test ($p = 0.008$). Other demographic and clinical differences of subjects among groups was evaluated with a two-way ANOVA in age, education level, CIAS, SAS, SDS, and BIS. There was significant main effect of IGD ($p = 0.006$) and smoking ($p < 0.001$) and IGD by smoking interaction ($p = 0.004$) on age. Significant main effect of IGD ($p < 0.001$) and smoking ($p = 0.003$) and no significant IGD by smoking interaction ($p = 0.06$) were also found in years of education (**Table 1**).

Duration of smoking and age at first smoking in smoking group was longer ($p = 0.01$, $p = 0.03$, respectively), than that in IGD-Smoking individuals. IGD-Smoking group had higher FTND score than smoking group ($p = 0.04$). The main effect of IGD on CIAS ($p < 0.001$), SAS ($p < 0.001$), SDS ($p < 0.001$), BIS ($p < 0.001$) was significant. CIAS ($p < 0.001$), SAS ($p < 0.001$), SDS ($p < 0.001$), BIS ($p = 0.039$) in smokers were lower than non-smokers.

Interaction Effects Between IGD and Smoking

Significant IGD and smoking interaction effects on ALFF were identified in the right medial pre-frontal cortex (MPFC) (i.e., orbital frontal gyrus and anterior cingulate cortex) extending to the ventral striatum, bilateral cerebellar and visual-related regions (i.e., lingual and calcarine gyrus and cuneus) as well as the left temporal gyrus (**Figure 1** and **Table 2**).

Effect of IGD on Healthy and Smoking Groups

The *post-hoc* pairwise comparison controlling for age, gender, educational level, and head motion (Bonferroni correction, $p < 0.0083$) demonstrated that ALFF showed no statistical differences between IGD group and healthy group (IGD group: 0.86 ± 0.03; Healthy group: 0.78 ± 0.03; $p = 0.441$) while IGD-Smoking group showed significantly lower ALFF when compared to smoking group (IGD-Smoking group: 0.76 ± 0.04; Smoking group: 1.13 ± 0.03; $p = 1.16 \times 10^{-12}$) in the right MPFC/ventral striatum (**Figure 2A**). In the bilateral cerebellar and visual-related regions, IGD group showed no statistical differences of ALFF with healthy group (IGD group: 1.35 ± 0.06; Healthy group: 1.55 ± 0.05; $p = 0.081$) while IGD-Smoking group showed significantly higher ALFF when compared to smoking group (IGD-Smoking group: 1.60 ± 0.07; Smoking group: 1.13 ± 0.05; $p = 2.32 \times 10^{-7}$, **Figure 2B**). As for the left temporal gyrus, IGD group showed no statistical differences of ALFF with healthy group (IGD group: 0.52 ± 0.02; Healthy group: 0.45 ± 0.02; $p = 0.20$) while IGD-Smoking group showed significantly lower ALFF when compared to smoking group

TABLE 1 | Demographic and clinical characteristics of four groups.

	IGD group ($n = 38$)	IGD-smoking group ($n = 34$)	HC group ($n = 60$)	Smoking group ($n = 46$)	*p*-value		
					IGD	Smoking	IGD* smoking
Age	19.66 ± 2.60	22.94 ± 2.93	22.29 ± 3.48	22.88 ± 2.78	0.006	<0.001	0.004
Gender (M/F)	26//12	33//1	41//19	31//15	–	–	0.008[a]
Education	10.74 ± 1.75	10.29 ± 2.10	14.76 ± 3.39	11.83 ± 2.50	<0.001	0.003	0.06
Duration of smoking	–	3.75 ± 1.88	–	5.18 ± 2.92	–	–	–
Age at first smoking	–	19.19 ± 2.83	–	17.70 ± 3.01	–	–	–
FTND	–	6.68 ± 2.07	–	6.28 ± 2.25	–	–	–
CIAS score	72.68 ± 10.27	81.26 ± 10.69	43.83 ± 10.83	49.93 ± 10.57	<0.001	<0.001	0.45
SAS score	48.89 ± 9.57	57.24 ± 11.56	40.53 ± 7.40	47.59 ± 9.93	<0.001	<0.001	0.66
SDS score	52.08 ± 9.41	58.00 ± 8.90	44.32 ± 8.62	50.65 ± 9.70	<0.001	<0.001	0.88
BIS-11 score	61.63 ± 8.19	64.12 ± 8.62	52.80 ± 6.91	55.67 ± 10.00	<0.001	0.039	0.88
BIS-attentional impulsiveness score	15.16 ± 2.69	15.97 ± 2.90	12.98 ± 2.30	13.13 ± 2.80	<0.001	0.24	0.41
BIS-motor impulsiveness score	20.63 ± 4.46	21.82 ± 3.64	17.92 ± 2.96	19.91 ± 4.00	<0.001	0.006	0.48
BIS-Non-planning impulsiveness score	26.16 ± 3.48	26.50 ± 4.66	21.90 ± 4.47	22.63 ± 5.62	<0.001	0.45	0.79

[a] *The Chi-square test showed significant sex differences within the four groups ($p = 0.008$).*

Values are expressed as mean ± standard deviation. The age, educational level, age at first cigarette, and duration of smoking are displayed in years. FTND, Fagerström Test of Nicotine Dependence; CIAS, Chen internet addiction scale; SAS, Self-rating Anxiety Scale; SDS, Self-rating Depression Scale; BIS-11, Barratt Impulsiveness Scale, version 11. The definition of educational level was the number of years of scholarship since primary school.

(IGD-Smoking group: 0.47 ± 0.03; Smoking group: 0.65 ± 0.02; $p = 1.00 \times 10^{-6}$, **Figure 2C**).

Effect of Smoking on Healthy and IGD Groups

The *post-hoc* pairwise comparison controlling for age, gender, educational level, and head motion (Bonferroni correction, $p < 0.0083$) also showed that ALFF in smoking group was significantly higher than healthy group in the right MPFC/ventral striatum ($p = 3.61 \times 1\ 0^{-15}$) and the left temporal gyrus ($p = 8.55 \times 10^{-10}$) while IGD-Smoking group showed no statistical differences of ALFF when compared to IGD group (*p*-value was 0.439 and 0.855, respectively) (**Figures 2A,C**). In the bilateral cerebellar and visual-related regions, smoking group showed significantly lower ALFF than healthy group ($p = 1.17 \times 10^{-7}$). Moreover, the ALFF difference between IGD-Smoking and IGD group ($p = 0.038$) did not survive the Bonferroni correction at $p < 0.0083$ (**Figure 2B**).

In addition, compared to the healthy control group, smoking caused significantly larger ALFF changes than IGD in the right MPFC/ventral striatum ($p = 5.55 \times 10^{-8}$, **Figure 2A**), as well as the left temporal gyrus ($p = 0.001$, **Figure 2C**). Although the difference between IGD and smoking group did not survive the Bonferroni correction at $p < 0.0083$, it was approaching significance ($p = 0.047$) in the bilateral cerebellar and visual-related regions (**Figure 2B**). However, ALFF in IGD-Smoking group was not significantly different with healthy group in these regions ($p = 1.00$).

Correlations Between ALFF and Clinical Characteristics

The ALFF values from significant clusters showing interaction effects were extracted to perform correlation analysis with clinical characteristics controlling for age, gender, educational level and head motion (**Figure 3**). We found that the participants in smoking group with higher ALFF values exhibited higher SAS ($r = 0.313$; $p = 0.043$), SDS ($r = 0.372$; $p = 0.015$) and motor impulsiveness dimension of BIS-11 ($r = 0.364$; $p = 0.018$) scores in the right MPFC/ventral striatum and SDS ($r = -0.349$; $p = 0.024$) scores in the bilateral cerebellar and visual-related regions, and higher SAS score ($p = 0.049$, $r = 0.305$) the left temporal gyrus. ALFF of other three groups did not show significant correlation with smoking related variables (i.e., duration of smoking, age at first smoking, and FTND), IGD related variables (CIAS) and questionnaire scores of SAS, SDS, BIS, and its three dimensions.

DISCUSSION

As far as we know, this is the first study to explore interaction between smoking and IGD on spontaneous brain activity using rs-fMRI. Our findings demonstrated that there were significant smoking by IGD interactions on ALFF in the right MPFC/ventral striatum, left temporal gyrus, and bilateral cerebellar and visual-related regions. Specifically, in the right MPFC/ventral striatum and left temporal gyrus, ALFF in smoking group was significantly higher than healthy group while there were no significant ALFF

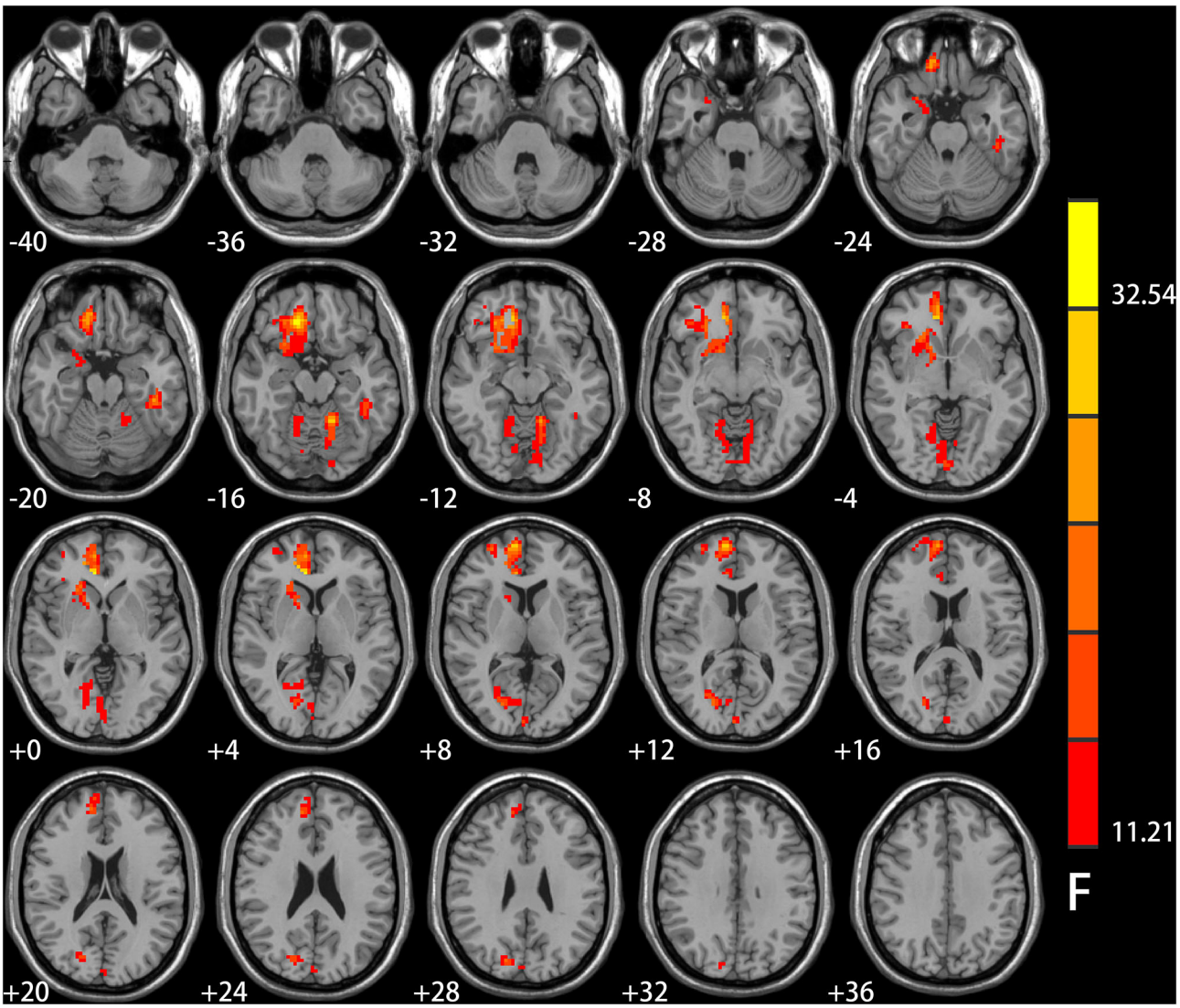

FIGURE 1 | Results of ANCOVA analysis controlling for age, gender, educational level, and head motion. Brain regions showed group differences among the four groups of healthy controls, smokers, internet gaming disorder (IGD), and IGD-Smoking individuals in amplitude of low-frequency fluctuation (ALFF) ($p < 0.05$, FWE-corrected). The brain regions mainly involved in the right medial pre-frontal cortex (MPFC, i.e., orbital frontal gyrus and anterior cingulate cortex) extending to ventral striatum, bilateral cerebellar, and visual-related regions (i.e., lingual and calcarine gyrus and cuneus) as well as the left temporal gyrus.

differences between IGD-Smoking group and IGD group. There were no significant ALFF differences between IGD group and healthy group while IGD-Smoking group showed significantly lower ALFF than smoking group. However, in the bilateral cerebellar and visual-related regions, ALFF in smoking group was significantly lower than healthy group while ALFF in IGD-Smoking group did not show significant difference with IGD group. There were no significant ALFF differences between IGD group and healthy group while IGD-Smoking group showed significantly higher ALFF than smoking group. Taken together, these findings suggest that smoking and IGD interact with each other while they work in human brain, especially in reward and motivation functions related regions.

Smoking group demonstrated significantly higher SAS, SDS, and impulsivity scores, which is consistent with previous studies that smoking was associated with anxiety and depression with smoking (34). Anxiety was one of the strongest predictors of nicotine intake and nicotine-seeking behavior (35) and significantly related to the severity of cigarette dependence and unsuccessful attempts to quit (36). Similar reports have been made of depression that compared with persons without depression, depressed individuals are more likely to smoke and relapse, less likely to quit (37). In turn, smoking appears to increase the risk for the development of depression (38). On the side of initiation and of the persistence of internet addiction, depression was also an important predicting factor

TABLE 2 | Regions showing significant amplitude of low-frequency fluctuation (ALFF) differences among the four groups of healthy control, smokers, internet gaming disorder (IGD), IGD-Smoking individuals ($p < 0.05$, FWE-corrected).

Identified brain regions	Peak coordinates (MNI)			Side	Peak F	Cluster size (voxels)
	X	Y	Z			
Medial pre-frontal cortex (i.e., orbital frontal cortex and anterior cingulate cortex)/ventral striatum	15	33	−15	R	36.81	863
Cerebellar and visual-related regions (i.e., calcarine, cuneus, and lingual gyrus)	−12	−51	−15	B	28.39	502
Inferior temporal gyrus	−42	−33	−21	L	25.28	71

Analysis of covariance (ANCOVA) controlling for age, years of education, and mean frame-wise displacement was performed to investigate the interaction between smoking and IGD on ALFF.

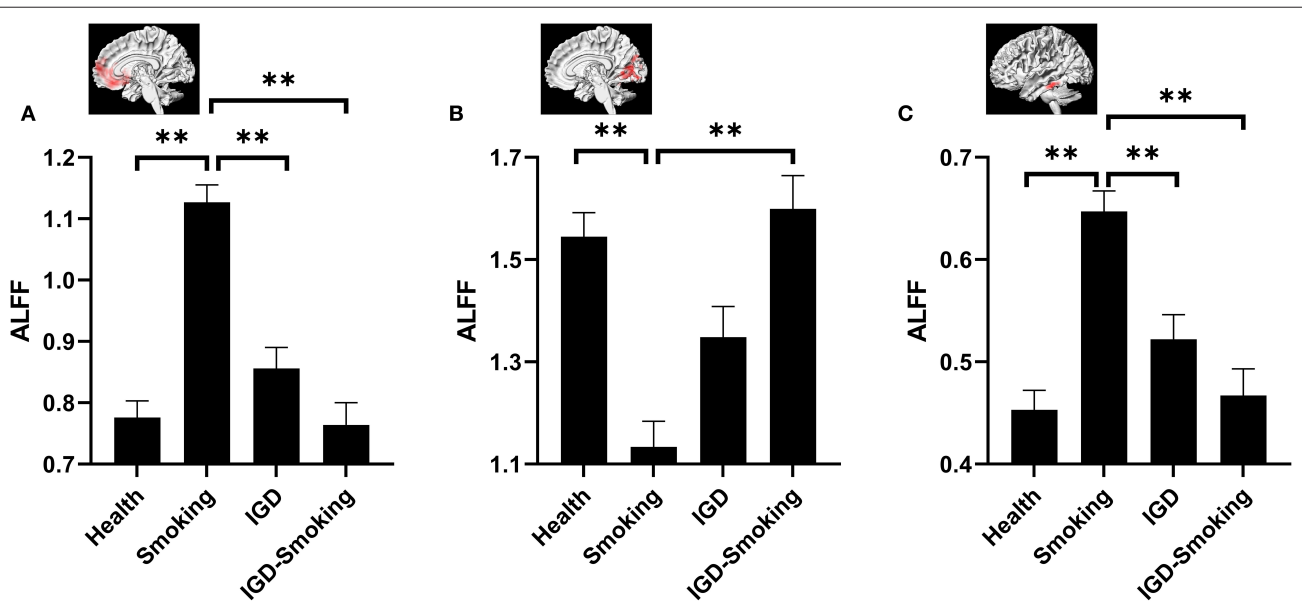

FIGURE 2 | *Post-hoc* analyses of ALFF values among the four groups. **(A)** The brain regions involved in the right MPFC/ventral striatum. ALFF in smoking group was significantly higher than healthy group ($p = 3.61 \times 10^{-15}$) while no significant ALFF differences between IGD-Smoking and IGD group were found ($p = 0.439$). There were no significant ALFF differences between IGD group and healthy group ($p = 0.441$) while IGD-Smoking group showed significantly lower ALFF than smoking group ($p = 1.16 \times 10^{-12}$). Moreover, ALFF in smoking group exhibited significantly higher value than IGD group ($p = 5.55 \times 10^{-8}$). **(B)** The brain regions involved the bilateral cerebellar and visual-related regions. ALFF in smoking group was significantly lower than healthy group ($p = 1.17 \times 10^{-7}$) while the difference between IGD–Smoking and IGD group did not survive the Bonferroni correction at $p < 0.0083$ ($p = 0.038$). No significant ALFF differences between IGD and healthy group was found ($p = 0.081$) while IGD-Smoking group showed significantly higher ALFF than smoking group ($p = 2.32 \times 10^{-7}$). **(C)** The brain regions involved the left temporal gyrus. ALFF in smoking group was significantly higher than healthy group ($p = 8.55 \times 10^{-10}$) while no significant ALFF differences between IGD-Smoking group and IGD group were found ($p = 0.855$). There were no significant ALFF differences between IGD group and healthy group ($p = 0.20$) while IGD-Smoking group showed significantly lower ALFF than smoking group ($p = 1.00 \times 10^{-6}$). Moreover, ALFF in smoking group exhibited significantly higher value than IGD group ($p = 0.001$). The graphs above the column group were the involved brain regions. $^{**}p < 0.05/6 = 0.0083$ (Bonferroni correction).

(9) and it increased linearly with YDQ score (39). In addition, there were significant correlations between internet addiction with depression and anxiety (40). Specific internet addicts showed higher social anxiety (41). In addition, impulsivity components differentially predicted tobacco use (42) and higher impulsivity determined by the BIS increased adolescents' odds of being smokers (43) and smokers reported higher impulsivity on the BIS-11 than never smokers (44). No significant correlation between SAS and SDS with IGD or IGD-Smoking in this study might be due to the small sample size.

The finding that in the right MPFC/ventral striatum and left temporal gyrus, smoking group had significantly higher ALFF than healthy group is consistent with the observation of increased activation in the ACC and superior temporal gyrus in the nicotine group compared with the controls (45). It suggested that compared to healthy group, these brain regions were more vulnerable to smoking. However, no significant ALFF

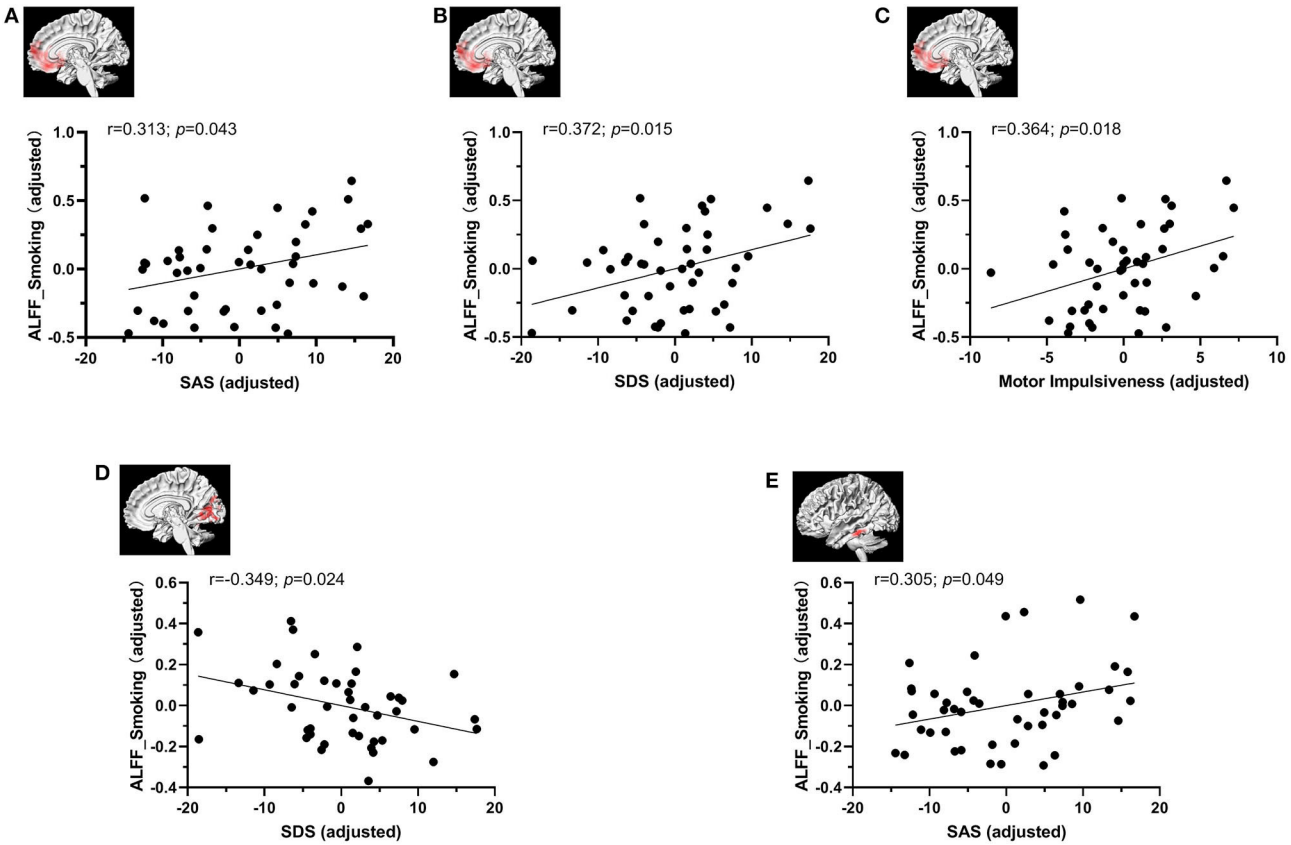

FIGURE 3 | The correlation analysis between ALFF values and clinical characteristics controlling for age, gender, educational level, and head motion. The ALFF value in the smoking group was associated positively with SAS **(A)**, SDS **(B)** and motor impulsiveness dimension of BIS-11 scores **(C)** in the right MPFC/ventral striatum. **(D)** The ALFF value in the smoking group had a trend toward negative correlation with SDS score in the bilateral cerebellar and visual-related regions of lingual and calcarine gyrus and cuneus. **(E)**The ALFF value in the smoking group was associated positively with SAS score in the left temporal gyrus.

differences between IGD-Smoking group and IGD group in these brain areas were found. In other words, when both IGD and smoking co-existed, ALFF of these brain regions were identical to that of the healthy control group. These two results demonstrated that when smoking occurred in the healthy and IGD group, the changes of the spontaneous brain activity were different between these two groups. It was reported that the youthful initiation of internet addiction in teenage students could be predicted by smoking, and substance use like smoking is likely to exacerbate internet addiction initiation and persistence (9). In addition, Sung et al. (24) proposed that smoking may associate with high risk of internet addiction. More interestingly, compared to schizophrenia non-smokers, schizophrenia smokers demonstrated reversed intrinsic brain activity in the pre-frontal cortex. In short, smoking had different effects on healthy and IGD group.

Additionally, in the same brain regions, we found no significant ALFF differences between IGD group and healthy group. Although many studies had found that IGD group had significant differences with healthy controls on the spontaneous brain activity in the right orbital frontal gyrus (46), the right ACC (19), and the temporal gyrus (47). The inconsistency with our finding may arise from the sample characteristics or correction method. For example, in this study, we using FWE correction in the two-way ANCOVA to find significant clusters and Bonferroni correction in the *post-hoc* pairwise comparison while the correction method was Alphasim correction in the report of Lin et al. (47). The study also revealed that IGD-Smoking group showed significantly lower ALFF than smoking group. Sung et al. (24) also suggested that compared with low-risk group for internet addiction, smoking rates were significantly higher among high-risk group for internet addiction. This cannot be explained that IGD causes smoking. It might be that they have the similar causal factors. This may make the phenomenon that IGD individuals in the internet cafes often tended to be smokers and the ALFF of IGD—Smoking group did not show significant difference with healthy controls better understood. To sum up, these results in this study illustrated that when healthy and smoking group were addicted to IGD, the changes of the spontaneous brain activity were different between these two groups.

In this study, there was significant interaction between IGD and smoking in the right MPFC/ventral striatum and left temporal gyrus. The MPFC is considered as promoting

goal-directed behavior by assessing the incentive-significant stimuli and choosing to generating the desired results (48–50). It integrates the body signals to help make decisions which involved in creating and maintaining the expectations of rewards associated with reinforcement (51). The activity of medial OFC and ACC was highly correlated with the subjective valuation of drug-related stimuli (52) and showed increased activity after individuals receiving a reward or before an expected reward immediately (53). The glucose metabolic activity of OFC in IGD adolescents also showed abnormal increase when compared with controls. (54). So the brain activity in the frontal gyrus might be perceived as a marker to reflect the reduced cognition and control ability of addiction (15) and enhanced reward sensitivity (46). Increased functional connectivity of OFC was also observed in cannabis users (55). However, in heroin-dependent individuals, regional homogeneity was diminished in the medial OFC (22). In methamphetamine users, metabolic activity of OFC was reduced (56). The medial OFC is a part of the limbic system, which is involved in decision making and expectation (57). In these studies, the OFC demonstrated conflicting results, which, on the one hand, may be due to the small sample size and different addictive substance with different mechanism. On the other hand, ALFF measures the average value over a period of time, which could not reflect the dynamic property of real brain signal. Further dynamic investigation will be developed to obtain more evidence to understand the potential neural mechanism behind addiction disorder.

Activation was also found in ACC in IGD group when compared to the control group in an event-related fMRI study (58). Another fMRI study found that global cerebral blood flow in IGD subjects appeared to be significantly higher in ACC which may related to attention and arousal mediation (59). ACC involves in making the information of emotion and motivation, regulation of reward-seeking behavior as well as attention salient (60, 61) and processing memory and encoding the motivation of substance cue (62, 63). It is crucial in monitoring the meaning of the stimulus relative to the target. Once the appetite cues are identified, ACC will determine whether and how strong the behavioral response is (64, 65). As for other kinds of addiction disorders, for instance, in alcohol use disorders activated the left cingulate gyrus and left superior temporal gyrus was founded (62). Significant activation of ACC was also observed in cocaine abusers (66). However, Volkow et al. found decreased metabolism in cingulate gyrus and OFC in cocaine abusers. Cocaine abusers also showed significant decreases in DA release which leads to decreased activation of reward circuits and further leads to compulsive drug administration to perpetuate cocaine use as a means to compensate for this deficit (67). Several studies demonstrated that the ACC is associated with selective attention (68, 69) which is a prerequisite for signaling reward (70). As a part of a corticostriatal circuit, the ACC plays an important role in stimulus–reward learning (71, 72). Once appetitive (and aversive) cues are identified by the visual cortex and motivational/attentional centers, ACC are activated to reinforce the processing (73). The ACC activation was also observed during cognitive processes which could be dysfunctional after anterior cingulotomy in human (74).

Increased fractional ALFF (fALFF) was found in the superior temporal gyrus and the volume of temporal lobe were significantly reduced in addiction persons (19). The primary function of the temporal lobe is regulating sensory perception including processing vision and auditory. The activated inferior temporal gyrus might serves as positive intensifying factor to reveal oneself contacting addiction behavior repetitively (19). What's more, the ALFF value in the smoking group was associated positively with SAS and SDS scores in these brain regions, indicating the more anxious, depressed, the higher ALFF in the smoking group. At the same while, the ALFF value in the smoking group was associated positively with SAS score in the left temporal gyrus. Given the function of these brain regions, we hypothesized that smoking might be due to anxiety, depression. Additionally, it also illustrates that ALFF can be regarded as a reliable marker in the exploration of the brain function.

In the bilateral cerebellar and visual-related regions of lingual and calcarine gyrus and cuneus, we found that ALFF in smoking group was significantly lower than healthy group. Activity in the bilateral cuneus and lingual gyrus was also found diminished in heroin-dependent individuals (22). However, ALFF in IGD–Smoking group did not show significant difference with IGD group. These two findings demonstrated that when smoking had different effects of spontaneous brain activity on healthy and IGD group. In addition, this study also illustrated no significant ALFF differences between healthy group and IGD group while IGD–Smoking group showed significantly higher ALFF than smoking group. All these results pointed out the interaction between IGD and smoking. A functional MRI study supported that the cerebellum serves as cognitive functions (75). Researchers have found a correlation between structural abnormalities in the cerebellum and the clinical manifestations of certain psychiatric disorders. The cerebellum has lots of functional connection with the brain, which, to some extent, contributes to regulating the cognition, emotions and thinking. There are also reports which found the cerebellar structural abnormalities is correlated with certain mental illness (76). The decreased activation of cerebellum of our study might suggest the impairment of cerebellum in IGD individuals and smokers with abnormality of cognitive functions. The cuneus involves in visual processing inhibitory control centers. The regional homogeneity of heroin-dependent individuals was found diminished in the bilateral cuneus (22). Moreover, the ALFF value in the smoking group had a trend toward negative correlation with SDS score in the bilateral cerebellar and visual-related regions of lingual and calcarine gyrus and cuneus, suggesting that smokers with lower ALFF values in these brain regions felt more depressed.

Whether smoking caused significant ALFF changes in healthy group but not in IGD group, or IGD caused significant ALFF changes in smoking group but not in healthy group, it demonstrated the interaction of the two factors and the different combined effect from the single effect of one factor. IGD was related to smoking (40). More interestingly, nicotine addiction effect independent of other addictions like gambling (77) and disease like schizophrenia (78) and IGD associated with harmful alcohol use among college students was also found (17, 79). Compulsive internet use might have a causal

relationship with changes in substance use in female (80). Yen proposed that the comorbidity of internet addiction and other substance addiction might indicate the predictive relationship between them (81). IGD and smoking might share the similar neurobiological mechanisms (8, 10). IGD and smoking is the typical representative of behavioral and substance addiction, respectively. The underlying mechanism about their different performance in different populations are still unknown, so further researches are need to determine.

Furthermore, in the significant brain areas of ANCOVA, we also found that compared to healthy controls, the increased ALFF in smoking group was significantly larger than IGD group in the right MPFC/ventral striatum. It illustrated that healthy controls might be more sensitive to smoking than IGD. The reason might be that in smokers, substance like nicotine or other aspects in cigarette worked while there wasn't any substance in IGD individuals.

This study has several limitations. First, the possible influences of alcohol cannot be excluded, as alcohol consumption was not quantitatively evaluated. Although most subjects self-reported no or seldom daily alcohol consumption, whether the subjects deceptively reported of alcohol dependence could not confirmed. Therefore, the possible effects of alcohol use on the interactions between smoking and IGD need to be studied in the future. Second, variables of age, sex and education level were not well-matched among the four groups although they went to the statistical procedure as controlling factors. Several studies showed sex-specific effects of cigarette smoking on rsFC (82) and IGD on ALFF and rsFC (83). Child abuse increases risk for substance use in part (84) and there were significantly differences in light and heavy smokers (20). Although they served as covariates, we cannot completely exclude the potential impacts of these factors. Third, no significant correlation remained after Bonferroni correction. This might be related to the small sample size and future research of large sample size is needed to help us to get deep understanding of the relationship between common scales of psychological disorder and IGD and Smoking. Finally, although this resting-state fMRI study investigated interaction between smoking and IGD on spontaneous brain activity, future task dependent examination will help us to have a better understanding of the neural mechanism of IGD and smoking.

In conclusion, we demonstrated that smoking and IGD were not independent and they actually interacted with each other on spontaneous brain activity, mainly characterized by significant interaction in the right MPFC (i.e., orbital frontal gyrus, anterior cingulate cortex and medial frontal gyrus) extending to the right ventral striatum, bilateral cerebellar and visual-related regions (i.e., lingual and calcarine gyrus and cuneus) as well as the left temporal gyrus. Our results may imply the fact that IGD people are more tended to get smoking addiction. It may also be possible to predict that smoking addiction person may be more easily to get internet addiction than healthy people. Our findings may have the possibility to improve our understanding of the latent neurological theory and mechanism by which several addictive factors work together on human brain.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Medical Ethics Review Board of Renji Hospital, Shanghai Jiaotong University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

FL and HL contributed to the study design. XH, YW, WD, YS and YZ collected the fMRI data. FL and XQ analyzed the MRI data and drafted the manuscript. All authors contributed to the article and approved the submitted version.

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Probabilistic Reversal Learning Deficits in Patients With Methamphetamine Use Disorder—A Longitudinal Pilot Study

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Methamphetamine use disorder (MUD) is increasing worldwide and commonly associated with learning deficits. Little is known the about underlying trajectories, i.e., how the affected higher-order cognitive functions develop over time and with respect to abstinence and relapse. A probabilistic reversal learning (PRL) paradigm was implemented to uncover the microstructure of impulsive choice and maladaptive learning strategies in 23 patients with MUD in comparison with 24 controls. Baseline data revealed fewer optimal choices and a pattern of altered learning behavior from negative and positive feedback in patients suggesting impairments in flexibly-adapting behavior to changes of reward contingencies. Integrating longitudinal data from a follow-up assessment after 3 months of specific treatment revealed a group-by-time interaction indicating a normalization of these cognitive impairments in patients with MUD. In summary, our study demonstrates behavioral correlates of maladaptive decision-making processes in patients with MUD, which may recover after 3 months of MUD-specific therapy paving the way for further learning-based interventions. Limited by a small sample size, the results of this pilot study warrant replication in larger populations.

Keywords: methamphetamine, crystal meth, probabilistic, reversal learning, longitudinal, psychotherapy

INTRODUCTION

Methamphetamine use disorder (MUD) has been a growing worldwide problem, and in the last decade, the incidence in Western Europe has increased rapidly (1). Behavioral approaches have been the mainstay of treatment, and although some behavioral interventions have increased retention rates (2), little is known about predictors that influence treatment responses and the trajectories of cognitive functioning in MUD.

Dysfunctional learning processes in the pathogenesis of addictive disorders have become apparent (3), which when paired with various neurobiological sequelae associated with MA may undermine treatment efficacy. There is considerable evidence for MA-related neurobiological

deficits (1, 4), including persistent gliosis and apoptosis in dopaminergic and serotonergic neurons (5) and abnormalities in morphology and function of fronto-striatal and limbic regions (6–9). Functionally, these changes have been linked to a broad range of cognitive impairments (10, 11), including multiple domains such as attention control, working memory and executive functions especially decision making (12–17).

Studies consistently show that maladaptive and impulsive decision making is common in MUD, where patients favor smaller immediate rewards over larger later rewards (12, 18–22). Although impulsive choice is influenced by the dynamic interaction of biases in delay and reward magnitude (23), it is unclear whether temporal discounting in MUD reflects deficits in processing and integrating reward contingencies and consequences. Adaptive decision making requires cognitive flexibility to maximize outcomes, whether to obtain reward or avoid punishment, and as learned drug-taking habits prevail despite devastating psychosocial consequences (24), it is critically important to identify the extent to which impulsive choice is related to deficits in processing and integrating outcomes/consequences.

Probabilistic reversal learning (PRL) paradigms enable the investigation on behavioral adaptation to changes in reward contingencies under uncertainty in the task environment (25). Thus, PRL can capture underlying deficits in behavioral flexibility (26), which requires updating choices when confronted with changes in the environment and neglecting rare events when environmental factors are stable (24). In PRL paradigms, participants must decide between two choices. Through trial-and-error, participants learn that one of the two choices is predominantly rewarded, whereas the other one is predominantly punished. The task is made more difficult by changing contingencies from time to time. Previously predominantly rewarded decisions are then more likely to lead to a punishment and vice versa. Optimal choice behavior is characterized by two strategies. First, participants should maintain the choice pattern that is predominantly associated with a reward despite the rare event of a punishment. These negative rare events must therefore be ignored. Second, participants must recognize a reversal or change in contingencies, where the former (correct) choice is predominantly punished. In this case, the strategy should be changed.

Previous studies have shown perseverative deficits in patients with a stimulant use disorder (27, 28), where difficulties in adapting behavior when contingencies change were exhibited. Another study showed that patients suffering from cocaine/crack or amphetamine dependence exhibited reduced reward-driven learning (24). In that study, stimulus-bound perseveration, a measure that shows the extent to which participants stick to their choice-making behavior regardless of the outcome, was greater in patients with substance use disorder (SUD) compared to controls. Although it is clear that SUD is associated with deficits in learning contingencies, a few studies have used PRL paradigms that can mirror these learning impairments in MUD. In addition, studies have shown that changes in cognitive function such as sustained attention can predict treatment outcomes (29, 30) or can improve with treatment (12); however, it is unclear whether addiction-specific treatment can improve longitudinal changes in cognitive flexibility and improve deficits in learning outcome contingencies in MUD.

This study, therefore, used a PRL task to investigate differences in learning mechanism associated with outcome contingencies in a sample of patients with MUD vs. healthy controls. Within the MUD group, changes in learning performance was tested before and after a 3-month addiction treatment program that included a combination of motivational interviewing, cognitive behavioral therapy, and psychoeducation. We hypothesized that patients would show an impaired ability to update their behavior whenever circumstances and contingencies changed compared to controls before treatment and that these aberrations would normalize post treatment.

METHODS

Participants

In- and outpatients were recruited at the University Hospital Dresden. Inclusion criteria for patients with MUD were 18–65 years of age; meeting the diagnostic criteria for MA abuse or dependence according to the International Classification of Diseases (ICD-10); abstinence from illicit drug use for at least 2 days, proven with negative urine screenings for MA, amphetamines, MDMA, opioids, and THC. Only patients, for whom MA was clearly the main problem substance, were included. Exclusion criteria were any medical conditions, (e.g., schizophrenia, severe depressive symptoms, limited physical mobility) that interfere with the capability to attend group therapy, i.e., the experienced scientific staff assessed that the

TABLE 1 | Sociodemographic and clinical data of participants.

	Patients	Controls	Statistics
Sample size	23	24	
Demographics			
Sex			$X^2 = 0.28$, df = 1, $p = 0.60$ (chi-square test)
Women	10 (43.5)	9 (36.0)	
Men	13 (56.5)	16 (64.0)	
Age (years)	30.4 ± 6.9	29.0 ± 5.5	$U = 327.5$, $p = 0.41$
Presence of own child	13 (56.5)	13 (52.0)	$X^2 = 0.01$, df = 1, $p = 0.75$ (chi-square test)
Lower secondary school leaving certificate or less	15 (65.2)	18 (72.0)	$X^2 = 0.26$, df = 1, $p = 0.61$ (chi-square test)
Clinical data			
MA dependence (years)	8.3 ± 5.4	n.a.	
Abstinence (days)	4.9 ± 4.0	n.a.	
Any psychiatric comorbidity	18 (78.0)	n.a.	

Participant characteristics at baseline (T1): All tests are based on the whole sample (N = 48) and complete data on all variables. Data are number (%) or mean ± SD. Two-sided significance was assumed at $p < 0.05$.

participants could at best be mildly affected by comorbid psychiatric symptoms.

For the control group, non-substance-abusing subjects (HCs) matched for age, sex, and education were recruited via advertisements placed on local community-based websites, which offered employment and volunteer opportunities. Participants were required to have no lifetime experience with any kind of stimulants (MA, amphetamines, MDMA, methylphenidate, cocaine, etc.) and have never been diagnosed with any psychiatric disorder including SUD. The final sample consisted of 23 MA-dependent patients and 24 HCs (**Table 1**). All participants provided written informed consent and received a compensation between 10 and 20€. The study was approved by the local ethics committee of the Technische Universität Dresden and carried out in accordance with the Declaration of Helsinki.

Study Design

All study patients received treatment as usual, i.e., they completed our manual-supported methamphetamine-specific standard program, which was established at our clinic and has since been positively evaluated in terms of its effectiveness (2) and feasibility (31). This program is for patients who endorse MA as their main problem substance and who are all sufficiently motivated to change their drug consumption, i.e., to significantly reduce their use or to remain abstinent. The manual (31, 32) consists of 15 modules and includes a combination of strategies (such as motivational interviewing, cognitive behavioral therapy, and psychoeducation) and accounts for behavioral and demographic aspects specific to MA (e.g., younger users relative to other substance users, high rates of polysubstance use, frequent use in social and party settings, and the motivation to use as a performance enhancement). The effectiveness of this manual in treating MA problems has been sufficiently well-established and can be easily implemented in everyday clinical practice. It takes into account special aspects of MUD compared with other SUDs, e.g., the high proportion of young patients and polyvalent substance abuse as well as the widespread use of methamphetamine as recreational drug and for alleged performance enhancement.

Research staff, independent of providing treatment, conducted the recruitment as well as baseline (T1) and follow-up (T2) assessments after about 3 months. The assessments included a PRL task and the collection of clinical and sociodemographic data using standardized questionnaires. At T1, MA usage patterns were assessed, including age of first MA use, total duration of MA use, and days of abstinence. Psychiatric comorbidities were recorded according to ICD-10 criteria.

As described previously (12), inpatients provided weekly urine samples, and additional drug screening was performed in cases of clinical suspicion or after prolonged absences (during inpatient stay). Patients were randomly assigned to drug screening with a probability of 1/6 on working days. The cutoff for a positive result for amphetamines and MA was set at 300 ng/ml. We defined relapse as any positive screening result.

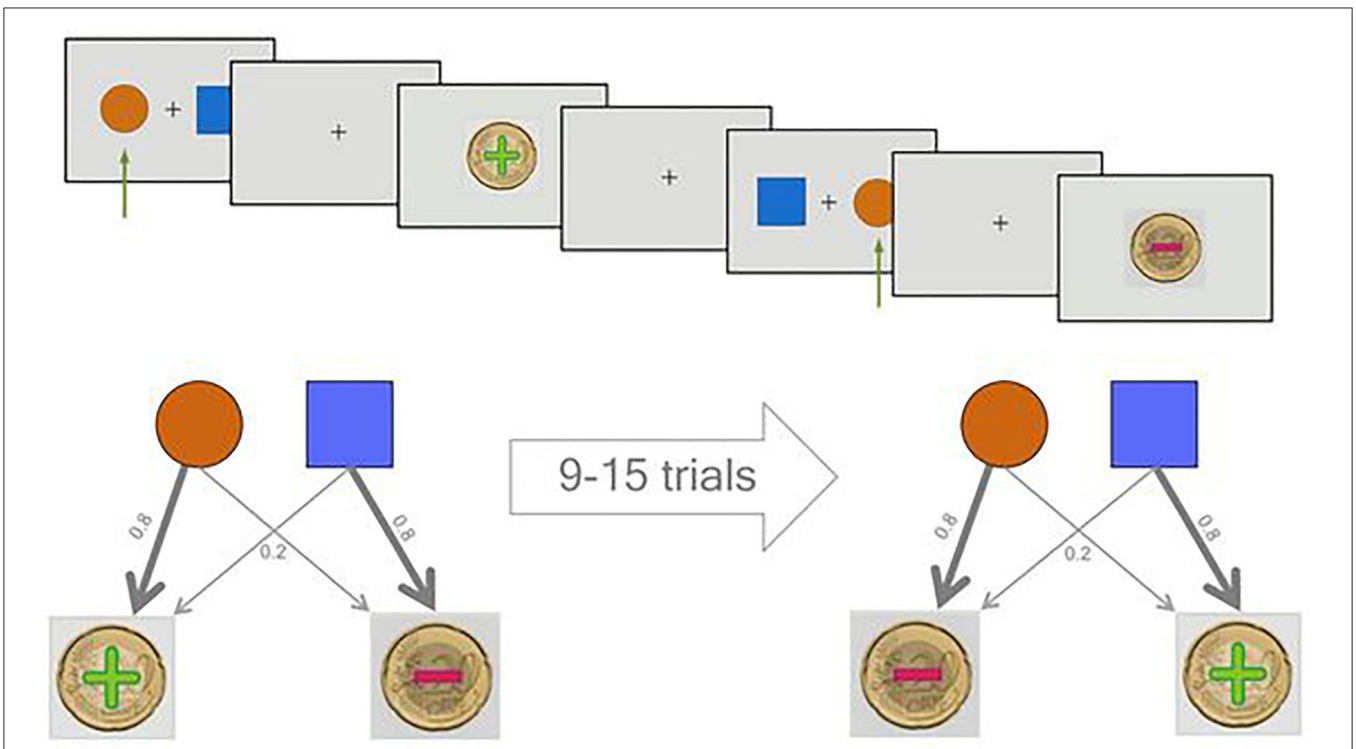

FIGURE 1 | Overview of the probabilistic reversal learning (PRL) task. In the upper half of the picture, two sample trials of the task are displayed. Two sample trials are shown. The green arrow shows the selection of the participant. The first decision leads to a reward and the second to a punishment. In the lower, the probabilistic character of the task is illustrated.

According to Petzold et al. (2), treatment was classified as "successful" if the patient attended at least 8 out of 15 group therapy sessions or was enrolled in a post-acute management program. Additionally, a single MA-positive test result during the course of treatment was allowed, provided the relapse was self-critically processed. The treatment was classified as "unsuccessful" if the therapy was prematurely terminated or if more than one relapse occurred.

The Task

In the PRL task, participants make choices between two options and receive positive or negative feedback based on their decision. One choice has a high probability of reward (e.g., 80%) and is called the correct option, and the other, the incorrect option, most likely (e.g., 80%) delivers a punishment.

Previous studies used adaptive task designs to increase the difficulty for more capable subjects. In these studies, the more rewarding option becomes the less rewarding one after a certain number of correct choices have been made (25, 33–35). For the less rewarding option, they set very close punishment and rewarding probabilities of 60 and 40%, respectively, which leads to very different expected values for different subjects. However, these modifications are too demanding for clinical populations, which frequently have cognitive impairments. To overcome these issues and make the task equally difficult for everyone, we set the reward and punishment probabilities for the correct option to 80 and 20%, respectively, and vice versa for the incorrect one (**Figure 1**). Moreover, we used a task design where blocks of 9–15 trials were fixed to accommodate the correct and incorrect options. We then shuffled the blocks for different participants and randomly assigned one of the cues (square or circle) to be the correct option on the first block. The reversal/contingency change was then applied such that the correct cue was switched to become the alternative cue at the beginning of each block. This design resulted in an approximately 8% chance of a contingency change from one trial to another.

Participants performed two rounds of training prior to data acquisition. The first training round was done with deterministic rewards and punishments, and the second round was a real practice with probabilistic rewards and reversals. Following the training, 11 blocks of fixed lengths were presented comprising 132 trials and 10 contingencies in total. Participants received/lost 20 cents as rewards/ punishments, displayed as Euro coins with positive and negative signs superimposed and the cumulative reward as a feedback after each trial. The task was implemented using the Psychophysics Toolbox extensions (36–38) in MATLAB, release 2017a (The MathWorks, Inc., Natick, MA).

Although different modeling techniques like reinforcement learning and hidden Markov models have been used to further investigate the mechanisms underlying decision processes, we decided on simple behavioral measures for pilot study.

TABLE 2 | Comparison of subsamples with successful vs. unsuccessful outcome.

	Successful outcome	Unsuccessful outcome	Statistics
Sample size	13 (56.5)	10 (43.5)	
Demographics			
Sex			$p = 0.09$ (Fisher's exact test)
Women	8 (61.5)	2 (20.0)	
Men	5 (38.5)	8 (80.0)	
Age (years)	30.1 ± 6.8	30.9 ± 7.3	$U = 70$, $p = 0.78$
Presence of own child	7 (53.8)	6 (60.0)	$p = 1.00$ (Fisher's exact test)
Lower secondary school leaving certificate or less	10 (76.9)	5 (50.0)	$p = 0.22$ (Fisher's exact test)
Clinical data			
MA dependence (years)	8.8 ± 6.3	7.7 ± 4.2	$U = 61.5$, $p = 0.85$
Abstinence [days]	4.2 ± 3.4	5.9 ± 4.6	$U = 81.50$, $p = 0.31$
SUD comorbidity	6 (46.2)	7 (70.0)	$p = 0.40$ (Fisher's exact test)
Psychiatric comorbidity except SUD	5 (38.5)	5 (50.0)	$p = 0.68$ (Fisher's exact test)
Treatment with antidepressant or antipsychotic	5 (38.5)	5 (50.0)	$p = 0.68$ (Fisher's exact test)
Family history of SUD	6 (46.2)	6 (60.0)	$p = 0.68$ (Fisher's exact test)

Patient characteristics at baseline (T1): All tests are based on the whole patient sample (N = 23) and complete data on all variables. Data are number (%) or mean ± SD. Two-sided significance was assumed at $p < 0.05$.

Statistical Analyses

We evaluated the differences between groups and subgroups regarding sociodemographic characteristics, clinical data, and behavioral measures with appropriate statistical tests depending on the type and distribution of variables and their sample sizes. The Shapiro–Wilk test, histograms, and normal quantile–quantile plots were used to judge normality.

For demographic and clinical data, we used Pearson's chi-square test for categorical variables, applying Fisher's exact test when needed and the unpaired *t*-test for continuous variables, applying the Mann–Whitney *U*-test when needed. Because only a few behavioral variables were normally distributed, we used the non-parametric Mann–Whitney *U*-test for all comparisons of behavioral measures for consistency.

Statistical significance was decided using an alpha level of 0.05. The exploratory longitudinal analysis was conducted using mixed effects models, which handle unequal sample sizes very well. We calculated effect sizes for the significant differences in baseline behavioral measures between controls and patients in the first measurement session.

RESULTS

Sample

Clinical and sociodemographic characteristics of patients and HCs are summarized in **Table 1**. There were no significant differences in sex, age, and education. Among the MA group,

seven were diagnosed with a cannabis use disorder, four with alcohol use disorder, two with polytoxicomania, five with borderline personality disorder, two with attention-deficit hyperactivity disorder, and three patients suffered from a depressive episode. Only one patient completed the entire study as an outpatient. Therefore, the effect of outpatient vs. inpatient treatment could not be analyzed. The duration of hospitalization was based on health insurance regulations, which cover treatment periods of 3–4 weeks for qualified drug detoxification. However, depending on comorbidities and treatment motivation, the length of stay can vary considerably. The mean duration of hospitalization was 22.5 ± 19.3 days.

Thirteen patients (56.5%) had a "successful" treatment outcome, i.e., enrollment in a post-acute management program or attending at least 8 out of 15 group therapy sessions with a maximum of one MA-positive test result. In 10 patients, the therapy was classified as "unsuccessful" since it was prematurely terminated or two or more relapses occurred (**Table 2**).

Behavioral Measures

We considered the number of correct choices (hits), irrespective of entailing a reward or a punishment, as a measure of performance. This measure was used to compare groups and to predict clinical outcome parameters. We also compared the number of times that participants switched from or continued selecting the previous choice after losing or winning.

At baseline, patients had fewer hits than controls (median of 80 and 89, respectively; one-sided Mann–Whitney *U*-test, $U = 129.5$, $p < 0.001$, $r = 0.77$). The effect size quantified by Pearson's r is related to the probability that one randomly selected patient has fewer number of hits than a randomly chosen control (39). Moreover, patients had fewer shifts after losing ($p < 0.01$; **Figure 2**), but more shifts after winning ($p < 0.01$; **Figure 3**) compared with controls.

Considering treatment outcomes, patients with a successful treatment had fewer hits ($p < 0.01$) than those with an unsuccessful one (**Figure 4**) and also tended to have fewer shifts after losing ($p = 0.07$; **Figure 2**) and more shifts after winning ($p = 0.07$; **Figure 3**) at baseline.

Longitudinal Analysis

Follow-up data at T2 could be acquired for 76% of controls and 65% of patients leading to a considerable reduction in the statistical power. Nevertheless, we performed an exploratory longitudinal analysis using mixed effects models to investigate effects of time, group, and their interaction. The analysis was done with *nlme* package in R (40). As shown in **Table 3**, we found a significant group effect and a trend for the interaction term: The main effect of group ($p = 0.001$) indicated that patients

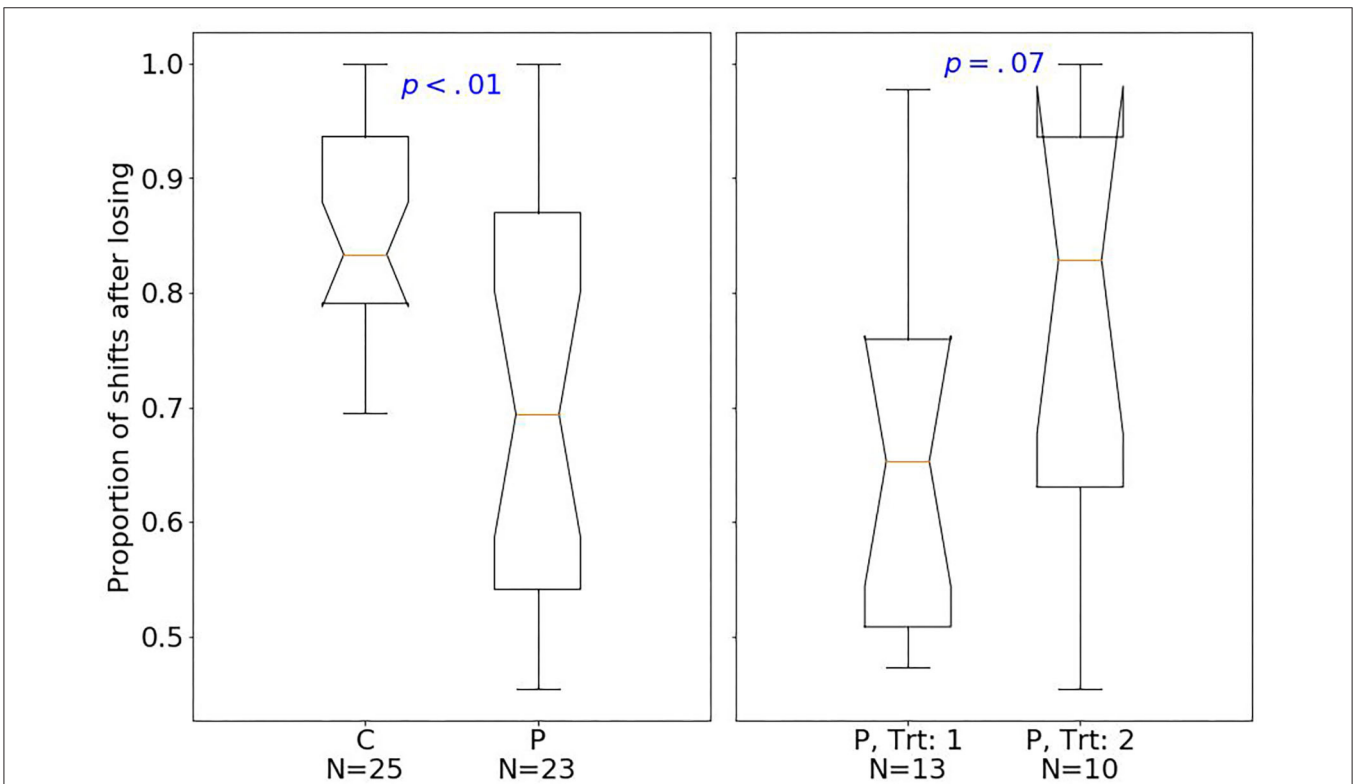

FIGURE 2 | Box plots of the PRL task. The horizontal line represents the median; the boxes extend to the first and third quartile, while whiskers extend to the max/min or the corresponding quartile + 1.5 IQR. C, healthy controls; P, patients with MUD; Trt_1, successful treatment outcome; Trt_2, unsuccessful treatment outcome. Box plots of proportion of shifts after losing.

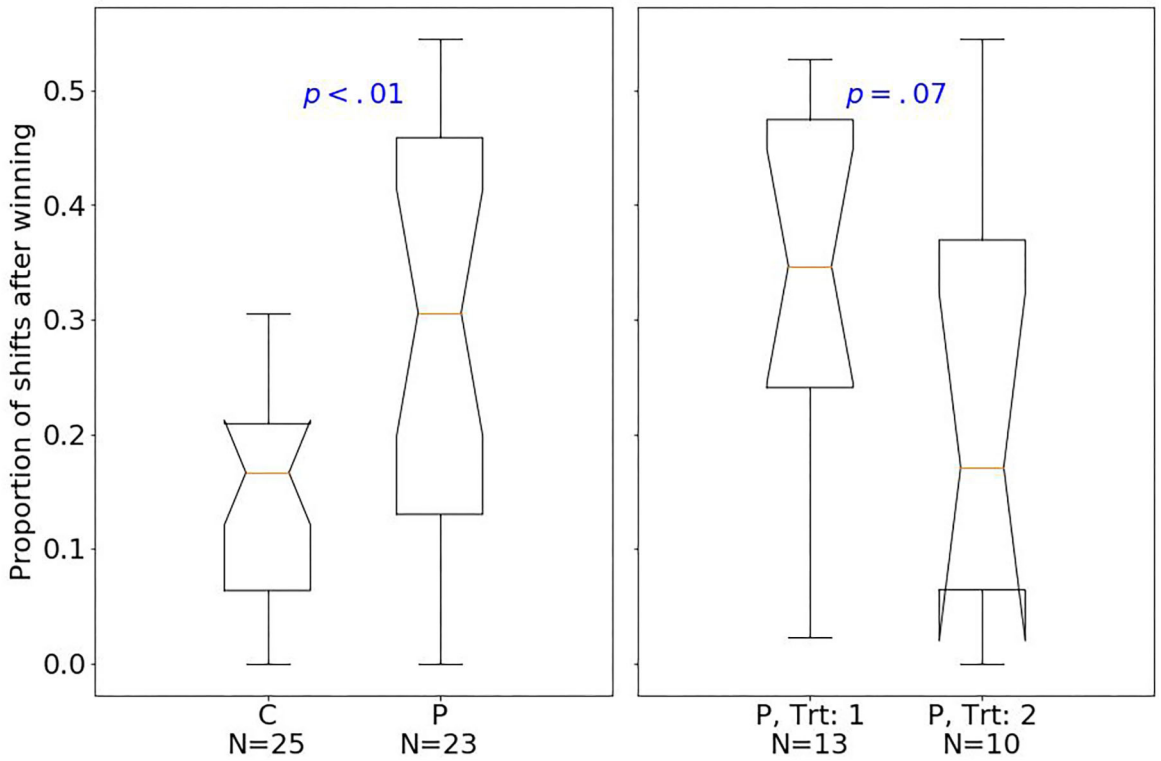

FIGURE 3 | Box plots of the PRL task. The horizontal line represents the median; the boxes extend to the first and third quartile, while whiskers extend to the max/min or the corresponding quartile + 1.5 IQR. C, healthy controls; P, patients with MUD; Trt_1, successful treatment outcome; Trt_2, unsuccessful treatment outcome. Box plots of proportion of shifts after winning.

exhibited a lower number of hits than controls. The almost significant time-by-group interaction ($p = 0.057$) indicated a possible improvement in the number of hits from T1 to T2 in patients but not in HC (**Figure 5**). We further conducted a multiple imputation, using the *mice* package in R (41), which suggests that the group effect is consistent, and statistically significant interaction effects might be found with a higher sample size (**Table 4**).

DISCUSSION

In this study, a typical clinical sample of German patients with MUD demonstrated a lower number of optimal choices than healthy controls in a PRL paradigm suggestive of overall maladaptive decision making. Patients showed greater difficulties in adjusting their behavior following a rule change compared with HC. This finding is in line with earlier studies suggesting similar deficits in patients suffering from amphetamine (24, 27) and cocaine (42) dependence from the United Kingdom. Using a combination of a "Go/No-Go" task with reversal learning, a study has shown maladaptive choices among cocaine and alcohol-dependent patients when contingency change. Here, the patients tended to increase stimulus-bound perseveration (43).

To differentiate the microstructure of behavior, we analyzed specific learning patterns from positive and negative reinforcement. Compared with controls, patients exhibited stimulus-bound perseveration in low-reward probability choices, where they maintained their choices after losing. Interestingly, patients with MUD continued to choose the wrong option despite the higher probability of receiving negative consequences but shifted their response to the wrong option after winning. In contrast, controls switched to the other option after losing more often than patients ("lose-shift") and more often stayed at the same option after winning ("win-stay behavior"). Intuitively, a reasonable strategy is to change response patterns after receiving a punishment and to maintain it after receiving a reward. To that extent, the responding pattern of patients can be considered closer to random decision making.

This learning pattern is in line with that of Ersche et al. (42), who found that individuals with cocaine dependence were impaired in both learning from negative and positive reinforcement. Kanen et al. (24) also showed diminished win-stay behavior in SUD; however, learning from negative feedback (lose-shift) was exhibited in SUD. The difference in results might be influenced by the salience of feedback. This study and the study of Ersche et al. implemented aversive negative outcomes; the loss of money or electric shock (respectively), while the feedback in Kanen et al. was an image of a sad red face, which presumably engenders less motivation. These results suggest the importance of salient and motivating punishments to drive

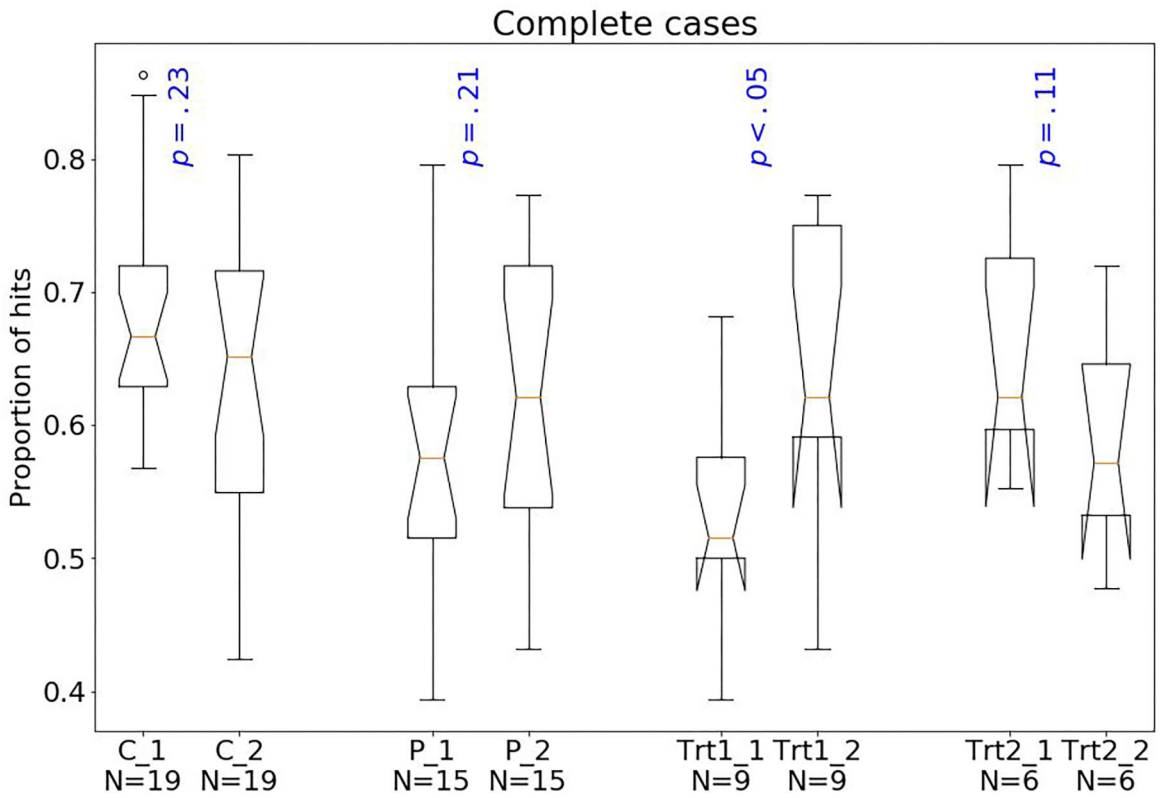

FIGURE 4 | Box plots of the PRL task. The horizontal line represents the median; the boxes extend to the first and third quartile, while whiskers extend to the max/min or the corresponding quartile + 1.5 IQR. C, healthy controls; P, patients with MUD; Trt_1, successful treatment outcome; Trt_2, unsuccessful treatment outcome. Box plots of proportion of hits.

TABLE 3 | Output of mixed effects model using nlme package in R.

	Value	Std. error	DF	*t*-value	*p*-value
(Intercept)	65.77	5.91	46	11.13	0.000
Time	14.28	8.50	32	1.68	0.103
Group	12.54	3.69	46	3.40	0.001
Time:group	−10.31	5.23	32	−1.97	0.057

learning and behavior. From a clinical perspective, impaired learning capacities from high-salient negative consequences highlight the ineffectiveness of punitive, interventions for SUDs.

Longitudinal Course

An analysis of the longitudinal course showed a main effect of group and an almost significant ($p = 0.057$) group-by-time interaction. After imputing missing values for T2, the interaction became significant. While the number of hits was not significantly different between T1 and T2 in controls, the performance improved in patients over time but did not reach statistical significance; however, the significant differences in performance between HC and patients with MUD at T1 were ameliorated at T2. The improvements in the MUD group were primarily driven by patients with better treatment outcomes. The almost significant group-by-time interaction and the lack of behavioral differences between groups at T2, suggests that the ability to update behavior when circumstances change can normalize in patients with MUD after 3 months of specific therapy. Since the sample size is small and some values had to be imputed, this interpretation should be treated with caution and requires confirmation in larger studies. On the other hand, the results fit plausibly to the existing data: Bernhardt et al. (12) showed that sustained attention deficits in patients with MUD could also normalize over a 3-month therapy period. Along those lines, Volkow et al. (17) and Wang et al. (44) found an improvement in motor and verbal memory. Moreover, even in short observation periods (i.e., 3 weeks), the performance of patients with MUD in neuropsychological tests including attention (45) and executive functioning (46) showed an improvement. Limited by the lack of a control group, effects on the causal role of abstinence on performance changes can hardly be concluded (12). In another longitudinal study, however, MA-dependent participants showed a normalization of global cognitive function compared to control subjects after an average abstinence of 1 year (47). These results might question the usefulness of treatment strategies based on contingency management at an early stage. Contingency-based strategies in

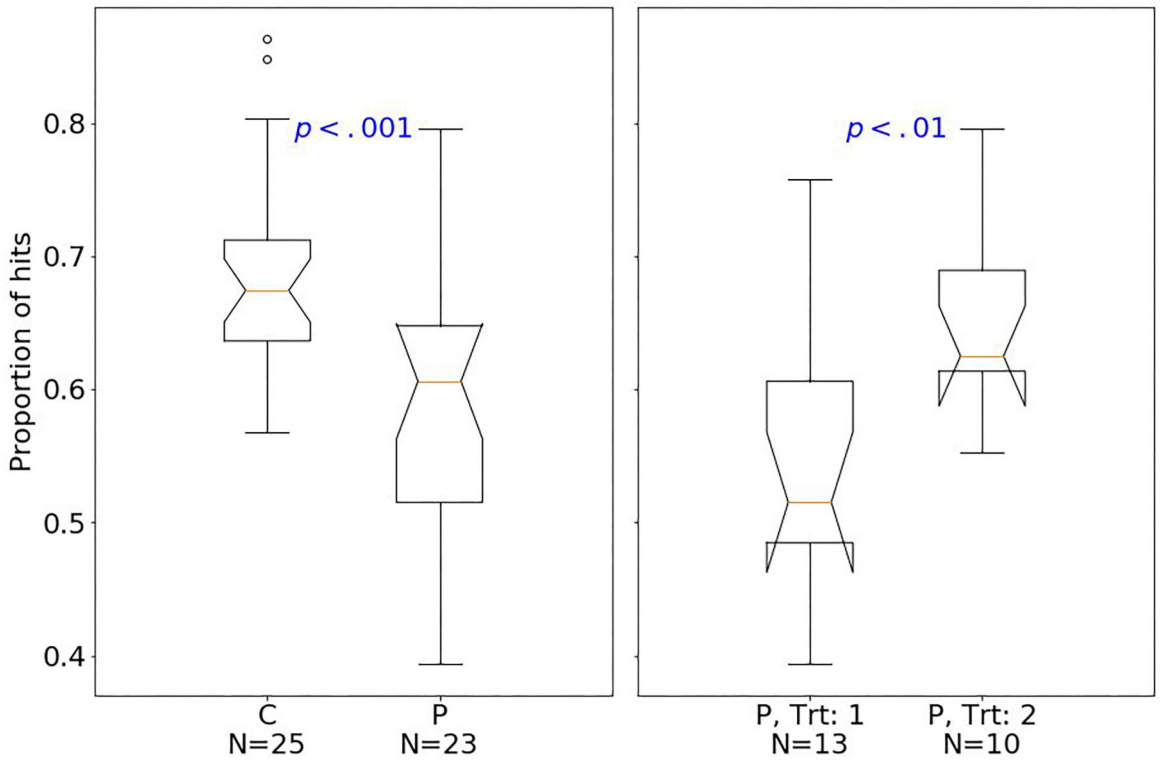

FIGURE 5 | Box plots of the PRL task. The horizontal line represents the median; the boxes extend to the first and third quartile, while whiskers extend to the max/min or the corresponding quartile + 1.5 IQR. C, healthy controls; P, patients with MUD; Trt_1, successful treatment outcome; Trt_2, unsuccessful treatment outcome. Box plots of proportion of hits.

TABLE 4 | The 95% confidence intervals from multiple imputation using mice package in R.

	2.5%	97.5%
Time	−7.79	26.37
Group	5.21	19.86
Time:group	−17.71	3.35

the treatment of MUD may be more successful once behavioral control through reward and punishment contingencies has normalized. Our study suggests that this could be the case after 3 months.

Predictors

Next, we explored whether certain patterns in PRL performance at T1 were associated with clinical outcome parameters. For this purpose, the group of patients was divided into "successful" (n = 13; 56.5%) and "unsuccessful" (n = 10) depending on the clinical outcome. This rate of successful courses is in the upper range of previously investigated, comparable therapies with success rates between 30 and 70% (2). This could be due to small sizes but could also reflect that targeting MA-specific behavior is an effective approach in intervention strategies. Various particularities of MA dependence compared to other substances are taken into account, including the young age of the persons concerned, the relation to the party scene, and the high importance of other addictive substances, and this approach should be extended in future studies with sufficient sample sizes. A comparison between both groups did not reveal any significant differences in demographic or clinical parameters, but the response pattern differed significantly and revealed unexpected results. Participants with unsuccessful treatment outcomes showed significantly better performance in the "proportion of hits" at T1 compared to participants with successful treatment outcomes and were on par with the performance of healthy controls. This pattern was also significant when comparing proportion of shifts after winning and shifts after losing. These effects were relatively large and constant in different analyses. To our knowledge, such a result has not yet been described in the literature.

It could be speculated that baseline impairments in decision making is not a predictor in treatment success, but rather, the cognitive capacity for improvement is important to sustain abstinence. The research, however, on baseline cognitive performance as a predictor for treatment outcome is mixed. In a study of alcohol use disorder, the baseline performance on a probability discounting task did not predict treatment outcome (48), whereas baseline executive function predicted treatment

retention in other studies (49–51). Another study showed that a subset of cognitive tasks can predict treatment success but not an overall composite score of cognitive performance (52). Notably, the relationship between cognition and abstinence was shown to be mediated by the improvements in coping skills learned in cognitive behavioral therapy sessions (53). As the successful treatment group in this study performed worse at baseline but showed the greatest improvement over time, the results suggest that perhaps, cognitive enhancement in PRL translates to other domains of behavioral control. This is in line with the goal of behavioral therapy to enhance learning skills that strengthen cognitive control to maintain abstinence (54, 55). The ability to flexibly adapt and learn new contingencies may be an important component of abstinence, as animal models of extinction show reductions in cue-elicited or drug-seeking responses in animals that have learned new associations with drug administration (56). Together the data suggest that the capacity for inhibitory control and learning new contingencies may reduce relapse. With a growing number of pharmaceutical agents for addiction designed to enhance cognitive performance (54), therapy that improves cognitive flexibility, in combination with medications may facilitate the requisite behavioral change needed to maintain abstinence. Another possibility is that individual variability of abstinence on brain function may drive differences in cognitive performance. Future studies examining these possibilities will greatly enhance treatment approaches for MUD and other use disorders.

Future studies examining these possibilities will greatly enhance treatment approaches for MUD and other use disorders.

Limitations

First, the sample size was small, and the sample had various comorbid conditions including multidrug abuse. Therefore, the study had limited power for between and within-subject analyses making it difficult to determine whether the decision-making impairments and progresses are related to comorbidities. In addition to other SUDs, depression, for example, can also change PRL (57). Second, no control condition for the MA-specific intervention was included. Therefore, we cannot differentiate specific therapeutic effects from unspecific abstinence effects.

On the other hand, our study has several strengths, exemplified by the longitudinal control group and a naturalistic sample of MA patients with comorbid psychiatric disorders and drug abuse histories.

CONCLUSION

Our study demonstrates behavioral correlates of maladaptive decision-making processes and an imbalanced learning from negative and positive feedback in patients with MUD. Because these perturbations may recover after 3 months of MUD-specific therapy, our findings warrant the development of further learning-based treatments.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the study was approved by the local ethics committee of the Technische Universität Dresden and carried out in accordance with the Declaration of Helsinki. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MP and MS designed the study. SP and MS developed the PRL task. AJ, CS, and MP contributed to study management, data collection and processing. AJ, SP, JP, MK, and CS analyzed the data. SP, MP, JP, AJ, MK, and MS wrote the manuscript. MK, MP, JP, and SP processed the revision process. MK thoroughly revised the language of the manuscript. All authors listed have made a substantial, direct and intellectual contribution to the work, approved it for publication, and reviewed the final manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Does an Over-Connected Visual Cortex Undermine Efforts to Stay Sober After Treatment for Alcohol Use Disorder?

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A fine-tuned interplay of highly synchronized activity within and between the brain's communities is a crucial feature of the brain's functional organization. We wanted to investigate in individuals with alcohol use disorder (AUD) the degree to which the interplay of the brain's community-architecture and the extended brain reward system (eBRS) is affected by drinking status (relapse or abstinence). We used Graph Theory Analysis of resting-state fMRI data from treatment seekers at 1 month of abstinence to model the brain's intrinsic community configuration and their follow-up data as abstainers or relapsers 3 months later to quantify the degree of global across-community interaction between the eBRS and the intrinsic communities at both timepoints. After 1 month of abstinence, the ventromedial PFC in particular showed a significantly higher global across-community interaction in the 22 future relapsers when compared to 30 light/non-drinking controls. These differences were no longer present 3 months later when the relapsers had resumed drinking. We found no significant differences between abstainers and controls at either timepoint. *Post hoc* tests revealed that one eBRS region, the ventromedial PFC, showed a significant global across-community interaction with a community comprising the visual cortex in relapsers at baseline. In contrast, abstainers showed a significant negative association of the ventromedial PFC with the visual cortex. The increased across-community interaction of the ventromedial PFC and the visual cortex in relapsers at timepoint 1 may be an early indicator for treatment failure in a subgroup of AUD patients.

Keywords: segregation, integration, modules, provincial hub, intrinsic, alcohol relapse marker, default mode network, participation coefficient

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INTRODUCTION

The brain is a complex functional network that is hierarchically organized in multiple levels of smaller subnetworks or communities nested within each other. A crucial feature of the brain's community organization - and an important requirement for efficient processing - is that the regions belonging to the same community are densely inter-connected, whereas the connections between the communities are sparse (1, 2). As a result, just a few distinct brain regions are engaged in information integration across the communities, which are otherwise functionally segregated (1–3).

This fine-tuned interplay between integration and segregation is a hallmark of the brain's functional organization when engaged in goal-driven behavior or cognitive tasks (4–6) and at rest (3, 7, 8). Integration and segregation are two opposite positions on a continuum of the brain's modus operandi. Any shift toward an extreme of the continuum is disturbing this fine-tuned balance leading to either over-integration, or over-segregation. Both over-integration and over-segregation have been shown to have significant effects on cognition and behavior in healthy populations (3, 9–12), but this concept also helps to understand psychiatric and neurological conditions like traumatic brain injury (13), schizophrenia (14), Alzheimer's disease (15, 16), and attention deficit/hyperactivity disorder (17).

Neurotransmitters like glutamate and ρ-aminobutyric acid (GABA) are intimately involved in orchestrating the excitatory/inhibitory interplay that shapes this within-community and between-community architecture of the brain (10, 12, 18–25). Chronic alcohol exposure and abstinence during treatment for alcohol use disorder (AUD) have been shown to result in neuroadaptations in GABAergic and glutamatergic synaptic transmission, especially in the brain reward system (26–31). These AUD related neuroadaptations have also consequences for the future course of the alcohol dependence (32–34).

Since research has shown an altered inhibitory and excitatory neurotransmitter tone in AUD individuals during the different stages of a typical alcohol use cycle (active use, withdrawal, remission), we reason that we might gain new insights into the effects of AUD on the brain by investigating the intrinsic community architecture in treatment seekers and how this community architecture relates to the extended brain reward system [eBRS, (35, 36)] and treatment outcome. To that aim, we modeled the brain's community organization and the degree of interaction between the communities in patients at 1 month of abstinence and 4 months into treatment when they were either abstinent or had resumed heavy drinking. We had two main hypotheses: 1. the interaction of the eBRS regions within its own community is differentially altered in relapsers (REL) and abstainers (ABS) when compared with healthy controls (CON), and 2. the interaction of the eBRS regions with the other communities differs between relapsers and abstainers when compared with controls.

METHODS

Participants

Included in this analysis were the resting-state functional MRI (rs-fMRI) data of 34 AUD individuals (mean age 41.7 of years; SD 9.9) obtained within 4 weeks of abstinence from alcohol while in outpatient treatment [timepoint 1 (TP1)]. They had been recruited from the San Francisco Veterans Administration Medical Center Substance Abuse Day Hospital and the Kaiser Permanente Chemical Dependence Recovery outpatient treatment clinics. Twenty-four of these AUD individuals (mean age 41.1 of years, SD 9.0) returned to undergo the same research protocol three months later (TP2). Demographics and clinical characteristics are given in **Table 1a** (baseline), and **Table 1b** (follow-up). Drinking status (abstinent or relapsed) of the AUD individuals at timepoint 2 determined their group membership for the purpose of this analysis. Twenty-two abstinent AUD individuals studied at timepoint 1 were future relapsers (REL), 14 of whom returned for the 3 month-follow-up; the other 12 AUD individuals were abstinent at both timepoints (ABS), and 10 of them returned for follow-up. Thirty light/non-drinkers were recruited as control participants from the local community (CON), 21 of whom came back for a follow-up. Some demographic data given in **Table 1a** was not available for all control participants since controls were recruited for three different but contemporaneous research studies that acquired slightly different demographic information.

All study participants were administered the screening section of the Structural Clinical Interview for DSM-5 Axis I disorders. All AUD individuals had moderate or severe AUD and no other moderate or severe substance use disorder. Exclusion criteria for all participants included a history of neurologic disorder, e.g., epilepsy, traumatic brain injury with loss of consciences $>$ 30 min, cerebrovascular disease, a history of general medical disease such as untreated hypertension, diabetes, hypo/hyperthyroidism, and of psychiatric diseases such as major depression, anxiety, trauma. In addition, all study participants had to have at least 5 min of clean rs-fMRI data left after cleaning the data from motion and physiological noise.

All participants were also assessed by a battery of interviews and standardized questionnaires that included the Beck Depression Inventory [BDI; (37)], Barratt Impulsiveness Scale [BIS; (38)], as well as standardized questionnaires assessing lifetime substance use (alcohol and other substances including tobacco). AUD individuals had a history of consuming at least 80 standard alcoholic drinks per months ($>$150 for men) for $>$ 6 years ($>$ 8 years in men) before treatment. Controls had consumed fewer than 60 standard alcoholic drinks in any month over lifetime (1 standard alcoholic drink contains 13.6 g of ethanol). The committees of human research at the University of California San Francisco and the VA Medical Center had approved the study and informed consent was obtained from each participant prior to any research procedures in accordance with the Declaration of Helsinki.

MRI Data

The MRI data were collected at the VA Medical Center San Francisco on a 3.0 T MRI scanner (Siemens Magnetom Skyra Syngo MR D13) using a 20 channel receive head coil. The study protocol included different types of structural imaging, as well as rs-fMRI. For this study were used: (a) A T1 weighted MPRAGE sequence with repetition time (TR) = 2,300 ms, echo time (TE) = 2.98 ms, flip angle 9^0, field of view (FOV) 192 × 256 × 256 mm^3, isotropic voxel size 1 × 1 × 1 mm^3, 256 slices per volume, acquisition duration = 5.28 min. (b) A T2 weighted TSE sequence with TR = 3,210 ms, TE = 11 ms, flip angle = 150^0, FOV = 230 × 230 × 54 mm^3, anisotropic voxel size 0.9 × 0.9 × 3 mm^3, 54 slices per volume, acquisition duration = 3.21 min. (c) A whole brain task-free echo planar imaging blood oxygen level dependent echo-planar 2D PACE sequence with TR = 2,020 ms, TE = 27 ms, 37 transverse slices (FOV = 1,320 ×

TABLE 1a | Demographic description of the three groups: Controls (CON), Abstainers (ABS), Relapsers (REL).

	TP1			TP2		
	CON	**ABS**	**REL**	**CON**	**ABS**	**REL**
N	**30**	**12**	**22**	**21**	**10**	**14**
Age [years]	44.2 (11.1)	37.1 (7.8)	43.9 (10.4)	42.6 (10.9)	38.1 (8.5)	43.2 (9.2)
Gender F|M [n|	12|18	8|4	8|14	7|14	6|4	5|9
Education [years]	15.9 (2.4)	14.7 (1.9)	14.8 (2.0)	16.1 (2.5)	14.7 (1.6)	15.6 (1.6)
1-year average drinks/month	6.1 (8.3)	367.0 (206.1)**	355.2 (243.8)**	7.5 (9.9)	329.6 (192.9)*	384.8 (297.4)**
Lifetime average drinks/month	8.7 (7.3)	183.9 (93.6)**	190.4 (98.6)**	7.9 (6.8)	160.9 (80.7)**	197.6 (111.8)*
Smokers no | current | former [n]	15|3|4	7|2|3	10|9|3	8|3|2	6|2|2	7|4|3
FTND total score	2.1 (2.0)	1.6 (2.3)	2.7 (2.1)	2.0 (1.8)	1.3 (1.5)	2.3 (2.2)
BIS-II-total score	55.1 (9.9)	65.8 (8.8)*	66.6 (11.7)**	54.4 (10.9)	62.3 (11.6)	63.1 (9.3)*
BDI_total score	3.1 (3.9)	12.0 (8.9)**	15.0 (7.5)**	2.6 (2.8)	7 (6.8)	9.9 (5.8)**
STAI_state score	23.9 (5.4)	37.5 (11.0)*	38.2 (12.6)**	25.3 (8.8)	35.7 (12.0)*	33.5 (9.6)
STAI_trait score	30.9 (8.9)	47.0 (11.7)**	47.5 (11.5)**	30.1 (6.8)	41.9 (10.6)*	38.8 (10.5)*

Mean value with SD in brackets; one asterisk = $p < 0.05$, two asterisks = $p < 0.001$ indicate the statistical level on which the two AUD groups differed from the controls as the reference group. The controls were pooled from three contemporaneously performed research studies, so not all demographic variables were consistently available. The variables other than age, gender and education were obtained from 22 controls at timepoint 1 and 9 controls at timepoint 2. Abbreviations: TP, timepoint; FTND, Fagerstrom Test for Nicotine Dependence; BIS-II, Barratt Impulsiveness Scale; BDI, Beck Depression Inventory; STAI State/STAI Trait, State-Trait Anxiety Inventory State/Trait.

TABLE 1b | Comparison of participants with and without follow-up.

	AUD at TP1		ABS at TP1		REL at TP1	
	With follow-up	**Dropouts**	**With follow-up**	**Dropouts**	**With follow up**	**Dropouts**
N	**24**	**10**	**10**	**2**	**14**	**8**
Age [years]	40.8 (8.9)	43.9 (12.1)	37.9 (8.4)	36.3 (5.5)	42.9 (9.1)	45.9 (12.7)
Gender F|M [n]	11|13	5|5	6|4	2|0	5|9	3|5
Education [years]	15.3 (1.8)	13.6 (1.9)*	15.1 (1.9)	13.0 (1.4)	15.5 (1.8)	13.8 (2.1)*
1-year average drinks/month	361.8 (255.6)	353.6 (154.1)	329.6 (192.9)	554.2 (220.3)	384.8 (297.5)	303.4 (96.1)
Lifetime average drinks/months	182.3 (99.79)	202.1 (87.4)	160.9 (80.7)	298.9 (77.5)*	197.6 (111.8)	177.9 (75.1)
Smokers no | current | former [n]	13|6|5	4|5|1	6|1|3	1|1|0	7|5|2	3|4|1
FTND total score	1.8 (2.0)	3.3 (2.1)	5	0.7 (1.5)*	2.4 (2.1)	3.0 (2.1)
BIS-II-total score	66.4 (10.6)	66.3 (11.4)	67.2 (9.2)	59.0 (1.4)	65.8 (11.9)	68.1 (12.1)
BDI_total score	14.4 (6.8)	12.9 (8.6)*	13.4 (9.1)	5.0 (2.8)	15.1 (8.4)	14.9 (6.0)
STAI_state score	37.4 (10.6)	39.4 (15.2)	38.8 (11.43)	31.0 (7.1)	36.4 (10.2)	41.5 (16.3)
STAI_trait score	46.9 (11.7)	48.2 (11.1)*	47.6 (12.7)	44.0 (4.2)	46.4 (11.4)	49.2 (12.2)*

Mean value with standard deviation in brackets; an asterisk indicates significant differences between dropouts vs. participants with follow up at $p < 0.05$. Abbreviations: TP, timepoint; FTND, Fagerstrom Test for Nicotine Dependence; BIS-II, Barratt Impulsiveness Scale; BDI, Beck Depression Inventory; STAI State/STAI Trait, State-Trait Anxiety Inventory State/Trait.

1,320 mm^2; Matrix size 88 × 88, descending acquisition without a gap, anisotropic voxel size 2.5 × 2.5 × 3.5 mm^3), 240 volumes and 8 min acquisition duration.

MRI Pre-processing

General pre-processing: All pre-processing steps of the MRI data were performed using SPM12 (https://www.fil.ion.ucl.ac.uk/spm/) running on MATLAB 2018b. First, the first ten volumes of the echo planar imaging (EPI) data were discarded reducing T1 saturation effects, leaving 230 volumes for analysis. Then the EPI data were slice time-corrected for descending acquisition and a first alignment of the EPI data was performed by using a two-pass procedure where the EPI images were first aligned to the first image of the series and then again aligned to the mean EPI image of the first run. Only the motion-parameter file and the mean image of that first alignment step were used for the further pre-processing steps. Next, the T2 weighted image was co-registered to the T1 weighted image and afterwards the mean EPI image co-registered to the T2 weighted image, whereby it was also indirectly co-registering the EPI image to the T1 weighted image. A second alignment step was then performed by which the original 230 EPI volumes were co-registered to the mean EPI image (now co-registered to the T1 weighted image) from the first alignment. Next, the T1 weighted image was segmented into gray, white and CSF tissue maps using the "New Segmentation" algorithm of SPM12. The DARTEL procedure (39) was run to create a study population specific template that was used for the subsequent normalization step in which the structural and

functional images were normalized to the MNI-space. During this step the functional and structural images were resampled to a $2 \times 2 \times 2\ mm^3$ isotropic voxel size and only minimally smoothed using an isotropic Gaussian kernel (FWHM 1 mm) in order to minimize possible spurious correlations for the subsequent graph theoretical analyses (40).

Denoising of the rs-fMRI Data and Computing Correlation Matrices

Conn (Version18b, https://www.nitrc.org/projects/conn/) running on MATLAB (MATLAB 2018b) was used to denoise the rs-fMRI data and to compute participant-specific Pearson correlation matrices. The Artifact Detection Toolbox (ART) as implemented in the pre-processing pipeline of Conn was used to identify motion corrupted outliers in the fMRI time-series (global-signal z-value threshold 5; subject-motion mm threshold 0.9). The anatCompCor method (41) was used to detect further low-frequency physiological confounding signals such as heart rate or respiration that can modulate the BOLD signal and influence the connectivity strength. The volumes identified as outliers were then censored by dummy-coding them as nuisance regressors and regressed out together with the confounding signals calculated by the CompCor method. Subsequently, the data were band-pass filtered (0.008–0.09 Hz), detrended (linear, quadratic and cubic), and despiked. We used *t*-tests and the *composite motion measure* implemented in ART and the number of motion corrupted outlier volumes as identified by the denoising procedure to assess that the three groups did not significantly differ in motion artifacts and confounding signals, see **Table 2**.

Graph Theoretical Analyses (GTA)

To specify nodes for the GTA, we used the Atlas of Intrinsic Connectivity of Homotopic Areas [AICHA, (42)]. This parcellation fulfills two mandatory requirements for GTA because each of its 384 ROIs is characterized by functional homogeneity of the constituting voxels and the parcellation covers the entire cortex and all subcortical structures (42). Since we wanted to investigate the interplay between functional integration and segregation in the brain of participants with AUD, we used both positive and negative BOLD-signal correlations to define the edges between the nodes. Integration corresponds to synchronized brain activity of a group of brain regions measured/operationalized as positive correlations of the BOLD signal between these regions. Segregation as the counterpart of integration corresponds best to anticorrelated BOLD signal activity between regions.

Defining the Extended Brain Reward System (eBRS) Using AICHA ROIs

Several well-established intrinsic connectivity networks, e.g., the executive-control network or the attention networks, have intrinsic activity patterns that are very similar to those that can be observed with task-based fMRI when the person is actively engaged in one of these activities (43). The eBRS, however, does not have such an intrinsic activity equivalent and in order to study it using rs-fMRI, it must be modeled. Using 62 ROIs of the AICHA parcellation, we built the following composites representing the nine regions of the eBRS (35, 36): left and right nucleus accumbens, anterior thalamus, amygdala, hippocampus and parahippocampal gyrus, anterior insula, lateral orbital prefrontal cortex, dorsolateral prefrontal cortex, ventromedial prefrontal cortex, and temporal pole). To compute region-specific GTA values for our analyses, we averaged the GTA values of the constituent AICHA ROIs of each of the nine eBRS composites (**Figure 1**).

Modeling the eBRS' Over-Integration or Disconnection on Community-Level With GTA

First Step: Defining the Group-Specific Community Configuration at Timepoint 1

To address our first hypothesis, we determined the underlying community configuration of for each of three groups at timepoint 1. For that purpose, we used a data-driven approach and ran 10,000 iterations of the community_Louvain algorithm ($\gamma = 1.6$ [Brain Connectivity toolbox (BCT), (44)] on each participant's individual connectivity matrix from timepoint 1 while simultaneously computing a participant-specific agreement matrix, which coded how often two nodes were allocated to the same community over the 10,000 iterations. Next, we computed three group-specific average agreement matrices which were then used as input to compute a group-specific consensus partition. A consensus partition allows to find the community structure that represents the community configuration characteristics that were most commonly shared by all participants and across all iterations. To that end, the Louvain algorithm was re-run with 10,000 iterations [Brain Connectivity toolbox (BCT), (44)] on the average agreement matrix of each group until the re-clustered agreement matrices converged into a single cluster solution for that group at timepoint 1.

Second Step: Determining the Degree of Global Across-Community Integration of Each of the 384 Nodes

All GTA measurements described in the two next sections were computed for each of the individual 384 nodes of the AICHA parcellation at first and then averaged [1] across the nodes that formed one of the nine regions of the eBRS as defined above and [2] across the nodes that were members of the same community.

The group-specific community configuration at timepoint 1 was used to inform the computation of the participant coefficient (PC) for each participant individually at timepoint 1 (with the participant's connectivity matrix from timepoint 1) and at timepoint 2 (with the participant's connectivity matrix from timepoint 2) according to the participant's group membership.

The PC [Brain Connectivity toolbox (BCT), (44)] measures to which degree a node is involved in the global across-community information integration. A node with a PC (= "positive" PC) value near 1 has a high number of connections with nodes from other communities and qualifies as a connector hub that facilitates the inter-community integration (2). When using signed weighted matrices, i.e., connectivity matrices with positive and negative correlations, it is possible to compute a

TABLE 2 | Results of the Student *t*-Tests for Group-Specific Differences in Motion Artifacts and other Physiological Confounds.

	TP1			TP2		
	Mean	**SD**	***p*-value**	**Mean**	**SD**	***p*-value**
Maximum voxel displacement in mm						
CON vs. ABS	0.304/0.254	0.190/0.098	0.38	0.327/0.272	0.181/0.183	0.41
CON vs. REL	0.304/0.346	0.190/0.158	0.37	0.327/0.296	0.181/0.143	0.61
ABS vs. REL	0.254/0.346	0.098/0.158	0.13	0.272/0.296	0.183/0.143	0.73
Number of motion corrupted outlier volumes						
CON vs. ABS	17.6/7.4	19.7/8.4	0.11	16.6/9.7	21.0/21.0	0.34
CON vs. REL	17.6/17.3	19.7/20.9	0.95	16.6/9.5	21.0/12.0	0.27
ABS vs. REL	7.4/7.3	8.4/20.9	0.14	9.7/9.2	21.0/12.0	0.98

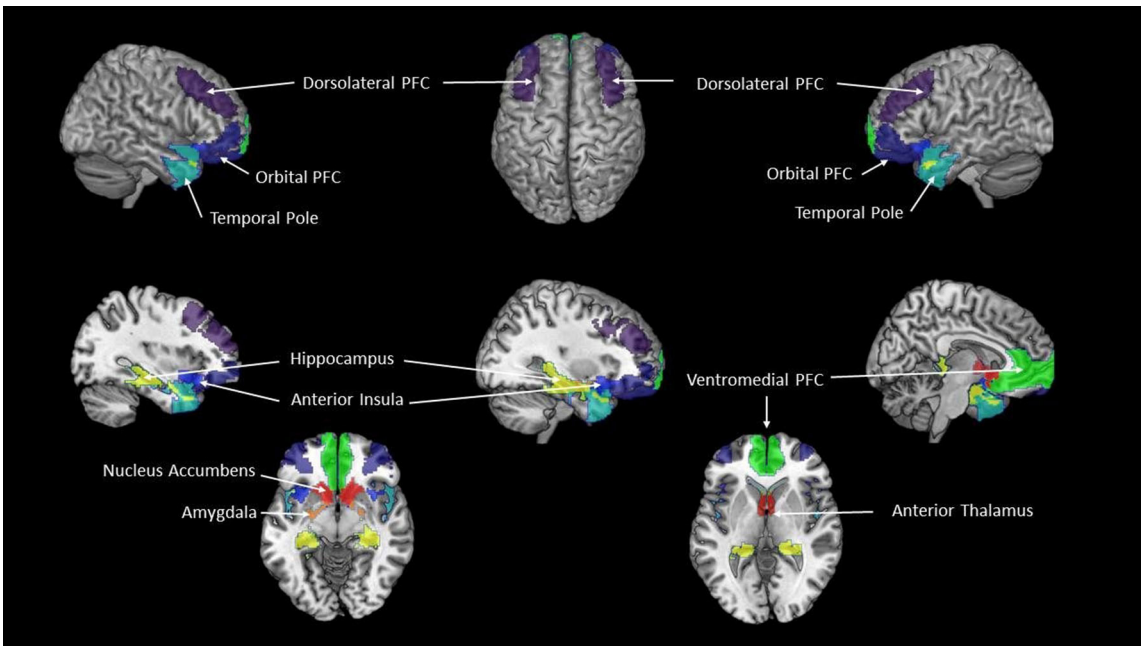

FIGURE 1 | The extended Brain Reward System (eBRS). The nine brain regions of the extended brain reward system–plum = dorsolateral PFC, dark blue = orbital PFC; cyan = temporal pole; pistachio-green = hippocampus; navy-blue = anterior insula; yellow = amygdala; orange-red = nucleus accumbens, dark red = anterior thalamus; lime-green = ventromedial PFC.

second PC value (= "negative" PC) for the negatively correlated edges of every individual node as well (45). A node with a "negative" PC value near 1 is highly segregated or even disconnected from the global inter-community information integration. The information of the "positive" PC value and the "negative" PC value can be combined to describe a node's overall importance for the communication between communities. In that context a connector hub is defined by a high "positive" PC and a "negative" PC near zero, whereas a disconnected or separated node is characterized by a high "negative" PC combined with a very low "positive" PC value (45).

To be able to quantify a node's engagement in the between-module information integration, we subtracted the "negative" PC value of each node from its "positive" PC value. A positive result of that subtraction indicates that the inter-community integrative role of that node is relatively higher than its role in separating the communities from each other, while a negative result indicates that the node is mainly segregated from the global inter-community integration. For the remainder of the paper, we call this new measurement we derived from the subtraction of the two PC values "global integration coefficient" or GIC.

In order to differentiate beneficial integration or segregation from maladaptive "over-integration" or "over-segregation," a reference value was defined, above or below which a node was considered "over-integrated" or "disconnected," respectively. We used our control participants as reference group. Specifically, we defined that a node showed over-integration whenever the

result of the subtraction of the "negative" PC value from the "positive" PC value (i.e., the GIC value) was significantly higher than the reference value of the same node in the controls. Vice versa, a node with a GIC value significantly lower than the same node's reference value in the controls qualified as "disconnected."

Third Step: Determining the Centrality of the eBRS Regions for Within-Community-Crosstalk

Another feature of an efficient network organization is that not all nodes belonging to the same community are equally important for the information integration within that community. Some nodes are more densely connected within their own community than the other nodes in the community to the effect that these nodes are central for the communication within the community. Such nodes are called "provincial hubs." However, as it is the case with connector hubs controlling the information integration across communities, too many nodes qualifying as provincial hubs within the same community make the network organization inefficient and are also an indicator of (community-intern) over-integration.

We used the "within-module-degree-z-score" algorithm [Brain Connectivity toolbox (BCT), (45)] to determine the eBRS regions' centrality for the information integration within their respective communities. A positive within-community centrality value characterizes a node highly connected with the other nodes of its community, whereas a negative within-community centrality value indicates a node that is mostly segregated from the within-community information integration (2, 44).

Statistics

As neither of the two PC values nor the values of the resultant GIC met the assumption for parametric *t*-tests, we used non-parametric Wilcoxon Rank Sum tests to test for significant differences between the three groups (significance level $p = 0.05$). Likewise, we used Spearman's rho to compute GIC associations between the regions of the eBRS and the communities within each group at each timepoint with the aim to determine the communities with whom the regions of the eBRS showed over-integration or segregation. To compute group differences in within-community centrality, we used Student *t*-tests (significance level $p = 0.05$). As we proposed specific hypotheses regarding the eBRS regions crosstalk within their own community and across the communities (i.e., we had *a priori* assumptions that did not mandate correction for multiple comparison), we will report significant results before as well as after correction for multiple comparisons. By reporting the uncorrected *p*-values, we follow Krauth's (46) recommendation for interpreting the *p*-value and let the readers decide for themselves whether the results are significant at the significance level they may find acceptable. To correct for multiple comparisons, we used the Benjamini-Hochberg formula with the significance level $q = 0.05$ (47). Effect sizes r for the Wilcoxon Rank Sum tests were computed using the formula of Rosenthal et al. (48, 49). Hedge's g was used to compute the effect size for *t*-tests because it outperforms Cohen's d with relatively small and unbalanced group sizes as was the case with our data.

RESULTS

Demographics

Table 1a shows the demographic characteristics of the three study groups at each timepoint. As expected, the two AUD groups had significantly higher scores at both timepoints than the controls for monthly drinks averaged over 1 year before treatment and over lifetime. The AUD individuals at timepoint 1 scored also significantly higher on self-reported impulsivity than the controls, whereas at timepoint 2 only the relapsers differed from the controls. Both drinking groups had significantly higher BDI scores (depressive symptomatology) than controls at timepoint 1, but only relapsers had higher BDI scores also at timepoint 2. State and trait anxiety scores were significantly higher than in controls for abstainers at timepoint 1 and timepoint 2; both scores were also significantly higher for relapsers at timepoint 1, but only the trait anxiety score remained higher than those of controls at timepoint 2.

Table 1b shows to what extent the AUD participants with three-months follow up differed from those who dropped out after timepoint 1.

Community Configurations by Group at Timepoint 1

We found an intrinsic community structure consisting of three communities in all three groups. The community structure was very similar for controls and relapsers (**Figure 2,** rows 1 and 2). Community 1 (blue color in **Figure 2**) covered bilateral superior, middle, inferior frontal and orbital regions, as well as the medial part of the prefrontal cortex, the temporal pole and the middle and inferior gyri of the temporal cortex, parts of the supramarginal and angular gyri and the cingulate cortex in the midline of the brain. Community 2 (green color in **Figure 2**) covered the caudal part of the frontal cortex over the motor and sensory cortex into the parietal lobe on the lateral part of the cortex also encompassing superior gyrus of the temporal cortex, parts of the insula and the rolandic operculum. Community 3 (red color in **Figure 2**) consisted of the occipital cortex only. The abstainers' community configuration differed from the two other groups in so far as the superior temporal gyrus, hand and face area of the motor cortex were also allocated to community 3 together with the visual cortex (**Figure 2**, row 3). Important in the context here is that the community allocation of the nine eBRS regions at timepoint 1 was identical for the three groups. The eBRS regions were all allocated to community 1 in all three groups except for relatively small parts of the dorsolateral prefrontal cortex located posterior to the middle frontal gyrus (**Figure 2**, last row). Therefore, group specific differences in the degree of the eBRS' global across-communities integration were not related to the slightly different community structure observed in controls/relapsers vs. abstainers at timepoint 1. The AUD related differences in whole-brain community configuration are the subject of separate paper.

Analysis of the eBRS' Global Integration Coefficients (GIC)

The exact GIC mean, median and interquartile values for the three groups at both timepoints are shown in **Table 3,** the results

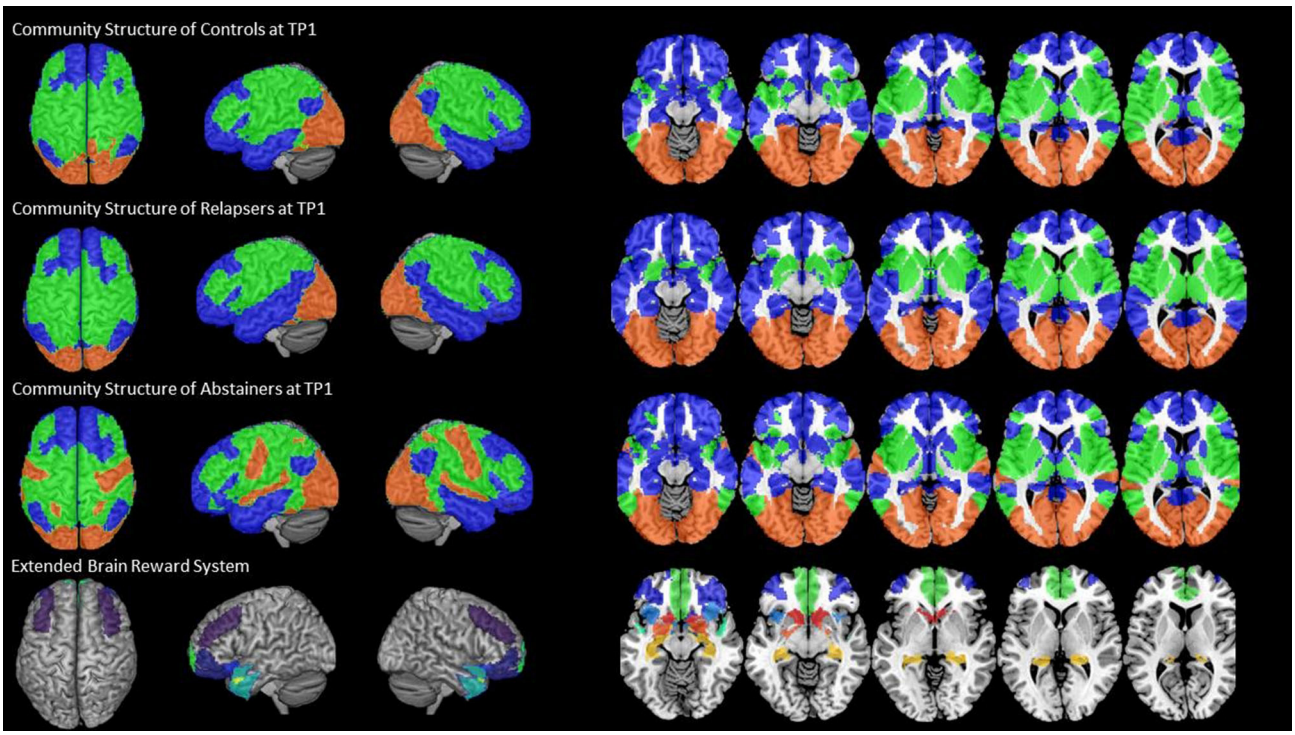

FIGURE 2 | The three-community configuration and the location of the eBRS in relation to the communities. The three-community configuration of the controls (CON), the relapsers (REL), and abstainers (ABS) found at baseline are shown in the first, respectively, second and third row, the location of the eBRS within the brain is and in relation to the three communities is shown in the bottom row. The majority of the eBRS regions were allocated within community 1 (blue color).

of the Wilcoxon Rank Sum tests are shown in the left half of **Table 4,** and **Figure 3** maps the GIC values on the eBRS regions for a graphical illustration of the findings. The controls' eBRS was clearly segregated from the inter-community information integration since they had negative values for all individual eBRS regions at both timepoints. Abstainers also showed negative GIC values for all of the eBRS regions, except for the dorsolateral PFC and the temporal pole at timepoint 2, indicating a general segregation of the eBRS from the inter-community-crosstalk at both timepoints. In contrast, relapsers presented with positive GIC values for six of the nine eBRS regions, the ventromedial PFC, orbital PFC, anterior insula, temporal pole, hippocampus, and nucleus accumbens at timepoint 1, which indicates a two-thirds majority of the eBRS regions was involved in the inter-community-crosstalk. However, at timepoint 2 only half these regions, the temporal pole, hippocampus and nucleus accumbens, still presented with positive GIC values in the relapsers. **Table 4** shows that several of these eBRS regions showed even significant over-integration in the relapsers at both timepoints when compared with the controls, but only the *p*-value for the ventromedial PFC at timepoint 1 survived the Benjamini-Hochberg correction at timepoint 1.

To investigate which one of the two other communities contributed to the ventromedial PFC's over-integration in the relapsers, we computed a Spearman correlation between the average GIC values of the communities with the GIC values of the ventromedial PFC. **Table 5** shows that the GIC values of the ventromedial PCF - as expected - were significantly positively correlated with its own community 1 in all three groups at both timepoints. However, the ventromedial PFC was negatively associated with the other two communities in the controls and abstainers, although these negative correlations reached statistical significance only in the abstainers for both communities at timepoint 1. In contrast to the two other groups, the GIC values of the relapsers' ventromedial PFC at timepoint 1 showed significant positive association with community 3 but not with community 2. This suggests an over-integration of the ventromedial PFC with the (occipital) community 3 in the relapsers at timepoint 1. At timepoint 2, the controls and the relapsers showed the expected association patterns – a significant positive correlation of the ventromedial PFC's GIC values with its own community– and essentially no associations with communities 2 and 3.

Within-Community Centrality

We found that the ventromedial PFC had the highest within-community centrality values in all three groups at both timepoints and together with the anterior insula it qualified as a provincial hub in all three groups (see **Table 6** for the exact mean and standard deviation values, right half of **Table 4** for the results of the *t*-tests, and **Figure 4** maps the within-community centrality values on the eBRS region for a graphical illustration of the findings). Three other features seem noteworthy: firstly, and as expected, the within-community configuration of the

TABLE 3 | The global integration coefficient of the extended brain reward system (eBRS) and its constituent brain regions for the controls (CON), relapsers (REL), and abstainers (ABS).

	Global Integration Coefficient		
	CON	**REL**	**ABS**
eBRS at TP1	−0.0364/−0.0441 (0.0432)	0.0034/−0.0030 (0.0609)	−0.0551/−0.0728 (0.0959)
eBRS at TP2	−0.0412/−0.0392 (0.0792)	−0.0137/−0.0108 (0.0552)	−0.0610/−0.0714 (0.1032)
Cortical regions of the eBRS at TP1	−0.0348/−0.0489 (0.0415)	0.0283/0.0038 (0.0685)	−0.0391/−0.0409 (0.0735)
Cortical regions of the eBRS at TP2	−0.0578/−0.0677 (0.0711)	−0.0314/−0.0246 (0.0667)	−0.0461/−0.0432 (0.0528)
Dorsolateral prefrontal cortex at TP1	−0.0084/−0.0076 (0.0812)	−0.0367/−0.0392 (0.0775)	−0.0116/−0.0211 (0.1472)
Dorsolateral prefrontal cortex at TP2	−0.0252/−0.0409 (0.1429)	−0.0534/−0.0527 (0.1316)	0.0065/0.0238 (0.1254)
Ventromedial prefrontal cortex at TP1	−0.0968/−0.0992 (0.1256)	0.0219/0.0328 (0.0953)	−0.1040/−0.1101 (0.1661)
Ventromedial prefrontal cortex at TP2	−0.1219/−0.1210 (0.1358)	−0.0316/−0.0073 (0.1290)	−0.1352/−0.1019 (0.2291)
Orbital prefrontal cortex at TP1	−0.0453/−0.0364 (0.0554)	0.0059/−0.0213 (0.0979)	−0.0196/−0.0218 (0.1430)
Orbital prefrontal cortex at TP2	−0.0549/−0.0331 (0.1225)	−0.057/−0.0600 (0.0533)	−0.0304/−0.0663 (0.1224)
Anterior insula at TP1	−0.0414/−0.0471 (0.0798)	0.0165/0.0055 (0.1132)	−0.0446/−0.0276 (0.14301)
Anterior insula at TP2	−0.0717/−0.0637 (0.0641)	−0.0164/−0.0082 (0.1162)	−0.0763/−0.0607 (0.1161)
Temporal pole at TP1	−0.0271/−0.0330 (0.0787)	0.0064/−0.0180 (0.1467)	−0.01577/−0.0036 (0.1464)
Temporal pole at TP2	−0.0154/−0.0019 (0.0607)	0.0019/−0.0067 (0.0797)	0.0046/−0.0018 (0.1522)
Subcortical regions of the eBRS at TP1	−0.0272/−0.0237 (0.0590)	0.0042/0.0132 (0.0903)	−0.0751/−0.0717 (0.1272)
Subcortical regions of the eBRS at TP2	−0.0203/−0.0229 (0.0982)	0.0083/−0.0062 (0.1073)	−0.0796/−0.0935 (0.1688)
Hippocampus at TP1	−0.0240/−0.0232 (0.0755)	0.0304/0.0229 (0.0942)	−0.0507/−0.0393 (0.1266)
Hippocampus at TP2	−0.0131/−0.0006 (0.0977)	0.0242/0.0264 (0.0835)	−0.0401/−0.0601 (0.1342)
Amygdala at TP1	−0.0229/−0.0357 (0.1342)	−0.0159/−0.0392 (0.1562)	−0.0484/−0.0898 (0.1907)
Amygdala at TP2	−0.0051/−0.0017 (0.1051)	−0.0191/−0.275 (0.1206)	−0.0455/−0.0789 (0.0709)
Anterior thalamus at TP1	−0.0405/−0.0523 (0.1279)	−0.0401/−0.0549 (0.1663)	−0.1185/−0.1125 (0.1807)
Anterior thalamus at TP2	−0.0476/−0.0650 (0.1563)	−0.0113/−0.0276 (0.1791)	−0.1242/−0.1407 (0.2403)
Nucleus accumbens at TP1	−0.0215/−0.0356 (0.1435)	0.0431/0.0797 (0.1507)	−0.0825/−0.1071 (0.2492)
Nucleus accumbens at TP2	−0.0257/−0.0213 (0.1671)	0.0395/0.0265 (0.1468)	−0.1085/−0.1358 (0.2829)

The table lists the mean, the median and the interquartil distance (in brackets) of the group-specific GIC values at each timepoint. The eBRS and its constituent brain regions were relatively over-integrated in the REL at TP1, since the REL group has generally the highest GIC values of all three groups at TP1 with exception of the GIC values of the dorsolateral prefrontal cortex.

controls (with ventromedial PFC, anterior insula and temporal pole all qualifying as provincial hubs) was stable across the two timepoints. Secondly, besides the anterior insula and ventromedial PFC, none of the eBRS regions in the relapsers qualified as a provincial hub at timepoint 1. Additionally, with exception of two frontal eBRS regions (the dorsolateral PFC and the orbital PFC, which both do not belong to the core BRS but are considered part of the extended BRS), relapsers also had always the lowest within-community centrality values of all three groups, with the differences for the ventromedial PFC and nucleus accumbens at timepoint 1 statistically significant when compared with the controls and surviving the subsequent Benjamini-Hochberg correction. Three months later, after the relapsers had resumed alcohol consumption, they still had globally lower within-centrality values than the controls with the anterior thalamus, the nucleus accumbens, and additionally the dorsolateral PFC being significantly lower, but only the *p*-value of nucleus accumbens also surviving the correction for multiple comparisons. Thirdly, abstainers at both timepoints had always the highest within-centrality values of the three groups with exception of the dorsolateral PFC and the orbital PFC, for which they had the lowest within-centrality values at both times. As a result, abstainers had more eBRS regions qualifying as provincial hubs than controls and relapsers at both timepoints. At timepoint 1, the ventromedial PFC, anterior insula, temporal pole, and the anterior thalamus qualified as provincial hubs. Three months later, the number of provincial hubs in the abstainers' eBRS was even higher with the ventromedial PFC, anterior insula, temporal pole, nucleus accumbens, amygdala, and hippocampus qualifying as provincial hubs at timepoint 2. The subsequent *t*-tests for differences in within-community-centrality between controls and abstainers at timepoint 2 showed significant group differences for the hippocampus and nucleus accumbens that survived the correction for multiple comparisons.

DISCUSSION

The aim of our rs-fMRI analyses was to investigate the degree to which relapse or abstinence in AUD treatment

TABLE 4 | List of significant group differences in Global Integration Coefficient (GIC) and Within Centrality of the eBRS at 1 month (TP1) into treatment and at follow-up 3 months later (TP2).

	Global Integration Coefficient			Within Centrality		
	CON vs. REL	**CON vs. ABS**	**REL vs. ABS**	**CON vs. REL**	**CON vs. ABS**	**REL vs. ABS**
eBRS at TP1	$p = 0.0013$ ($r = 0.446$)*		$p = 0.0136$ (r = 0.423)	$p < 0.0001$ ($g = 1.276$)*	–	$p = 0.0002$ ($g = 1.449$)*
eBRS at TP2	–	–	–	$p = 0.0039$ ($g = 0.381$)*	–	$p < 0.0001$ ($g = 1.872$)*
Cortical regions of the eBRS at TP1	$p = 0.0022$ ($r = 0.425$)*	–	–	$p = 0.0175$ ($g = 0.691$)	–	–
Cortical regions of the eBRS at TP2	–	–	–	–	–	–
Dorsolateral prefrontal cortex at TP1	–	–	–	–	–	–
Dorsolateral prefrontal cortex at TP2	–	–	–	$p = 0.0246$ ($g = 0.763$)	–	$p = 0.0027$ ($g = 1.302$)*
Ventromedial prefrontal cortex at TP1	$p < 0.0001$ ($r = 0.571$)*	–	$p = 0.0026$ ($r = 0.532$)*	$p < 0.0001$ ($g = 1.345$)*	–	$p = 0.0003$ ($g = 1.188$)*
Ventromedial prefrontal cortex at TP2	$p = 0.0040$ ($r = 0.487$)	–	$p = 0.0242$ ($r = 0.460$)	–	–	–
Orbital prefrontal cortex at TP1	$p = 0.0485$ ($r = 0.274$)	–	–	–	–	$p = 0.0409$ ($g = 0.776$)
Orbital prefrontal cortex at TP2	–	–	–	–	–	–
Anterior insula at TP1	$p = 0.0201$ ($r = 0.322$)	–	–	–	–	–
Anterior insula at TP2	$p = 0.0451$ ($r = 0.339$)	–	–	–	–	–
Temporal pole at TP1	–	–	–	–	–	–
Temporal pole at TP2	–	–	–	–	–	$p = 0.0269$ ($g = 1.079$)
Subcortical regions of the eBRS at TP1	–	–	$p = 0.009$ ($r = 0.448$)	$p < 0.0001$ ($g = 1.197$)*	–	$p < 0.0001$ ($g = 1.578$)*
Subcortical regions of the eBRS at TP2	–	–	$p = 0.0207$ ($r = 0.472$)	$p = 0.0014$ ($g = 1.141$)*	$p = 0.0083$ ($g = 1.086$)*	$p < 0.0001$ (g =2.307)*
Hippocampus at TP1	$p = 0.0103$ ($r = 0.356$)	–	$p = 0.0136$ ($r = 0.423$)	–	–	–
Hippocampus at TP2	–	–	–	–	$p = 0.0054$ ($g = 1.075$)*	$p = 0.0027$ ($g = 1.321$)*
Amygdala at TP1	–	–	–	–	–	–
Amygdala at TP2	–	–	–	–	–	$p = 0.0278$ ($g = 0.936$)
Anterior thalamus at TP1	–	–	–	–	–	–
Anterior thalamus at TP2	–	–	–	$p = 0.0215$ ($g = 0.778$)	–	$p = 0.0494$ ($g = 0.753$)
Nucleus accumbens at TP1	$p = 0.0109$ ($r = 0.353$)	–	$p = 0.0065$ ($r = 0.467$)*	$p < 0.0001$ ($g = 1.521$)*	–	$p < 0.0001$ ($g = 1.755$)*
Nucleus accumbens at TP2	–	–	$p = 0.0242$ ($r = 0.458$)	$p < 0.0001$ ($g = 1.578$)*	$p = 0.004$ ($g = 1.191$)*	$p < 0.0001$ ($g = 2.747$)*

The results of the Wilcoxon rank sum tests used to test for group differences in the not-normally distributed GIC values and the corresponding effects sizes r (in brackets) computed following the formula of Rosenthal et al. (48) are listed on the left side of the table. The results of the Student t-tests used to test for group differences in the normally distributed Within Centrality and the corresponding effect sizes (Hodge's g) are listed on the right side of the table. Asteriks indicate p values that stay significant at q = 0.05 after Benjamini-Hochberg correction for multiple comparisons.

seekers affects the brain's intrinsic community structure. In particular, we hypothesized that 1. the interaction of the eBRS within its own community is differently altered in relapsers and abstainers when compared with control participants, and 2. the interaction of the eBRS regions with the other communities (between-community crosstalk) differs between relapsers and abstainers when compared with controls. In the subsequent sections we discuss to what extent our findings supported our two hypotheses and lead up to our main conclusions.

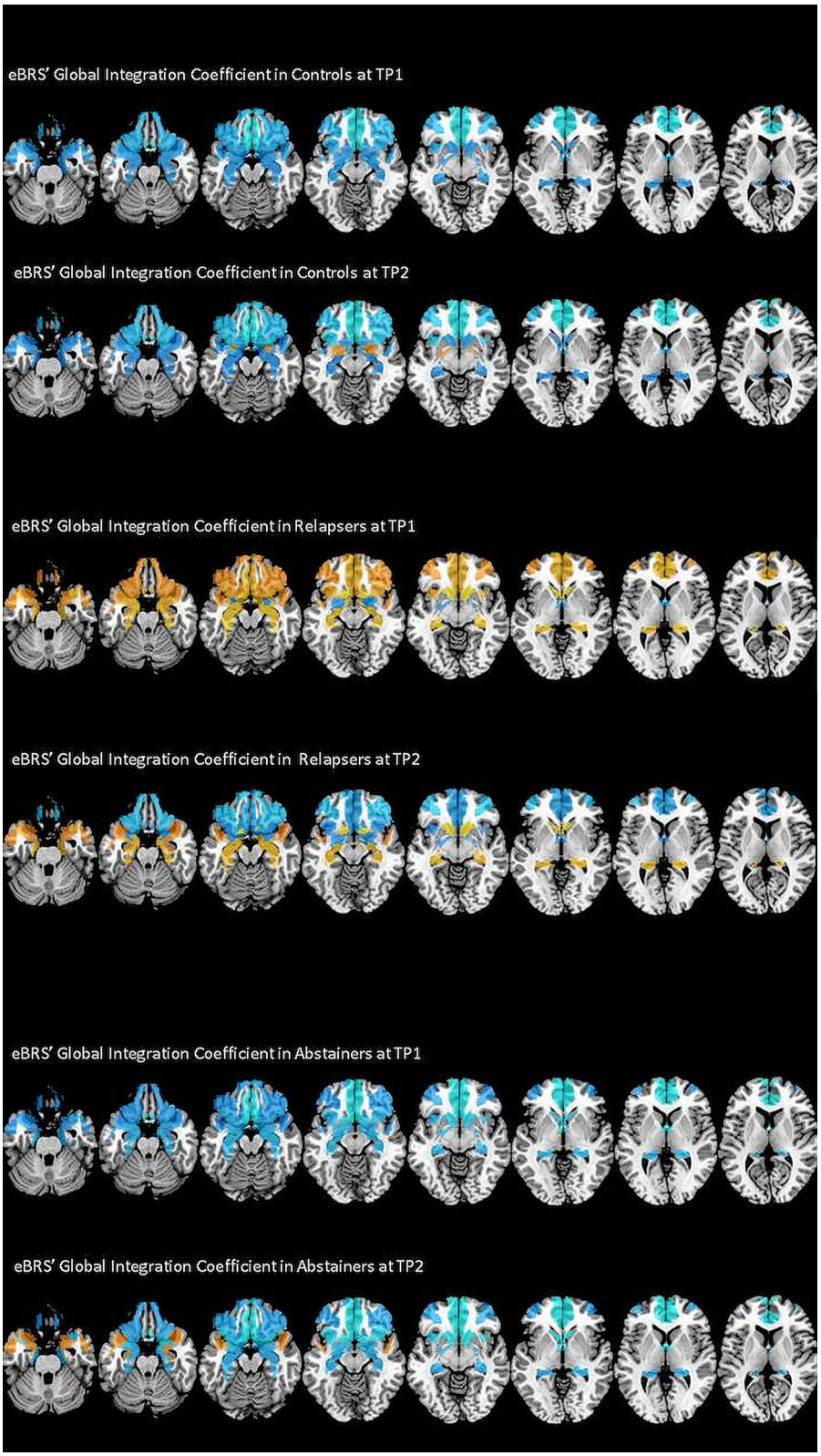

FIGURE 3 | The degree of global integration of the nine eBRS regions at 1 month into abstinence ad 3 months later. eBRS regions with a positive GIC value are coded in orange – yellow (range 0–0.05) and eBRS regions with a negative GIC value are coded in blue – light blue (range 0–0.1). When compared with the controls and abstainers, relapsers showed over-integration (= positive GIC values) in almost all eBRS regions, while the eBRS in the two other groups was mostly disconnected (= negative GIC values) from the global across-community interaction.

TABLE 5 | Results of the *post-hoc* tests – Over-Integration of the ventromedial PFC in relation to the three community-structure.

		Ventromedial Prefrontal Cortex		
		Community 1	**Community 2**	**Community 3**
TP 1	CON	$\rho = 0.687$; $p < 0.0001$**	$\rho = -0.231$; $p = 0.220$	$\rho = -0.226$; $p = 0.229$
	REL	$\rho = 0.636$; $p = 0.0015$*	$\rho = -0.135$ $p = 0.563$	$\rho = 0.476$ $p = 0.0251$*
	ABS	$\rho = 0.608$; $p = 0.036$*	$\rho = -0.664$; $p = 0.018$*	$\rho = -0.622$; $p = 0.031$*
TP 2	CON	$\rho = 0.735$; $p = 0.0001$**	$\rho = -0.129$; $p = 0.579$	$\rho = -0.036$; $p = 0.876$
	REL	$\rho = 0.737$; $p = 0.0027$*	$\rho = -0.055$; $p = 0.852$	$\rho = 0.007$; $p = 0.982$
	ABS	$\rho = -0.988$; $p = < 0.0001$**	$\rho = -0.515$; $p = 0.128$	$\rho = -0.551$ $p = 0.098$

Significant Spearman's ρ correlations are highlighted with one asterisk for the significance level < 0.05 and with two asterisks for the significance level < 0.001. The ventromedial PFC in the CON was negatively but not significantly correlated with communities 2 and 3 at both timepoints (TP). REL showed significant positive correlations for ventromedial PFC and community 3 at timepoint 1, indicating over-integration, but not anymore at timepoint 2. The ventromedial PFC in the ABS was negatively and significantly correlated with communities 2 at timepoint 1 but not anymore at timepoint 1.

TABLE 6 | The within centrality of the extended brain reward system (eBRS) and its constituent brain regions in the controls (CON), relapsers (REL), and abstainers (ABS) at 1 month (TP1) into treatment and at follow-up 3 months later (TP2).

	Within Centrality		
	CON	**REL**	**ABS**
eBRS at TP1	0.0049 (0.2003)	−0.2436 (0.1778)	0.0359 (0.2017)
eBRS at TP2	−0.1523 (0.2194)	−0.2391 (0.2397)	0.1471 (0.1448)
Cortical regions of the eBRS at TP1	0.1733 (0.1843)	0.0290 (0.2384)	0.1341 (0.2176)
Cortical regions of the eBRS at TP2	0.1473 (0.1743)	0.1022 (0.2610)	0.1171 (0.1293)
Dorsolateral prefrontal cortex at TP1	−0.1114 (0.4122)	−0.0794 (0.5655)	−0.3486 (0.5492)
Dorsolateral prefrontal cortex at TP2	−0.1067 (0.4851)	0.2491 (0.3454)	−0.3347 (0.4669)
Ventromedial prefrontal cortex at TP1	0.8596 (0.2856)	0.3953 (0.4138)	0.8757 (0.3856)
Ventromedial prefrontal cortex at TP2	0.7934 (0.3303)	0.5350 (0.4612)	0.8445 (0.5252)
Orbital prefrontal cortex at TP1	−0.1600 (0.4266)	−0.1255 (0.3833)	−0.4341 (0.4233)
Orbital prefrontal cortex at TP2	−0.3087 (0.3559)	−0.3080 (0.5440)	−0.5214 (0.3037)
Anterior insula at TP1	0.2710 (0.4351)	0.0259 (0.5111)	0.3529 (0.5579)
Anterior insula at TP2	0.2173 (0.5129)	0.0805 (0.5947)	0.2776 (0.3284)
Temporal pole at TP1	0.0072 (0.4142)	−0.0704 (0.4172)	0.2247 (0.4153)
Temporal pole at TP2	0.1414 (0.4298)	−0.0455 (0.3643)	0.3197 (0.2966)
Subcortical regions of the eBRS at TP1	−0.2054 (0.3359)	−0.5843 (0.2875)	−0.0868 (0.3452)
Subcortical regions of the eBRS at TP2	−0.2185 (0.3901)	−0.6659 (0.3955)	0.1845 (0.3250)
Hippocampus at TP1	−0.2466 (0.3918)	−0.4128 (0.5059)	−0.1500 (0.5297)
Hippocampus at TP2	−0.2821 (0.3681)	−0.3535 (0.3253)	0.1320 (0.4208)
Amygdala at TP1	−0.1762 (0.5847)	−0.4825 (0.6132)	−0.1466 (0.5626)
Amygdala at TP2	−0.2966 (0.6699)	−0.4582 (0.7446)	0.1793 (0.5773)
Anterior thalamus at TP1	−0.3836 (0.6392)	−0.6222 (0.7669)	0.2164 (0.5956)
Anterior thalamus at TP2	−0.0951 (0.6114)	−0.6823 (0.9323)	−0.0853 (0.5315)
Nucleus accumbens at TP1	−0.0151 (0.5071)	−0.8199 (0.6019)	−0.2672 (0.6515)
Nucleus accumbens at TP2	−0.2003 (0.6036)	−1.1694 (0.6302)	0.5119 (0.5847)

The table lists the mean and the standard deviation (in brackets) of the normally distributed group-specific within centrality values at each timepoint. With exception of the dorsolateral prefrontal cortex and the orbital prefrontal cortex, abstainers had generally the highest Within Centrality values of all three groups, whereas relapsers had the smallest Within Centrality values.

Within-Community Analysis

Our fist hypothesis that the interaction of the eBRS within its own community is differently altered in relapsers and abstainers when compared with control participants was confirmed. Analyzing the role of the eBRS regions and the whole eBRS within their own community revealed significant differences between abstainers,

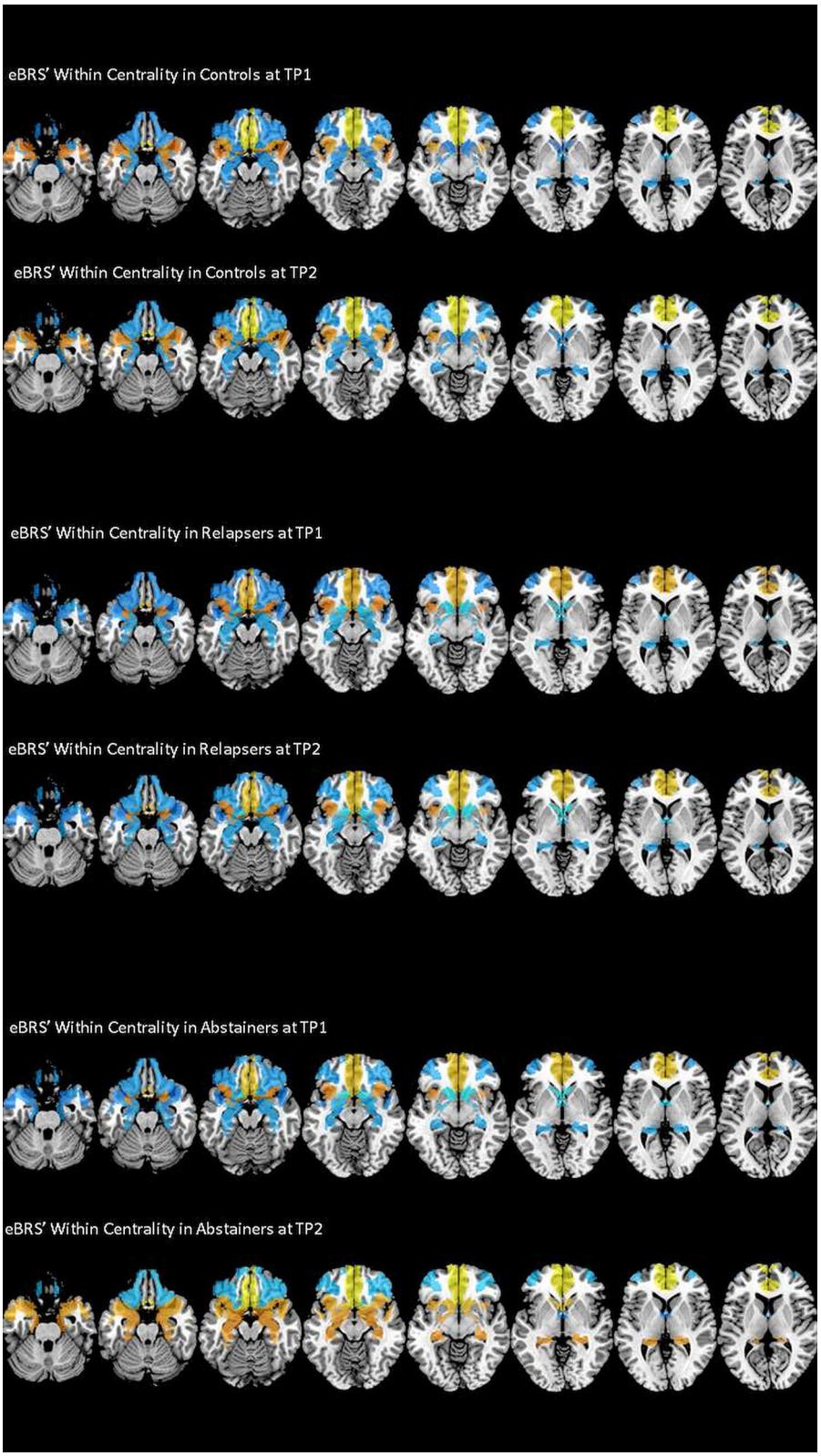

FIGURE 4 | Within Centrality of the nine eBRS regions at 1 month into abstinence ad 3 months later. eBRS regions with a positive Within Centrality value are coded in orange – yellow (range 0–0.8) and eBRS regions with a negative Within Centrality value are coded in blue – light blue (range 0–1). Relapsers had generally lower Within Centrality values than the two other groups at both timepoints, abstainers showed significantly higher Within Centrality values in subcortical regions, especially in the hippocampus and nucleus accumbens, than controls and relapsers at the 3 months follow-up.

relapsers, and controls at timepoint 1, which were even more accentuated at timepoint 2. The controls had a middle position between the two AUD groups with the AUD groups showing an opposite pattern since abstainers had generally the highest within-centrality values when relapsers had generally the lowest within-centrality values or vice versa. Only the ventromedial PFC and anterior insula qualified as provincial hubs in all three groups at both timepoints, suggesting that these two eBRS regions were central for the within-community crosstalk regardless of group membership or time. While the within-community configuration of the controls was stable across the two timepoints, the abstainers underwent a prominent within-community re-configuration. As a result, they had two times more eBRS regions qualifying as provincial hubs than the controls at timepoint 2. Additionally, while the eBRS regions' within-community configuration between controls and abstainers was not significantly different at timepoint 1, 3 months later, hippocampus and nucleus accumbens had qualified as new additional provincial hubs in the abstainers but both regions had also significant higher within-centrality surviving the Benjamini-Hochberg correction. In contrast to the abstainers, relapsers stayed relatively stable with the exception of the dorsolateral PFC showing an increase in within-centrality to the effect that this region newly qualified as a provincial hub at timepoint 2. Since this eBRS region had consistently negative within-centrality values in controls and abstainers (and qualified in the abstainers even as a hub for between-community crosstalk at timepoint 2) and the dorsolateral PFC's prominent role in executive control (50), this change in the relapsers might be rather an indicator for a maladaptive than beneficial process.

Across-Community Analysis

Our second hypothesis pertained to the eBRS regions' global crosstalk with other communities, specifically that we would find significant differences between the three groups. The eBRS regions of controls and abstainers showed the expected negative GIC values, reflecting relative segregation from the inter-community-communication at both timepoints. In contrast, relapsers had generally higher GIC values than the other two groups and even positive GIC values in six of the nine eBRS regions (with the ventromedial PFC surviving multiple comparison corrections) at timepoint 1, which shows that these eBRS regions were engaged in the global information integration across communities early in treatment. The subsequent *post hoc* correlation analyses showed a positive association of the ventromedial PFC in relapsers with community 3, which was practically identical with the occipital cortex. This positive association is all the more remarkable as the ventromedial PFC is a core region of the default mode network (DMN), the most salient feature of which is its anticorrelated activity with task-positive networks like the visual network. Controls and abstainers precisely showed that negative association, equivalent with segregation of the ventromedial PFC and the visual cortex in these two groups during rest.

The meaning of the brain's intrinsic brain activity and how it generates these patterns of dynamic syn- and desynchronization is still not fully understood. However, it has been shown that this intrinsic activity is also present during active task-performance (43), and that it shapes the functional network architecture during tasks (51). Consequently, the finding that the ventromedial PFC of the relapsers seemed to be involved in global information integration with the visual cortex already during rest may suggest that the relapsers have something like an intrinsic processing advantage whenever it is necessary that the ventromedial PFC and - by virtue of the ventromedial PFC's role as the most prominent provincial hub within the eBRS – the other eBRS regions have to actively interact with regions of the visual cortex.

However, there is evidence that this intrinsic over-connectivity of the ventromedial PFC with the visual cortex found in the relapsers is not at all a processing advantage but may rather make them more vulnerable to relapse. It has repeatedly been shown that AUD individuals have a higher degree of brain activation in the medial PFC, the orbitofrontal cortex, anterior cingulate cortex, insula, and striatum in response to alcohol cues (52, 53), and that this increased activation predicts relapse in detoxified AUD individuals (54). Also, the primary and secondary visual cortices respond with significant higher activation when confronted with alcohol vs. neutral cues (53). Therefore, stronger interactions between visual cortex and ventromedial PFC, together with structural alterations in the prefrontal cortex generally reported in AUD individuals such as our study participants that may interfere with top-down control (55–59), and put an individual at greater risk of relapse.

That relapsers might have something like a maladaptive processing advantage–particularly for alcohol-related cues – was also demonstrated by EEG (60). The N170 is an event related potential reflecting a very early, bottom-up, and subconscious process in the visual cortex. It is commonly associated with visual face processing but has also been found for stimuli that related to other kinds of expertise knowledge (61, 62). Compared with abstainers, relapsers showed a significantly higher N170 amplitude in a Go-NoGo experimental setting when instructed to focus attention on the neutral cue and to ignore the alcohol-related cue (60). That increased N170 was specific for the condition in which the alcohol-related cue had to be ignored. When the cue was presented with a neutral beverage cue (i.e., a picture of a pitcher and a glass with orange juice instead of a picture with a beer bottle and a full glass of beer), the N170 amplitude did not differ between relapsers and abstainers (60). Based on these findings the authors concluded that a heightened N170 amplitude in response to a to-be-ignored alcohol cue is specifically related to a high relapse risk.

Another aspect of the medial PFC-visual cortex over-integration pattern found in treatment-seekers while they are still abstinent corroborates the clinical relevance. The ventromedial PFC is a core region of the DMN, and it was – together with the other eight eBRS regions – consistently allocated to community 1 in the relapsers. One of the most striking features of this community was how much it resembled the DMN in its anatomical topography. Our finding that the DMN interacts with the visual cortex in AUD individuals is supported by another

study, Fede et al. (63) used a machine learning prediction analysis and found that the connectivity between the DMN and the visual networks predicted reliably the AUD severity (measured by AUDIT scores) in adults with problematic drinking patterns. While our two AUD groups did not significantly differ in AUD severity, the stronger crosstalk between DMN and visual networks (i.e., their over-integration) at 1 month of abstinence while in treatment was associated with a negative treatment outcome within the subsequent 3 months.

Study Limitations

One limitation of the study is certainly the unbalanced group sizes and relatively small sample size of the abstainers at both timepoints. We had only 34 AUD individuals with acceptable data for timepoint 1, and only 24 of them had also acceptable data for the second timepoint. The reason for the relatively modest sample size is 2-fold: One, the rate of relapse within the first 6 months after treatment is substantial, so that we had many more relapsers than abstainers in our study, and two, motion artifacts can seriously compromise the data quality of all types of fMRI analyses, because even small head movements can falsely increase BOLD signal correlations. To assure the high quality of our analyses, we therefore employed rigorous censoring and denoising procedures and only used rs-fMRI data of participants with at least 5 min of data. Although all three groups had a similar proportion of data excluded for excessive motion or other noise, the abstainer sample was already small to start with.

We found characteristic differences in intrinsic network organization in future relapsers and successful abstainers early in treatment. However, we do not know whether these two groups did already differ in their intrinsic network organization when starting the treatment or whether the differences resulted from different neuroplastic alterations during the 1st month of sobriety. We also are not able to say whether these intrinsic network organization differences are not just the result of different rates for neuroplastic re-configuration over time, resulting in potentially similar configurations had the relapsers been able to stay sober for a longer time.

Conclusion

This study focussed on how the eBRS interacts with the regions within its own community and with the other intrinsic communities of the brain. It showed that the eBRS of the abstainers was not significantly different from that of the controls in the degree of across-community-crosstalk at either timepoint; however, the abstainers demonstrated a significant re-configuration of the eBRS regions' involvement in the crosstalk within their own community 1 month into treatment in so far as that the eBRS had a far more central role within its own community than it had in the other two groups. This re-configuration was even more pronounced 3 months later during abstinence. In contrast, the relapsers 1 month into treatment showed an over-integration of most of the eBRS regions when compared with the controls, especially a significant over-integration of the ventromedial PFC with the visual cortex while still abstinent. We suggest that this over-integration contributed to the relapsers' subsequent inability to remain abstinent and, as such, is an early indicator of subsequent treatment failure. Three months later after having relapsed to heavy alcohol consumption, the relapsers' community configuration was no longer over-integrated relative to that of the controls, and the relapsers (mal)functioned again in regard to substance abuse as they had before treatment.

DATA AVAILABILITY STATEMENT

The datasets for this article are not publicly available because they were created as part of Veteran's Administration approved research. Requests to access the datasets should be directed to the senior author (dieter.meyerhoff@ucsf.edu).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Human Research Protection Program IRB; (Formerly CHR) University of California San Francisco and SF VA Medical Center (SF VAMC). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DM and AM contributed conception, design of the study, contributed to manuscript revision, read, and approved the submitted version. AM performed the analyses and wrote the first draft of the manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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The Relationship Between Serum Cytokine Levels and the Degree of Psychosis and Cognitive Impairment in Patients With Methamphetamine-Associated Psychosis in Chinese Patients

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Background: Cytokine levels can be changed in methamphetamine (METH) use disorders (MUDs) and primary psychosis. The present study assessed serum levels of some kinds of interleukins (ILs) in METH-associated psychosis (MAP) and their relationships with psychotic symptoms and cognitive dysfunction.

Methods: Serum IL-2R, IL-6, IL-8, and IL-10 levels were examined by chemiluminescence assays in MAP patients ($n = 119$) and healthy controls ($n = 108$). The Positive and Negative Syndrome Scale (PANSS) and Montreal Cognitive Assessment (MOCA) were administered.

Results: Serum levels of IL-6 and IL-8 were significantly increased in MAP patients (all $p < 0.05$). There was a negative relationship between IL-2R levels and PANSS positive (P) subscale scores ($r = -0.193$, $p = 0.035$). IL-6, IL-8 and IL-10 levels were all negatively correlated with the naming, delayed recall and orientation subscores on the MOCA ($r = -0.209$, $p = 0.022$; $r = -0.245$, $p = 0.007$; $r = -0.505$, $p < 0.001$, respectively).

Conclusions: Our results indicate that immune disturbances are related to MAP and that IL-2R, IL-6, IL-8, and IL-10 are associated with the severity of psychotic symptoms and cognitive function impairment.

Keywords: cognitive, methamphetamine, psychosis, serum cytokine, addiction

INTRODUCTION

Methamphetamine (METH), a drug in the class of amphetamine-type stimulants (ATS), has been a global health issue for the past several decades, with an estimated 29 million people using ATS in 2017 (1). From 2010 to 2018, the number of registered ATS users has increased from 0.36 million to 1.35 in China (2). METH use places a heavy burden on families, society and the health care system.

Methamphetamine-associated psychosis (MAP) resulting from methamphetamine use, such as schizophrenia, depression, and other neuropsychiatric disorders (3–5). Moreover, patients with MUD have cognitive function deficits relative to healthy control subjects, including impairments of attention, executive function, and working memory (6). Most studies on MUDs have focused on neurotoxic effects, and the role of neuroinflammation in these toxic effects has been identified as important. METH exposure induces the release of a number of proinflammatory markers, including interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor-alpha (TNF-α), that cause neuroplasticity in the brain (7).

MUD also results in dysregulation in the peripheral immune system, leading to an imbalanced expression of cytokines. The expression of pro- and anti-inflammatory cytokines has been implicated in METH-related neuronal injury, which may be associated with MUD (8). Some studies have found that peripheral cytokines penetrate the blood-brain barrier directly via active transport mechanisms or indirectly via vagal nerve stimulation (9) and can relay messages responsible for inducing changes in motor function and motivation.

Many studies have examined the relationships between serum cytokine levels and the degree of psychosis in patients with primary mental disorders, such as schizophrenia and mood disorders (10, 11). However, studies examining MAP and cytokine levels are rare. The aim of our study was to examine the association of the intensity of psychiatric symptoms and the exacerbation of cognitive functions with imbalance in the immune system, which is reflected by serum cytokine concentrations, in MAP patients.

MATERIALS AND METHODS

Subjects

Patients were recruited from Wuhan Mental Health Center (WMHC), a Wuhan city-owned psychiatric hospital. All patients met the following inclusion criteria: age 20–64 years and Han Chinese; confirmed diagnosis of MAP based on the Diagnostic and Statistical Manual of Mental Disorders 5 (DSM-5) by the consensus of two psychiatrists. A diagnosis of MAP was made if the following symptoms were present: (1) prominent hallucinations or delusions, a score $\geq$4 on at least one Positive and Negative Syndrome Scale (PANSS) (12) psychosis item (delusions, conceptual disorganization, hallucinatory behavior, grandiosity, or suspiciousness/persecution); (2) symptoms that met the criterion developed during or soon after substance intoxication or withdrawal or after exposure to METH, and the psychotic symptoms lasted for more than 2 weeks; (3) psychotic symptoms that were not part of a psychotic disorder (such as schizophrenia, schizophreniform disorder, or schizoaffective disorder, and if the psychotic symptom onset was prior to substance or medication use or persist longer than 1 month after substance intoxication or withdrawal, then another psychotic disorder is likely); (4) psychotic symptoms that do not only occur during delirium; and (5) use of METH at least once per week during the 3 months prior to enrollment and a positive urine screen for METH. The patients had not received any antipsychotic drugs, immunomodulators or antioxidants for at least 12 months before entry into the study.

Healthy participants were recruited in Wuhan by advertisements. None of them had taken any immunomodulators, antipsychotic drugs, or antioxidants during the 1 year before the study. None of the healthy subjects had a personal or family history of psychiatric disorder.

Subjects with primary psychosis, polydrug abuse or major medical conditions were excluded.

The study was approved by the WMHC Ethics Committee, and written informed consent was obtained from the participating patients and healthy individuals before enrollment, which is when the data were retrospectively collected.

Clinical Measurements

The severity of psychotic symptoms was estimated with the PANSS. Cognitive functions were assessed using the Montreal Cognitive Assessment (MOCA) (13). Two psychiatrists who had simultaneously attended a training session before the use of the PANSS and MOCA administered these measures.

Investigation of IL-2R, IL-6, IL-8, and IL-10 Levels in Serum

Blood samples were drawn from 7 to 9 a.m. after an overnight fast. The serum was separated by centrifugation and then stored at −70°C. Serum IL-2R, IL-6, IL-8, and IL-10 concentrations were analyzed with chemiluminescence assays. The reference ranges of IL-2R, IL-6, IL-8, and IL-10 were 223–710 U/mL, 0–5.9 pg/mL, 0–62 pg/mL, and 0–9.1 pg/mL, respectively.

Wuhan CMLabs is an independent clinical laboratory that holds a laboratory accreditation certificate from the China National Accreditation Service for Conformity Assessment (CNAS Number: MT0324), the College of American Pathologists certification (CAP Number: 8997670, AU-ID: 1734258) and biosafety laboratory certification, Hubei, China (BLS-2[2020]01-01-001).

Statistical Analysis

Differences between patients and healthy controls were evaluated using independent *t*-tests for continuous variables and chi-square tests for demographic and clinical variables. Given that the IL data were not normally distributed in the two groups (Shapiro-Wilk test; IL-2R: $z = 0.127$, $p = 0.000$; IL-6: $z = 0.327$, $p = 0.000$; IL-8: $z = 0.268$, $p = 0.000$; IL-10: $z = 0.352$, $p = 0.000$ for patients; IL-2R: $z = 0.933$, $p = 0.000$; IL-6: $z = 0.569$, $p = 0.000$; IL-8: $z = 0.819$, $p = 0.000$; IL-10: $z = 0.877$, $p = 0.000$ for controls), a nonparametric test (Mann-Whitney test) was used for comparisons between the two groups.

We performed all correlation analyses using Pearson's correlation coefficient. We performed a multivariate regression analysis for each serum cytokine concentration, correcting for confounding variables, including age, education level and duration and frequency of METH use.

RESULTS

Demographic Data

The study included 119 MAP inpatients with psychotic disorders, and the mean age of the patients was 34.5 ± 8.7 years. The mean age of the 108 controls was 35.3 ± 9.5 years. Age, gender, marital status and education did not differ significantly between the two groups; further details are shown in **Table 1**. There were insignificant correlations between gender, age and marital status, and serum IL-2R, IL-6, IL-8, and IL-10 levels are shown either for the whole group or for the two groups separately. However, the frequency of METH use and the time of onset psychotic symptoms after METH use were significantly correlated with IL-8 ($r = 0.293$, $n = 119$, $p = 0.001$, and $r = 0.203$, $n = 119$, $p = 0.027$).

TABLE 1 | Demographics of patients and healthy control subjects.

	Cases ($n = 119$)	Healthy controls (108)	t or χ^2 or F	p-value
Gender			1.365	0.243
Male	102	98		
Female	17	10		
Marital status			0.154	0.985
Not married	56	52		
Married	49	42		
Divorced	12	12		
Widowed	2	2		
Age (years)	34.5 ± 8.7	35.7 ± 9.5	−0.956	0.34
Education (years)	11.0 ± 2.7	10.5 ± 2.8	1.155	0.25
Age of onset METH use (years)	25.7 ± 7.2			
Duration of METH use (years)	8.0 ± 4.1			
Frequency of METH use (times per week)	4.9 ± 5.1			
Time of onset psychotic symptoms after METH use (years)	3.9 ± 3.2			
PANSS total score	87.2 ± 18.7			
P subscore	25.5 ± 11.1			
N subscore	15.8 ± 9.5			
G subscore	46.7 ± 9.5			
MOCA total score	21.48 ± 2.49			
Visuospatial/executive subscore	3.43 ± 1.26			
Naming subscore	2.93 ± 0.28			
Attention subscore	1.29 ± 0.62			
Language subscore	1.18 ± 0.58			
Abstraction Subscore	1.38 ± 0.52			
Delayed recall subscore	2.20 ± 1.45			
Orientation subscore	5.92 ± 0.46			

METH, Methamphetamine; PANSS, Positive and Negative Syndrome Scale; P, the positive scale; N, the negative scale; G, the general scale; MOCA, the Montreal Cognitive Assessment.

Serum Measures

Table 2 shows the comparison of the mean serum cytokine levels in MAP patients with those in normal controls.

Serum IL-2R Levels

The serum IL-2R levels were higher in the MAP patients {median [quartile range (QR)]: 338.0 (168.0)} than in the normal controls [median (QR): 380.5 (161.3); ($z = -2.38$; $p = 0.017$)]. In the multivariate regression analysis, however, this difference was not significant after adjusting for covariance in gender, age, marital status and education ($p = 0.07$) (**Table 2**).

Serum IL-6 Levels

Serum IL-6 levels were higher in the MAP patients [median (QR): 3.8 (3.6)] than in the controls [median (QR): 3.3 (4.0); ($z = -2.69$; $p = 0.007$)]. In the multivariate regression analysis, this difference was still consistent after adjusting for covariation in age, gender, marital status and education ($p = 0.037$) (**Table 2**).

TABLE 2 | Multivariate regression analyses of the association between serum cytokine concentration and MAP, as measured in patients compared to healthy controls.

Variables		Standardized regression coefficients: Beta regression coefficient	t	p-value
IL-2R	(Intercept)		5.461	0.000
	Gender	−0.128	−1.940	0.054
	Age	−0.038	−0.554	0.580
	Marital status	−0.074	−1.084	0.279
	Education	0.035	0.531	0.596
	Diagnosis	0.121	1.821	0.070
IL-6	(Intercept)		1.185	0.237
	Gender	−0.044	−0.668	0.505
	Age	−0.028	−0.409	0.683
	Marital status	0.081	1.192	0.235
	Education	0.104	1.560	0.120
	Diagnosis	−0.139	−2.096	0.037*
IL-8	(Intercept)		0.776	0.439
	Gender	−0.030	−0.461	0.645
	Age	0.027	0.397	0.692
	Marital status	0.110	1.636	0.103
	Education	0.133	2.013	0.045*
	Diagnosis	−0.174	−2.649	0.009*
IL-10	(Intercept)		3.652	0.000
	Gender	−0.055	−0.830	0.408
	Age	0.011	0.159	0.873
	Marital status	0.112	1.635	0.103
	Education	−0.130	−1.942	0.053
	Diagnosis	−0.009	−0.142	0.887

METH, Methamphetamine.
**Statistically significant with p-value < 0.05.*

Serum IL-8 Levels

Serum IL-8 levels were insignificantly different between the MAP group [median (QR): 163 (413)] and the control group [median (QR): 154.5 (164.8); ($z = -0.852$; $p = 0.394$)]. In the multivariate regression analysis, this difference was significant after gender, age, marital status and education were used as covariates ($p = 0.009$) (**Table 2**).

Serum IL-10 Levels

Serum IL-10 levels were not significantly different between the MAP group [median (QR): 3.45 (1.85)] and the control group [median (QR): 3.47 (1.74); ($z = -0.211$; $p = 0.833$)]. In the multivariate regression analysis, this difference was still consistent after adjusting for covariation in gender, age, marital status and education ($p = 0.887$) (**Table 2**).

The Relationship Among Cytokine Levels in Patients and Controls

Correlation analysis showed a significant and positive relationship between serum IL-6 and IL-8 levels in the MAP patients ($r = 0.363$, $p < 0.001$) and healthy controls ($r = 0.476$, $p < 0.001$) (**Figure 1**).

The Relationship Between IL-2R, IL-6, IL-8, and IL-10 Levels and Clinical Symptoms

PANSS scores were found to have a statistically significant negative correlation with IL-2R levels (positive subscore: $r = -0.193$, $p = 0.035$). Correlations were found between IL-6, IL-8, and IL-10 levels and some clinical parameters, especially the severity of cognitive impairment. There was a significant negative relationship between serum IL-6 levels and MOCA scores (naming subscore: $r = -0.209$, $p = 0.022$). IL-8 levels were also found to have significant negative relationships with the delayed recall subscores on the MOCA ($r = -0.245$, $p = 0.007$), frequency of METH use ($r = 0.293$, $p = 0.001$), and time of psychotic symptom onset after METH use ($r = 0.203$, $p = 0.027$). There were significant and negative relationships between IL-10 and the orientation subscores on the MOCA ($r = -0.505$, $p < 0.001$). Further details are shown in **Table 3**.

Cytokine Levels and the Severity of Clinical Symptoms

To further confirm the correlations between IL levels and the severity of psychotic symptoms, patients were placed in one of two subgroups based on their mean scores on the PANSS positive (*P*) subscale (25.5 ± 11.1), the negative (*N*) subscale (15.8 ± 9.5) and the general psychopathology (G) subscale (46.8 ± 9.5). When the patients were classified on the basis of their average PANSS *P* subscale scores, there was a trend toward lower IL-2R levels in the patients with high *P* subscale scores ($n = 54$) than in those with low *P* subscale scores ($n = 65$) (393.9 ± 150.8 vs. 333.6 ± 132.8 U/ml, respectively; $z = -2.4$, $p = 0.017$). IL-2R levels were lower in the patients with high G subscale scores ($n = 47$) than in those with low G subscale scores ($n = 72$) (399.7 ± 148.5 vs. 335.7 ± 135.9 U/ml, respectively; $z = -2.4$, $p = 0.017$).

To corroborate the correlations between IL levels and cognitive dysfunction, patients were placed in one of two subgroups based on the mean MOCA visuospatial/executive subscores (3.4 ± 1.3), naming subscores (2.9 ± 0.3), attention subscores (1.3 ± 0.6), language subscores (1.2 ± 0.6), abstraction subscores (1.4 ± 0.5), delayed recall subscores (2.2 ± 1.5), and orientation subscores (5.9 ± 0.5). Higher IL-8 levels were observed only in the patients with low delayed recall subscores ($n = 70$) compared with those with high delayed recall subscores ($n = 49$) (462.4 ± 656.1 vs. 165.2 ± 185.7 pg/ml, respectively; $z = -3.4$, $p = 0.001$).

DISCUSSION

This is the first study to evaluate the relationships between the severity of psychotic symptoms, cognitive abilities and serum levels of cytokines in MAP patients. The results of this study indicated that (1) serum levels of IL-6 and IL-8 were increased in MAP patients; (2) IL-2R levels had a negative correlation with the intensity of positive psychotic symptoms, but IL-6 and IL-8 levels did not; (3) cognitive dysfunction was present in the MAP patients (MOCA total scores of 26 and higher are generally considered normal); and (4) the severity of different types of cognitive impairment was positively correlated with IL-6, IL-8, and IL-10 levels. These results agree with the aforementioned findings indicating altered immune response activity in MAP patients (14–18).

The serum IL-2R levels in MAP patients were higher than normal in our study. Few studies have reported the relationship between IL-2R and MAP, but several studies have found that *in vivo* and *in vitro* exposure to METH resulted in changes in IL-2 levels. IL-2 plays a critical role in the immune response as a T-cell growth factor. Most studies have reported that serum IL-2 levels were reduced following METH exposure (18–20), which might result from decreased spleen, thymus, and peripheral T-lymphocyte cellularities (21). In METH-treated mice, there were decreases in the ratios of spleen and thymus weight to body weight, which indicated that METH induced immunosuppression and decreased IL-2 production by splenocytes (18). Moreover, IL-2 levels were lower in splenocytes from mice treated with concanavalin A (Con A) and METH than in splenocytes from mice treated with only Con A, which might be because *in vitro* METH exposure suppresses the induction of specific cytotoxic T-lymphocytes and alters the function of lymphocytes and NK cells (18, 19). Further study showed that exposure to METH induced mitochondrial dysfunction in the form of marked decreases in mitochondrial membrane potential, increased mitochondrial mass, enhanced protein nitrosylation and diminished protein levels of complexes I, III, and IV of the electron transport chain. These changes paralleled the reduction in IL-2 secretion and T cell proliferative responses after METH treatment (22). However, the finding of lower IL-2R levels in our present study was in conflict with the results of other studies (23, 24). This inconsistency may be due to the clinical status of the patients; for example, the participants in the Loftis study were adults in early recovery from METH dependence; in contrast, our participants were MAP inpatients, and their duration and frequency of METH use were different (23). In addition, the

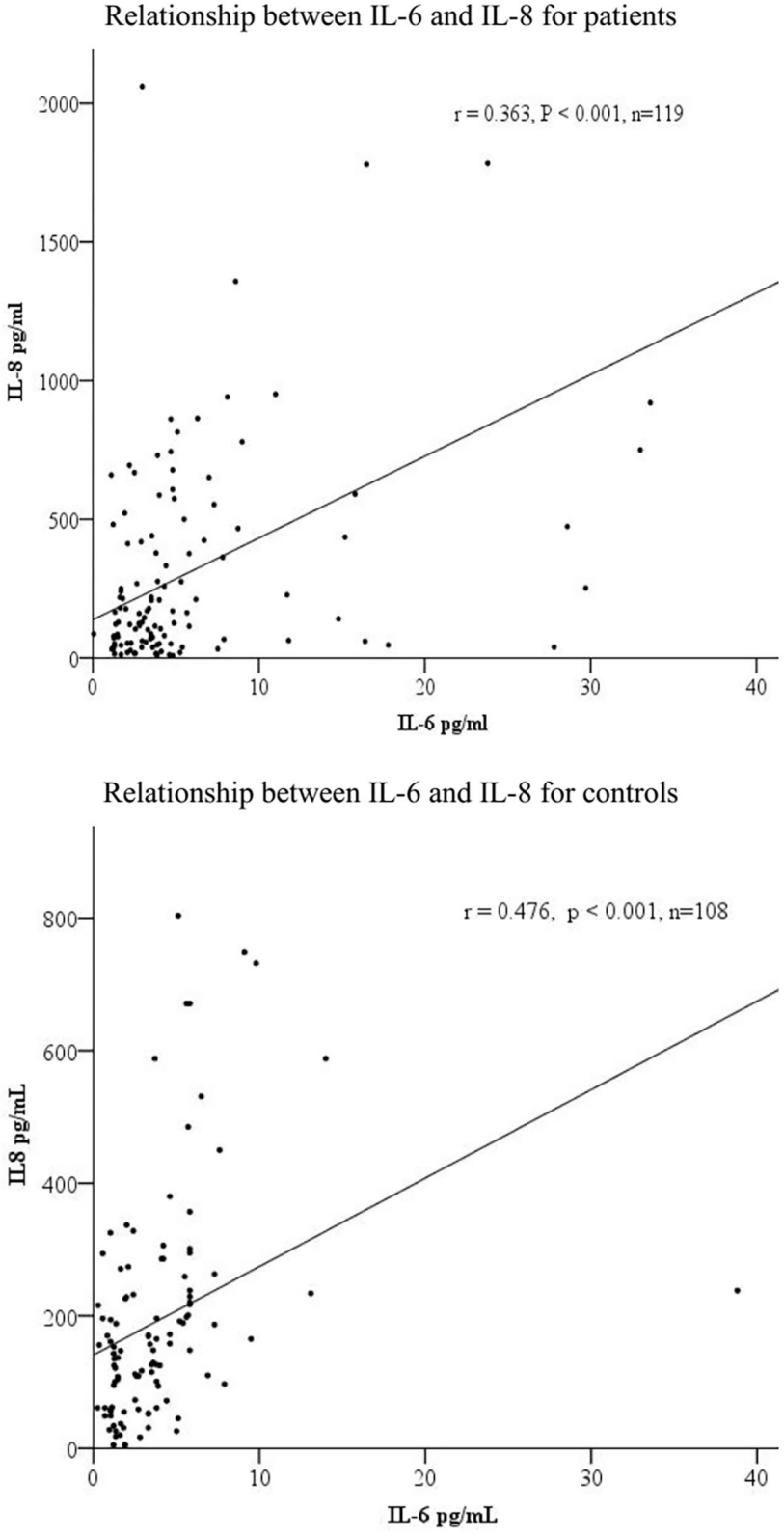

FIGURE 1 | The correlations among serum IL-6 and IL-8 levels in patients ($n = 119$) and controls ($n = 108$).

TABLE 3 | Pearson's correlations between clinical parameters and serum cytokine concentrations.

Clinical parameters		IL-2R	IL-6	IL-8	IL-10
P subscore	Correlation coefficient	−0.193*	0.062	0.081	−0.176
	p-value	0.035	0.505	0.382	0.056
N subscore	Correlation coefficient	−0.073	−0.047	−0.014	0.021
	p-value	0.430	0.613	0.878	0.825
G subscore	Correlation coefficient	−0.083	0.095	0.113	0.022
	p-value	0.367	0.302	0.221	0.813
Visuospatial/ executive subscore	Correlation coefficient	0.076	0.139	0.188*	0.130
	p-value	0.409	0.131	0.040	0.159
Naming subscore	Correlation coefficient	−0.009	−0.209*	−0.167	0.029
	p-value	0.923	0.022	0.069	0.751
Attention subscore	Correlation coefficient	−0.083	0.115	0.113	−0.031
	p-value	0.372	0.212	0.221	0.739
Language subscore	Correlation coefficient	−0.032	−0.008	−0.064	−0.024
	p-value	0.734	0.928	0.491	0.797
Abstraction subscore	Correlation coefficient	0.014	−0.053	−0.031	−0.062
	p-value	0.877	0.566	0.737	0.504
Delayed recall subscore	Correlation coefficient	−0.069	−0.045	−0.245*	0.134
	p-value	0.458	0.626	0.007	0.147
Orientation subscore	Correlation coefficient	−0.031	0.025	0.093	−0.505*
	p-value	0.742	0.789	0.317	0.000
Age of onset METH use	Correlation coefficient	−0.108	0.003	0.005	0.169
	p-value	0.243	0.974	0.956	0.067
Duration of METH use	Correlation coefficient	0.113	0.044	0.113	−0.112
	p-value	0.221	0.635	0.222	0.227
Frequency of METH use	Correlation coefficient	0.157	0.186*	0.293*	0.006
	p-value	0.087	0.043	0.001	0.948
Time of onset psychotic symptoms after METH use	Correlation coefficient	0.012	0.033	0.203*	−0.027
	p-value	0.894	0.719	0.027	0.767

METH, Methamphetamine; PANSS, Positive and Negative Syndrome Scale; P, the positive scale; N, the negative scale; G, the general scale; MOCA, the Montreal Cognitive Assessment.

**Statistically significant with p-value < 0.05.*

samples in some studies were brain tissue from mice, while our study used samples of peripheral serum in humans, which might have caused differences.

Another finding in this study was the significant negative relationship between IL-2R levels and PANSS positive subscale scores. These results suggest a possible correlation between IL-2R and the psychotic symptoms of MAP patients. There are few studies in this area, but the results of our present study agree with the findings in schizophrenia patients (25, 26). Studies have demonstrated that IL-2 increases dopamine (DA) turnover in the prefrontal cortex (27) and stimulates the release of DA in rat striatal cells (28). Higher CSF levels of IL-2 in neuroleptic-free schizophrenia patients could result in elevated DA neurotransmission and may contribute to psychotic symptoms (29). Moreover, IL-2 dose-dependently modulated the release of endogenous DA in a biphasic pattern, increasing release from striatal neurons at lower concentrations and inhibiting release at high concentrations, suggesting that the different IL-2 levels may play different roles in the pathophysiological process underlying psychotic symptoms (30).

We found that IL-6 and IL-8 levels were increased in MAP patients. Some studies have found that METH-activated microglia increase IL-6 and IL-8 expression in astrocytes (17, 31). mGluR5 receptor activity may be altered by METH, which activates the Akt/PI3K cascade. This may further activate the phosphorylation of IκB-α via the kinase activity of IKK, releasing the free form of heterodimeric NF-κB. Finally, active NF-κB translocates from the cytoplasm to the nucleus and promotes the transcription of IL-6 and IL-8 (17, 31). Another study showed that the PI3-Akt pathway and ERK1/2 mediated the induction of IL-6 and IL-8 by METH (15, 16). These findings were based on brain samples; however, METH has been shown to cause blood-brain barrier damage (32–34) and monocyte infiltration into the central nervous system (CNS) (35), and this, along with elevated levels of inflammatory markers, might indicate that peripheral cytokine levels are similar to CNS cytokine levels.

In our study, the interesting result was the consistent link between IL-6 and IL-8 levels and specific domains of cognitive performance. We found that increased IL-6 could be associated with naming impairments and that IL-8 could be associated with memory. Many studies have shown that abnormal IL-6 and IL-8 levels may be associated with cognitive dysfunction. Blood levels of IL-6 are significantly elevated in individuals with Alzheimer's disease (36, 37), and polymorphisms in the IL-6 gene that decrease plasma IL-6 levels may be associated with a lower risk of developing Alzheimer's disease (38). Increased serum concentrations of IL-6 and IL-8 have been associated with poor performance in memory, speed and motor function domains and predict increased risks of cognitive decline in an elderly general population (39). As levels of IL-8 increased, African Americans demonstrated slower reaction times on a delayed memory task (40). In addition to their roles in neurodegenerative processes, apoptosis and excitotoxicity, cytokines are capable of influencing neurotransmitter (41) and neuroendocrine responses (42) that subserve cognition and can directly modulate neuronal and glial cell function (43). These results indicate that the levels of peripheral IL-6 and IL-8 might be important biological indicators associated with the severity of cognitive dysfunction. It might be helpful to evaluate the severity of psychosis and develop a therapeutic strategy for MAP.

There were some limitations in this study. The elevated cytokine levels in MAP could easily be attributed to the psychotic

condition instead of the MUD. We will design 3 groups, MAP, MUD without psychosis and healthy, in the next study to address this problem. There are few articles about serum cytokine levels in MAP, and in some articles about patients with schizophrenia, the sample size may have been insufficient, which raises the risk of spurious findings when making multiple comparisons. Moreover, the ability to extrapolate to larger human populations may be important given differences in drug use patterns and aspects of immune function across different ethnic populations.

CONCLUSIONS

This study showed that IL-6 and IL-2R levels were elevated in MAP patients. Positive psychotic symptoms were found to negatively correlate with IL-2R, while the frequency of METH use and cognitive function were correlated with IL-6 levels. Such results suggest that immune disturbance may be involved in MAP and may have a relationship with more severe psychopathology in MAP.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Wuhan Mental Health Center Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

GW, QD, XY, and HZ were responsible for the study concept and design. XY and XL contributed to the acquisition of patient' clinical data. XZ and HZ performed the serum cytokine analysis. QX, HZ, and GW assisted with data analysis and interpretation of findings. XY drafted the manuscript. GW and QD provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2020.594766/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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BRIEF RESEARCH REPORT
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The Relationship Between Behavioral Inhibition and Behavioral Activation Systems, Impulsiveness, and Internet Gaming Disorder Among Students of Different Ages

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Background and Aims: Internet Gaming Disorder (IGD), which is an underlying symptom classified as a behavioral addiction disorder, has many related social problems that have garnered the bulk of attention in recent research. However, its psychological/behavioral mechanism is still unclear. The purpose of the current study was to explore the relationship between IGD and impulsivity which is regulated by behavioral inhibition and activation among different age groups.

Methods: A total of 1,525 students completed Young's Internet Addiction Test (IAT), Online Game Addiction Questionnaire, the Behavioral Inhibition System/Behavioral Activation System (BIS/BAS) scales, and the Barratt Impulsiveness Scale-11 (BIS-11).

Results: (1) The prevalence of IGD in different ages students was 10.3%. (2) BIS, BAS-Fun seeking (BAS-F), and BIS-11 scores indicated that there were significant differences between IGD and non-IGD individuals. (3) BIS showed significant differences in different grades (i.e., between undergraduates and middle school students with IGD; $F = 3.10$, $p < 0.05$). (4) The IAT scores demonstrated a significant positive correlation with BIS scores ($r = 0.375$, $p < 0.05$) among undergraduates with IGD; IAT scores of high school students with IGD were negatively correlated with BAS-Reward (BAS-R) ($r = -0.28$, $p < 0.05$).5)BIS and BIS-11 together could explain 14.9% variance of IGD among the college group; BAS-R and BAS-F together could explain 16.7% variance of IGD among the high school group ($p < 0.05$).

Conclusions: The relationships between IGD and BIS, BAS-F, BAS-D, BIS-11 scores differed due to the age group of individuals tested.

Keywords: students with IGD, BIS, BAS, impulsivity, different grades

INTRODUCTION

The "45th China Internet Development Statistics Report" which was published by the China Internet Network Information Center (1), showed that mobile Internet users account for an absolute majority of Internet users, accounting for 99.3 percent, while mobile Internet game users account for 59 percent of mobile Internet users. With the rapid development of society and widespread access to free public Wi-Fi and the ubiquity of smart phones, online games have become an important part of the private lives of college, high school, and middle school students. However, online gaming is a double-edged sword, which not only brings joy but may also make gamers unable to extricate themselves from the virtual world, causing harm to their physical and mental health. According to statistics, Internet addiction (IA) was the most common addiction type among Asian students, with the majority of IA being reported as online gaming addiction. This disorder can harm to the community, families, and individuals and is especially detrimental to the physical and mental health of students (2–4). The Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5) (5), incorporated Internet Game Disorder (IGD) into the category of psychiatric disorders that require further study, encouraging more IGD studies to be conducted.

Addictive behavior has often been explained by Gray's reinforcement sensitivity theory (RST) (6), which refers to an individual's sense of reward and punishment. Additionally, Gray argues that there are individual differences in sensitivity, and that these differences stemmed from two basic brain systems that control human behavior: the Behavioral Inhibition System (BIS) and the Behavioral Activate/ Approach system (BAS). The BIS is sensitive to punishment, novelty, and negative emotions, which may be related to activation within the hippocampus and the amygdala (7). Conversely, the BAS is sensitive to rewards and is thought to be related to positive emotions, such as happiness, which may be related to the striatum (8). The current assessment tool of the RST are BIS/BAS Scales (9), which includes the BIS and BAS scales. The BAS scale is divided into the BAS-Reward (BAS-R), BAS-Drive (BAS-D), and BAS-Fun seeking (BAS-F) subscales. Studies have shown that BAS scores are important predictors of drug abuse and alcohol addiction (10, 11). However, it is possible that IA could have a different pathogenesis than other dependences. Researchers believe that IA is more likely to be associated with BIS rather than BAS (12). For instance, in a study of college students, BIS and BAS-F scores were higher in students with IA than in students with alcohol dependence (13). Park (14) found that BIS and BAS-F scores independently predicted IA behavior in the adolescents. Yet, more recent studies have yielded inconsistent results. Specifically, a separate adolescent IA study found that elevated BAS and BAS-F scores were high-risk factors of IA (15). Furthermore, another study showed that BAS-D, BAS-R and impulsivity increased the risk of smartphone addiction (16). Given these findings, it is possible that the purpose of surfing the internet and ages have not been distinguished by previous studies. Therefore, the relationship between BIS/BAS and IA was inconsistent due to different these factors (Note: IA include IGD here).

The core symptoms of IGD is that patients are of the direct impairment of their social functioning due to online gaming but are unable to control their game-playing behavior. This would suggest an impulse control disorder that does not involve drug or alcohol (17). Studies have shown that there is a significant positive correlation between IA and impulsivity (18, 19), that can predict IA (20), and that 90% of IA falls under the classification of IGD. Impulsiveness is generally considered to be correlated with BAS (21). Slessarev et al. believed that negative emotions could regulate the relationship between BIS (Behavioral Inhibition System) and self-control (22).

The purpose of this study was to explore the relevance of RST and impulsivity in students with IGD in different age groups. This was done in an effort to determine psychological behavior mechanism underlying IGD, which would provide a theoretical basis for future psychological interventions. We hypothesized that impulsivity is a susceptibility factor for IGD in different age groups, and RST system plays a synergistic role.

METHODS

Participant Selection

Data was collected using questionnaire surveys administered by two psychiatric postgraduates from September 10th to December 30th 2016. Participants were 1,600 students, aged 12–26 years, from Guizhou University of Technology,Guizhou Medical University, Guiyang Vocational and Technical College, Guiyang 31st Middle School, Guiyang 34th Middle School, and Guiyang 39th Middle School, and were chosen via stratified random cluster sampling. A total of 1,525 valid questionnaires were collected, representing a 95.3% response rate. The sample was composed of 878 males (57.6%) and 647 females (42.4%). Data was split into three categories: 794 college students, 316 high school students, and 415 middle school students.

The criteria for being placed in the IGD group were (1) IAT score> 50 points; (2) the main purpose of the Internet was for playing online games; (3) the average daily online game time was 4–6 h for more than a year. (4) Diagnostic of IGD. Students who meet the above criteria were diagnosed with IGD by attending psychiatrists in accordance with the diagnostic criteria for IGD in DSM-5 (5). We must also exclude the following rules: (1) Severe mental disorder, such as schizophrenia and major depression disorder; (2) severe cognitive dysfunction.

Instruments

Young's Internet Addiction Test (IAT) (23)

The IAT is composed of 20 items rated on a 5-point scale, (1 = *very rarely*, 5 = *very frequently*). All item scores are summed to obtain an IAT total score. A total of 50 points and below indicates no problem with IA; 50–79 indicates mild IA; 80–100 indicates severe IA.

Self-Made Game Questionnaire

The questionnaire contains three questions: (1) What is the main purpose of your Internet use? (2) Which of the following

do you spend the most time on? (A. Game, B. Other), and (3) How long have you played games each day over the past year? If the answers to the first two questions were "game," the time spent was 4–6 h/day, the subject's IAT score> 50 points, and the individual was classified as exhibiting online game addiction.

BIS/BAS Scales (24)

The 24 item BIS/BAS Scales which were compiled by Carver and White (9), were translated into Chinese and tested reliability and validity by our preliminary study. The scales have demonstrated satisfactory reliability and validity. Our revised BIS/BAS scale was composed of four dimensions, the total Cronbach's α coefficient of the scales was 0.80, with each Cronbach's α coefficient of the BIS (5 items), BAS-R (6 items), BAS-D (4 items), BAS-F (3 items) was 0.67,0.77,0.68 and 0.59, respectively. The Chinese edition of BIS/BAS Scales consists of 18 items and 4 fillers rated on a 4-point Likert scale from "*totally agree*" to "*totally disagree.*"

Barratt Impulse Scale 11th Edition (BIS-11)

The Yang Huiqin revised BIS-11 (25) was used. The scale contains 30 questions, and Cronbach's α was 0.8.

Statistical Analysis

All the data was entered into an Excel database. A *t*-test was used to examine the differences in BIS/BAS and BIS-11 among individuals with and without IGD. In students with IGD, an ANOVA was used to examine the differences of BIS/BAS and BIS-11 among middle school students, high school students, and college students. All comparison tests were two-tailed. The relationship between IAT scores and scores of BIS, BAS-R, BAS-D, BAS-F, and BIS-11 was analyzed by Pearson correlation analysis. Regression analysis was conducted to test the roles of BIS-11, BIS, and all of the BAS-subscales as predictors of IAT scores. All statistical analyses were conducted using IBM SPSS Statistics for Windows Version 22.

RESULTS

The Prevalence of IGD in Middle School Student and College Student

Utilizing criteria of IGD, 157 participants were classified as having IGD, accounting for 10.3% of the total. Of these, 66 were college students, 48 were high school students, and 43 were middle school students, aged 12–22 years.

Comparison of BIS/BAS and BIS-11 Between Students With IGD and Without IGD

Table 1 shows that there were significant differences in BIS, BAS-F, and BIS-11 between students with and without IGD ($p < 0.001$), with scores of students with IGD being greater in the BIS, BAS-F and BIS-11.

Comparison of BIS, BAS-R, BAS-D, BAS-F, and BIS-11 Among 157 Students of Different Ages With IGD

As can be seen in **Table 2**, each of the dimensions of BAS and impulsivity, exhibited no significant differences among the students of different ages with IGD. However, BIS scores of the college student group, were significantly higher than those of the high school and the middle school students ($p < 0.05$).

Correlation Analysis

The correlation between IAT score and BIS, BAS-R, BAS-D, BAS-F, and BIS-11 of 157 IGD students in different age stages was examined. There was not a significant correlation between IAT and BIS, each of the dimensions of BAS and BIS-11 among middle school students. There was a significant negative correlation between IAT and BAS-R among high school students ($r = -0.28$, $p < 0.05$). There was a significant positive correlation between IAT scores and BIS in college students ($r = 0.375$, $p < 0.05$).

Roles of BAS/BIS and BIS-11 as Predictors of Addiction Among 157 Students of Different Ages With IGD

Regression analysis was conducted to test the roles of BIS, each of the dimensions of BAS and BIS-11 scores as predictors of IAT scores (**Table 3**).

The predictive variables of the college group model were BIS and BIS-11.

The predictive variables of the high school group model were BAS-R and BIS-F.

As can be seen from **Table 3**, the R2 model of the high school group was the larger, BIS and BIS-11 together could explain 14.9% variance of IGD among the college group, BAS-R and BAS-F together could explain 16.7% variance of IGD among the high school group. These two models demonstrated statistical significance ($p < 0.05$).

DISCUSSION

The primary objective of this study was to explore different risk factors for IGD among students of different ages. The BIS, each of the dimensions of BAS and impulsivity were compared between students with and without IGD by using the BIS/BAS questionnaire and the BIS-11. If we don't differentiate between ages, consistent with previous research, results indicated that there were significant differences between the IGD group and the non-IGD group in BIS, BAS-F, and impulsivity, with IGD showing elevated scores on these measures (13, 14, 19, 20).

Previous research has shown that BIS/BAS can change with age, with significant age effects observed for BIS and BAS-D (26). Similarly, the current study also found that the relationship between BIS/BAS and IAT is inconsistent among students with IGD of different age groups. For instance, The BIS scores of college students were highest of the three groups. Furthermore, analysis of the correlation between IAT scores and BIS/BAS, showed that IAT scores had a significant positive correlation

TABLE 1 | BIS/BAS and BIS-11 scores of students with and without IGD.

	BIS M ± *SD*	BAS-R M ± *SD*	BAS-D M ± *SD*	BAS-F M ± *SD*	BIS-11 M ± *SD*
IGD ($n = 157$)	15.34 ± 2.53	16.77 ± 2.32	11.23 ± 2.21	8.58 ± 2.00	71.7 ± 9.41
Non-IGD ($n = 1,368$)	14.27 ± 2.63	16.66 ± 2.36	11.22 ± 2.55	7.73 ± 1.77	64.1 ± 10.45
T	3.59***	0.41	0.02	3.96***	6.65***

****P < 0.001.*

TABLE 2 | BIS/BAS and BIS-11 scores of students with IGD in different age groups.

	BIS M ± *SD*	BAS-R M ± *SD*	BAS-D M ± *SD*	BAS-F M ± *SD*	BIS-11 M ± *SD*
Middle school student ($n = 43$)	19.63 ± 3.59	15.88 ± 2.65	10.95 ± 2.26	11.56 ± 2.23	70.95 ± 5.62
High school student ($n = 48$)	19.79 ± 3.11	16.08 ± 2.52	11.35 ± 1.95	10.94 ± 2.21	71.58 ± 5.48
College student ($n = 66$)	20.89 ± 2.29	16.76 ± 2.08	11.36 ± 2.33	11.70 ± 2.42	71.65 ± 5.11
F	3.10*	2.07	0.53	1.60	0.25
LSD	3>1,2				

**p < 0.05, LSD3 > 1,2 means that college students have the highest score in the total score of the BIS scale, with a significant higher score compared to that of high school students, and a significant higher score compared to that of middle school students.*

with BIS among the college student IGD group, which indicated that college students had higher scores on BIS, and more likely that they suffered from IGD. We speculate those who score highly on BIS would experience more negative emotions, such as anxiety and depression, and may be more likely to escape into the virtual world of online games. Zhang (27) have also confirmed that College students' online game addiction is more related to emotion. Further regression analysis revealed that BIS and BIS-11 together could explain 14.9% variance of IGD among the college group. Mediating model found that impulsivity is directly related to online game addiction, and the effect of BIS on online game addiction works partly through impulsivity in our previous study (28). Additionally, IAT scores of high school students were significantly negative correlated with BASR. That is, the higher their BAS-R score, the less likely they are to have IGD, which means that getting more praise in the real world is a protective factor for IGD. Further regression analysis revealed that BAS-R and BAS-F together could explain 16.7% variance of IGD among the high school group. For high school students, the characteristics of network gaming itself might be one of causes leading to IGD. Internet games are extremely fun and meet the desire to achieve in the virtual world, especially for teenagers who are under learning pressure without enough leisure time and sport. But our study surprisingly found the IAT scores of middle school students were no correlated with BAS/BIS and impulsivity. We speculate that this difference is due to different psychopathological mechanisms of IGD at different ages. What's more, because our subjects excluded IGD patients with depression, we also did not examine the effect of mood on impulsivity, but control mechanisms might also depend on emotional factors (29).

The deficiency of this study lies in three point. Firstly, our sample size of IGD in different age groups was small; Secondly, the object of this study who is 157 IGD students of different ages which was 12–22 years, including adolescents and young adults here, so it is varied in the social and education environment, peer relationships, available leisure times; Last, the diagnostic criteria for IGD in DSM-5 have shortcomings, such as different item sensitivities, unclear cause-and-effect relationship with gaming, and difficulty in manipulating severity (30), which could have influenced the results. So we will add IGD subjects in different age groups, and explore the impact of impulsivity and BIS, each of the dimensions of BAS on IGD by incorporating social demographic characteristics and emotional state in future study.

TABLE 3 | BIS/BAS regression analysis of adolescent Internet addiction in different periods.

Model	*R*	R^2	Adjusted R^2	*F*
College group	0.464	0.215	0.149	3.284*
High school group	0.506	0.256	0.167	2.890*
Middle school group	0.178	0.032	−0.099	0.243

**p < 0.05.*

CONCLUSIONS

In the current study of students with IGD of different age, there were different possible psychological mechanisms contributing to the results. This suggests that further research should be carried out on patients with IGD in different age groups, so that more targeted interventions can be developed.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board at Guizhou Province People's Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

HX designed the study and wrote the protocol. XT, YZ, and JC conducted literature searches, provided summaries of previous research studies, and conducted the statistical analysis. MP provided revision of the manuscript. QZ edited and organized the manuscript. All authors contributed to and have approved the final manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Structural Imaging-Based Biomarkers for Detecting Craving and Predicting Relapse in Subjects With Methamphetamine Dependence

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Background: Craving is the predictor of relapse, and insula cortex (IC) is a critical neural substrate for craving and drug seeking. This study investigated whether IC abnormalities among MA users can detect craving state and predict relapse susceptibility.

Methods: A total of 142 subjects with a history of MA dependence completed structural MRI (sMRI) scans, and 30 subjects (10 subjects relapsed) completed 4-month follow-up scans. MA craving was measured by the Visual Analog Scale for Craving. Abnormalities of IC gray matter volume (GMV) between the subjects with and without craving were investigated by voxel-based morphometry (VBM). The receiver operating characteristic (ROC) analysis was performed for the region-of-interest (ROI) of IC GMV to assess the diagnostic accuracy.

Results: By comparing whole-brain volume maps, this study found that subjects without craving ($n = 64$) had a significantly extensive decrease in IC GMV (family-wise error correction, $p < 0.05$) than subjects with craving group ($n = 78$). The ROI of IC GMV had a significantly positive correlation with the craving scores reported by MA users. The ROC analysis showed a good discrimination (area under curve is 0.82/0.80 left/right) for IC GMV between the subjects with and without craving. By selecting Youden index cut-off point from whole model group, calculated sensitivity/specificity was equal to 78/70% and 70/75% for left and right IC, respectively. By applying the above optimal cut-off values to 30 follow-up subjects as validations, the results showed a similar sensitivity (73–80%) and specificity (73–80%) for detecting craving state as model group. For predicting relapse susceptibility, the sensitivity (50–55%) was low and the specificity (80–90%) was high.

Conclusions: Our study provides the first evidence that sMRI may be used to diagnosis the craving state in MA users based on optimal cut-off values, which could be served as MRI bio-markers and an objective measure of craving state.

Keywords: structural imaging, biomarkers, craving, relapse, methamphetamine use

INTRODUCTION

Drug addiction is a chronic brain disease marked by high rates of relapse even after months or years of abstinence, long after self-reported craving and withdrawal have abated (1–3). The psychostimulant methamphetamine (MA) is a highly addictive and widely abused drug that has no available pharmacotherapies at the present (4). A core challenge for treating MA and other drug addiction is the high propensity for relapse. Relapse prevention treatment should be aimed at helping to control craving (5). However, reliable and valid biomarkers that accurately predict relapse and developmental processes in craving are still not available. These facts highlight the need for further insight into the neurobiology of drug craving might be gained by exploiting variations in the magnitude of cravings, which will facilitate appropriated medical interventions to prevent relapse among drug dependent individuals.

Since the report of being able to stop smoking without cravings by insular lesions (due to stroke) among smoking patients, extensive evidence indicates that drug addiction and craving have been associated with alterations in the insula cortex (IC). The IC has a key role in rebalancing the homeostatic imbalance, such as deprivation from drugs and reward cues (6, 7). The essential role of the insula in craving across drug categories provides a strong mechanistic foundation for exploring structural imaging-based biomarkers for detecting craving in methamphetamine addiction (6–8). Furthermore, exploring the neural correlates of IC as a predictor of relapse and craving state will provide the basis for future treatment strategies of relapse prevention.

Neuroimaging markers play an important role in gaining a deeper insight into the neurobiological basis of drug addiction. Structural brain abnormally among long-term drug users may reflect pathophysiologic processes of the addiction which may in turn act as treatment response markers to guide treatment options. The role of the insula in addiction and craving is evident. However, it is still unclear whether morphological alterations of the insular cortex constitute a state characteristic of the addiction that is present only during the course of the addictive disorder, or a trait marker that underlies a neurobiological vulnerability to the addiction that is present even during periods of sobriety. Investigating the individuals currently present subjective craving for MA and those currently without subjective craving for MA may be a particularly useful approach to shed light on this issue or to provide a necessary step toward identifying addiction traits. Thus, we assessed whether IC could be used as a predictor of relapse and craving state in this study.

MATERIALS AND METHODS

Subjects

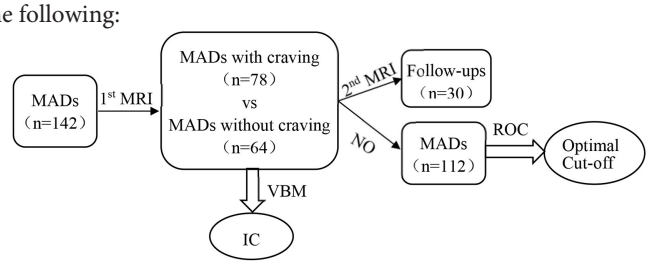
A total of 142 subjects with a history of MA use from two rehabilitation centers (the Kangda Voluntary Drug Rehabilitation Center in Hunan province and the Department of Addiction Medicine, Hunan Brain Hospital) were recruited between June 2013 to June 2015. Inclusion criteria: (1) aged between 18 and 42 years old; (2) being able to give voluntary informed consent; (3) met the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (9) criteria for MA dependence (MAD); and (4) had no history of neurological illness or other major physical diseases. Exclusion criteria: (1) met the DSM-IV criteria for any other substance dependence (excluding nicotine) at any time; (2) presence of a major medical or neurological illness; (3) a lifetime history of a psychotic disorder; (4) a history of traumatic head injury, pregnancy; or (5) contraindications for MRI.

The study protocol was approved by the Ethics committee of the Second Xiangya Hospital, Central South University, Hunan, China (No. S095, 2013) and carried out in accordance with the Declaration of Helsinki.

Procedures

All subjects underwent clinical and drug use history assessments by two qualified psychiatrists. All subjects were diagnosed for MA dependence with the Structured Clinical Interview for DSM-IV (9). Visual Analog Scale for Craving (VASc) (10) was used to measure MA craving. Subjects scored their craving by VASc right before the MRI scan on a scale from 0 (no craving) to 10 (extreme craving). The "craving group" was defined as subjects who had craving scores ≥ 1, and the "without craving group" was defined as subjects who had craving scores $= 0$. To maintain sobriety during the program, subjects were screened for the presence of drugs via urine toxicology. Study procedures occurred after 2 weeks of detoxification treatment. All subjects ($n = 142$) were given written informed consent to participate in a clinical interview session, the first MRI session, and a follow-up phone interview at 4-month to invite subjects ($n = 30$) for the second MRI session. At follow-up visit, all subjects were asked about their current living and mental situation and whether they had used any of the following substances in the past months: hallucinogens, stimulants, ketamine, or heroin (confirmed by a urine test). Relapse was defined as any use of these substances. During the follow-up, 20 subjects remained abstinent from drugs (with the exception of nicotine) from the time of treatment to 4-month follow-up, 10 subjects relapsed (mainly used MA).

The timeline/flowchart the study design is shown in the following:



MRI Data Acquisition

All MRI data were acquired with a 3.0-T Siemens Magentom Trio scanner (Siemens, Erlangen, Germany) at the Magnetic Resonance Center of Hunan provincial People's Hospital, China. The structure T1-weighted image was acquired without gap, and the number of slices = 176, repetition time = 2,000 ms, echo time = 2.26 ms, field of view = 256 × 256 mm^2, flip angle = 8°, matrix size =256 × 256, slice thickness = 1 mm.

TABLE 1 | Demographic and drug use characteristics between groups of subjects with and without MA craving.

Characteristic (Mean ± SD)	Subjects with MA craving ($n = 78$)	Subjects without MA craving($n = 64$)	*P*-value
Demographics			
Age (years)	29.0 ± 5.17	29.4 ± 5.91	0.64*
Education (years)	11.8 ± 2.63	11.6 ± 2.58	0.70*
Male/Female, No.	68/10	56/8	0.95#
MA Use and smoking characteristic			
Craving scores	2.4 ± 2.61	0	
Age of first MA use (years)	23.7 ± 7.10	24.4 ± 6.21	0.39*
Smoking duration (years)	11.5 ± 4.78	11.8 ± 6.45	0.71*
Cigarettes smoked per day	20.5 ± 10.04	19.0 ± 10.39	0.38*

#*Indicates p-values for Chi-squared test.* **Indicates p-values for two-sample t-tests.*
SD, standard deviation.

Data Analysis

All subjects' structural T1-weighted image data was processed using the statistical parametric mapping software (SPM12, https://www.fil.ion.ucl.ac.uk/spm-statistical-parametric-mapping/) on the Matrix Laboratory MATLAB R2010a platform (Mathworks, Sherborn, MA, USA) according to the following steps: (1) Segmented: the structural T1-weighted image were segmented into gray matter (GM), white matter, and cerebral-spinal fluid based on the "new-segment" tool implemented in SPM8, and both the native space and DARTEL (Diffeomorphic Anatomical Registration Through Exponentiated Lie) imported versions of the tissues were generated. (2) Custom template creation: DARTEL was applied to conduct spatial normalization (11). This procedure achieves more accurate inter-subject registration of images by registering individual structural images to an asymmetric customized T1-weighted template based on different samples, rather than a standard T1-weighted template. (3) Smoothing: smoothed (8 mm full-width at half-maximum, FWHM), modulated, and spatially normalized gray matter images were generated using the Montreal Neurological Institute (MNI) template.

Statistical Analysis

The bilateral insular cortex was selected as a mask from the automated anatomical labeling (AAL) template using WFU_Pickatlas software (12, 13). A two-sample *t*-test of the insular cortex volumes was conducted in SPM12 using a full factorial model with age, gender, educational level, the number of cigarettes smoked per day and whole brain volume as covariates. All statistical maps were thresholded at $p < 0.05$ with family-wise error (FWE), and a minimum cluster size of 50 voxels was significant. Cerebral regions displaying significant differences in the two-sample *t*-test between the two groups were identified as ROIs (ROI: region-of-interest). The mean volumes of the ROIs were measured for the subjects with craving using the MATLAB program. The Pearson correlation analysis test was performed to investigate the correlation between age, years of education, craving score, and the mean volume in each ROI using SPSS 20.0.

ROC Analysis

The receiver operating characteristic (ROC) curve is a graphical plot that summarizes the sensitivity and specificity of a marker for distinguishing subject groups. We applied ROC analysis to evaluate the sensitivity and specificity of the mean gray matter volume changes in the ROIs in order to assess their performance in distinguishing the two groups. As a lot of subjects lost to follow at the second MRI scan, the total of 30 follow-up subjects separated from others. Therefore, subjects were divided into two groups: the model (training) group ($n = 112$, lost to follow) and the validation group ($n = 30$, followed-up). Using area under the ROC curve (AUC) in the assessment of classification model performances is sometimes biased for small sample sizes, and different cross-validation approaches are often used for model validation (14–16). To mimic more datasets of model groups, 112 patients were further randomly divided into four groups of 28 patients and repeated for 30 random partitions (total 120 groups). The ROC analysis was performed for the IC GMV obtained in the model group to evaluate diagnostic performance for detecting craving state. The 2 × 2 contingency tables were constructed for the validation group based on IC GMV smaller vs. larger than the optimal cut-off values and a corresponding number of MADs without vs. with craving, and non-relapse vs. relapse to calculate sensitivity and specificity.

RESULTS

Demographic and Clinical Characteristics

Demographic and drug use characteristics of groups of MA users with and without MA craving are shown in **Table 1**. It shows that there were no significant differences in age, gender, education levels, age of first use MA and smoking history between these two groups. All methamphetamine dependent (MAD) subjects were cigarette smokers. The MA craving group had an average of 4.5 ± 1.84 (mean ± SD) scores in MA craving by VASc from 1 to 10 (low to high). Fifty percentage of them reported medium to high levels of MA craving (scored ≥ 5). Among 30 subjects who returned for a 4-month follow-up, 10 of them had relapsed. The average MA abstinence day for relapsers was 34.0 ± 3.41 (mean ± SD) days.

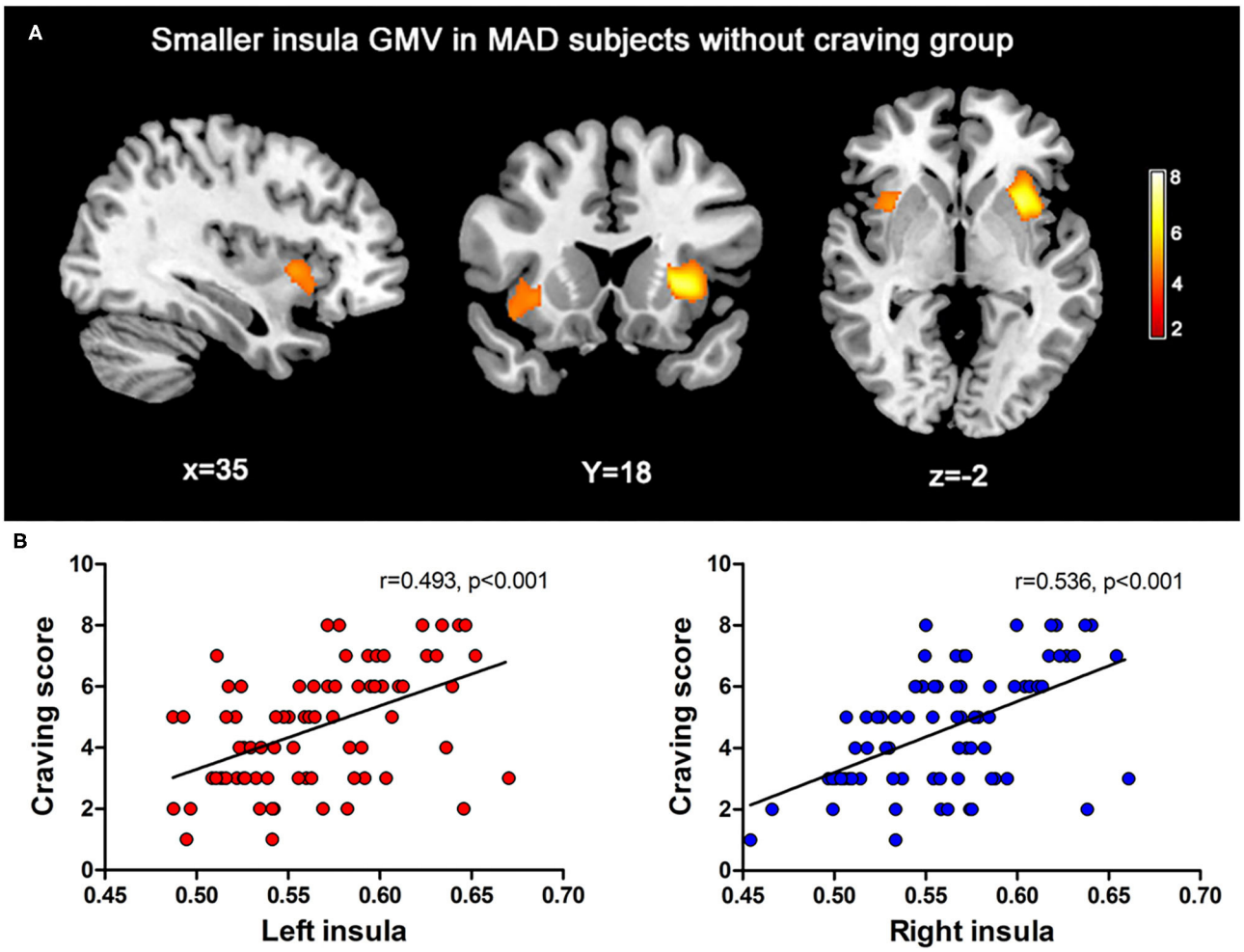


FIGURE 1 | (A) The maps for the MADs without craving had extensive significantly decreased in IC GMV (FWE correction, $p < 0.05$) than the MADs with craving. **(B)** The scatter plot of the correlation obtained between regions of interesting of IC GMV with the craving scores reported by the MADs at the first MRI scan.

Decreased Gray Matter Volumes in the Bilateral Insular Cortex in MAD Subjects Without MA Craving

By voxel-based morphometry (VBM) analysis, a two-sample *t*-test revealed significant differences in the GMV of the IC between the two groups. The group of MAD subjects without craving had decreased GMVs in the bilateral IC compared with the group of subjects with MA craving, which are shown in **Figure 1A**. Details of smaller gray matter volume (GMV) in subjects without MA craving compared to subjects with MA craving are also shown in **Table 2**.

Decreased Insular GMV Among MAD Subjects Without MA Craving

Among the two brain regions selected as ROIs, the MAD subjects without MA craving group (left: 0.55 ± 0.04; right: 0.54 ± 0.03) showed significantly lower ($p < 0.05$) mean of gray matter volumes in the insular cortex (IC) relative to the MADs with craving group (left: 0.59 ± 0.04; R: 0.58 ± 0.03). As shown in **Figure 1B**, a positive correlation was found between the volumes of the IC and the craving score in the MADs with craving group (left: $r = 0.493$, $p < 0.001$; right: $r = 536$, $p < 0.001$).

Detecting Craving State and Predicting Relapse in MAD Subjects

The ROC analysis for 120 random 28-patients model groups and also for whole model group MADs (**Figure 2**) showed that there was a good discrimination [area under the curve (AUC)=0.82/0.80 left/right] for IC GMV between the MADs with and without craving. By selecting the Youden index cut-off point from the whole model group, calculated sensitivity/specificity was equal to 78/70% and 70/75% for left and right IC, respectively.

TABLE 2 | Smaller GMV in MAD subjects without MA craving compared to subjects with MA craving.

Brain region	Cluster size (No. Voxel)	MNI coordinates (mm)			*t*-statistic
		X	Y	Z	
Left insula	276	−36	−21	10	5.61
Right insula	87	39	18	−12	5.22

GMV, gray matter volume; MAD, methamphetamine dependence; MA, methamphetamine.
Minimum cluster size of voxels: 50.
Family-wise error correction, $p < 0.05$.

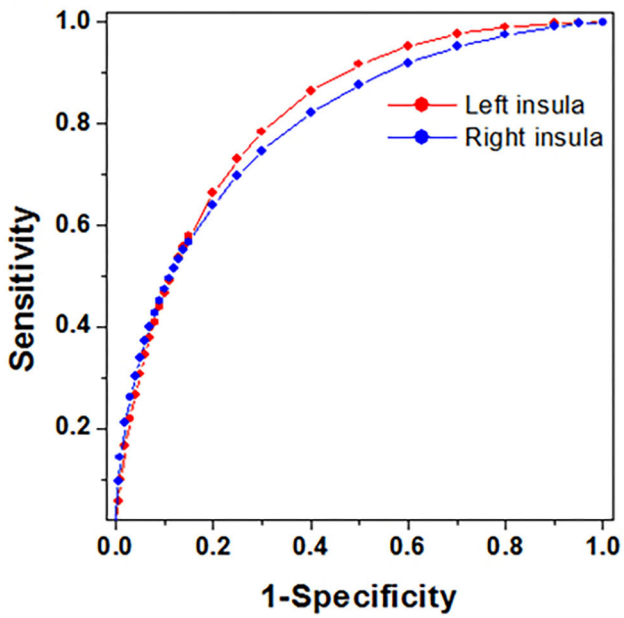


FIGURE 2 | The plots of ROC curves obtained from whole model group MADs. All the sensitivity and specificity were calculated based on Youden index cut-off point.

TABLE 3A | The number of follow-up MADs had IC GMV smaller and larger than the optimal cut-off values, and corresponding number of self-reported craving scores at the 1st MRI scan.

(Left IC, Right IC) 1st MRI	Craving state @ 1st MRI	
	VASc = 0	VASc > 0
Smaller than cutoff value	(11, 12)	(3, 4)
Larger than cutoff value	(4, 3)	(12, 11)

TABLE 3B | The number of follow-up MADs had IC GMV (measured at 2nd MRI) smaller and larger than the optimal cut-off values, and corresponding number of MADs with relapse and non-relapse.

(Left IC, Right IC) 2nd MRI	Relapse	
	Non-relapse	Relapse
Smaller than cutoff value	(11, 10)	(1, 2)
Larger than cutoff value	(9, 10)	(9, 8)

By applying the above optimal cut-off values to 30 follow-up MADs as validations, 2 × 2 contingency tables were constructed for MADs with and without craving (reported at the 1st MRI, **Table 3A**), and relapse and non-relapse (evaluated at the 2nd MRI, **Table 3B**). The results from the contingency tables showed a very similar sensitivity (73–80%) and specificity (73–80%) for detecting craving state as a model group. However, there was a low sensitivity (50–55%) and high specificity (80–90%) for predicting relapse susceptibility.

DISCUSSION

The current study provides the first preliminary evidence that structural MRI could be used to diagnosis the craving state in subjects with methamphetamine dependence (MAD).

We found decreased GMV in the bilateral insular cortex in MAD subjects without MA craving, compared with those who experienced MA craving. Furthermore, we found a positive correlation was found between the GMV of the bilateral IC and the craving score in the MADs with craving group. The gray matter reduction in the bilateral insular in MA dependent patients who experienced no MA craving may indicate the damage to this brain region after long-term use of MA. Among cigarette smoking patients with stroke, the damage to the insula led to an abrupt and profound disruption of dependence on cigarette smoking (7). On the one hand, the decease of drug craving could be considered as evidence of a "cure" for addiction. On the other hand, it may represent the damage of the brain among these chronic drug users. While the high levels of drug craving may represent the less damage to the brain in MA or other drug dependent subjects.

These results stimulate questions about why and how the insula plays an important role in addiction. There has been an increasing number of preclinical and clinical studies exploring the underline specific neural mechanisms about the functions of the insula in addiction. A brain functional connectivity study found that, examination of connectivity with left insula revealed increased connectivity during smoking cue related craving (by smoking videos) compared with food cue related craving (food videos) in the right anterior and posterior insula (17). However, a resting state functional magnetic resonance imaging (rs-fMRI) study assessed changes of resting state connectivity in the insula

by treatment of repetitive Transcranial Magnetic Stimulation (rTMS) among treatment-seeking alcohol dependent subjects, and showed no efficacy of rTMS targeting the insula in alcohol addiction (both rTMS and control groups showed a reduction of heavy drinking) (18).

In this study, structural MRI is also used to detect craving state and predict relapse in MAD subjects. The ROC analysis showed that there was a good discrimination (with high sensitivity and specificity) for the GMV of the IC between the MADs with and without craving. Also, there was a high specificity but low sensitivity for predicting relapse susceptibility. The insula has been linked to conscious interoception, emotional experience, and decision- making. A critical underlying theme of this conceptual framework is that a primary goal for the addicted drug users is to obtain the effects of the used drug, and the insula may contribute to how drug users' feeling, memory, and decision about taking drugs (19). A line of research demonstrated the role of the insula in nicotine (20), alcohol (21) and drug (22) addictive behavior. Furthermore, the insula also works as a control system in addiction, involving in weighing the pursuit of certain rewards against uncertain negative consequences, e.g., deciding to take drug or not (23). The current results raise an assumption that if the structure of the insula has been damaged, MA dependent patients' ability of to feel, remembering, or decide to take MA may be disabled. Correspondingly, their craving for MA has been decreasing. However, in addition to neural predictors, there are also clinical and biological factors that predict relapse risk for drug relapse (24). Thus, this study showed low sensitivity for predicting relapse susceptibility.

LIMITATIONS

This study had several limitations. First, only about 20% (30/142) of subjects were included in the 4-month follow-up. In other words, the majority of MA users in this study were lost to follow. Second, this study only included subjects with of MA dependence history. We didn't compare insular GMV between MAD subjects and healthy control subjects who had no drug use history. Third, we only assessed self-reported MA craving by the Visual Analog Scale for craving, and assessed craving as a state rather than a trait. A comprehensive assessment of psychological (as a state) and physical craving (as a trait) should be more accurate. Furthermore, subjects in this study were from drug rehabilitation centers after detoxification treatment. Thus, they may experience less craving for MA than those MA users from the general population. Fourth, the two groups of subjects were matched in age, gender, education levels, age of first use MA, years of cigarette smoking and cigarettes smoked per day (a pack per day on average). However, this study didn't assess nicotine dependence and the impact of nicotine dependence on insular GMV. Fifth, this study only recruited <15% female subjects. So, we didn't assess gender differences in the alternation of insular GMV among MA users. In the future, we will conduct a larger sample follow-up study to investigate how common, specific insular and other brain regions' structural features in MAD patients with and without MA craving are related to clinical implications.

CONCLUSION

Our study provides the first preliminary evidence that structural MRI could be used to diagnosis the craving state in MAD subjects based on optimal cut-off values, which could be served as MRI bio-markers and an objective measure of craving state. The smaller IC GMV could alter the insula network, which is critical for the loss of addiction. Although the structural MRI had low sensitivity to diagnose non-relapse, it had high specificity for predicting of relapse. These results from the study may provide valuable insight into clinical diagnosis and treatment for MA and other drug users.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The study protocol was approved by the by the Ethics committee of the Second Xiangya Hospital, Central South University, Hunan, China (No. S095, 2013) and carried out in accordance with the Declaration of Helsinki. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CQ and YL conceived the study, did the literature review, and drafted the report. CQ, XF, and SF did statistical analyses. CQ, QW, AX, and ZF collected the data. YL took the lead in writing the manuscript. JT, WH, JL, and TL interpreted the data and commented on the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Impulsivity and Response Inhibition Related Brain Networks in Adolescents With Internet Gaming Disorder: A Preliminary Study Utilizing Resting-State fMRI

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Background and Aims: Internet gaming disorder (IGD), as a relapse disease, has become a common mental health problem among Asian teenagers. Functional connections in the prefrontal lobo-striatum affect changes in impulsivity and inhibition. Therefore, exploration of the directional connections of the relevant brain regions in the prefrontal-striatal circuit and the synchronization level of the two hemispheres will help us to further understand the neural mechanism of IGD, which can provide guidance for the development of prevention and intervention strategies.

Methods: Twenty-two adolescents with IGD, recruited through various channels, composed the IGD group. Twenty-six subjects, matching age, gender, and education level, were included in a recreational internet game users (RGUs) control group. Impulsivity and response inhibition were tested via general questionnaire, the Internet Addiction Test (IAT), the Barratt impulsivity scale-11 (BIS-11), and a Stroop color-word task. A Granger causality analysis (GCA) was used to calculate the directional connection between the prefrontal and striatum with the dorsolateral prefrontal cortex (DLPFC) as a region of interest (ROI). We chose voxel-mirrored homotopic connectivity (VMHC) to determine brain hemisphere functional connectivity in the prefrontal-striatal circuits.

Results: We found significant differences in impulsivity between the IGD group and RGU group, with members of the IGD group exhibiting higher impulsivity. Additionally, the response inhibition of adolescents with IGD in the Stroop color-word task was impaired. There was a significant difference in the directed connection of the left DLPFC and dorsal striatum between the IGD group and the RGU group.

Conclusions: This study confirmed the role of prefrontal-striatal circuits in the neural mechanism of IGD in adolescents. In the IGD group, bilateral cerebral medial orbitofrontal

cortex (mOFC) synchronization was significantly reduced, which indicated that mOFC signal transmission in both hemispheres of the brain might be affected by impulse behavior and impaired response inhibition.

Keywords: internet gaming disorder (IGD), prefrontal-striatal circuits, functional magnetic resonance imaging (fMRI), voxel-mirrored homotopic connectivity (VMHC), effective connectivity

INTRODUCTION

The "42nd China Internet Development Statistics Report," published by the China Internet Network Information Center, showed that the number of Internet users in China had reached 802 million, with an internet penetration rate of 57.7% by June 2018. The diversification of online games and game content was more obvious. With the popularity of the Internet, internet gaming disorder (IGD) (1) has had a considerable impact on public health.

IGD refers to the continuous and repeated use of the Internet to participate in games with others, resulting in clinically significant damage or pain. The Diagnostic and Statistical Manual of Mental Disorders (fifth edition) has classified IGD as a disease that requires further study. The 11th edition of the International Classification of Diseases (ICD-11) was released in June 2018, with game addiction being added to the list of behavioral addiction disorders. Adolescents and children are the main target population of online games. A systematic review of more than 50 studies worldwide (2) suggested that IGD is more common in adolescents.

Not everyone who comes into daily contact with online games develops IGD (3). Palaus et al. (4) collected the results of 116 scientific studies and found that online games could optimize attention resources, improving working memory and visual-spatial skills. This suggests that online games do not necessarily result in negative consequences and that IGD susceptibility varies among individuals (5). Therefore, this research recruited recreational online game users (RGUs) as a control group. While RGUs play online games for entertainment, they are not addicted and do not meet the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM−5) for IGD (6). A comparison between RGUs and individuals with IGD is beneficial to further understand online game addiction and the cause of addiction.

The abnormal psychological processes and behavior observed in individuals with IGD appear related to high levels of impulsivity and dysfunctional response inhibition (7). Some researchers define impulsiveness as a tendency to act too early and without careful consideration (8). Response inhibition refers to the ability to suppress responses that do not meet current demands or inappropriate behaviors. Impaired response inhibition is considered to be an important cause of impulsiveness (9). Some studies (10, 11) have observed abnormal activation of brain areas associated with impulsivity and inhibitory function in individuals with IGD. However, differences in brain functionality, based on changes in neural circuits, were not enough to explain abnormalities in external behavior.

Within the functional connectivity of anterior cingulate cortexes during the resting state, in contrast to the controls, the individuals with IGD showed increased connectivity with posterior cingulate, medium cingulate, midbrain, nucleus accumbens and supplementary motor area, but reduced connectivity with prefrontal cortex, temporal lobe, and occipital lobe. The IGD group had lower gray matter volume in the left orbitofrontal cortex, left medial prefrontal cortex, bilateral insula, left posterior cingulate cortex, and left supplementary motor area (12). These changes in brain structure might result in uncontrolled behavior and online gambling urges.

Some studies (13, 14) have reported dysfunction in frontal-striatal circuits in individuals with addiction. The prefrontal cortex and corpus striatum are regulated via dopaminergic connections. Video games might cause a rapid increase in striatal dopamine (15), making prefrontal-striatal circuits more likely to be activated. Response inhibition is primarily related to brain areas in the prefrontal cortex. Specifically, the hyper-direct and indirect pathways in the frontal-striatal circuits might be responsible for the inhibition of dominant responses. Impulsivity is also affected by prefrontal-striatal circuits, which play an important role in activating and inhibiting impulsive behavior (16).

One study (17) selected the dorsolateral prefrontal cortex (DLPFC) and ventral striatum as regions of interest to investigate the role of the prefrontal-striatal circuits in substance addiction. A previous study (18) had reported differences in rest-state functional connectivity (RSFC) of the prefrontal-striatal circuits in substance users. This is consistent with the conclusion of IGD studies (19). Further studies (20) have identified an association between RSFC and cognitive control deficits in patients with IGD. While abnormal cognitive control in patients with IGD has been shown to be associated with changes in the DLPFC, the current study focused only on the strength of the functional connections between the prefrontal cortex and the striatum. It is not clear whether the striatal system demonstrates "high impulsiveness," the prefrontal system demonstrates "low control," or a combination of dysfunction in these two aspects is responsible for IGD behavior. Therefore, this study intended to explore the direct brain network between the prefrontal cortex and striatum in adolescents with IGD, in an effort to determine causal effects and direction between nodes (21). According to previous studies (7, 8, 22, 23), the DLPFC, which plays an important role in inhibition control, was selected as a region of interest (ROI). Further, a Granger causality analysis (GCA) was used to determine the connection efficiency between DLPFC and striatal regions. Simultaneously, using voxel-mirrored homotopic connectivity (VMHC), the current study detected

brain hemisphere differences in the striatum and prefrontal cortex synchronicity of the two groups.

This study hypothesized that, compared with RGUs, adolescents with IGD would exhibit significant differences in behavioral performance on an impulsivity index and Stroop color-word task, and that impulsivity would be correlated with Internet Addiction Test (IAT) scores (24). We also hypothesized that there would be significant differences between the two groups on connection efficacy of the prefrontal-striatal circuits, and in the VMHC values of related brain regions. The purpose of this study was to explore the prefrontal-striatal circuits and resting state functional connectivity, which are related to impulsiveness and response inhibition, in adolescents with IGD, so as to provide neurological explanations for online gaming behaviors in this population, and to provide new imaging targets for the clinical diagnosis and intervention of IGD.

METHODS

Participant Selection

We conducted IAT questionnaire screening in middle schools in Guizhou Province. And we also screened through the Internet IAT questionnaire. A professional psychiatrist diagnosed adolescents with IAT>50 in Guizhou Provincial People's Hospital. Ninety-one adolescents (12–18 years of age), regardless of gender, were invited. Sixty-one adolescents declined the invitation, and 30 adolescents accepted the invitation. The criteria for being included in the IGD group were (1) an IAT score $\geq$ 50 (4, 5); (2) the main purpose of using the Internet was for playing online games; (3) an average daily online game time of 4–6+ h for more than 2 years (4, 5); (4) the symptoms met the diagnostic criteria for IGD in the DSM-5; (5) being right-handed. The criteria for being included in the RGU group were (1) IAT score $<$ 50 points; (2) an average daily online game time of 2–4 h for more than a year (4, 5); (3) being right-handed. The exclusion criteria were as follows: (1) psychoactive substance dependence, including coffee and cigarettes, schizophrenia, or other organic brain diseases; (2) severe cognitive dysfunction, such as head trauma history, cerebrovascular disease, or epilepsy; (3) other mental disorders; (4) taking medicine 1 week before the examination.

Strict quality control was carried out in this study. We have uniformly trained the study staff and had consistent test for all questionaires. Middle and high school students who met the screening standards in the psychiatric department of our hospital were selected from October 2016 to September 2018. A total of 30 eligible subjects were selected to be included in the IGD group. Matching the IGD group in terms of gender, age, and years of education, we recruited junior and senior high school students through online advertisement. A total of 30 subjects that met these criteria were selected to be included in the RGU group.

This study was approved by the ethics committee of the Guizhou Provincial People's Hospital. All of the subjects and their statutory guardians in the study were informed of the process and purpose of the study before the study started and provided written informed consent.

Instruments

IAT (24)

The IAT is composed of 20 items rated on a 5-point scale, (1= *very rarely*, 5 = *very frequently*). All of the item scores are summed to obtain an IAT total score. A total of 50 points and below indicates no problem with internet addiction (IA); 50–79 indicates mild IA; 80–100 indicates severe IA.

Self-Designed Online Game Questionnaire

The questionnaire contained four questions:(1) How long have you been connected to the Internet? (2) What is the main purpose of your internet use? (3) Which of the following do you spend the most time on? (A. Game, B. Other), and (4) How long have you played games each day over the past year? If the answers to questions 2 and 3 were "game," the time spent was 4–6+ h/day, and the subject's IAT score $>$ 50 points, the individual was classified as exhibiting IGD.

Barratt Impulse Scale, 11th Edition (BIS-11)

We used the Chinese version of BIS-11. The Chinese version of BIS-11 Scale were revised by Zhou et al. (25). This instrument rates on a 4-point scale three dimensions: attentional impulsivity (AI), motor impulsivity (MI), and non-planning impulsivity (NI). These can measure attention, cognitive stability, motor impulse, perseverance, continence, and cognitive complexity. The total Cronbach's α coefficient for the scales was 0.76, with each Cronbach's α coefficient of the AI, MI, NI being 0.56, 0.66, and 0.69, respectively.

Stroop Color-Word Task

Participants performed six runs of the Stroop color-word interference task (26) by the Eprime 2.0 program, in which participants reported the ink color of congruent or incongruent color-word pairs [e.g., "red" written in red (congruent) or blue (incongruent) ink]. Subjects were asked to press the digital corresponding key as soon as possible to the color stimulus (red = 1, green = 2, blue = 3). Runs consisted of 105 stimuli, presented for 1,300 ms, with an inter-trial interval of 350 ms, including 6 incongruent events that were presented pseudo-randomly every 13–16 congruent stimuli.

Image Acquisition

Images were acquired with a Siemens Trio 3T magnetic resonance imaging (MRI) system (Siemens, Erlangen, Germany) using a 32-channel phased-array head coil. Special care was taken to minimize head motion with the use of an earplug and head cushion that also allowed subjects to maintain a comfortable position during scanning. T1-weighted localized images were scanned by vertical lines of the anterior commissure and posterior commissure lines, and then the structural and functional images were scanned in the same position. High-resolution T1-weighted anatomical images were used for the structural image, repetition time/echo time [TR/TE] 1,500/2.83 ms, flip angle 7°, field of view [FOV] 25.6 × 25.6 cm, 256 × 256 matrix, 176 slices. Functional images were collected using an echo-planar image gradient-echo pulse sequence (TR/TE 1,650/27 ms, flip angle 60°, FOV 22 × 22 cm, 64 ×

64 matrix, the total volume = 105, the total scanning time = 173.25 s, 5 mm effective slice thickness with 1 mm gap, 25 slices).

Statistical Analysis

Psychological and Behavioral Data

Relevant parameters were recorded and analyzed by using the Statistical Package for the Social Sciences 21.0 (SPSS 21.0). To understand the differences between the different mean values, an independent-samples *t*-test was used for comparison between groups. Further, a paired sample *t*-test was used for comparison within group. Pearson correlation analysis was used to investigate the relationship between variables.

Functional MRI (fMRI) Data

fMRI data were preprocessed and analyzed using SPM12 (Statistical Parametric Mapping, https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) and DPABI (Data Processing & Analysis of Brain Imaging, http://rfmri.org/dpabi), and SPM12 was used based on MATLAB R2013b (matrix & laboratory, https://www.mathworks.com/) (27, 28).

All of the data were preprocessed by SPM 12. Runs with motion in excess of 2.0 mm displacement and 2 degrees rotation were rejected. Four cases of RGUs and 8 cases of IGD were excluded. A total of 22 individuals with IGD (5 females) and 26 RGUs (7 females) were included in the final fMRI analyses. The first 10 images were excluded to ensure steady-state longitudinal magnetization, and the remaining images were then corrected for temporal differences and head motion. The realigned datasets were normalized to Montreal Neurological Institute (MNI) space. The T1 structural images adopted DAETEL registration, and performs spatial smoothing of Gaussian filtering with a full width and half height of 6 mm. Finally, the image was de-linearly drifted, and the whole brain signal did not return. The filtering range was 0.01∼0.1 Hz. A 6-mm full-width-half-maximum Gaussian kernel was used for data smoothing.

The GCA is an appropriate method based on multiple linear regression for investigating whether the past value of one time series can correctly predict the current value of another (29). This method has been applied in previous fMRI studies to reveal causal effects among brain regions. In the current study, multivariate GCA was conducted using the Resting-State fMRI Data Analysis Toolkit (REST, http://www.restfmri.net), according to methods described in previous studies. Signed-path coefficients are employed to reveal the Granger causal effects among key nodes of intrinsic connectivity networks (ICNs) within each hemisphere, which are considered to be normally distributed and can be used in parametric statistical analysis for group-level inference.

According to prior literature, an ROI must be determined. Using multiple research paradigms and impulsivity scales, early behavioral studies (7) have confirmed the lack of impulsivity characteristics and inhibitory control ability of individuals with IGD. Inhibitory control circuits are mainly related to the relevant brain regions of the prefrontal cortex, and the DLPFC, anterior cingulate cortex and ventrolateral prefrontal cortex in an important node of these inhibitory control circuits (22). Abnormal inhibitory control in patients with IGD was associated with changes in the DLPFC. In individuals with IGD, functional connections between the DLPFC and the audio-visual, motor, and other brain regions have been shown to be altered (8). The connections between left DLPFC, ventral medial prefrontal cortex, and parietal cortex were weakened (23). Research showed that ventral prefrontal cortex activity was decreased in addictive behavior (22). In IGD patients, the degree of activation of the anterior cingulate gyrus is positively correlated with the desire for online games (30). Therefore, DLPFC, anterior cingulate cortex and ventrolateral prefrontal cortex was intended to be used as an ROI for effective connection analysis in this study.

Seed selection: the seed was designated as the DLPFC, and Neurosynth (http://www.neurosynth.org/) was used with DLPFC as the keyword. The locations of DLPFC in the meta statistical chart were obtained. The coordinates of peak points on the left were x = −46, y = 38, and z = 30. The coordinates of peak on the right were x = 42, y = 38, and z = 32. After selecting voxels with the highest z-scores within each cluster on the functional map, the final ROI were constructed by drawing spheres with centers as the seed point and a radius of 6 mm. Positive/negative connection values mean the degree to which one brain area affects another brain area. The larger the absolute value, the stronger the effect.

Global differences in VMHC (Symmetric Voxel Homotopic Connection) were examined across the whole brain (31). VMHC is an index proposed by researchers such as Stark and Zuo to measure the synchronization of the two hemispheres, by calculating the strength of the functional connection between a voxel and its counterpart in the opposite hemisphere (32). It reflects the synergy of left and right brain signal activities. The higher the VMHC value, the better the balance of functional activity between the cerebral hemispheres. The lower the VMHC value, the worse the balance of functional activity between the cerebral hemispheres. For each subject, the VMHC was computed as the Pearson correlation coefficient between each voxel's residual time series and that of its symmetrical interhemispheric counterpart. Correlation values were then Fisher z-transformed to improve normality. The resultant values constituted the VMHC and were used for group analyses.

RESULTS

Psychological Data Analysis

A total of 22 individuals with IGD (5 females) and 26 RGUs (7 females) participated in our behavior data collection. In the IGD group, mean IAT scores were 59.59 ± 7.58, mean age 14.1 ± 1.8 years old, education years 8.5 ± 1.5 years. In the RGU group, mean IAT scores were 39.05 ± 8.66, average age 13.9 ± 1.5 years old, education years 7.9 ± 1.5 years. There were no statistically significant differences in age, gender, or education level between the two groups ($p > 0.05$).

An independent sample *t*-test was used to compare the psychological data of two groups. The IAT and BIS-11 impulsivity scores of the IGD group on AI, MI, and NI factors were higher than those in the RGU group, and the differences were statistically significant (**Table 1**).

TABLE 1 | Differences between the IGD and RGU groups on the IAT and BIS-11.

Items	IAT M ± SD	BIS-11 M ± SD	AI M ± SD	MI M ± SD	NI M ± SD
IGD ($n = 22$)	59.59 ± 7.58	61.45 ± 7.52	16.18 ± 2.87	20.23 ± 3.37	25.05 ± 3.87
RGU ($n = 26$)	38.42 ± 8.41	51.19 ± 8.44	13.08 ± 2.94	17.46 ± 4.03	20.65 ± 3.94
t	9.09***	4.41***	3.69**	2.55*	3.88***
p	< 0.001	< 0.001	0.001	0.014	< 0.001

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

TABLE 2 | Correlation analysis of IAT and BIS-11.

Items	IAT	BIS-11	AI	MI	NI
IAT	1				
BIS-11	0.42**	1			
AI	0.44**	0.82**	1		
MI	0.25	0.79**	0.52**	1	
NI	0.36*	0.82**	0.54**	0.40	1

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

TABLE 3 | Differences between the IGD and RGU groups in the Stroop task.

Items	Condition	IGD ($n = 22$) M ± SD	RGU ($n = 26$) M ± SD	*t*	*p*
RT (ms)	CC	679.0 ± 105.4	558.1 ± 118.3	3.71**	0.001
	IC	850.6 ± 125.0	740.3 ± 129.6	2.98**	0.005
	Stroop	171.6 ± 73.6	182.2 ± 81.6	−0.47	0.640
Accuracy (%)	CC	96.1 ± 2.0	97.0 ± 1.8	−1.64	0.107
	IC	54.4 ± 21.3	75.0 ± 15.7	−3.85***	< 0.001
	Stroop	−41.7± 20.6	−22.0 ± 15.6	−3.68***	< 0.001

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Correlations Between IAT and BIS-11

IAT scores were positively correlated with BIS-11 total scores on the AI and MI factors (**Table 2**).

Stroop Task Differences Between the IGD and RGU Groups

Compared with the RGU group, the IGD group demonstrated longer reaction times (RTs) on congruent (CC) stimuli. At the same time, the IGD group had longer RT and lower accuracy on incongruent (IC) stimuli. The accuracy of the Stroop effect (IC-CC) exhibited a statistically significant difference between the two groups. However, the RT of the Stroop effect (IC-CC) demonstrated no difference between the two groups (**Table 3**).

Overall comparison found that both groups exhibited a Stroop effect. It can be seen that the accuracy of IC stimulus was lower than CC stimulus ($t_{IGD} = 10.93$, $t_{RGU} = 11.38$, $p < 0.05$) and IC stimulus had longer RT ($t_{IGD} = -9.47$, $t_{RGU} = -7.17$, $p < 0.05$; **Table 3**).

GCA Differences Between the IGD and RGU Groups

In this study, there is no significant difference in anterior cingulate cortex and ventrolateral prefrontal cortex area. We compared connection efficiency between the two groups of the DLPFC and the brain regions related to the prefrontal-striatal circuits. Compared with the RGU group, the dorsal striatum (caudate, putamen) exhibited significant differences in the directed junction values of the left DLPFC (the coordinate of peak, x = 18, y = 27, z = 6, $t = -5.1928$, voxel-level $p < 0.001$, cluster level $p < 0.05$, cluster size = 52). In the RGU group, the dorsal striatum had a positive connection value to the left DLPFC, a one-way positive effect. The activity of the dorsal striatum stimulates the DLPFC. In the IGD group, the connection value was a negative, one-way effect, with dorsal striatum activity inhibiting the left DLPFC (**Figure 1**).

VMHC Between the IGD and RGU Groups

Compared with the RGU group, the VMHC value of the IGD group demonstrated a significant reduction. The descending region was located in the medial orbital frontal lobe/gyrus rectus (the coordinate of peak, x = −6, y = 27, z = −15, $t = -4.34$, $p < 0.001$, cluster size = 89; **Figure 2**).

VMHC values in abnormal brain areas of the two groups were extracted, and it was found that VMHC values in the IGD group were negatively correlated with age ($r = -0.58$, $p < 0.05$). The RGU group did not exhibit this tendency ($r = 0.27$, $p > 0.05$). The correlation between the two groups was significantly different. The VMHC value of IGD patients was negatively correlated with MI ($r = -0.44$, $p < 0.05$; **Figure 3**).

VMHC values in abnormal brain areas of the two groups were extracted, and it was found that VMHC values in the RGU group were negatively correlated with RT on IC conditions ($r = -0.39$, $p < 0.05$). The IGD group did not have this tendency ($r = 0.13$, $p > 0.05$). Under CC condition, VMHC value in RGU group was negatively correlated with RT ($r = -0.39$, $p < 0.05$). The IGD group did not have this tendency ($r = -0.03$, $p > 0.05$; **Figure 4**).

DISCUSSION

Analysis of Psychological Data Results Between the IGD and RGU Groups

In this study, the impulsivity scores of subjects in the IGD group were significantly higher than those in the RGU group, and the total score of the IAT, as a standard for assessing the

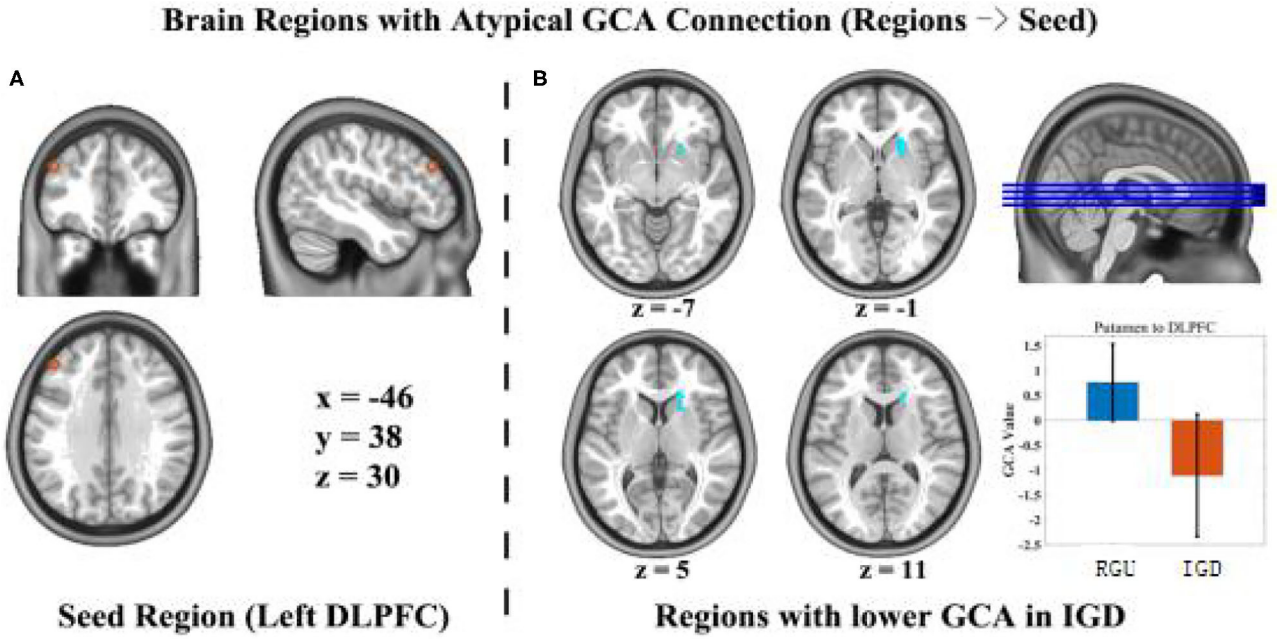

FIGURE 1 | Differences in GCA results between the IGD group and RGU group during resting state. $p < 0.05$ (AlphaSim correction). Voxel size = 3 × 3 × 3 mm^3, the orange circle is the seed point of the DLPFC left-brain position **(A)**. The blue circle represents an efficient connection with the seed point to abnormal brain areas **(B)**.

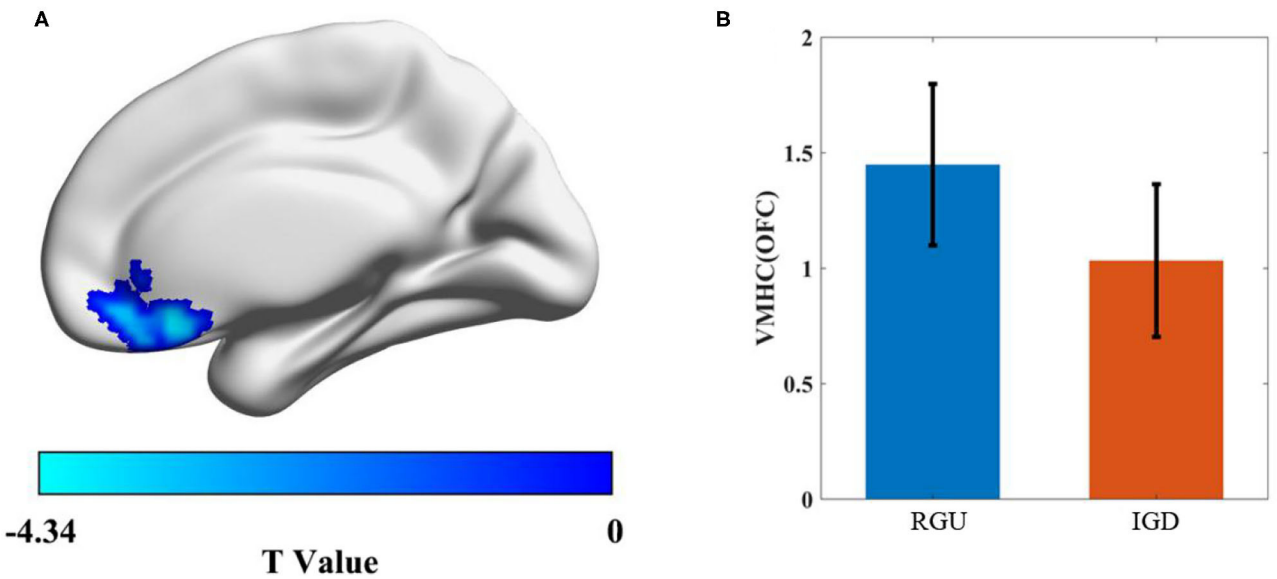

FIGURE 2 | Differences in VMHC value between the IGD and RGU groups durinresting state. $p < 0.05$ (AlphaSim correction). Voxel size = 3 × 3 × 3 mm^3, the blue color represents the VMHC decreased area in the IGD group compared with the RGU group **(A)**. Histogram **(B)** shows the mean and standard deviation of VMHC values in the two groups.

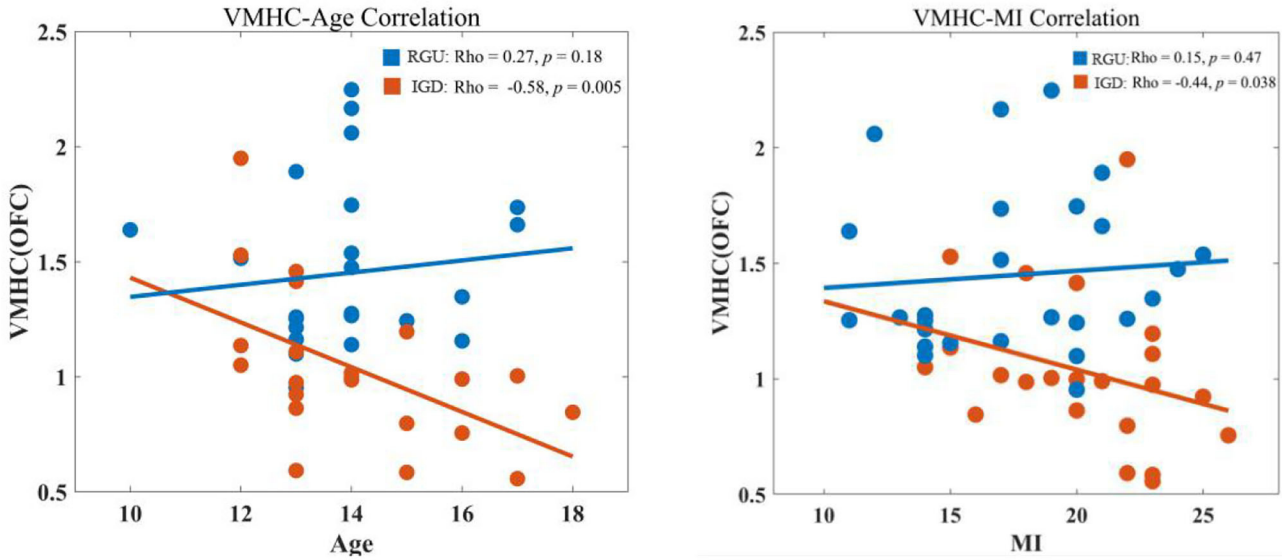

FIGURE 3 | Correlation analysis of VMHC value with age and MI. The left shows the correlation analysis between age and VMHC values of the two groups. The right shows the correlation analysis between the MI and VMHC values of the two groups.

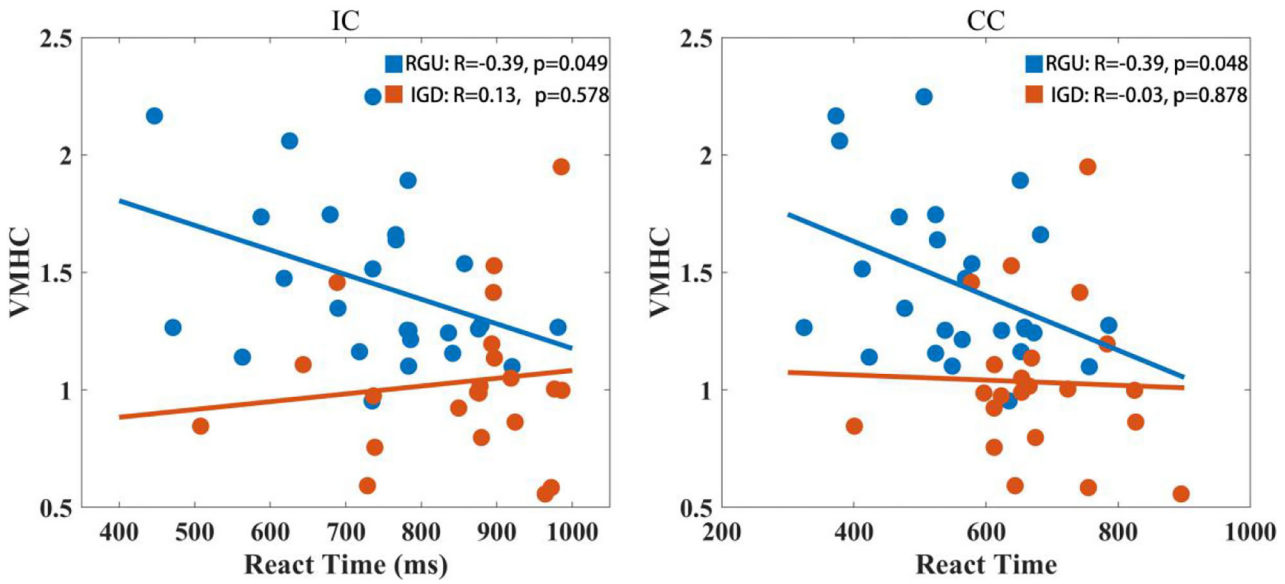

FIGURE 4 | Correlation analysis of VMHC value with RT. The left shows the correlation analysis between RT and VMHC values of the two groups in the IC condition. The right shows the correlation analysis between RT and VMHC values of the two groups in the CC condition.

severity of IGD, was positively correlated with impulsivity scores, which was essentially consistent with previous studies (33, 34). Impulsivity has often been associated with addiction, which may be a risk factor for IGD (34). High impulsivity scores show that the participant's impulse control is damaged (35). The degree of addiction can also affect the level of impulsivity, which results in individuals with IGD making impulsive decisions more easily and without considering the consequences (36). As such, these individuals are unable to overcome the impulsivity of playing online games. Many phenomena indicate that individuals with IGD, as a behavioral addiction, exhibit poor impulsive control.

Analysis of Stroop Task Results Between the IGD and RGU Groups

Comparison of Stroop data showed that the IGD group required significantly longer to react than their RGU counterparts under IC and CC conditions, indicating that the IGD group needed

more time to distinguish font color when performing a color-word task. This tendency shows that response inhibition is impaired in the IGD group. IGD group, under the stimulus of IC, demonstrated a lower accuracy than the RGU group, which indicates that individuals with IGD face double interference. This illustrates that it is difficult for these individuals to overcome conflict and interference, making it easier to make mistakes, when dealing with complex tasks, such as making decisions in life.

Both groups demonstrated significant Stroop effects, but the comparison between the two groups was not statistically significant. Both groups were more likely to make mistakes and perform worse in more complex and conflicting tasks. These results are consistent with previous results of substance addiction research, such as cocaine addiction (37), alcohol addiction (38), and nicotine addiction (39), which provides behavioral evidence for the similarity between IGD and substance addiction.

GCA Results Between the IGD and RGU Groups

Compared with the RGU group, there was a significant difference in connection efficacy between the left DLPFC and the dorsal striatum in the IGD group, which indicates that the prefrontal striatum circuits of adolescents with IGD adolescents were partially damaged. It is speculated that the left DLPFC and dorsal striatum play a more important role in the formation of IGD. Previous research has shown an abnormality in the resting state functional connectivity of the frontal lobe-striatal circuit in a population of individuals who abused drugs (18) and individuals with IGD (19, 20), providing a link between the abnormal function of the connection and cognitive control deficits. The experimental results try to fill the blank about the connectivity between the prefrontal cortex and the striatum adolescents with IGD. The high impulse of the nigrostriatal system affects the normal execution of response inhibition function in the prefrontal cortex. Thus, teenagers with IGD repeatedly may engage in online games.

In normally functioning prefrontal-striatal circuits, the DLPFC is successfully activated during the response inhibition process (30, 40). In the IGD group, the connection efficiency value between the dorsal striatum and the left DLPFC was negative, specifically indicating that dorsal striatum activity suppressed the left DLPFC. The striatum has been previously associated with impulsivity and the reward system (41, 42). Playing online games can activate the striatum (43). This means that, due to striatum activation, activity in the left DLPFC is inhibited, which may cause individuals with IGD to be unable to suppress their impulse to play online games (44). This is more likely to lead to impulsive, prolonged gaming behavior. The effective connection of the dorsal striatum and left DLPFC was a positive value. The activity of the dorsal striatum stimulates the DLPFC, which shows that left the DLPFC would be likely to help the individual to inhibit response processing successfully in the RGU group (45).

Electronic game activity increases striatal dopamine release (15). According to substance addiction studies (16, 46), combined with experimental results, we speculate that online games represent a high-level reward stimulus in individuals with IGD. Dopamine could activate the reward pathway of the striatum in the brain, resulting in insufficient regulation of impulsivity, which may lead to prefrontal lobe executive inhibitory control failure (41). This then will lead to more impulsive behaviors. However, this theory still requires more research.

VMHC Results Between the IGD and RGU Groups

The results of the current study showed that VMHC values in the IGD group were significantly lower than those of the RGU group, which was located in the medial orbitofrontal cortex (mOFC). A previous study (47) compared an IGD group with a healthy control group (the average playing time was <2 h per day). In addition to differences in OFC brain areas, it was found that VMHC values in the middle and superior frontal gyrus were also significantly reduced. However, differences in these brain areas were not observed in this experiment when compared with the RGU group. This indicates that the medial OFC plays a more important role in the IGD process than the superior frontal gyrus and the middle frontal gyrus. The OFC is located in the prefrontal cortex region of the prefrontal striatum circuits, which is involved in the processing of emotion regulation, craving, decision-making, and compulsive behaviors (48). In a study of the VMHC in substance use (32), abnormal OFC synchronization in the left and right hemispheres of the brain was observed in individuals with addiction.

In this experiment, the VMHC value of the mOFC in patients with IGD was significantly negatively correlated with MI, which is an index of motor impulsivity, indicating that adolescent impulsive behavior would affect OFC signal transmission in both hemispheres of the brain. OFC promotes an individual's tendency to goal-oriented behaviors by evaluating the expected results of stimulus and behavior choices. High-impulsivity adolescents with IGD may have impaired OFC assessment function and difficulty in making correct decisions.

In the experiment, the VMHC value of mOFC in the IGD group was negatively correlated with age, while a separate study showed that the duration of heroin use was negatively correlated with the VMHC value of the DLPFC (31). The reason for this may be that older teenagers with IGD may use online games for a longer time, leading to a gradual decline in VMHC value. It may also be that patients with IGD and their parents lack relevant knowledge of IGD, resulting in not seeking timely medical treatment. However, our research group only diagnosed IGD without collecting the disease time of subjects, which will be improved in future studies.

In this study, VMHC values of the mOFC were extracted and correlated with behavioral data obtained in the Stroop color word task. It was found that under the IC and CC conditions, VMHC values of the RGU group were negatively correlated with RT, while those of the IGD group were not correlated. The mOFC is involved in regulating negative emotional states (49). This study speculates that mOFC signal transmission on both sides of the brain might affect emotional processing in the task when RGUs

performed the Stroop task, and that the correlation between emotion and response inhibition promoted response inhibition in RGUs. No such link was found in adolescents with IGD, who exhibited lower response suppression. However, the current research (50–52) only stays at the activation level. The role of the functional connection of the mOFC and the mOFC signal synchronization level on both sides of the brain is still unclear in emotional regulation, which needs to be examined by future empirical research.

LIMITATIONS

Owing to technical reasons, our scanning parameters were outdated, making the research results limited. The scanning time of the resting state fMRI used in this study was <3 min. This might affect the reliability of resting-state fMRI connectivity estimates. Additionally, the sample size in the current study was small. We used G-Power to calculate the sample size and initially planned to recruit 77 subjects. As IGD adolescents were not interested in anything than Internet games and also did not think that IGD was a problem for them. So although many adolescents had IGD, but they were not willing to participate in our study. In this study, some teenagers also were reluctant to cooperate with MRI because of their schoolwork/previous commitments. At the same time, part of the collected data was not available because the patient's head motion did not conform to the later image analysis. Therefore, more subjects should be recruited in the future to improve the examination process and further confirm the conclusions of this study. The cause of abnormal effective connection value and VMHC value needs to be explained in combination with more structural imaging results. In these follow-up studies, diffusion tensor imaging data can be collected to further explain abnormal functional connectivity of homologous brain regions in adolescents with IGD. A prior ROI was used for to determine connection efficacy analysis in our study. If an independent component analysis was used to determine brain regions with differences between the two groups, and the ROI was determined this way, more meaningful connections could be found. Finally, this experiment only included IGD and RGU groups. If future research can include a healthy control group that does not play online games, the comparison of the three groups can better explain the pathogenesis of IGD from the biological point of view. In the future, we will recruit more IGD adolescents through schools and the Internet, and combine with diffusion tensor imaging to further explain the abnormal functional connection between the two hemispheres of IGD adolescents. Next, we will also conduct psychological interventions on IGD patients to further confirm the conclusions of this study through functional brain changes before and after the intervention.

CONCLUSIONS

There were significant differences in IAT and impulsivity scores between the IGD and RGU groups, with the IGD group exhibiting higher scores. This indicates that adolescents with IGD have addiction problems and high impulsivity. At the same time, adolescents with IGD demonstrated longer reaction time, lower accuracy, poor response inhibition, and impaired impulse control in the Stroop task. Compared with the RGU group, the efficacy of connections between the left DLPFC and the dorsal striatum in the IGD group were significantly different. It was speculated that online game activities may stimulate the reward pathway in adolescents with IGD at high levels, leading to the failure of normal response inhibition processing and the occurrence of impulsive behaviors. The VMHC value of the mOFC in the IGD group was significantly lower than that in the RGU group, indicating that the mOFC plays an important role in the prefrontal-striatal circuits. Impaired impulsive behavior and response inhibition in adolescents might affect mOFC signal transmission in both hemispheres of the brain.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of The Guizhou Provincial People's Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

HX designed the study and wrote the protocol. JC, YZ, and XL conducted literature searches and provided summaries of previous research studies and conducted the statistical analysis. RW and CT provided technical support. QZ edited and organized the manuscript. All authors contributed to and have approved the final manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The Evaluation of Cognitive Impairment in Alcohol-Dependent Patients Through RBANS Combined With ERPs

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Background: Recently, the cognitive impairment of patients with alcohol dependence has attracted more and more attention. The combination of Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) and event-related potentials (ERPs) for evaluating the degree of cognitive impairment in patients with alcohol dependence has not undergone enough in-depth investigation.

Method: Sixty patients with alcohol dependence were selected as alcohol-dependence group, whereas 40 healthy volunteers served as a normal control group. The original scores of the RBANS sub-items, the incubation period, and volatility of ERPs between the two groups were compared, and the correlation among the above indicators in the alcohol-dependence group was further analyzed.

Results: The RBANS test showed that the original scores of speech function, attention function, delayed memory, and immediate attention in the alcohol-dependence group were significantly lower than those in the normal control group. Compared with the normal control group, the latencies of P200 and P300 in the alcohol-dependence group were significantly prolonged, and the amplitude of P200 and P300 was significantly reduced. Correlation analysis between RBANS and ERPs in alcohol-dependence group showed that immediate attention score was positively correlated with P300 and P200 amplitude, visual breadth score was positively correlated with P200 latency, and attention function score was negatively correlated with P300 latency.

Conclusion: As RBANS scale was highly correlated with the results of ERPs, the combined use of these two scales may serve as an objective basis for early diagnosis of cognitive impairment in patients with alcohol dependence.

Keywords: alcohol dependence patient, repeatable battery neuropsychological status (RBANS), event-related potentials, cognitive impairment, evaluation

INTRODUCTION

Alcohol is a neurosuppressant that passes through the blood–brain barrier with a neurotoxic effect. Long-term alcohol use disorder (AUD) leads to lower blood flow to the frontal lobe, hippocampus, decreased oxygen metabolism, and reduced volume, which eventually leads to neuronal damage. This is the main cause of behavioral changes and cognitive impairment in clinically dependent patients (1, 2). Alcohol dependence has become one of the most serious public health problems. It was estimated that 50–80% of alcohol-dependent people had a certain degree of cognitive impairment (3), mainly in terms of memory, executive ability, visual spatial tasks, attention, learning ability, etc. (4). The cognitive impairment of alcohol-dependent people has serious adverse effects on the patient's social function and quality of life (5). Early detection of cognitive impairment in patients and early intervention can effectively delay the progression of disease in alcohol-dependent people. Therefore, the early assessment of cognitive impairment in alcohol-dependent people is an important part in controlling the harm caused by alcohol.

Currently, neuropsychological scales, including Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), the Mini-Mental State Examination, Montreal Cognitive Assessment, and functional neuroimaging techniques (6–9), are used for the cognitive function assessment of alcohol-dependent people. Among them, RBANS, which is used to assess neuropsychological state, is sensitive to cognitive state in an equivalent form, taking a short time about 20 to 30 min (10). Originally used as a cognitive screening tool for people with Alzheimer disease, RBANS has been extended to a variety of neurological and mental disorders including AUD samples (11, 12). However, owing to operation of the test subjects, the educational levels, and compliance of the testee, the cognitive function of alcohol-dependent patients assessed by the neuropsychological test scale by far may have certain subjectivity and bias, so it is unable to accurately and effectively reflect the cognitive function of the subjects. Therefore, to carry out a comprehensive quantitative assessment, the use of objective examination for the cognitive function of alcohol-dependent people is the premise of early diagnosis.

With the development of cognitive neuroscience, scholars have applied various objective evaluation tools to reveal the characteristics of neurocognitive impairment in patients with alcohol dependence. Event-related potentials (ERPs) are highly sensitive in identifying neurocognitive impairments even when no behavioral impairment is detected (13). As a more objective and sensitive electrophysiological index, it exhibits the abnormality of brain electrical activity and then reflects the change of cognitive function. Therefore, ERPs have obvious advantages and irreplaceability clinically. The classic (narrow) ERPs are mainly composed of the exogenous components P100, N100, and P200 and the endogenous components N200 and P300 (14, 15). The main measurement indices in cognitive function studies are the latent period and amplitude, which represent the degree of effective resource participation in the brain's perception of input information. Among them, P300 is a representative index to record the cognitive processing process, which provides an important basis for clinical diagnosis and treatment (16). Usually induced by the classical oddball paradigm, P300 reflects complex psychocognitive activities. It is generally accepted that the decrease in the P300 amplitude is a sign of susceptibility to AUD (17). The P300 latent period is an evaluation of the brain's cognitive processing of information, which reflects brain efficiency to a certain extent. However, at clinical level, although ERPs is clearly highly sensitive and predictive, its specificity is poor (18).

In the past, many scholars discussed the cognitive impairment of alcohol dependence via neuropsychological tests and ERPs (19–22), and abnormalities in the ERPs could be a candidate of specific neuropsychological trait marker for AUD people. Although several of them have combined neuropsychological tests with ERP assessment as a comprehensive tool to assess alcohol-dependent cognitive function and to explore the correlation between clinical phenomenology and neuroelectrical physiology, they either did not consider the educational level of cases or did not recruit enough subjects. Specifically, apart from making up for the deficiencies in previous studies, this is the first study using two evaluation tools simultaneously to conduct two-angle testing of cognitive impairment of alcohol dependence in Chinese, aiming to link the clinical characteristics of cognitive impairment with characteristics of neuroelectrical physiology, thus revealing the neuropsychological and electrophysiological mechanisms of cognitive impairment of alcohol dependence and providing evidence-based support for early diagnosis and treatment of alcohol dependence.

In this study, the cognitive impairments of alcohol-dependent patients were evaluated by the combined scale of RBANS and ERPs. We also analyzed the correlation between the two evaluation indicators, which identified whether the RBANS scale and ERPs could be verified with each other and provided an objective basis for early identification and diagnosis of cognitive impairment of alcohol-dependent people.

METHODS

Subjects

Alcohol-dependent patients in the inpatient department of the Psychiatry Department of the Second People's Hospital of Hunan Province from October 2017 to February 2018 were selected. Inclusion criteria were as follows: (1) all cases met the diagnostic criteria for alcohol dependence in the 4th Revision (DSM-IV-TR) of the *Diagnostic and Statistical Manual of Mental Disorders*; (2) those whose education level was junior high school or greater, aged 18–60 years, Han nationality; (3) those who completed acute withdrawal treatment for 7 days with no withdrawal symptoms. Exclusion criteria were as follows: (1) those having past and current history of brain injury, cerebral mental illness and other mental disorders; (2) those having substances other than nicotine dependence prior to entering the group; (3) those having consciousness disorders and delirium; (4) those who could not cooperate with the examination because of severe heart, liver, and kidney dysfunction; (5) pregnant and lactating women; and (6) those who exited the experiment.

The normal controls who met the inclusion criteria were the fixed or temporary employees of the Second People's Hospital of Hunan Province. Inclusion criteria were as follows: (1) those whose education level were junior high school or greater, aged 18–60 years, Han nationality; (2) all subjects had no history of alcohol dependence and a history of mental illness. Exclusion criteria were as follows: (1) those having past and current history of brain injury, cerebral mental illness, and other mental disorders; (2) those having substance other than nicotine dependence prior to entering the group; (3) those having consciousness disorders and delirium; (4) those who could not cooperate with the examination because of severe heart, liver, and kidney dysfunction; (5) pregnant and lactating women; and (6) those who withdrew from the experiment. This study informed both groups of subjects consenting to participate and was approved by the Ethics Committee of the Second People's Hospital of Hunan Province. Self-made general information of these recruited subjects included general demographic characteristics, average daily alcohol consumption, alcohol dependence years, alcohol dependence family history, and the daily amount of smoking.

Written informed consent was obtained from subjects recruited in both the alcohol-dependence group and the health control group, and the clinical trial passed the review of the Ethics Committee of the Second People's Hospital of Hunan Province.

RBANS Scale

The study used RBANS (Randolph 1998 version) (23) with a total of 12 entries, which were digital breadth, coding test, picture naming, word fluency test, graphic reproduction, line positioning, vocabulary learning, story learning, vocabulary recall, vocabulary re-recognition, story recall, and graphical recall. RBANS can evaluate the cognitive level of the normal population and the degree of impairment of patients' cognitive function. The scale is widely used in the study of cognition abroad. This 30 min test is conducted in a quiet and undisturbed environment. Two alcohol addiction and Internet addiction physicians who have experienced the rating scale evaluation training will evaluate the subjects simultaneously. The correlation efficient between two physicians assessing the subjects is 0.93. Among them, a physician is randomly selected to inform the subjects of the instructions for each item. A clear and uniform speech of physician is required to ensure that subjects hear all of the instructions clearly. Two doctors are responsible for recording the original scores of 12 items simultaneously and finally take the mean value of the original scores of the 12 items. Twelve mean values were then transformed into five scale scores consisting of immediate memory, visual breadth, speech function, attention function, and delayed memory as the RBANS score for subjects. All subjects completed the test calmly, and the test will be canceled once abnormal performances, such as dizziness and vomiting, appear in subjects.

ERPs Assays and Its Determination

Both the studies of alcohol-dependence group and the health control group were completed in the soundproof environment of the electrophysiological room. The room temperature was maintained at around 25°C. First, the experimental process was explained to the subjects, and the subjects were asked to take a seat and keep clear-headed and relaxed. Pretests including all the procedures in the formal experiment mentioned below were conducted before each formal examination, and the subjects began the formal experiments after they mastered the requirements of the experiment. In this study, the MEB9200K detector of Japanese optoelectronics was selected for the data recording, which placed the recording electrode on the Cz point. The reference electrode was double earlobe (A1, A2). The front of the head was grounded by the center patch electrode. All the electrode impedance was <5 KΩ. Stimulation was induced in the oddball paradigm. A pseudorandom sequence of deviant stimuli (15%) and standard stimuli (85%) was presented binaurally by a STIM 2 sound generator (Compumedics, El Paso, TX, USA), and 300 stimuli were presented binaurally through earphones. Each stimulus had a duration of 100 ms (10 ms rise and fall times) with uniform intertrial intervals of 1,250 ms. Target stimulation and non-target stimulation were 2,000 Hz, 80 dB of pure sound, and 1,000 Hz, 60 dB of pure sound, respectively. The probability ratio of target stimulation and non-target stimulation was 1/5. The subjects were asked to do the key press reaction responding to the target stimulus. The subjects with response time of <800 ms were averaged and analyzed, and those with response times of more than 800 ms were considered error responses. The analysis index was the incubation period and amplitude of P300 and P200, and the incubation period of N100 and N200 at Cz point. Component peaks were identified as the maximum voltage within the ranges as follows: N100 (maximum negative voltage from auditory tone to 150 ms), N200 (maximum negative voltage from 150 to 350 ms), and P300 (maximum positive voltage from 250 to 600 ms). Curry 7 software (Compumedics) was then used for processing electrophysiological signals offline. Recordings were down-sampled to 250 Hz, and data were then filtered using a referenced frequency of 0.3 to 30 Hz. Data were further segmented into 1,000-ms epochs, which all segments with voltage $> \pm 70\ \mu V$ were automatically discarded.

Statistical Analysis

All data were analyzed by the SPSS Statistics 23.0 statistical software and R project (version 3.6.1, https://www.r-project.org/). The measurement data were represented in $\overline{X} \pm S$. The independent-sample t-test was used to compare RBANS cognitive function score, ERP amplitude, and incubation period between the alcohol-dependent group and the normal group. The correlations between the indicators were analyzed by partial correlation analysis using R package ggm. The visualization of significantly correlated indicators was performed by R package ggplot2 and ggstatsplot. $P < 0.05$ was considered statistically significant. After adjusting by Bonferroni correction in the independent-sample t-test, P-values divided by the number of comparisons were considered statistically significant.

RESULTS

The General Information of Patients

The alcohol-dependence group had 60 male cases, with an average age of 42.33 ± 7.57 years. The control group had 40 male cases, with an average age of 42.03 ± 6.61 years. The alcohol-dependence group had an average age at first drinking of 16.83 ± 2.14 years, an average daily drinking of 217.7 ± 32.63 g/d alcohol, average alcohol dependence years of 11.30 ± 6.94 years, and an average alcohol dependence questionnaire score of 20.13 ± 6.21. Compared with the general data of the alcohol-dependence group and the normal group, the differences between the age, the years of education, and the daily smoking volume group are not statistically significant ($P > 0.05$), as shown in **Table 1**.

Comparison of RBANS Original Score Between the Alcohol Dependence Group and the Normal Control Group

Bonferroni correction was conducted by the number of comparisons. $P < 0.01$ was considered statistically significant. Compared with the normal control, the original scores of speech function, attention function, delayed memory, and immediate attention in the alcohol-dependent group are significantly reduced (**Figure 1A**; $t = -2.918$, $t = -3.426$, $t = -3.822$, $t = -12.928$; $P = 0.004$, 0.001, 0.000, 0.000, respectively); Compared with the normal control, there is no significant difference in the original score of visual breadth in the alcohol-dependent group ($t = -1.895$, $P = 0.054$), as seen in **Table 2**.

The Correlation Between the First Drinking Age, Alcohol Dependence Years, Daily Alcohol Consumption, and RBANS Scores in the Alcohol Dependence Group

The correlation between the first-drinking age, the age at alcohol dependence, and the amount of daily alcohol consumption in the alcohol-dependence group and the RBANS scores are shown in **Table 3**. Notably, items from RBANS scores have no statistically significant correlation with the three alcohol-related factors.

Comparison of ERPs Between the Alcohol Dependence Group and the Normal Control Group

Bonferroni correction was conducted by the number of comparisons. $P < 0.008$ was considered statistically significant. The results show that the incubation period of P300 in the alcohol-dependence group is significantly longer than that of the normal control group (**Figure 1B**; $t = -6.986$, $P = 0.000$). The differences in the incubation periods of N100 and N200 in the two groups are not statistically significant ($t = -0.716$, $t = -1.208$, $P < 0.05$), as seen in **Table 4**. The correlation between the first-drinking age, the age at alcohol dependence, and the amount of daily alcohol consumed in the alcohol-dependence group and the ERPs is shown in **Table 5**.

Correlation Between RBANS Scores and ERPs in the Alcohol Dependence Group

The correlations between RABNS scale scores and the components of ERPs were analyzed in the alcohol-dependence group by partial correlation analysis that has adjusted for confounding covariates, including age, education, age at first drinking, daily drinking, alcohol dependence years, and the daily amount of smoking, as seen in **Tables 6**, **7**. The results show that the visual breadth is positively correlated with the P200 incubation period (**Figure 2A**, $r = 0.278$, $P = 0.043$). The immediate attention in the alcohol-dependence group is positively correlated with the amplitudes of P300 and P200 (**Figures 2B**,**C**; $r = 0.282$, $r = 0.307$; $P = 0.041$, 0.025). The attentional function is negatively correlated with the P300 latent period (**Figure 2D**, $r = -0.338$, $P = 0.013$).

DISCUSSION

In this study, we confirmed that the alcohol-dependence group had significant impairments in immediate attention, attentional function, delayed memory, and speech function. Further, an abnormality of P300 in alcohol-dependent people was observed, which was characterized by an extended incubation period and a decrease in amplitude. A significantly positive

TABLE 1 | General demographic data and alcohol use in the alcohol-dependence group and the normal control group.

	Alcohol-dependence group ($n = 60$)	Normal control group ($n = 40$)	*F*	*p*
Age (year)	42.33 ± 7.57	42.03 ± 6.61	0.044	0.834
Education (year)	9.00 ± 1.85	9.58 ± 2.11	2.058	0.155
Age at first drinking (year)	16.83 ± 2.14	—	—	—
Daily drinking converted into pure alcohol (g/d)	217.7 ± 32.63	—	—	—
Alcohol dependence years (year)	11.30 ± 6.94	—	—	—
Alcohol dependence questionnaire score (#)	20.13 ± 6.21	—	—	—
The daily amount of smoking (stick/d)	22.28 ± 10.34	21.03 ± 9.68	0.355	0.553

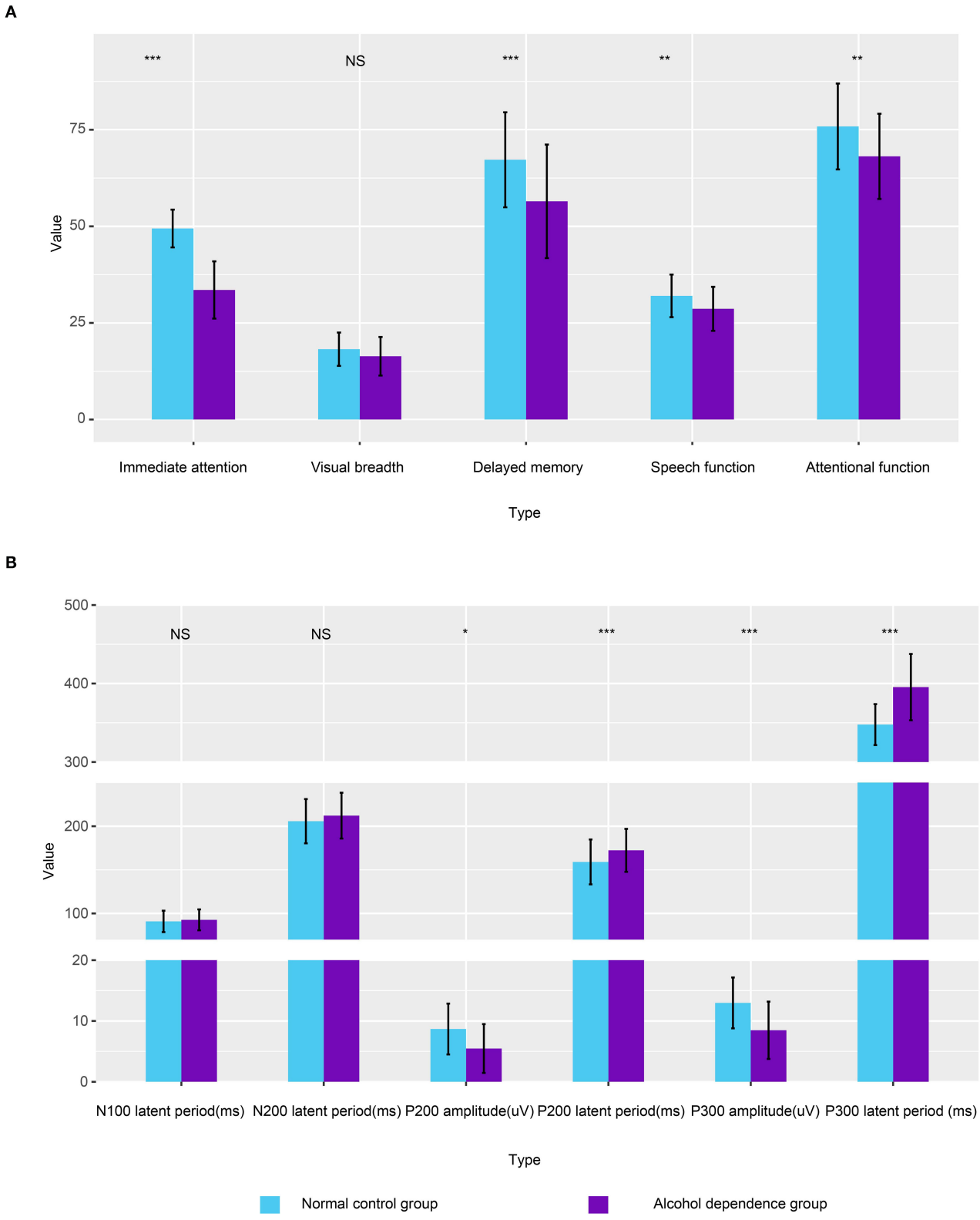

FIGURE 1 | Column plot depicting the differences of alcohol-dependence group and the normal control group. **(A)** The correlation between the first-drinking age, alcohol dependence years, daily alcohol consumption, and RBANS scores in the alcohol-dependence group. **(B)** Comparison of ERPs between the alcohol-dependence group and the normal control group. Error bars refer to the SEM of each variable.

TABLE 2 | Comparison of the original score in RBANS (mean ± SD).

	Alcohol-dependence group	Normal control group	*t*	*p*
Visual breadth	16.37 ± 5.00	18.20 ± 4.31	−1.895	0.054
Speech function	28.65 ± 5.70*	32.00 ± 5.51	−2.918	0.004
Attentional function	68.12 ± 11.02*	75.85 ± 11.12	−3.426	0.001
Delayed memory	56.47 ± 14.69*	67.23 ± 12.30	−3.822	0.000
Immediate attention	33.53 ± 7.41*	49.43 ± 4.89	−12.928	0.000

*$p < 0.01$.

TABLE 3 | Analysis of the correlation between the first-drinking age, year of alcohol dependence, daily alcohol consumption, and RBANS scores in the alcohol-dependence group.

	First-drinking age	Year of alcohol dependence	Daily alcohol consumption
Visual breadth	−0.031	0.054	0.020
Speech function	0.079	−0.108	0.004
Attentional function	−0.019	0.189	0.119
Delayed memory	−0.145	−0.154	−0.056
Immediate attention	0.009	0.069	0.224

*$p < 0.05$.

TABLE 4 | Comparison of event-related potentials between the alcohol-dependence group and normal control groups (mean ± SD).

	Alcohol-dependence group	Normal control groups	*t*	*p*
N100 latent period (ms)	92.62 ± 11.98	90.85 ± 12.26	0.716	0.476
N200 latent period (ms)	212.12 ± 26.29	205.73 ± 25.34	1.208	0.230
P200 latent period (ms)	172.30 ± 24.64*	159.03 ± 25.73	2.593	0.010
P200 amplitude (μV)	5.47 ± 4.01*	8.68 ± 4.18	−3.853	0.000
P300 latent period (ms)	395.43 ± 42.25*	347.68 ± 26.06	6.986	0.000
P300 amplitude (μV)	8.47 ± 4.71*	12.97 ± 4.18	−5.012	0.000

*$p < 0.008$.

TABLE 5 | Analysis of the correlation between the first-drinking age, year of alcohol dependence, daily alcohol consumption, and ERPs in the alcohol-dependence group.

	First-drinking age	Year of alcohol dependence	Daily alcohol consumption
N100 latent period (ms)	0.197	0.204	−0.181
N200 latent period (ms)	−0.051	−0.033	−0.004
P200 latent period (ms)	−0.159	−0.049	−0.050
P200 amplitude (μV)	−0.092	−0.051	0.066
P300 latent period (ms)	−0.108	−0.036	−0.075
P300 amplitude (μV)	−0.113	−0.117	0.031

*$p < 0.05$.

correlation was finally obtained between items in ERPs and RBANS scale, indicating the interconnection between these two assessment tools.

Previous studies have already shown that alcohol impaired an individual's attention function, creating a "short-sighted" effect, as a result of drinkers being able to pay only limited attention to some of the clues (9). In addition, under the influence of impaired attention ability, the activating and monitoring balance system of drinkers was disrupted, which further affected their cognitive and behavioral abilities (7, 24). Moreover, some scholars found that alcohol-dependent people could not completely reverse their impaired cognitive function even after temperance (25, 26). These studies supported that alcohol dependents may have multidisciplinary cognitive impairments. Our study provided further evidence that alcohol-dependent people had significant impairments in immediate attention, attention function, delayed memory, and speech function, indicating that the severity of cognitive impairment in alcohol dependents may be involved in language, attention function, and other cognitive fields.

Further, previous studies supported that the incubation period of P300 of patients with alcohol dependence was extended, and amplitude of P300 was reduced, compared to healthier people, indicating that their information-processing capacities were worse, and their cognitive functions were decreased compared with that of healthy subjects (27, 28), which was consistent with our findings. These findings suggest that auditory P300 could serve as a trait marker for alcohol dependents. Moreover, Park et al. showed that the P300 amplitude in the parietal area and the central region of the alcohol-dependence group was lower than that of the normal group (29). Restricted to the experimental design in this study, future research is expected to locate the different regions of subjects to improve the reliability and comprehensiveness of results.

The study also found no significant difference in the incubation period of N200 in the alcohol-dependent group compared to the normal group, which was consistent with Crego's report (30). However, there was an opposite view that the incubation period of N200 was prolonged in alcohol dependents. Maurage et al. compared ERPs in binge drinkers and normal subjects, showing no significant difference in baseline values

TABLE 6 | Correlation analysis between RBANS scores and ERPs in alcohol-dependence group (*r*).

	P300 latent period	P300 amplitude	P200 latent period	P200 amplitude	N100 latent period	N200 latent period
Immediate attention	−0.204	0.282*	−0.175	0.307*	−0.107	−0.151
Visual breadth	−0.006	−0.079	0.278*	−0.144	−0.248	0.248
Speech function	0.130	−0.139	0.046	−0.113	−0.004	0.110
Attentional function	−0.338*	0.077	−0.098	0.135	0.206	−0.103
Delayed memory	−0.001	0.004	0.217	0.002	−0.226	0.175

*$p < 0.05$.

TABLE 7 | Correlation analysis between RBANS scores and ERPs in alcohol-dependence group (*p*).

	P300 latent period	P300 amplitude	P200 latent period	P200 amplitude	N100 latent period	N200 latent period
Immediate attention	0.143	0.041*	0.211	0.025*	0.444	0.280
Visual breadth	0.966	0.576	0.043*	0.304	0.073	0.073
Speech function	0.355	0.321	0.741	0.420	0.978	0.430
Attentional function	0.585	0.013*	0.337	0.484	0.464	0.139
Delayed memory	0.978	0.994	0.990	0.119	0.210	0.103

*$p < 0.05$.

for the latency periods of N200, P300, and P100 in binge drinkers, but a significant increase in the incubation period when measured again 9 months later. It was believed that the latency changes caused by severe AUD in a short period of time were similar to alcohol dependence, reflecting a pathological slowdown in information processing (31). In other words, the body intake of a large amount of alcohol in a short period of time may induce obvious brain dysfunction.

Although many previous studies on the cognitive function of alcohol dependence have similar and robust conclusions about P300 and N200, there were few reports on N100 and P200 in alcohol dependence. Considered an exogenous component, N100 occurs without effortful task demands. N100 is positioned in the primary auditory cortex and modulated by attention, as a reliable index to evaluate the subjects' selective attention and working memory (32). P200 may be produced in the primary and secondary auditory cortex, mainly reflecting the early stages of perceptual processing (33). Our results showed that there was no significant difference between the incubation period of N100 component between the alcohol-dependence group and the normal group, but a significant difference between the incubation period and the amplitude of P200. These results suggested that the P200-related sensory cortex of alcohol-dependent people may be impaired, leading to impaired perception and attention function in patients.

Our study, first, combined the RBANS scale with ERPs to evaluate cognitive impairment in alcohol-dependent Chinese. The correlation analysis showed that the incubation period of P300 in alcohol-dependent group was negatively correlated with attention function, and amplitudes of P300 and P200 were positively correlated with immediate attention score. Previous study has reported a decrease in visual N100 amplitudes in individuals with AUD (34). Consistently, our finding indicated that the incubation period of N100 was positively correlated with visual breadth. These results showed that with the increased amplitude of P300 and P200, the immediate attention function of alcohol dependents was aggravated. With the extended incubation period of P300 and P200, attention function and visual breadth were gradually alleviated and aggravated, respectively. Notably, Kim and Lee (35) observed that in 25 alcohol-dependent patients the incubation period of P300 was prolonged and the cognitive ability screening test score decreased, in line with our results (35). This suggested that the latency period of P200, P300, and amplitude of P300 had good consistency in regard to the scale scoring results, verifying each other on cognitive function tests.

Although our study was not yet certain whether ERPs were directly involved in the cognitive process, based on the correlation between ERPs and RBANS scores, ERPs with multicomponent had its unique advantages in comprehensively understanding the electrophysiological characteristics in cognitive impairment among alcohol-dependent people, providing a relatively objective criteria for the diagnosis of cognitive impairment in alcohol dependents. However, there are also several limitations to this study. One major limitation is that the study included only male participants, which limits the generalizability of the results. Another limitation is that this study is a cross-sectional study without longitudinal observation of alcohol dependents. Moreover, the alcohol-dependence group and normal control group are not strictly intelligence quotient

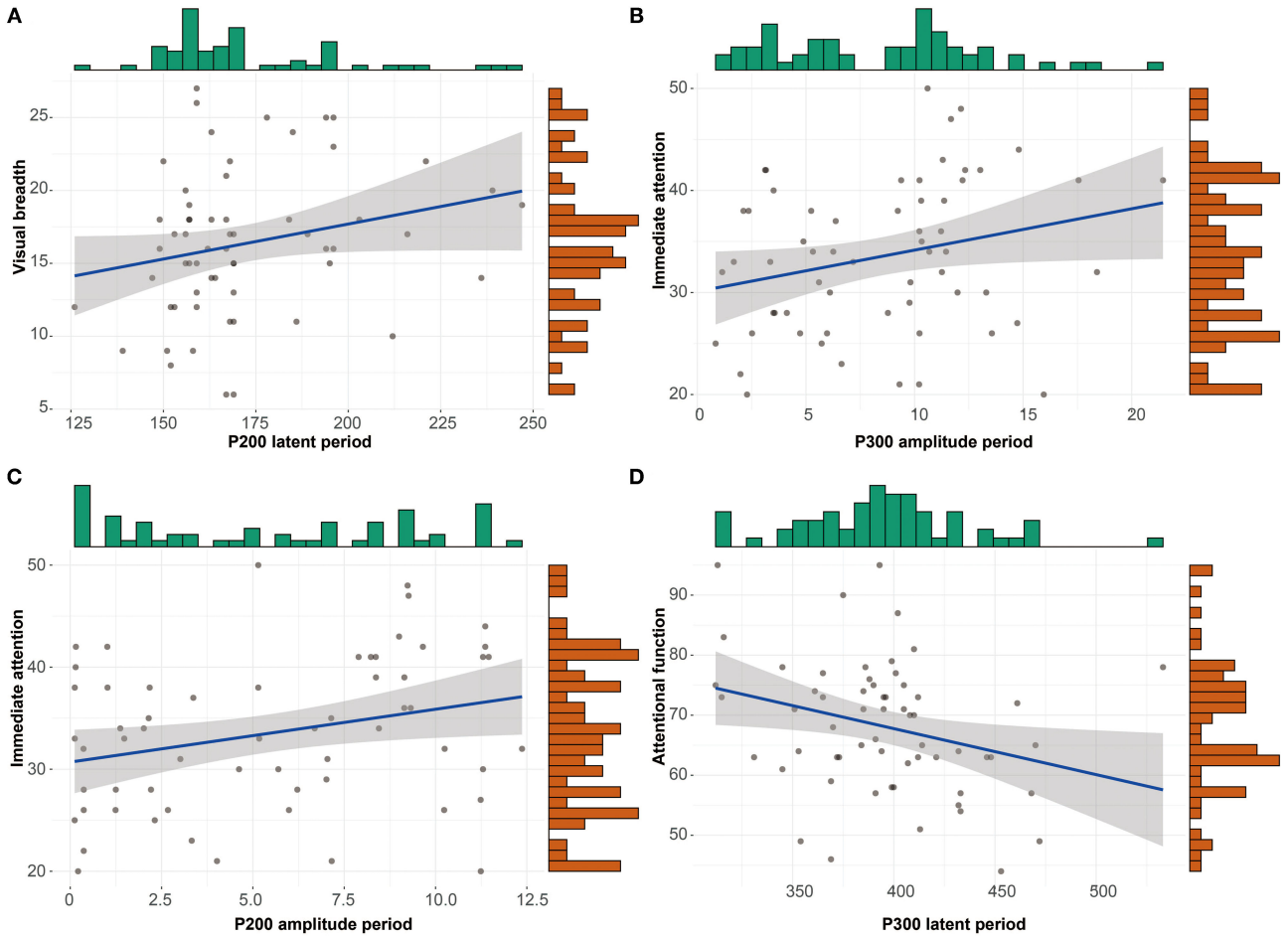

FIGURE 2 | Scattering plot depicting the correlation between items in RBANS and items in ERPs with statistical significance. **(A)** Correlation between visual breadth and P200 latent period. **(B)** Correlation between immediate attention and P300 amplitude period. **(C)** Correlation between immediate attention and P200 amplitude period. **(D)** Correlation between attentional function and P300 latent period.

(IQ) linked, and education year is insufficient to represent the IQ level of subjects that could potentially influence the accuracy of results. Recently, imaging is of great significance to the prevention, diagnosis, and treatment of cognitive impairment in alcohol dependents. But there are no imaging data used for the assessment of cognitive function in this study, and it needs to be further strengthened.

In summary, alcohol dependents have different degrees of cognitive impairment, which was characterized by immediate memory, attention function, delayed memory, and speech function. Using ERPs, alcohol-dependent cognitive impairment was characterized by an extended incubation period of P200, P300, and a decrease in amplitude. This study verified that ERPs and RBANS scales had good consistency in the evaluation of cognitive impairment, such that ERPs may be used as an objective and reliable neuroelectrophysiological index to assess cognitive impairment in alcohol-dependent people. Combined scale of RBANS and ERPs provided an objective basis for early diagnosis of alcohol dependence cognitive impairment.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

This study was approved by the Ethics Committee of the Second People's Hospital of Hunan Province. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XuZ and HC conceptualized and designed the research. ZL, WZ, MZ, and XL prepared the assessment tools. JX, JY, and XC performed the experiments. CH, SH, and XuZ undertook the statistical analysis. HC and XuZ wrote the first draft of the

manuscript and contributed to the final manuscript. All authors critically reviewed the content, approved the final version for publication, and made substantial contributions to this study.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Functional Connectivity of Nucleus Accumbens and Medial Prefrontal Cortex With Other Brain Regions During Early-Abstinence Is Associated With Alcohol Dependence and Relapse: A Resting-Functional Magnetic Resonance Imaging Study

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Background: Alcohol dependence (AD) is a chronic recurrent brain disease that causes a heavy disease burden worldwide, partly due to high relapse rates after detoxification. Verified biomarkers are not available for AD and its relapse, although the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC) may play important roles in the mechanism of addiction. This study investigated AD- and relapse-associated functional connectivity (FC) of the NAc and mPFC with other brain regions during early abstinence.

Methods: Sixty-eight hospitalized early-abstinence AD male patients and 68 age- and education-matched healthy controls (HCs) underwent resting-functional magnetic resonance imaging (r-fMRI). Using the NAc and mPFC as seeds, we calculated changes in FC between the seeds and other brain regions. Over a follow-up period of 6 months, patients were measured with the Alcohol Use Disorder Identification Test (AUDIT) scale to identify relapse outcomes (AUDIT $\geq$ 8).

Results: Thirty-five (52.24%) of the AD patients relapsed during the follow-up period. AD displayed lower FC of the left fusiform, bilateral temporal superior and right postcentral regions with the NAc and lower FC of the right temporal inferior, bilateral temporal superior, and left cingulate anterior regions with the mPFC compared to controls. Among these FC changes, lower FC between the NAc and left fusiform, lower FC between the mPFC and left cingulate anterior cortex, and smoking status were independently associated with AD. Subjects in relapse exhibited lower FC of the right cingulate anterior cortex with NAc and of the left calcarine sulcus with mPFC compared to non-relapsed subjects; both of these reductions in FC independently predicted relapse. Additionally,

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FC between the mPFC and right frontal superior gyrus, as well as years of education, independently predicted relapse severity.

Conclusion: This study found that values of FC between selected seeds (i.e., the NAc and the mPFC) and some other reward- and/or impulse-control-related brain regions were associated with AD and relapse; these FC values could be potential biomarkers of AD or for prediction of relapse. These findings may help to guide further research on the neurobiology of AD and other addictive disorders.

Keywords: alcohol dependence, relapse, relapse severity, predictor, rest-functional magnetic resonance imaging, functional connectivity

INTRODUCTION

Alcohol use disorder (AUD) is a major burden on public health. It not only contributes to physical diseases and mental disorders but also increases the incidence of traffic accidents and impulsive violence (1). Approximately 240 million adult people worldwide are estimated to suffer from AUD (2). Globally, alcohol may cause 5.3% of all deaths and 5.1% of disability-adjusted life years (DALYs) lost (3), and is the 3rd highest risk factor of disease and 7th highest risk factor when considering both deaths and DALYs together (4). According to the criteria of the DSM-IV, AUD may be categorized as either alcohol dependence (AD) or alcohol abuse. A systematic review showed that for males, the overall lifetime and current prevalence of AD in mainland China were 4.7 and 4.4%, respectively; for females, the corresponding data were below 0.1% (5). One of the main reasons for its large disease burden worldwide is that AD is a chronic encephalopathy with a high relapse rate (6). Prospective studies indicated that the relapse of AD is high (i.e., higher than 60% in most studies) (7, 8).

Consequently, there is an urgent need to explore the predictive factors for development and progression of AD, including pathogenesis and relapse. However, most studies on the diagnosis and prognosis of AD have focused on clinical features, such as drinking history, clinical symptoms, and compliance with treatment (9–11). These studies documented inconsistent evidence and did not adequately clarify the neurobiological etiology of AD and its relapse.

The rapid development of the non-invasive technique of magnetic resonance imaging (MRI), with associated advantages of safety, and high resolution, has provided an excellent window for studying brain mechanisms of mental disorders *in vivo*, including addiction (12, 13). When neuroimaging studies began, most morphometric studies observed that the structure of gray and white matter had abnormal changes in subject with AD (14). Meta-analysis results indicated, among all gray matter damage, a significant reduction in corticostriatal-limbic loops, such as the striatum, dorsal lateral prefrontal cortex, and anterior cingulate cortex. Gray matter reduction in nucleus accumbens (NAc) region was negatively associated with the duration of AD, while shrinkage of mPFC regions was related to lifetime drinking consumption (15). Some studies have gradually focused on functional MRI (fMRI) studies. For example, Cservenka et al. (16) reported that youth with a family history of alcoholism differed from healthy controls (HCs) with respect to functional connectivity (FC) between NAc and medial prefrontal cortex (mPFC) (16). Wrase et al. observed lower activation of the NAc region in the execution of a monetary gain task and increased activation in alcohol-related cues among AD patients but not HCs (17). These cross-sectional neuroimaging studies documented evidence that both structural and functional changes in some brain regions, especially in regions of frontostriatal circuits (including the NAc and mPFC), are associated with AD. Some reviews of the mechanisms of addiction in neuroimaging, including AD, suggested that the NAc and mPFC may play a key role in adjusting the balance between goal-directed, craving, habit and, compulsion on the macroscale level (18). Meanwhile, on the mesoscale or microscale levels of tracing neurons and synapses, alterations of corticostriatal-limbic systems, including mesolimbic glutamate, dopamine, and gamma-amino-butyric acid (GABA) pathways, are associated with addictive behavior (19). Disrupted function of reward circuity is proposed to be a key mechanism of AD and other forms of addiction (20). It is widely accepted that the NAc plays a crucial role in the reward circuit (21). With the development of addiction, changes in the reward pathway are shifted from reward-driven behavior at the early stage to loss of reward at the later stage (22). Meanwhile, other models of addiction suggested that substance use is initially goal-directed behavior, aiming at improving sleep, regulating emotion or prompting relationships with others, and finally develops into habitual or compulsive behavior (23). The changes from goal-directed to compulsive behavior may result from the structure or function of the brain after long-lasting drug use. The NAc and mPFC have been regarded as crucial roles in the reward-learning-execution pathway (24).

Relatively few studies have investigated brain functional connectivity patterns to predict future relapse. Beck et al. found, for the alcohol-cue stimuli task in relapsers compared with abstainers, lower FC between the midbrain and the left mPFC (25). Cope et al. used a survey to show that higher activation in the NAC could predispose individuals to earlier substance use during 3 years follow-up (26). Camchong et al. suggested that, compared to abstainers, diminished resting-state synchrony of relapsers in execution of the Go-No-go task between the executive control networks, reward circuit and visual network may predict relapse after a 6-month follow-up period (27). These

longitudinal studies mainly focused on task-status fMRI and determined the importance of the NAc and mPFC to relapse prognoses, while some researchers indicated that alcohol craving not only appears in response to alcohol-cue stimuli but also occurs in a resting state; thus, further study of the changes in rest-fMRI (r-fMRI) is needed (28, 29).

Considering the importance of the NAc and mPFC in the reward circuit and in the cognitive execution function of addiction, based on prior evidence implicating the NAc and mPFC as regions of interest (ROIs) or seeds, this study aimed to investigate the AD- and relapse-associated FC changes with other brain regions during early abstinence using a 6-month follow-up longitudinal design.

MATERIALS AND METHODS

This longitudinal cohort study was conducted in the mental health center of a general hospital from September 2015 to September 2019 and approved by the Ethics Committee of West China Hospital of Sichuan University in 2016 (NO.22). Informed written consent was obtained from every participant in this study.

Participants

One hundred thirty-six male participants (68 alcohol-dependent patients and 68 HCs) enrolled in this study. All participants were all right-handed and of Han nationality. All recruited patients experienced hospitalized detoxification and were in the early stage of abstinence. Each patient met DSM-IV criteria of AD according to the Structured Clinical Interview for DSM-IV Axis I Disorders-Patient Edition (SCID-I/P) administered by psychiatric clinicians (30). During screening, participants provided information about their past, current, and recent drinking, including consumption quantity and frequency. Exclusion criteria for AD cases in this study were a history of neurological illness (or trauma) and a history of past or present substance use disorders except alcohol or tobacco dependence, intelligence quotient < 70, age of <18 years and or more than 55 years. Matched health control subjects in age and education were also recruited from the general community. HCs were assessed and selected with the diagnostic interview of DSM-IV Axis I Disorders-Nonpatient Edition (SCID-NP). The exclusion criteria for controls were psychotic disorders, emotional disorders, substance-use disorders except tobacco dependence, or having a first-degree relative with psychotic disorders.

Baseline Measurements

A series of self-conducted questionnaires were used to collect demographic information (including age, education years, and smoking status) and clinical characteristics [including data related to alcohol use (including the age of the participant's first drink and years of alcohol use)].

Alcohol use in recent years was measured using a Chinese version of the Alcohol Use Disorder Identification Test (AUDIT). The AUDIT is a short screening tool consisting of 10 questions (total score ranged from 0 to 40) to estimate severity of alcohol consumption in the preceding year (31, 32). Different language versions (including a Chinese version) have been validated worldwide (33).

MRI scans were acquired during early abstinence (5–12 days after stopping drinking) using a 3.0 T (Achieva; Philips, Amsterdam, the Netherlands) scanner equipped with an *Invivo* HD eight-channel high-resolution head coil. Foam padding and earplugs were used to minimize head movement and scanner noise. During examination, participants were instructed to rest quietly with their eyes closed and to keep their minds blank without falling asleep (confirmed by participants immediately after finishing MRI scans).

Approximately 8 min of resting-state echo-planar imaging (EPI) scans were acquired for each participant with 38 axial slices and 240 volumes (TR = 2.0 s, TE = 3.0 ms; flip angle = 90°; matrix 256 × 256; FOV 24 × 24 cm^2; and voxel size = 3.75 × 3.75 × 4 mm^3). High-resolution T1-weighted anatomical images with 188 contiguous axial 1 mm thick slices were also acquired (TR = 8.1 ms, TE = 3.7 ms; flip angle = 7°; matrix 256 × 256; and FOV 25.6 × 25.6 cm^2).

Outcomes

AD patients were regularly followed up at 1, 3, and 6 months after the baseline survey. During the follow-up interview, all participants were asked "Have you had alcohol again since the latest discharge from the detoxification hospital?" Subjects who replied "yes" were further interviewed for their time (months) of the first drink after discharge and alcohol consumption of the most severe month after discharge using a revised Chinese 1-month version of AUDIT (34). To assess the relapse severity in the month with the heaviest drinking after the baseline or after the last follow-up interview, the timeframe of the scale was revised from 1 year to 1 month. According to previous studies on the optimal cutoff of the AUDIT score to screen for alcohol use disorders (31, 33), previously detoxified patients were categorized into the relapse group if the total AUDIT score was ≥ 8 in any follow-up interviews, and other patients were included in the non-relapse group.

Imaging Data Acquisition

Functional Connectivity Analyses

Baseline imaging data were preprocessed by the Data Processing Assistant for Resting-State fMRI (DPARSF) (35) software toolbox in MATLAB (MathWorks, Natick, MA, USA). After removing the first ten time points, the data were slice-time corrected, realigned and normalized to the Montreal Neurological Institute (MNI) template and resampled to 3 × 3 × 3 mm^3. Nuisance covariates containing Friston-24 motion parameters, white matter and cerebrospinal fluid (CSF) were regressed out (36). The residual time series underwent bandpass filtering within a frequency range of 0.01–0.10 Hz (37), which reflected mainly neuronal oscillations and could eliminate high-frequency physiological noise. Data were smoothed with a Gaussian kernel (full-width half-maximum: 6 6 6 mm). Head motion parameters of all images were <1.5 mm, and the rotation was $<1.5°$. No significant differences between groups were calculated for framewise displacement, in line with Power et al. (38). FC is one type of processed r-fMRI data that can be used to examine the

correlation between a seed voxel and other voxels in the whole brain. The NAc (Talairach coordinates x = 0, y = 10, z = −10, radius = 10 mm) (39) and mPFC (Talairach coordinates x = 0, y = 42, z = 18, r = 20 mm) (40) were defined as seeds to explore FC changes in relation to the rest of the whole brain in a voxel-wise manner.

Statistical Analyses

Imaging Data Comparison

Two sample *t*-tests contained in DPARSF were used to estimate the differences in baseline FC values between alcohol-dependent patients and HCs, and between the relapse and the non-relapse group, respectively. The confounding effects of age, education years, and smoking status were adjusted as covariates. Additionally, to identify the association between FC and the symptom severity of relapse (AUDIT scores) in the relapse group, a series of partial correlation analyses was conducted in DPARSF by adjusting for age, education years, and smoking status. Statistical inferences were made with $p < 0.001$ for multiple comparisons at the voxel level, adjusted using cluster-level threshold familywise error (FWE) based on Gaussian Random Field; $p < 0.05$ indicated statistical significance for bidirectional (i.e., positive and negative) explorations.

Logistic Regression

Each participant's baseline FC values that were significantly different between patients with AD and controls or between relapse- and non-relapse category participants, were extracted for further logistic regression to determine FC indictors independently associated with AD or independently predictive of relapse. Binary logistic regression (forward: conditional) was performed using demographic data including age and education years, clinical characteristics (including smoking status and, for relapse category participants, baseline AUDIT scores), as well as extracted FC values as independent variables. To represent the classifying or predictive power of the regression models and each FC independently associated with AD or relapse, the area under the receiver operating characteristic (ROC) curve (AUC) was employed in the next steps.

Linear Regression

Each patient's baseline FC values, which were significantly correlated with the severity of relapse in partial correlation analysis in the relapse group, were extracted for further linear regression to determine FC indictors independently associated with the severity of relapse. Multivariate linear regression (stepwise) was conducted using demographic data (age and education years), clinical characteristics (smoking status and baseline AUDIT scores) and the correlated FC values as independent variables.

The rates (95% confidence intervals, 95% CI) and means (±standard deviation, SD) of demographic variables and outcomes were used to derive descriptive statistics. Student's *t*-tests for quantitative data and χ^2 tests for categorical data were used to calculate differences between groups. Regression analyses outlined above were conducted in SPSS 25.0 (IBM Corp., Armonk, NY, USA) using a two-tailed α level criterion of 0.05.

RESULTS

Sample Characteristics

The sample characteristics are illustrated by the data displayed in **Table 1**. Age (AD group: 39.97 ± 9.00 years; HC group: 38.03 ± 9.53 years) and education years (AD group: 12.81 ± 3.42; HC group: 13.91 ± 3.54) were equivalent between AD and HC groups. The rate of smoking among AD cases (91.04%) was higher than that of HC (38.24%) ($\chi^2 = 41.73$; $P < 0.001$). Possible confounding effects of age, education years, and smoking status were adjusted as covariates. Mean first drinking age and drinking years of AD were 19.65 ± 5.40 and 20.79 ± 8.71 years. Among

TABLE 1 | Sample demographics and their clinical characteristics based on baseline- and follow-up surveys.

	AD[a] ($n = 68$) Mean ± SD[e]	HC[b] ($n = 68$) Mean ± SD[e]	t^f/χ^{2g}	Relapse[c] ($n = 35$) Mean ± SD[e]	Non-relapse[d] ($n = 32$) Mean ± SD[e]	t^f/χ^{2g}
Demographic variables						
Age (years)	39.97 ± 9.00	38.03 ± 9.53	−1.22	40.97 ± 9.18	38.97 ± 8.95	0.90
Education years	12.81 ± 3.42	13.91 ± 3.54	1.85	12.51 ± 3.41	13.09 ± 3.51	−0.69
Smoking status (%)	62 (91.04%)	26 (38.24%)	41.73***	31 (88.57%)	30 (93.75%)	0.55
Clinical characteristics						
First drinking age	19.65 ± 5.40			18.71 ± 5.16	20.88 ± 5.45	−1.67
Years of drinking	20.79 ± 8.71			22.36 ± 8.79	18.50 ± 8.38	−1.51
Family history of alcohol drinking (%)	15 (22.06%)			7 (20.00%)	8 (25.00%)	0.24
Consumption in the last month (g)	158.92 ± 70.91			168.40 ± 65.45	144.24 ± 77.67	−0.27
Consumption at the last drinking period (g)	95.11 ± 66.58			22.17 ± 3.62	23.54 ± 3.30	−1.81
AUDIT[h] score based on baseline survey	28.13 ± 8.22			28.36 ± 8.01	27.96 ± 8.77	−1.85
AUDIT score based on follow-up survey	–			25.26 ± 10.23	0.44 ± 1.34	13.62***

*[a]AD: alcohol dependence; [b]HC: healthy controls; [c]Relapse: those who relapsed among alcohol-dependent individuals and had AUDIT scores ≥ 8 at the follow-up survey; [d]Non-relapse: those who did not relapse among alcohol-dependent individuals and had AUDIT scores < 8 at the follow-up survey. [e]SD: standard deviation; [f]t: values of student's t-test; [g]χ^2: values of Chi-square test; [h]AUDIT: Alcohol Use Disorder Identification Test. ***$P < 0.001$.*

AD patients 15 individuals (22.06%) had a positive family history of drinking. The mean AUDIT score of patients with AD at the baseline survey was 28.13 ± 8.22. Based on the 6-month follow-up survey, 35 patients (52.24%) relapsed. Among 32 non-relapsers, there were 28 cases with AUDIT scores of 0 and 4 cases with AUDIT score of 1–6. No significant differences were estimated in age, education years, smoking status or the clinical characteristics mentioned above, between the relapse and non-relapse groups. As expected, the most severe AUDIT score at the follow-up survey was higher in the relapse group than observed in the non-relapse group.

FC Associated With AD

Compared to HCs, the FC values of seeds of the NAc with the left fusiform ($t = -5.27$, x = −33, y = −39, z = −24, $P_{FWE} < 0.001$), bilateral temporal superior gyrus ($t = -4.53$, x = 36, y = −9, z = 3, and $t = -4.76$, x = −54, y = −24, z = 15, $P_{FWE} < 0.001$) and right postcentral ($t = -5.07$, x = 9, y = −24, z = 48, $P_{FWE} < 0.001$) (**Figure 1A**; **Supplementary Table 1**), and the FCs of seeds of the mPFC with the right temporal inferior ($t = -4.62$, x = 45, y = −45, z = −3, $P_{FWE} < 0.001$), bilateral temporal superior gyrus ($t = -5.37$, x = −66, y = −30, z = 9 and $t = -5$, z = 51, y = −30, z = 24, $P_{FWE} < 0.001$) and left cingulum anterior ($t = -5.46$, x = 3, y = −12, z = 33, $P_{FWE} < 0.001$) of the AD group lower significantly (**Figure 1B**; **Supplementary Table 2**). Logistic regression revealed lower FC between the NAc and left fusiform cortex. In addition, lower FC was evident between mPFC and left cingulate anterior cortex, and smoking status - both were independently associated with AD. The AUCs (95% CI) of logistic regression models using all three indicators, two FC indicators, only the FC between the NAc and left fusiform, or only the FC between the mPFC and left cingulate anterior cortex to differentiate AD cases and HCs were 0.91 (0.86, 0.96), 0.85 (0.78, 0.92), 0.77 (0.69, 0.85), and 0.77 (0.70, 0.85), respectively (**Table 2**; **Figure 1C**).

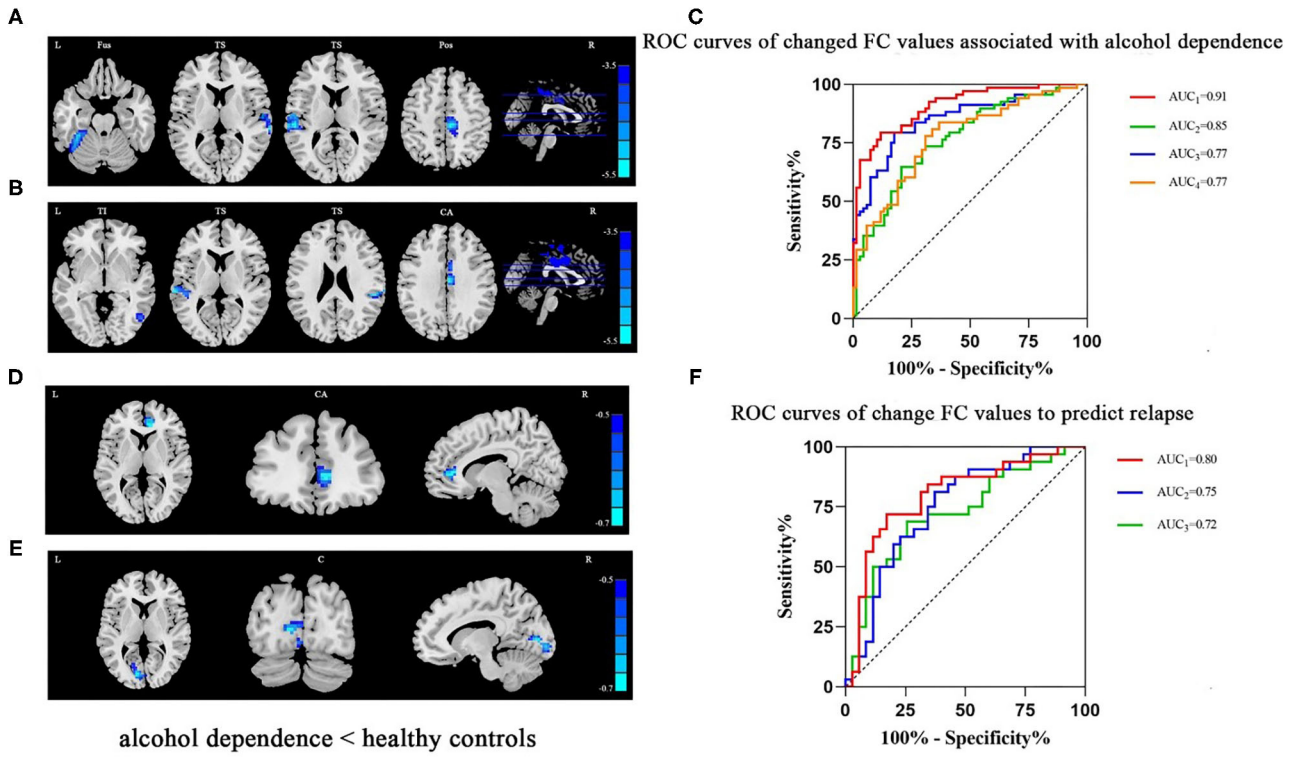

FIGURE 1 | (A) In patients with AD, lower FC was detected in the left fusiform, bilateral temporal superior and right postcentral areas (seed: NAc). **(B)** In patients with AD, lower FC was detected in the right temporal inferior, bilateral temporal superior and left cingulum anterior areas (seed: mPFC). **(C)** The ROC curves of logistic regression models using all three indicators (smoking status and two FC indicators, $AUC_1 = 0.91$) or two FC indicators ($AUC_2 = 0.85$), only FC between the NAc and left fusiform ($AUC_3 = 0.77$), or only FC between the mPFC and left cingulum anterior ($AUC_3 = 0.77$) to differentiate AD cases and HCs. **(D)** Among patients with AD who had relapsed based on the follow-up survey, lower FC in the right cingulum anterior (seed: NAc) was detected. **(E)** Among patients with AD who had relapsed, lower FC in the left calcarine (seed: mPFC) was detected. **(F)** The ROC curves of logistic regression models using both FCs ($AUC_1 = 0.80$), only FC between NAc and right cingulum anterior ($AUC_2 = 0.75$), or only FC between mPFC and left calcarine sulcus ($AUC_3 = 0.72$) to predict relapse. Clusters were labeled with the Automated Anatomical Labeling (AAL) atlas. The *t* maps of **(A,B)** were drawn with a threshold of $p < 0.001$ at the voxel level and $P_{FWE} < 0.05$ at the cluster level. Though the results of **(D,E)** could not exist relative to the above threshold, *t* maps of these results were shown with a threshold of $p < 0.016$ at the voxel level and $P_{FWE} < 0.05$ at cluster level. The color bar indicates voxel-wise *t* values. lower FC is shown in blue. The brighter the blue, the lower the FC. FC, functional connectivity; NAc, nucleus accumbens; mPFC, medial prefrontal cortex; L, left; R, right; Fus, fusiform; TS, temporal superior gyrus; Pos, postcentral; TI, temporal inferior; CA, cingulum anterior; C, calcarine sulcus; ROC, receiver operating characteristic; AUC, area under curve; AD, alcohol dependence.

TABLE 2 | Multivariate logistic regression models using three indicators to AD ($n = 136$).

Variables	β	S.E.	Wald	OR[a] (95% CI[b])
Smoking status	2.90***	0.57	25.87	18.24 (5.96, 55.83)
NAc_fusiform_L	−6.31**	1.89	11.14	0.002 (0.001, 0.07)
mPFC_cingulum anterior_L	−4.16**	1.38	9.13	0.02 (0.001, 0.23)

*[a]OR: adjusted odds ratio which was based on binary logistic regression (forward: conditional) of alcohol dependence (yes vs. no) as a dependent variable, and age, education years, changes of FC as independent variables; [b]95% CI: 95% confidence interval; AD, alcohol dependence; NAc, nucleus accumbens; mPFC, medial prefrontal cortex; L, left; R, right; **P < 0.01; ***P < 0.001.*

TABLE 3 | Multivariate logistic regression models to predict relapse ($n = 67$).

Variables	β	S.E.	Wald	OR[a] (95% CI[b])
NAC_cingulum anterior_R	−3.40*	1.60	6.26	0.02 (0.001, 0.42)
mPFC_calcarine_L	−3.59*	1.53	5.55	0.03 (0.001, 0.55)

*[a]OR: adjusted odds ratio which was based on the binary logistic regression (forward: conditional) using relapse (yes vs. no) as a dependent variable, and age, education years, AUDIT scores at baseline survey and FC changes as independent variables; [b]95% CI: 95% confidence interval; NAc, nucleus accumbens; mPFC, medial prefrontal cortex; L, left; R, right; *P < 0.05.*

FC Values Predicted AD Relapse

Compared with the non-relapse category, the baseline FC values between the NAc and right cingulate anterior cortex seeds ($t = -3.95$, x = 9, y = 39, z = 6, $P_{FWE} < 0.016$) (**Figure 1D**; **Supplementary Table 3**) and between the mPFC and left calcarine sulcus seeds ($t = -3.79$, x = −12, y = −81, z = 6, $P_{FWE} < 0.016$) (**Figure 1E**; **Supplementary Table 4**) of the relapse group were significantly lower. Logistic regression revealed that both of these FC values independently predicted AD relapse. Based on the AUCs (95% CI) of logistic regression models using both FC values, the FC value between NAc and right cingulate anterior cortex, and the FC value between mPFC and left calcarine sulcus to relapse were 0.80 (95% CI: 0.69, 0.91), 0.75 (0.63, 0.87), and 0.72 (0.60, 0.85), respectively (**Table 3**; **Figure 1F**).

Results of FC Values Correlated With Relapse Severity and Its Predictors

Partial correlation analyses yielded that respective FC values of the NAc with the left cingulate anterior cortex ($r = -0.66$, x = −6, y = 39, z = 0, $P_{FWE} < 0.001$) (**Figures 2A,C**; **Supplementary Table 5**) and FC of mPFC with the right frontal superior gyrus ($r = -0.67$, x = 18, y = 3, z = 57, $P_{FWE} < 0.001$) and with the left precentral ($r = -0.64$, x = −27, y = −15, z = 63, $P_{FWE} < 0.001$) (**Figures 2B,D,E**; **Supplementary Table 6**) in the early-abstinence stage were negatively correlated with relapse severity based on the follow-up survey. Linear regression showed lower FC between the mPFC and right frontal superior gyrus, and education years were independently predictive of the severity of relapse (**Table 4**).

DISCUSSION

According to the hypothesis that AD is accompanied by an abnormal reward circuit and cognitive execution, which is mostly based on the findings of previous neuroimaging and neurobiological research, this study selected the NAc and mPFC as seeds to investigate resting-state FC associated with AD and its relapse after detoxification. The first part of this study is based on the baseline cross-sectional case-control data collected from a relatively larger sample (compared to previous neuroimaging studies on AD) and found that AD was associated with several lower FC values. The second part of this study is based on longitudinal data of 67 AD patients who were hospitalized and underwent detoxification and found that some lower FC values during early abstinence were predictively associated with relapse during the 6-month follow-up. These findings add to our scientific knowledge regarding neuroimaging features and biomarkers of AD and its relapse.

Regarding the lower FC between the NAc and fusiform cortex among AD patients, we propose that dysfunctional visual encoding has an influence on individuals' attention bias in the activation of the reward circuit, which could be associated with impulsive drinking behaviors in relation to cue salience. Although the defined role of the fusiform cortex is currently unclear, previous evidence suggests that its activity may be essential for encoding visual objects (such as faces, scenes, and words) and subsequent memory (41, 42). Zehra et al. found that alcohol use disorder patients showed lower activation in regions of ventral and dorsal attention networks (such as insula, parietal, and prefrontal cortex) compared with health controls (43). In addition, Hill et al. have suggested that alcohol may contribute to the hypofunctional fusiform of adolescents with a family history of AD during the execution of the eyes task of Baron-Cohen (44). Therefore, the lower FC between the NAc and fusiform cortex may indicate that the functional consistency between the reward circuit and visual encoding has been abnormally changed in AD patients. The lower FC between the mPFC and cingulum anterior may reflect the dysfunction of a crucial brain network among AD patients. A default mode network (DMN) among brain regions, including the cingula anterior, mPFC and precuneus, has been identified as the most crucial brain network in the resting state, although it is deactivated during executive task performance utilizing frontoparietal regions (45). Previous research on addiction indicated that individuals with hypoactivation in the mPFC and cingulum anterior showed deficits in response inhibition and in impulse control, which could indirectly increase the individual's susceptibility to substance use by activating stress and reward centers (46, 47). For the hypothesis of response inhibition deficits and impulse control failure in the neurobiological mechanisms underlying AD, some structural imaging studies, including a diffusion tensor imaging (DTI) study that found diminished white matter integrity in cingulate and frontal areas among individuals with AUD (48). Our evidence for lower FC between the mPFC and cingulate anterior cortex provides further functional imaging evidence. The lower FC values associated with AD found in our study are likely mostly related to the etiology of

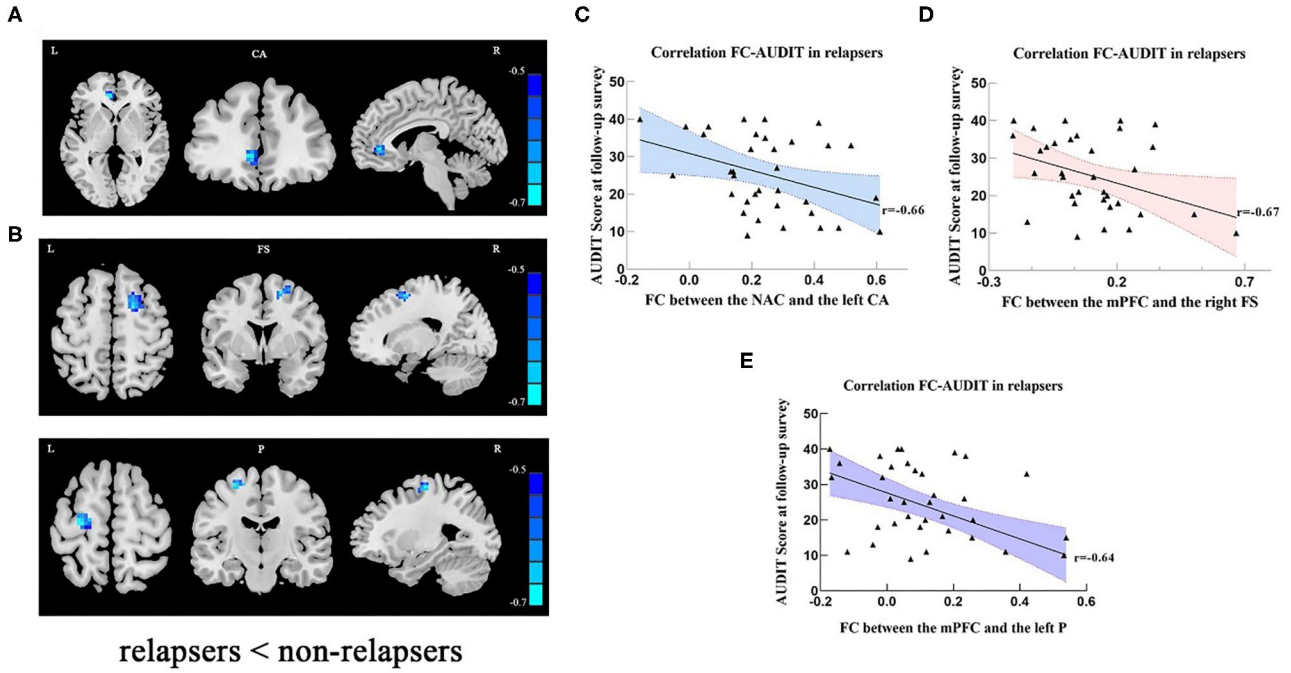

FIGURE 2 | (A) In relapse cases, lower FC was detected in left cingulate anterior cortex (seed: NAC). **(B)** In relapse cases, lower FC was detected in right frontal superior and left precentral areas (seed: mPFC). The scatter plots show that relapse severity was negatively associated with FC between NAc and left CA **(C)**, FC between mPFC and right FS **(D)**, and FC between the mPFC and left P **(E)**. Clusters were labeled with the Automated Anatomical Labeling (AAL) atlas. The *t* maps were drawn with a threshold of $p < 0.001$ at the voxel level and $P_{FWE} < 0.05$ at the cluster level. The color bar indicates voxel-wise *t* values. lower FC is shown in blue. The brighter the blue, the lower the FC. FC, functional connectivity; NAc, nucleus accumbens; mPFC, medial prefrontal cortex; L, left; R, right; CA, cingulum anterior; FS, frontal superior gyrus; P, precentral; AUDIT, alcohol use disorder identification test.

TABLE 4 | Multivariate linear regression analysis to predict relapse severity ($n = 35$).

Variables	R^{2a}	S.E.	*t*	β (95% CI)[b]
Education years	0.43	0.45	−4.04	−1.84 (−2.34, −0.59)**
mPFC_frontal superior_R		8.07	−4.32	−34.84 (−51.44, −19.16)***

*[a]R^2: adjusted R^2 of determination coefficient; [b]β (95% CI): regression coefficient (95% confidence interval) which was based on the multivariate linear regression (stepwise) using relapse severity (AUDIT score at follow-up) as the dependent variable, and age, education years, AUDIT scores at baseline survey and changes FC as independent variables; NAc, nucleus accumbens; mPFC, medial prefrontal cortex; L, left; R, right; **P < 0.01; ***P < 0.001.*

AD rather than brain impairment due to alcohol use, although a potential causal relationship between changes in brain FC and alcohol use cannot be verified because these findings are based on baseline cross-sectional case-control data.

It is especially encouraging that this study found that lower FC values between the NAc and cingulate anterior cortex and between the mPFC and calcarine sulcus, independently predicting relapse. The NAc and cingulate anterior cortex are primarily involved in processing motivational information, salience of emotion, cravings and cognitive control in the mechanism of addiction (49, 50). The calcarine sulcus plays a primary role in the visual cortex, not only accepting signals directly from neural fibers from the retina but also projecting extensively to the cerebral cortex, which is associated with recognizing text, memorizing, identifying objects, differentiating distance and determining relationship with others. Limitations in cognitive processes may cause an associated reduction in the amount of specific visual signal encoding and finally lower the probability of recognition (51, 52). Therefore, the connection of the mPFC and calcarine sulcus has an important role in recognition memory and information processing, which are involved in the reward circuit, visual cortex, and cognitive control. Previous studies have also found subtle deficits in the function of these regions that may significantly predict craving and relapse in the progression of addiction (53). We also found that relapse severity was significantly predicted by a lower education level and lower connectivity between the mPFC and right frontal superior gyrus. Lower education as a predictor of the severity of relapse is consistent with previous evidence, which indicated that those who have a lower education level have more limited cognition in relation to health issues (54). The prefrontal cortex is a core region for inhibitory control (55, 56). Its subregions, such as the mPFC, frontal superior cortex and cingulate anterior cortex, usually make differential contributions to decision making. The ventral prefrontal cortex, where the mPFC is located, mainly suppresses inappropriate risky choices, while the dorsal region including the frontal superior and cingulate anterior cortices often promotes more profitable risk-associated choices (57).

Thus, it is possible that the disruption of the connection between these areas may contribute to inappropriate risky choices such as heavy drinking. Accordingly, the findings of those lower FC values during early-abstinence predictively associated with relapse and its severity are not only helpful for determining potential biomarkers for predicting AD relapse but also contribute to research on the neurobiological etiology of AD.

LIMITATIONS

There are several limitations in the present study that were not mentioned previously. Participant's parents should be interviewed by uniform tools helpful to exclude Axis I psychiatric disorders, and we also should exclude Axis II psychiatric disorders in patients or their parents. This study was based on a priori selection of ROIs (the NAc and mPFC) as seeds, which cannot analyze FC alterations in the whole brain and may lead to observational bias. Additional whole brain network analysis studies based on larger samples should be explored to elucidate the neuroimaging perspective on mechanisms of addiction. Another disadvantage of this study is that the fMRI scan data were limited to a single scan at early-abstinence stage, which may be satisfactory for predictive association analysis but obviously not for examination of longitudinal neuroimaging changes after detoxification for both groups of relapse and non-relapse. Because relapse is considered as a return to unhealthy behaviors and negative consequences (58) this study used the optimal cutoff of the AUDIT score to screen for alcohol use disorders to define relapse. Nevertheless, some individuals characterized as non-relapsers in this study, especially those who returned to mild drinking, might relapse by the time of longer-term follow-up. Pharmacological treatment of withdrawal symptoms and complications of AD during hospitalization is complex due to varied types and doses of drugs, which may have impacts brain FC. In the current study, we did not have a sufficient sample size to control simply for those possible confounding effects. It is notable, however, that Tao et al. indicated a diversity of medicines used in AD treatment have no significant association with relapse (34). Smoking status has not been well-matched between AD cases and HCs, although smoking status was controlled as a covariate in data analysis. This is a thorny issue given the frequency co-existence of tobacco and alcohol use, and the impact of smoking on brain function is difficult to exclude entirely in this context. This study is inclusion of only male subjects, a research location socio-cultural issue compared to other international sites, due to a lack of recruitment of female participants. Becker et al. reported different changes in the brain after exposure to drugs in relation to biological sex (59). Some neuroimaging alterations associated with alcohol dependence and relapse have been found in this study. However, a further research of neurochemical changes in microscale also is mandatory.

CONCLUSION

This study found that values of FC between selected seeds (i.e., the NAc and the mPFC) and some other reward- and/or impulse-control-related brain regions were associated with AD and relapse; these FC values could be potential biomarkers of AD or for predicting relapse after detoxification. These findings represent a meaningful stimulus for further research on the neurobiology of AD and other addictive disorders.

DATA AVAILABILITY STATEMENT

We are happy to share the data of the present study with other researchers for academic use on request under the regulations of the Ministry of science and technology of China, and West China Hospital of Sichuan University. Researchers may contact the primary investigators (Wan-jun Guo, guowjcn@163.com) for data sharing.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2021.609458/full#supplementary-material

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Characterizing the Structural Pattern of Heavy Smokers Using Multivoxel Pattern Analysis

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Background: Smoking addiction is a major public health issue which causes a series of chronic diseases and mortalities worldwide. We aimed to explore the most discriminative gray matter regions between heavy smokers and healthy controls with a data-driven multivoxel pattern analysis technique, and to explore the methodological differences between multivoxel pattern analysis and voxel-based morphometry.

Methods: Traditional voxel-based morphometry has continuously contributed to finding smoking addiction-related regions on structural magnetic resonance imaging. However, voxel-based morphometry has its inherent limitations. In this study, a multivoxel pattern analysis using a searchlight algorithm and support vector machine was applied on structural magnetic resonance imaging to identify the spatial pattern of gray matter volume in heavy smokers.

Results: Our proposed method yielded a voxel-wise accuracy of at least 81% for classifying heavy smokers from healthy controls. The identified regions were primarily located at the temporal cortex and prefrontal cortex, occipital cortex, thalamus (bilateral), insula (left), anterior and median cingulate gyri, and precuneus (left).

Conclusions: Our results suggested that several regions, which were seldomly reported in voxel-based morphometry analysis, might be latently correlated with smoking addiction. Such findings might provide insights for understanding the mechanism of chronic smoking and the creation of effective cessation treatment. Multivoxel pattern analysis can be efficient in locating brain discriminative regions which were neglected by voxel-based morphometry.

Keywords: smoking addiction, multivoxel pattern analysis, voxel-based morphometry, machine learning, structural magnetic resonance imaging

INTRODUCTION

Tobacco smoking in the form of cigarettes continues to be the leading cause of preventable illness and mortality in the world (1). China, the largest producer of tobacco, is estimated to contain 311 million individuals who are current smokers, with 295 million men and 16 million women, respectively (2). Chronic smoking is known to correlate with a series of diseases including stroke, lung cancer, hepatocellular carcinoma, and vascular dysfunctions (3–7). Related neuroimaging studies also suggested that the numerous toxic chemicals contained in a cigarette, especially nicotine, could promote potential brain afflictions in chronic cigarette smokers (8, 9).

Apart from these serious public health problems, 78% of smokers who expressed willingness to quit smoking reported a relapse situation in China, and the percentage in America is currently 80% (10, 11), indicating the ineffectiveness of existing cessation treatments. Poor treatment outcome for smoking cessation may result from a lack of awareness of the mechanism behind smoking addiction and the available biomarkers that characterize heavy smokers (12, 13).

Structural magnetic resonance imaging (sMRI), which visualizes the central neural system with high-resolution in a non-invasive way, provides indispensable spatial information in the procedure of identifying such biomarkers (i.e., discriminative brain regions). For the past decade, univariate approaches such as voxel-based morphometry (VBM) have continuously been applied on sMRI studies (14, 15). In these studies, morphological abnormalities are consistently discovered in heavy smokers in some brain regions, including the prefrontal cortex, anterior cingulate cortex, thalamus, and the insula (9, 14–18).

Despite the accumulative results reported, VBM, as a traditional univariate approach, has its inherent limitations in identifying spatial patterns that exist in a certain population (e.g., a group of heavy smokers). Generally, VBM has the multiple comparisons problem (19), which causes a loss of sensitivity and overlooks the dependency of the focal set of voxels in localizing informative regions relevant to specific brain abnormalities (20). Multi-voxel pattern analysis (MVPA), which utilizes a multi-variate technique and is driven by machine learning algorithms, provides a sensitive and different approach in identifying group-wise differences. MVPA takes multiple voxels into account and considers patterns across a group of voxels that may respond weakly but consistently differently between conditions (21, 22). Therefore, MVPA can be sensitive in distinguishing different experimental conditions. MVPA has become favorable in neuroimaging research for its ability to detect subtle anatomical differences (23–25).

The field of utilizing MVPA to discover structural abnormalities in heavy smokers remains less active. Notably, in an sMRI study based on support vector machine (SVM), the authors (26) used the mean gray matter volumes (GMVs) of 1,024 self-defined brain regions as input features to the SVM to identify the most discriminative regions by finding the most informative features in the SVM. Using average brain region GMVs as features may be an over simplistic way to characterize the anatomical structure of the neural system. The subtle difference within the same self-defined region may be overlooked. In this study, in order to discover smaller regions that reflect the experimental conditions of interest, we used a searchlight algorithm by moving a searchlight window through the volume of the brain, to sample the gray matter values of voxels as the input features to our linear classifier (SVM). A searchlight algorithm is able to preserve subtle differences between a group of voxels while a linear SVM can detect such subtle differences by efficiently defining a boundary that maximally separates two classes (e.g.,: heavy smokers and healthy non-smokers) of samples.

With regard to the peer studies on smoking addiction and the limitations of the current univariate approach, in this study, we aimed to: (1) explore the most discriminative gray matter regions between heavy smokers and otherwise healthy controls (HCs) with a data-driven MVPA technique and sMRI data; and (2) explore the methodological difference between MVPA and VBM. We hypothesized that MVPA would be a more sensitive method in locating group-wise brain differences than VBM.

METHODS

Participants

The study adhered to the Declaration of Helsinki and was approved by the Medical Ethics Review Board of Zhongnan Hospital of Wuhan University. After a complete description of the study, written informed consent was obtained from the subjects.

Sixty-eight right-handed subjects (39 heavy smokers and 29 healthy non-smoker control subjects) were recruited via advertisement flyers and referrals for MRI studies. All subjects were screened for psychiatric and non-psychiatric medical disorders using the Mini International Neuropsychiatric Interview (27). All participants underwent an interview session followed by an inclusion procedure. The inclusion criteria for both groups were: the absence of any history of medical (e.g., cardiac disease) or neurological (e.g., stroke) disorders, intellectual disability, drug abuse or dependence (other than nicotine dependence for heavy smokers), or psychiatric disease; none of the subjects reported the daily consumption of alcohol or experienced social consequences secondary to alcohol intake. Heavy smokers were defined as those who met the DSM-IV criteria for nicotine dependence, had smoked at least 20 cigarettes daily in the past 5 years, and had no period of smoking abstinence longer than 3 months. The severity of the heavy smokers' nicotine addiction was measured using the Fagerström Test for Nicotine Dependence (28). The healthy control subjects in this study were defined as those who had a history of smoking no more than five cigarettes in a lifetime.

Data from two heavy smokers and one non-smoker were excluded for not meeting the above criteria. As a result, 37 heavy smokers (male: 30; female: 7) and 28 healthy non-smoking control subjects (male: 21; female: 7) were included in this study. More detailed information on the subjects in each group is presented in **Table 1**. Demographic data were compared between the two groups by using two-sample *t*-tests or a Chi-square test

TABLE 1 | Demographic and clinical characteristics of the heavy smokers and HCs.

Measure	HS	HC	*P*-value
Number	37	28	
Age (SD)/years	47.18 (7.22)	43 (9.62)	0.96[a]
Gender (male/female)	29/8	20/8	0.77[b]
Years of education (SD)	9.24 (2.16)	11.67 (4.72)	0.36[a]
Lifetime cigarette usage (years)	25.34 (9.23)	–	–
Age at first cigarette use (years)	21.02 (5.38)	–	–
Average cigarette per day	35.13 (10.70)	–	–
FTND score	8.89 (0.68)	–	–

Data are presented as mean (standard deviation). HS, heavy smokers; HC, healthy controls. FTND, Fagerström Test for Nicotine Dependence; a, two-sample two tailed t-test; b, Chi-square test.

in the Statistical Package for Social Science, version 19 (SPSS Inc., USA). The threshold level of significance was set as $p < 0.05$.

MR Acquisition

All participants underwent a high-resolution 3dn-MTC T1-weighted structural scan using a Siemens Trio 3.0-Tesla MR scanner (Erlangen, Germany) with a standard birdcage head coil. sMRI images were obtained using a MPRAGE pulse sequence with the following parameters: repetition time = 25 ms; echo time = 4.51 ms; flip angle = 25; acquisition matrix = 256 × 256; and slice thickness = 1 mm with a 1 mm gap.

Image Preprocessing

The raw DICOM images of sMRI were converted to the NIFTI format using MRIcron (University of South Carolina, Columbia, SC, USA, http://www.mricro.com). The following preprocessing steps were then performed to obtain gray matter (GM) maps using VBM8 toolbox (http://dbm.neuro.unijena.de/vbm) in SPM8 (Version 6313, Wellcome Department of Imaging Neuroscience, London, UK, http://www.fil.ion.ucl.ac.uk/spm) on MATLAB R2013a. Firstly, the sMRI images were registered to the Montreal Neurological Institute (MNI) stereotactic space and resampled to a 1.5 mm isotropic voxel spacing. Secondly, the co-registered images were segmented into three types of tissues, namely GM, white matter, and cerebrospinal fluid. Thirdly, a study-specific template was created using the high-dimensional Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL) algorithm and with the predefined templates in the VBM8 toolbox. Next, in order to preserve the total volume of each brain tissue, the segmented images were modulated using non-linear deformation which can compensate for the effect of spatial normalization. This step multiplied the spatially normalized gray matter by its relative volume before and after spatial normalization (29). At last, the images were smoothed with an 8-mm full-width-half-maximum Gaussian kernel.

MVPA

An MVPA technique combining a searchlight algorithm and a linear SVM was used to classify prominent regions that distinguished heavy smokers from HCs. Generally, by moving a searchlight region through the brain volume, one can continuously map the information content regarding the experimental conditions of interest in the brain (30). The procedure of our MVPA method is as follows. The smoothed GM maps computed from the data preprocessing step were used as inputs in the MVPA. Firstly, at each voxel (Vi) of the GM images in the normalized space, a three-dimensional cubical region size of 3 × 3 × 3 centering at Vi was identified. For each subject, the gray matter volume values of all 27 voxels (at a specific voxel position) were extracted and converted into a high-dimensional vector and used to construct the feature matrix. To train and test the SVM, a leave-one-out (LOO) cross-validation (CV) strategy was adopted, which excludes one subject as a testing set each time and trains the classifier using the remaining subjects. As a result, two feature matrices M_{F1*S} and M_{F2*S} were retrieved as the training set and testing set (F1 = 64, F2 = 1, S = 27), respectively. F1 and F2 indicate the number of subjects in the two sets respectively, and S indicates the number of voxels of the feature matrix. Next, the training set was fed into the SVM implemented in the LIBSVM toolbox (http://www.csie.ntu.edu.tw/~cjlin/Libsvm). In each training set, a nested 5-fold CV was applied to determine the optimized parameter C (regularization) and g (gamma for radius basis function) for testing. That is, each time one-fifth of the training set was selected as a testing sample and the classifier was built upon the remaining data. Parameters that produced the highest accuracy across these 5-folds was identified as the optimized C and g. The identified C and g were then used in the corresponding testing procedure. Finally, the accuracy of the trained classifier was assigned to the chosen voxel Vi. After repeating this procedure on every voxel, a three-dimensional accuracy map denoting the classification ability between heavy smokers and HCs for all voxels was obtained.

To evaluate the statistical significance of the experimental results, we converted the accuracy map to a *p*-value map under an assumption of binomial distribution. Detailed information about the conversion procedure can be found in another publication (25). A connected domain algorithm was conducted on the *p*-value map to produce clusters with significant predictive power, whose threshold was set at $p < 0.001$ with more than 50 adjacent voxels (24).

Post hoc Analysis of MVPA Analysis Results

To further explore the difference of GMVs between heavy smokers and HCs, a *post hoc* analysis was carried out, with which a further VBM analysis was conducted within the brain clusters detected by MVPA. The analysis was corrected for multiple comparisons using family wise error (FWE) at the cluster level ($p < 0.05$). To do this, firstly the GMVs of the brain regions detected by the MVPA were extracted using the MarsBar toolbox (http://www.mrc-cbu.cam.ac.uk/Imaging/marsbar.html). Then, with age and gender as co-variates, voxel-wise two independent samples *t*-tests were further performed in these regions to determine the significant differences between heavy smokers and HCs ($p < 0.05$, FWE-corrected, two-tailed).

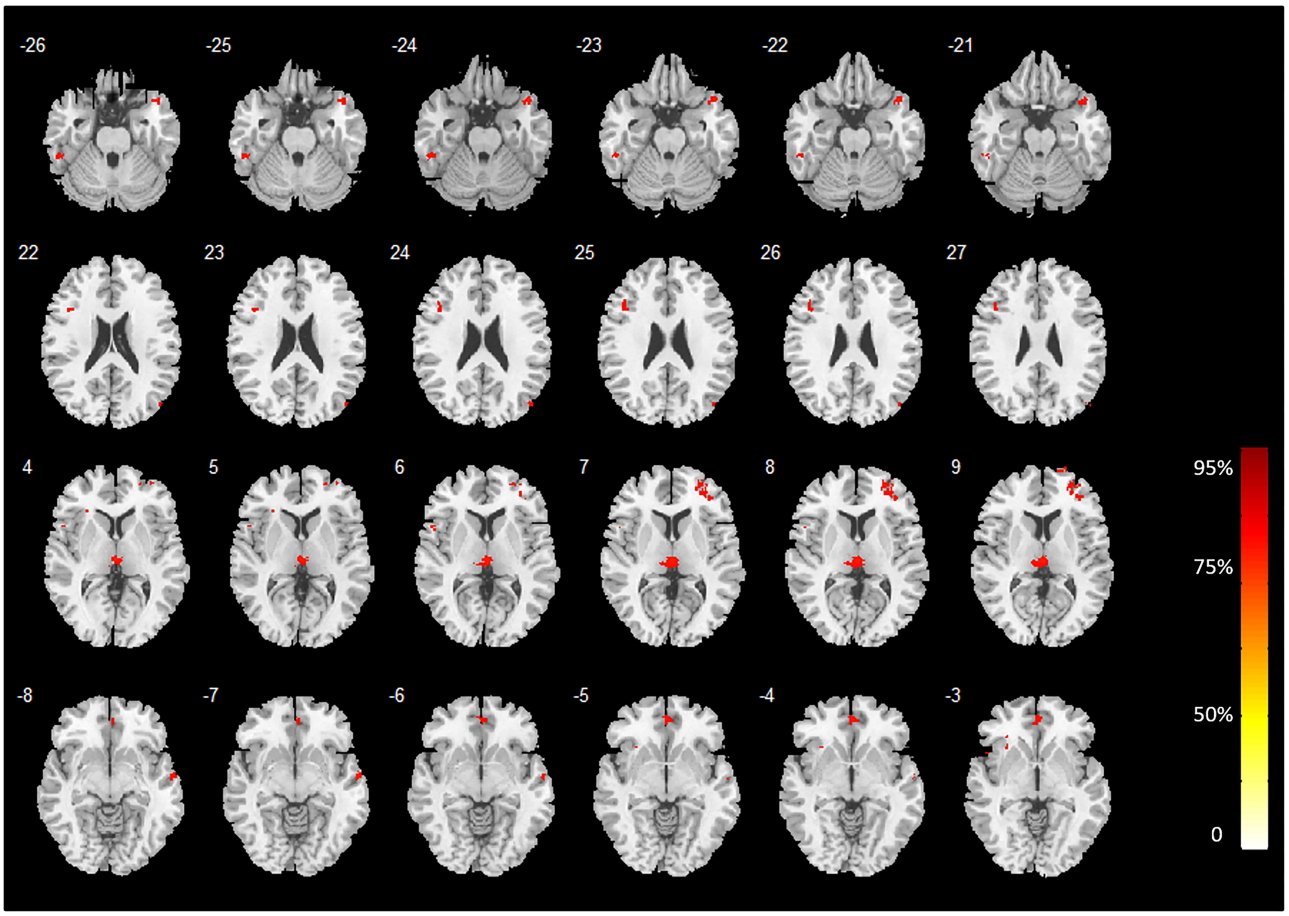

FIGURE 1 | Brain regions with high classification accuracy identified by MVPA. The color bar indicates the classification accuracy values of the whole brain GM voxels. The image is displayed in the neurologic convention, with the left side corresponding to the left brain.

Whole Brain VBM Analysis

In order to compare the methodological differences between MVPA and VBM, a whole brain VBM analysis was conducted. With age and gender as co-variates, the whole brain GM maps of heavy smokers and HCs were fed to VBM. The same threshold values of *post hoc* analysis were applied in order to conduct a comparison between whole brain VBM analysis and MVPA. Discriminative regions discovered by the whole brain VBM were defined as clusters with a cluster size > 50 voxels and a p-value < 0.001.

ROC Analysis

To evaluate the ability of MVPA or VBM in distinguishing the smokers from the healthy controls, we performed ROC analysis based on the brain regions with significant differences. Firstly, we used the average gray matter values of brain regions as features to establish an SVM model using 5-fold cross validation. Then the ROC analysis was performed with the predicted probability of each participant in the procedure of cross validation. The area under the ROC curve (AUC) denotes the ability of classifying heavy smokers from healthy controls.

RESULTS

Demographic Characteristics

Heavy smokers did not differ significantly with HCs in terms of age ($p = 0.96$), years of education ($p = 0.36$), or gender ratio ($p = 0.77$). For heavy smokers, the average FTND score was 8.89 ± 0.68 (range, 8–10; median, 9) and the average number cigarettes per day was 35.13 ± 10.70, which indicated a relatively high dependence on cigarettes. Detailed demographic information for both groups can be found in **Table 1**.

MVPA and *post hoc* Analysis

The discriminative regions recognized by our MVPA technique without covariates regression before classifier training are shown in **Figure 1**. Our proposed technique yielded a voxel-wise accuracy of at least 81% for classifying heavy smokers from HCs. Several cortical and subcortical regions demonstrated a strong classification ability with GM differences between heavy smokers and HCs. These regions were primarily located at the temporal cortex and prefrontal cortex, occipital cortex, thalamus (bilateral), insula (left), anterior and median cingulate gyri (ACG and MCG), and precuneus (left).

TABLE 2 | Brain regions with high predictive accuracy identified by MVPA and the corresponding *post-hoc* (VBM) results, which was corrected for multiple comparisons using family wise error (FWE) at the cluster level ($p < 0.05$).

Brain regions (AAL)	Cluster size (voxels)	Peak MNI coordinates			Peak *t*-value
		X	Y	Z	
Temporal_Pole_Mid_R	78	42	7.5	−37.5	3.69
Occipital_Inf_L	64	−36	−73.5	−0.5	−3.87
Calcarine_L	525	1.5	−79.5	−3	−4.62
Frontal_Med_Orb_L	89	−13.5	63	−3	3.91
Postcentral_R	622	24	−37.5	81	4.17
Temporal_Inf_L	143	−54	−39	−19.5	−4.12
Lingual_L	197	1.5	−78	−1.5	−4.32
Cuneus_L	317	−1.5	−97.5	18	4.19
Angular_R	449	45	−75	40.5	3.89
Occipital_Sup_L	294	−13.5	−99	31.5	4.30
Frontal_Mid_R	217	31.5	42	9	4.50
Parietal_Sup_R	98	15	−72	70.5	3.95

AAL, anatomical automatic labeling; MNI, Montreal Neurological Institute; L, left hemisphere; R, right hemisphere; acc, accuracy; HS, heavy smokers; HC, healthy controls. Peak t-value, post-hoc (VBM) results, conducted on brain regions detected by MVPA and corrected for multiple comparisons using family wise error (FWE) at the cluster level ($p < 0.05$). Positive t-value indicates GM volume in HS is significantly smaller than HC while negative value indicates the opposite.

Two-sample *t*-tests (*post hoc* analysis) of GMVs revealed that 16 out of the 18 brain regions identified by MVPA demonstrated a significant GMV decrease in heavy smokers as compared to healthy controls ($p < 0.05$, FWE-corrected, two-tailed). Notably, the inferior temporal gyrus (left) and cerebellum were found with a significantly increased GMV in heavy smokers than in HCs. The peak accuracy values of these clusters and the corresponding *t*-values which were derived from the *post hoc* analysis are reported in **Table 2**. The peak *t*-value was defined as the maximum *t*-value found in the extracted cluster. A positive *t*-value indicated that the GM volume in HS was significantly smaller than HC in a specific region while a negative value indicated the opposite. The brain regions recognized by VBM are shown in **Figure 2**.

The ROC analysis results are shown in **Figure 3**. The ROC results indicate that MVPA outperformed VBM in differentiating the heavy smokers from the HCs (AUC, 0.81 vs. 0.78; sensitivity, 0.82 vs. 0.79; specificity, 0.77 vs. 0.77; positive predictive value (PPV), 0.72 vs. 0.71; negative predictive value (NPV), 0.85 vs. 0.82).

VBM Analysis

As summarized in **Table 3**, whole brain VBM analysis revealed that six brain regions demonstrated a significant decrease or increase of GMV in heavy smokers than in HC. These regions

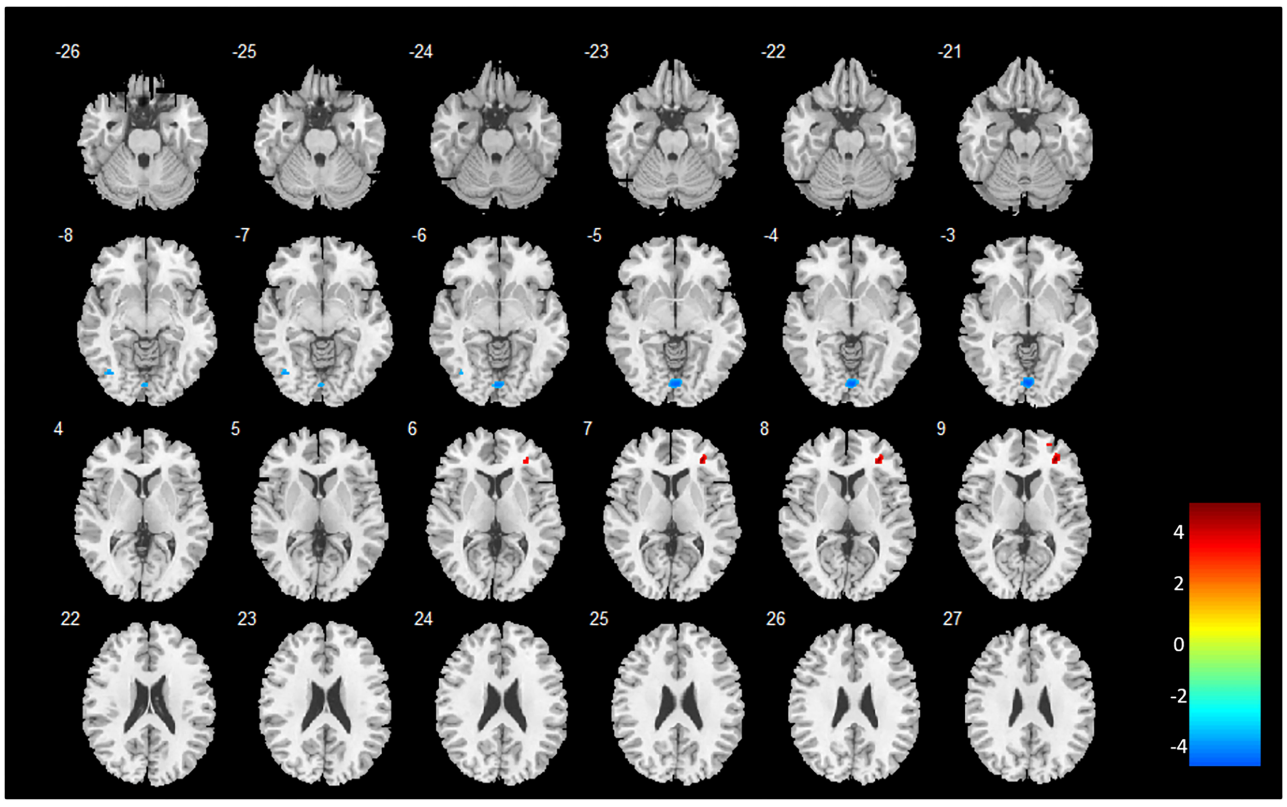

FIGURE 2 | Brain regions identified by whole brain VBM analysis. The color bar indicates the peak t-values of the maximum voxel found in the extracted cluster. A positive *t*-value indicates that the GM volume in HS is significantly smaller than HC in a specific region while a negative value indicates the opposite. The image is displayed in the neurologic convention, with the left side corresponding to the left brain.

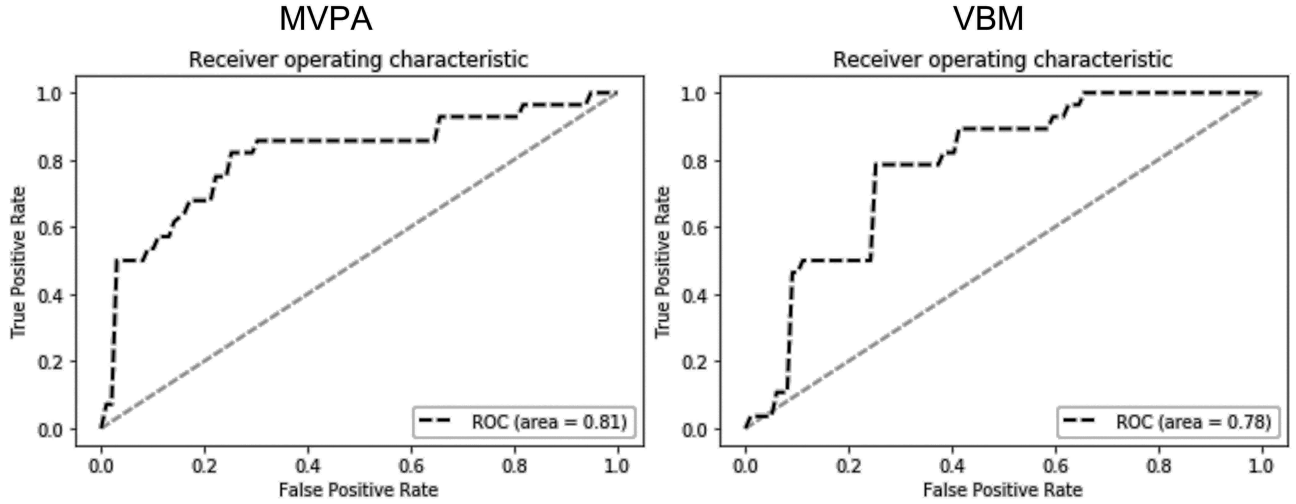

FIGURE 3 | The ROC analysis results of MVPA (left) and VBM (right). The results (AUC, 0.81 vs. 0.78; sensitivity, 0.82 vs. 0.79; specificity, 0.77 vs. 0.77; PPV, 0.72 vs. 0.71; NPV, 0.85 vs. 0.82) indicate that MVPA outperformed VBM in differentiating the smokers from healthy controls by using the GMVs of the detected brain regions as the features.

TABLE 3 | Brain regions detected by VBM with a significant decrease or increase GMV in heavy smokers than HC.

Brain regions (AAL)	Cluster size (voxels)	Peak MNI coordinates			Peak *t*-value
		X	Y	Z	
HS < HC					
Right cerebrum	129	31.5	40.5	9	5.313
Left cerebrum	73	−13.5	−99	31.5	3.63
Occipital_Sup_R	100	21	−93	46.5	3.88
Precuneus_L	50	−12	−49.5	51	3.93
HS>HC					
Occipital_Inf_L	58	−40.5	−70.5	−9	−3.64
Lingual_L	355	−1.5	−79.5	−1.5	−4.39

AAL, anatomical automatic labeling; MNI, montreal neurological institute; L, left hemisphere; R, right hemisphere; HS, heavy smokers; HC, healthy controls. t-value, positive t-value indicates GM volume in HS is significantly smaller than HC while negative value indicates the opposite. The cluster size is more than 50 voxels and the threshold is set as $p < 0.001$, uncorrected.

were located in the occipital, precuneus lingual, and cerebrum. The peak *t*-value was defined as the maximum *t*-value found in the extracted cluster. A positive *t*-value indicated that the GM volume in HS was significantly smaller than HC in a specific region while a negative value indicated the opposite.

DISCUSSION

Traditional univariate studies prior to this research have constantly discovered that heavy smokers shared similar GM alterations in the cingulum, thalamus, cerebellum, prefrontal gyrus, and precuneus (9, 15–18, 31). In this study, these regions were identified by MVPA as discriminative regions between heavy smokers and HCs. In a following *post hoc* analysis on these particular regions, our analysis results also demonstrated significant GM decrease in several areas including the temporal cortex, prefrontal cortex, thalamus, ACG and MCG, and precuneus in heavy smokers, indicating a strong correlation between GM alteration in regional GM deficiency and chronic smoking. In addition, our MVPA result also revealed that regions (insula, cerebellum) which were seldomly reported in traditional VBM analysis, may participate in the chronic smoking mechanism. The resulting contrast between MVPA and VBM may indicate the methodological differences between these two methods.

In our study, the bilateral thalamus were identified by MVPA with high accuracy. Particularly, in the *post hoc* analysis, the right thalamus was found to have significant GMV loss in heavy smokers. The observation of GMV deficiency in heavy smokers may primely result in the cognitive impairment reported in several studies (8, 17), as the thalamus relays information between the cerebral and different subcortical cortexes (32). In addition to cognitive impairment, the thalamus has been identified as a brain region with a relatively high density of nicotine acetycholine receptors (α4β2* nAChRs) (23, 33, 34). Frequent nicotine binding activities could be the leading reason for the GMV decrease in the thalamus. Such a finding also provides insight for formulating more effective withdrawal treatment. In a related Positron Emission Computed Tomography (PET) study (33), the author suggested that maintaining α4β2* nAChRs in the desensitization state may be a possible way to alleviate withdrawal symptoms.

The cingulum, bidirectionally connected with the medial temporal lobes, was another region widely reported to be associated with smoking addiction in univariate studies. In this study, the bilateral cingulum could discriminate heavy smokers from HCs with at least an accuracy of 75%. In the corresponding

post hoc analysis, both the ACG and MCG demonstrated significant GM loss in heavy smokers. As a region connected to sites repeatedly implicated in cognitive control, the ACG is involved in executive function behaviors, such as inhibitory control and conflict resolution (35), which may play an important part in the process of quitting smoking.

The prefrontal area was another discriminative region identified by MVPA with high accuracy. Especially, in the *post hoc* analysis, the inferior and middle areas were found with significant GMV loss in heavy smokers as compared to HCs. As the prefrontal cortex is associated with concentration, emotion, and other higher brain functions, our findings with the prefrontal cortex may partially explain the decreased attention and impairment of working memory reported in heavy smokers (36–39).

Among other regions detected by MVPA, the insula presented relatively high predictive accuracy and stood out as a discriminative region. Acting as a critical neural substrate for addiction including nicotine dependence (40), the insula plays a potential role in the decision-making task that is associated with relapse to drug use (41) and cue-induced drug urges (14, 42, 43). However, the insula was not reported in our whole brain VBM analysis nor in other univariate sMRI studies. In contrast, our finding of the insula was in accordance with another multi-variate sMRI study deploying SVM (26). The fact that the insula along with the ACG are part of the salience network (SN) (44), a network that mediates one's subjective feelings, is thought to be one of the solutions to developing effective treatment plans. A previous fMRI study discovered that damage to the insula could disrupt addiction to cigarette smoking which in turn may help improve smoking cessation outcomes (40). Besides, evidence of the insula's role in mediating addiction urges may partially justify why the insula stands out as a discriminative region.

Notably, nicotine binding activity in the cerebellum, a region that was identified by our MVPA method with high accuracy (82%) has also been reported in several biochemical studies (45, 46). Heavy smokers showed a greater density of nicotine binding in the cerebellum as compared to non-smoking individuals (47), and brain blood flow in the cerebellum was increased by cigarette smoking (45, 46). This evidence lends support to the fact that decreased cerebellar GMV is associated with smoking addiction. However, like the insula and other regions, significantly lower GMV was not found in the cerebellum in heavy smokers more than in healthy controls in the whole brain VBM analysis. Moreover, as indicated by sMRI studies deploying ML techniques (26, 48), the insula and cerebellum were found to participate in the mechanism of chronic smoking. Such differences may be attributed to the fundamental difference between MVPA and traditional univariate analysis.

To be specific, MVPA fixes what was considered a disadvantage in traditional univariate methods. Conventional univariate analysis methods try to separately find voxels that show a statistically significant response to the experimental/physical conditions by deriving the average of the chosen voxels in all subjects, which assumes that the covariance across neighboring voxels is not informative about the experimental conditions under examination (49). Such assumption leads to the following inadequacies. Firstly, low response voxels that still carry important information could therefore be dismissed. Secondly, a consistent spatial pattern is neglected by simply averaging all voxels. MVPA uses a different solution to boost accuracy. Instead of uniformly averaging all voxels in VBM, MVPA assigns a weighted average in different conditions. This operation allows MVPA to discover spatial patterns neglected by VBM. Further, MVPA tries to optimize these weights by involving data-driven machine learning techniques. The evidence that the regions showing correlation to chronic smoking also implied that MVPA could be more sensitive than conventional univariate methods. In this view, MVPA can be better than VBM in discovering specific patterns in the chronic smoking population.

The study still presents the following limitations. Firstly, the sample size in the current study is relatively small. In a future study, more data should be collected in order to construct a more robust classifier and to verify our findings. Secondly, a more comprehensive dataset combining different modalities, such as fMRI and diffusion tensor imaging, would be helpful in exploring the mechanism of discriminative ability of such regions in heavy smokers.

In this study, MVPA was applied to structural MRI to identify the brain regions in discriminating heavy smokers from healthy individuals. The anatomical deficiency in heavy smokers was mainly discovered in the insula, ACG, MCG, prefrontal cortex, precuneus, and cerebellum, which are highly involved in the addiction of chronic smoking. These findings in accordance with previous results (45, 47, 48, 50) might provide insights for understanding the mechanism of chronic smoking and effective cessation treatment. Such insights also indicate the potential of using MVPA in future neuroimaging research. Moreover, the comparison between VBM and MVPA revealed that MVPA can be efficient in locating brain discriminative regions which were neglected by VBM.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: The data for this study are not publicly available because Zhongnan Hospital of Wuhan University, the center from which the data were collected, does not agree to make the data publicly accessible. Requests to access these datasets should be directed to huangb@szu.edu.cn.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical Ethics Review Board of Zhongnan Hospital of Wuhan University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

YY, JZ, GD, and GW: conception and design. GD and GW: administrative support. GW: provision of study materials or patients. YY, JZ, GD, GW, and SW: collection and assembly of data. YY, JZ, GW, and SW: data analysis and interpretation. All authors: manuscript writing. All authors final approval of manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Treatment Response Prediction and Individualized Identification of Short-Term Abstinence Methamphetamine Dependence Using Brain Graph Metrics

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Background: The abuse of methamphetamine (MA) worldwide has gained international attention as the most rapidly growing illicit drug problem. The classification and treatment response prediction of MA addicts are thereby paramount, in order for effective treatments to be more targeted to individuals. However, there has been limited progress.

Methods: In the present study, 43 MA-dependent participants and 38 age- and gender-matched healthy controls were enrolled, and their resting-state functional magnetic resonance imaging data were collected. MA-dependent participants who showed 50% reduction in craving were defined as responders to treatment. The present study used the machine learning method, which is a support vector machine (SVM), to detect the most relevant features for discriminating and predicting the treatment response for MA-dependent participants based on the features extracted from the functional graph metrics.

Results: A classifier was able to differentiate MA-dependent subjects from normal controls, with a cross-validated prediction accuracy, sensitivity, and specificity of 73.2% [95% confidence interval (CI) = 71.23–74.17%), 66.05% (95% CI = 63.06–69.04%), and 80.35% (95% CI = 77.77–82.93%), respectively, at the individual level. The most accurate combination of classifier features included the nodal efficiency in the right middle temporal gyrus and the community index in the left precentral gyrus and cuneus. Between these two, the community index in the left precentral gyrus had the highest importance. In addition, the classification performance of the other classifier used to predict the treatment response of MA-dependent subjects had an accuracy, sensitivity, and specificity of 71.2% (95% CI = 69.28–73.12%), 86.75% (95% CI = 84.48–88.92%), and 55.65% (95% CI = 52.61–58.79%), respectively, at the individual level. Furthermore, the most accurate combination of classifier features included the nodal clustering coefficient in the right orbital part of the superior frontal gyrus, the nodal local efficiency in the right orbital part of the superior frontal gyrus, and the right triangular part of the inferior frontal gyrus and right temporal pole of middle temporal gyrus. Among these,

the nodal local efficiency in the right temporal pole of the middle temporal gyrus had the highest feature importance.

Conclusion: The present study identified the most relevant features of MA addiction and treatment based on SVMs and the features extracted from the graph metrics and provided possible biomarkers to differentiate and predict the treatment response for MA-dependent patients. The brain regions involved in the best combinations should be given close attention during the treatment of MA.

Keywords: methamphetamine dependence, support vector machine, classification, treatment response, graph metrics

INTRODUCTION

Abuse of the synthetic psychostimulant, methamphetamine (MA), has gained international attention as the most rapidly growing illicit drug problem worldwide (1). In the 2017 World Drug Report, ∼37 million people have become addicted to MA. MA abuse is usually accompanied by various health consequences, such as depression, anxiety, psychosis, cardiovascular disease, and human immunodeficiency virus, hepatitis B virus, hepatitis C virus that are spread by blood and sex behaviors due to the social environmental factor of MA use. Besides, a variety of secondary social costs were found, such as disruptions to family, school and work life, and violent behavior and drug-related crime (2, 3). Furthermore, frequent MA use can alter the release and activity of monoaminergic neurotransmitters, dopamine, norepinephrine, and serotonin and other unknown mechanisms on the central nervous system (CNS), which can be called neurotoxicity (4). The MA-induced harm on CNS further results in significant psychiatric withdrawal symptoms. Typical psychiatric symptoms include persecutory delusions, auditory hallucinations, and loss of insight that difficultly distinguish from some mental illnesses such as schizophrenia, and MA-induced withdrawal symptoms usually include anhedonia, hypersomnia, irritability, anxiety, aggression, and intense cravings for MA (5, 6). In general, the severity of these clinic symptoms depends on the amount of MA consumed.

MA abuse is termed as brain-based diseases given the neural mechanisms and clinical performance of MA use (7–9), which imply that the brain should contain information about an individual's current disease status and prognosis (10). At present, the diagnosis of drug use disorders mainly relies on descriptive signs and symptoms, according to the fourth or fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-IV* or *DSM-5*) criteria, in which a single "methamphetamine use disorder" with an added severity specification was listed (11). However, MA addicts, especially those with less severe symptoms, may deliberately mislead about the diagnosis because of their resistance to treatment. Therefore, an objective method to help diagnose MA addiction at the individual level is worth exploring. On the other hand, although there are many treatments available currently, few yielded convincing results of MA dependence (12, 13). At present, treatments of MA abuse are mainly based on two types: psychosocial treatments and pharmacologic treatments. Commonly used psychosocial treatments include cognitive behavioral therapy (14), contingency management (15, 16), motivational interviewing (17), and so on. Meanwhile, researchers explored many medications of MA treatment that frequently target dopaminergic, serotonergic, GABAergic, and/or glutamatergic brain pathways such as dexamphetamine (18, 19), methylphenidate (20, 21), naltrexone (22, 23), topiramate (24, 25), and so on. Therefore, it makes sense to predict the therapeutic effect before treatment, in order to allow doctors to identify the optimal treatment plan at the individual level. In this study, we defined MA-dependent participants with 50% reduction in craving as responders to treatment.

MA abuse was found to be associated with structural and functional alterations of the brain. In a study that used functional magnetic resonance imaging (fMRI), Zhang et al. researched the resting-state functional connectivity (RSFC) between seeds [region of interest (ROI)] selected with a higher value of ReHo and the whole-brain voxels and found different RSFC performance when compared with healthy controls (HCs) (26). Furthermore, in the studies conducted by Kohno et al. (27, 28), the midbrain and dorsolateral prefrontal cortex were chosen as ROIs, referring to previous research results. Kohno et al. reported that MA addicts differ from HCs in many ROI-related RSFCs, such as RSFC between the midbrain and caudate. These studies selected different ROIs based on different research aims, eventually presenting different results. Hence, RSFC based on ROI selection could not reveal the full spatial patterns of brain changes, and more suitable methods for determining differences in neural network activity are needed. Graph–theory–based complex network analysis is a non-invasive MRI-based tool that provides a powerful framework for examining the topological properties of brain networks and with no need for ROI selection. With this method, the nodes represent the brain regions, and the edges represent the anatomical or functional connections (FCs) among brain regions (29–32). The present study comprehensively examined the graph theoretical properties of the network structure after MA abuse, because it is unclear which graph metric is the most neurologically informative.

Equipped with machine learning techniques, researchers could make progress in identifying and predicting the treatment response for patients at the individual level. The support vector machine (SVM) is a multivariate pattern classification algorithm based on machine learning, and this could iteratively improve the performance in uncovering the relationships between variables

by training classifiers (33). SVMs determine the hyperplane to separate multivariate features of two classes, allowing samples to be well-divided into two groups (such as patients and control groups). In studies that applied SVMs to identify MA-dependent patients, researchers have used features, including arterial spin labeling (34), task state fMRI data (35), heart rate extracted from MA-induced electrocardiogram (36), and differentially expressed genes (37). Compared to these methods, the present study selects features from various graph metrics. These graph metrics described the characteristics of brain networks from various aspects and comprehensively captured the functional information of the brain.

The present study aims to develop possible biomarkers for MA diagnosis and treatment prediction on an individual level, so that it provides a deeper understanding of the addiction and treatment of MA from the perspective of diagnosis and treatment prediction based on the graph–theory analysis and SVMs. The results of the present study represent an important step toward the development of traditional tools in imaging diagnostic identification, as well as the personalized treatment approaches for MA abstinence treatment in the future.

MATERIALS AND METHODS

The present study was carried out in accordance with the Drug Clinical Trial Management Regulations and approved by the Ethics Committee of the Second Xiangya Hospital of Central South University, which make decisions based on the Helsinki Declaration and International Ethical Guidelines for Biomedical Research Involving Human Subjects. An informed consent was obtained from each patient.

Patient Population

In the present study, 43 MA-dependent participants (31.81 ± 8.50 years old, 28 males) and 38 age- and gender-matched HCs (35.21 ± 7.84 years old, 25 males) were recruited. The MA participants were collected from three drug rehabilitation centers in Hunan Province. In China, drug addicts are forced to receive treatment in the drug rehabilitation center for at least 2 years. During this period, they have no access to any drugs. In our study, MA participants were also forced to serve a 2-year sentence at the rehabilitation center. All MA patients volunteered to participate in the experiment and were compensated for their time with 1-month reduction of their sentence. All subjects reported a detailed drug use history during the face-to-face interview. MA addicts were included with positive urine test for MA, negative urine test for other drugs, and the diagnosis of addiction based on the *DSM-IV*. Subjects who had comorbid or history of psychiatric illness (e.g., schizophrenia and major depression unrelated to drug withdrawal), neurological disorder (e.g., multiple sclerosis, Parkinson disease, other primary degenerative brain diseases, and any brain infections or neoplasms), head trauma, or major chronic medical illnesses (e.g., diabetes, uncontrolled hypertension, and heart disease) were excluded. In addition, subjects with pregnant or contraindications to MRI (e.g., metallic, electronic devices, or implants) were excluded. Patients who met the aforementioned criteria were involved in the present study. HCs were recruited through WeChat, QQ, and poster with the same inclusion and exclusion criteria applied for MA users.

Information on the demographics was gathered before the brain imaging was performed (e.g., age, gender and educational attainment). Furthermore, the Fagerström Test for Nicotine Dependence (FTND) and the Alcohol Use Disorder Identification Test (AUDIT) were collected to evaluate for cigarette smoking and alcohol use. The severity of present MA craving was measured before the fMRI, with the Methamphetamine Craving Questionnaire (MCQ) adapted from the Heroin Craving Questionnaire.

All MA subjects underwent two examinations. The first examination was performed from 1 week to 1 month after abstinence (MA1 group), and the second examination was performed at ∼1.5 years after abstinence (MA2 group). During this abstinence period, MA-dependent participants had no access to anything related to MA and received different types of drug treatment, including only traditional Chinese medicine ($n = 12$), only antidepressants and antianxiety medications ($n = 10$), both of these ($n = 19$), and placebo ($n = 2$) for 2 months after the first MRI examination. Before each MRI examination, these patients completed the MCQ.

MRI Data Acquisition and Preprocessing

All MRI data were acquired using a 3-T Siemens Skyra MRI scanner (Magnetom Skyra, Siemens, Germany) equipped with a 32-channel head coil. The structural T1-weighted three-dimensional magnetization-prepared rapid acquisition with gradient echo was acquired with the following parameters: 176 sagittal slices, slice thickness = 1 mm, gap = 0 mm, field of view (FOV) = 256 × 256 mm, repetition time (TR) = 1,450 ms, echo time (TE) = 2.03 ms, inversion time (TI) = 900 ms, flip angle = 30°, and voxel size = 1 × 1 × 1 mm^3.

Resting-state functional MRI scans were acquired using the following parameters: 36 axial slices, thickness = 4 mm, FOV = 220 × 220 mm, TR = 2,000 ms, TE = 30 ms, flip angle = 80°, and 225 volumes. All subjects were placed in the supine position with eyes open and foam padding between their head and the head coil to minimize head motion.

The resting-state fMRI data were preprocessed using the DPABI (version 3.0, http://rfmri.org/dpabi) software, which include the slice timing, realignment, spatial normalization, regression, smoothing, and filter. For functional image preprocessing, the first 10 volumes were discarded to avoid signal instability. Slice timing was conducted to correct the remaining volumes, and head realign was used to reject abnormal subjects who had more than 2 mm of motion or 2.0° of rotation. The realigned images were transformed to the Montreal Neurological Institute standard space using the DARTEL algorithm. Then, the signals were regressed out, including the global signal, cerebrospinal fluid signal and white matter signal, and the 24 head motion parameters. Afterward, the images were smoothed with the Gaussian kernel using the full width at half maximum of 6 mm and bandpass temporal filtered (0.01–0.1 Hz) to reduce the low-frequency drift and physiological high-frequency noise, including the breath and heartbeats (26).

Calculation of Graph Metrics

A total of 15 graph metrics were calculated using the GRETNA software (version 2.0.0, https://www.nitrc.org/projects/gretna/), which included seven global network metrics: clustering coefficient, shortest path length, global efficiency, local efficiency, assortativity, synchronization, and hierarchy, and eight nodal network metrics: nodal clustering coefficient, nodal shortest path length, nodal efficiency, nodal local efficiency, degree centrality, betweenness centrality, community index, and participant coefficient. The Anatomical Automatic Labeling template was used to extract the average time series of the blood oxygen level–dependent (BOLD) signals in each brain region. There were 90 regions across the whole brain. Next, the correlation between each pair of the regional time series of the BOLD signal was calculated as FCs, resulting in 4,005 ($90 \times 89/2$) FC values, excluding the self-correlation. Then, these FCs were used to calculate the graph metrics for each subject, with a sparsity threshold ranging from 0.05 to 0.50, in steps of 0.05. For features extracted from graph metrics, each subject has one value with the whole brain for global network metrics and 90 values with 90 brain regions for nodal network metrics. Afterward, these features were reduced into the top features in dimension that could be entered into the SVMs also with the same sparsity threshold.

Statistical Analysis

The statistical analysis was performed on the graph metrics ($7 \times 1 + 8 \times 90$, 727 features) to extract the top features using the GRETNA software. General linear model was performed between the MA1 group and HC group, with education level, FTND, and AUDIT as the covariables. Repeated-measures analysis of variance (ANOVA) was performed between the MA1 group and MA2 group for each graph metric. A strict false discovery rate of 0.05 was applied to the analysis results as the multiple-comparisons correction. Then, features that significantly differed between groups were considered as the top features and entered into the SVMs. The top features that differed between the MA1 group and HC group were extracted from these two groups and entered into the classifier for identifying those with MA addiction. In addition, another classifier for predicting the treatment response received top features extracted from the MA1 group, which differed between the MA1 group and MA2 group.

SVM-Based Classification

Two classifiers were conducted with one for investigating the best top feature combination to differentiate patients from controls and the other for investigating the best top feature combination to predict the treatment response of MA addicts. This was performed using the SVMs implemented in the LIBSVM classification library (38, 39). The adopted linear kernel allowed for the interpretation of the weight vector (i.e., the relative importance of each feature in the classifier). Then, the weight vectors were subsequently used to rank the importance of the features in identifying MA. The SVMs were initially trained using all top features derived from the graph metrics. In order to identify the most informative feature, the least important feature (in terms of weight vector) was removed after each round of SVM training, and a new SVM was trained with the remaining features. This process was repeated until only a single feature remained. The accuracy of the classifier was recorded at each stepwise removal. Leave-one-out cross-validation was used, in which one subject was iteratively left out as the testing target, and the remaining samples were used to train the SVMs. Each SVM was repeated for 1,000 times (40, 41).

A patient was termed as a responder if the MCQ scores exhibited a 50% decrease or more. The percentage drop of the MCQ score was calculated, as follows:

$$d = \frac{\mathrm{MCQ_{first}\text{-}MCQ_{second}}}{\mathrm{MCQ_{first}}}. \quad (1)$$

According to the value of d, the MA-dependent participants were divided into two groups: responder group and non-responder group. Then, the SVM was used to classify whether the MA-dependent participant would be a responder after the abstinence treatment.

RESULTS

Patient Population

In the process of image preprocessing, nine MA-dependent participants and three HCs could not survive from the realignment, ultimately resulting in the involvement of 34 MA-dependent participants and 35 HCs in the present study. The demographic characteristics of the MA and HC groups are presented in **Table 1**. Among the 34 MA-dependent participants, there were 21 responders and 13 non-responders, and their demographic characteristics are presented in **Table 2**.

Classification Performance of MA-Dependent Patients

After the general linear model was performed, 23 top features remained, and all these top features were entered into the SVMs. Through the stepwise removal of the top features, the investigators were able to arrive at an optimal combination, which identified patients in 73.2% [95% confidence interval (CI) = 71.23–74.17%] of the cases (**Table 3A**). The progressive removal of the first 20 top features (in order of increasing feature importance) increased the classification accuracy, and this was the result of the reduced overfitting in SVMs. The most accurate combination of classifier features included the nodal efficiency in the right middle temporal gyrus (MTG.R), and the community index in the left precentral gyrus (PreCG.L) and cuneus (CUN.L), in which the community index in PreCG.L had the highest importance. The SVM accuracy decreased when additional top features were added or subtracted from the optimal combination. The accuracy and evolved top features of each SVM are shown in **Figure 1**.

Classification Performance of Treatment Response Prediction

After the ANOVA was performed, 12 top features remained, and all these top features were entered into the SVMs. Through the

TABLE 1 | Clinical characteristics of the participants (mean ± SD or median [IQR]).

Characteristics	MA1 ($n = 34$)	MA2 ($n = 34$)	HC ($n = 35$)	*T* value	*P*-value
Age (years)	32.62 ± 8.80	—	35.14 ± 7.94	−1.25	0.22
% Male	58.8	—	62.9	0.12	0.81
Education level (years)	8.87 ± 2.49	—	11.19 ± 2.78	−3.65	0.001
Duration of MA use (years)	4 (2.88,6.75)	—	—	—	—
Average abstinence (days)	21.5 (8.75,45.25)	406.50 (331.75,447.50)	—	5.09	<0.001
FTND	4.74 ± 2.29	—	2.34±2.55	−4.10	<0.001
AUDIT	3.5 (0,8)	—	0 (0,3)	−2.62	0.009
MCQ	55.50 (5.50,61.00)	25.50 (0,32.25)	—	−4.78	<0.001

TABLE 2 | Demographic characteristics of the responders and non-responders of MA-dependent participants [mean ± SD or median (IQR)].

Characteristics	Responders ($n = 21$)	Non-responders ($n = 13$)	*P*-value
Age (years)	30.81 ± 8.27	35.54 ± 9.15	0.13
% Male	92.31%	61.9%	0.002
Education level (years)	9.28 ± 1.74	8.19 ± 3.35	0.22
FTND	4.71 ± 2.41	4.76 ± 2.16	0.95
AUDIT	4 (0,8)	3 (0,9)	0.85

TABLE 3A | Individual identification of MA-dependent patients.

N patients (*N* HC)	Accuracy	Sensitivity	Specificity
69 (35)	73.2% (95% CI: 71.23–74.17%)	66.05% (95% CI 63.06–69.04%)	80.35% (95% CI: 77.77–82.93%)

stepwise removal of the top features, the investigators were able to arrive at an optimal combination, which identified patients in 72.2% (95% CI = 69.28–73.12%) of the cases (**Table 3B**). The progressive removal of the first eight top features (in order of increasing feature importance) increased the classification accuracy. The most accurate combination of classifier features included the nodal clustering coefficient in the right orbital part of superior frontal gyrus (ORBsup.R), the nodal local efficiency in ORBsup.R, the right triangular pat of the inferior frontal gyrus (IFGtriang.R), and right temporal pole of the middle temporal gyrus (TPOmid.R). Among these, the nodal local efficiency in TPOmid.R had the highest feature importance. **Figure 2** presents the accuracy and evolved top features of each SVM.

In order to further validate the classification performance (i.e., generalization) of the classifiers, these results were then replicated in a separate test group of patients including 10 MA-dependent patients (four responders and six non-responders) and 10 HCs. These features differentiated the independent test data with the accuracy, sensitivity, and specificity of 67.7% (95% CI = 65.7–69.69%), 61.7% (95% CI = 58.69–64.81%), and 73.65% (95% CI = 70.82–76.48%), respectively, and predict the responders with the accuracy, sensitivity, and specificity of 67.2% (95% CI = 65.40–69.40%), 58.2% (95% CI = 55.1–61.36%), and 76.2% (95% CI = 73.35–79.05%).

DISCUSSION

In the present study, the investigators filtered informative features about MA addiction and treatment by the training of classifiers using the SVM combined with the graph metrics, which could provide possible biomarkers to identify and predict the treatment response for MA-dependent patients at the individual level.

In the classifier for identifying MA-dependent patients, it was found that the combination of nodal efficiency (MTG.R), community index (PreCG.L), and community index (CUN.L) exhibits the best performance. In the graph theory, the nodal efficiency for a given node characterizes the efficiency of the parallel information transfer of that node in the network and quantifies the importance of this node for the communication within the network (42). In addition, the community index indicates which community or modularity a given node belongs to. Changes in the community index generally suggest the changes of locally connected clusters or modules implicated in specialized information processing (43). Both of these were used to describe the location and efficiency of nodes in the information transfer. As for the regions observed in present study, the middle temporal gyrus was previously described as the dorsal visual streams that encoded the spatial information among objects (44). A previous study also reported an activation in response to less complex visual images in the posterior right middle temporal gyrus when healthy adult participants received MA, and it was concluded that MA may change the relative sensitivity of higher-order sensory processing (45). The precentral gyrus is well-recognized as the primary motor cortex, and this was reported to have a reduced activation, when compared to HCs, in performing the emotion-matching task with fearful non-face images (46). The reduced gray matter of the left precentral gyrus was consistently observed in study of Hall et al. (47). In addition, Van Hedger et al. (48) reported the greater activation in the bilateral cuneus when exploring the neural responses correlated to the visual–auditory stimuli paired with MA in healthy people. These three brain regions were all involved in the sensory and emotion processing. The

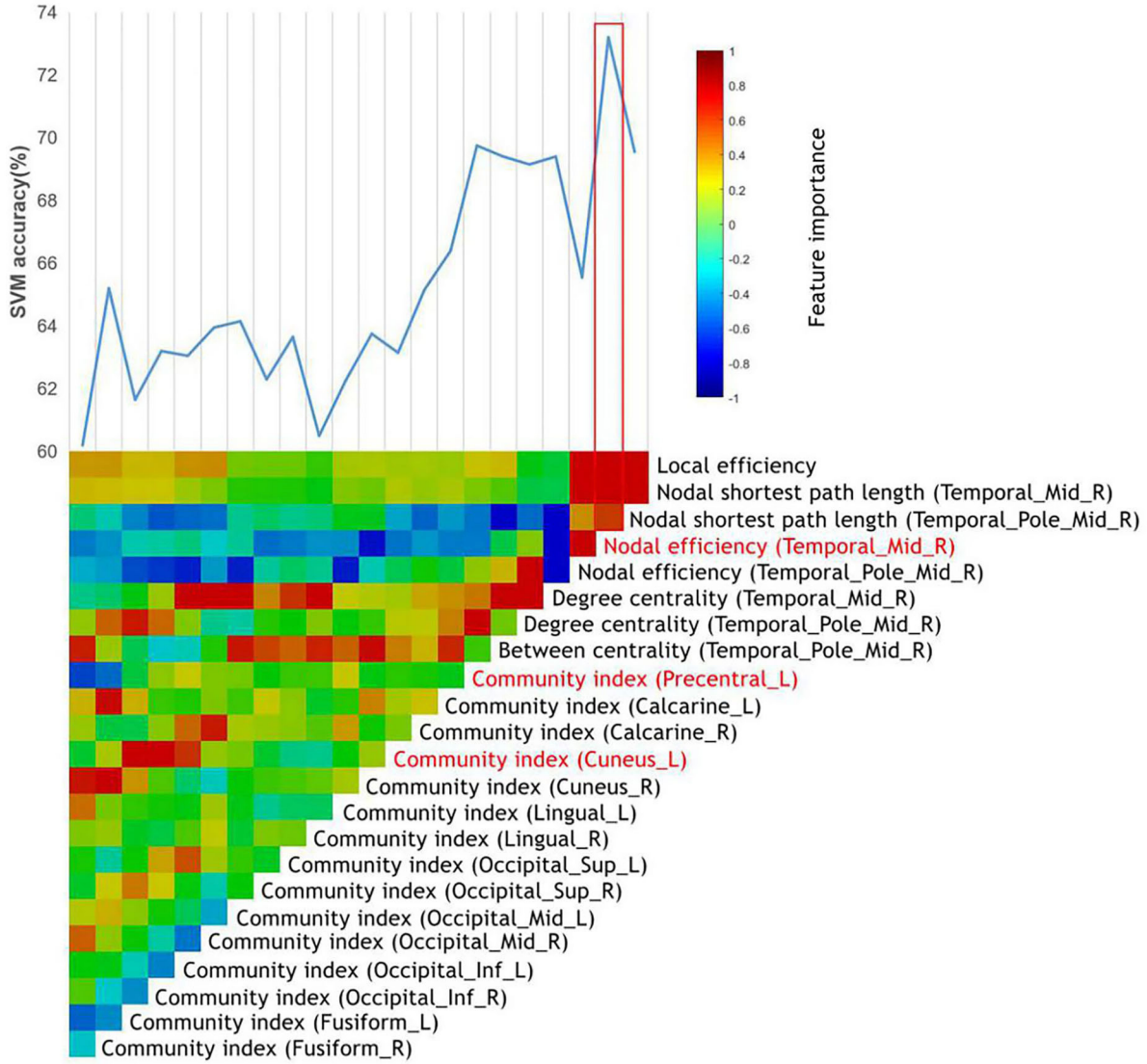

FIGURE 1 | The identification of MA-dependent patients using the graph metrics. The vertical color bar represents the relative feature importance of all top features when used together in training the SVM to differentiate MA-dependent patients. The stepwise removal of the least influential feature was repeated until a single top feature remained. At each stage of the feature removal, the accuracy of the retrained SVMs was recorded above the color bars. The most influential feature combination was outlined in red, which included the top features highlighted in red.

altered nodal efficiency or community index of these regions may affect the correction and efficiency of information translation and damage the integrity of sensory and emotional pathway. These abnormalities are partly correlated to the negative emotions and hallucinations of MA patients. Interestingly, these present results mainly revealed the sensory and emotion processing-related brain regions, instead of the dopaminergic reward circuits (49), providing support to the possibility that sensory effects could also become conditioned (50). Furthermore, it was speculated that the diagnosis of MA is more closely correlated to the sensory and emotion function and that the combination of features mentioned above could be a potential subjective predictable biomarker for MA abuse.

TABLE 3B | Individual prediction of responders to the abstinence treatment of MA-dependent patients.

N patients (*N* responders)	Accuracy	Sensitivity	Specificity
34 (21)	72.2% (95% CI = 69.28–73.12%)	86.75% (95% CI = 84.48–88.92%)	55.65% (95% CI = 52.61–58.79%)

On the other hand, in the classifier for predicting the responders of MA-dependent patients, the investigators found that the combination of nodal clustering coefficient (ORBsup.R), nodal local efficiency (ORBsup.R), nodal local

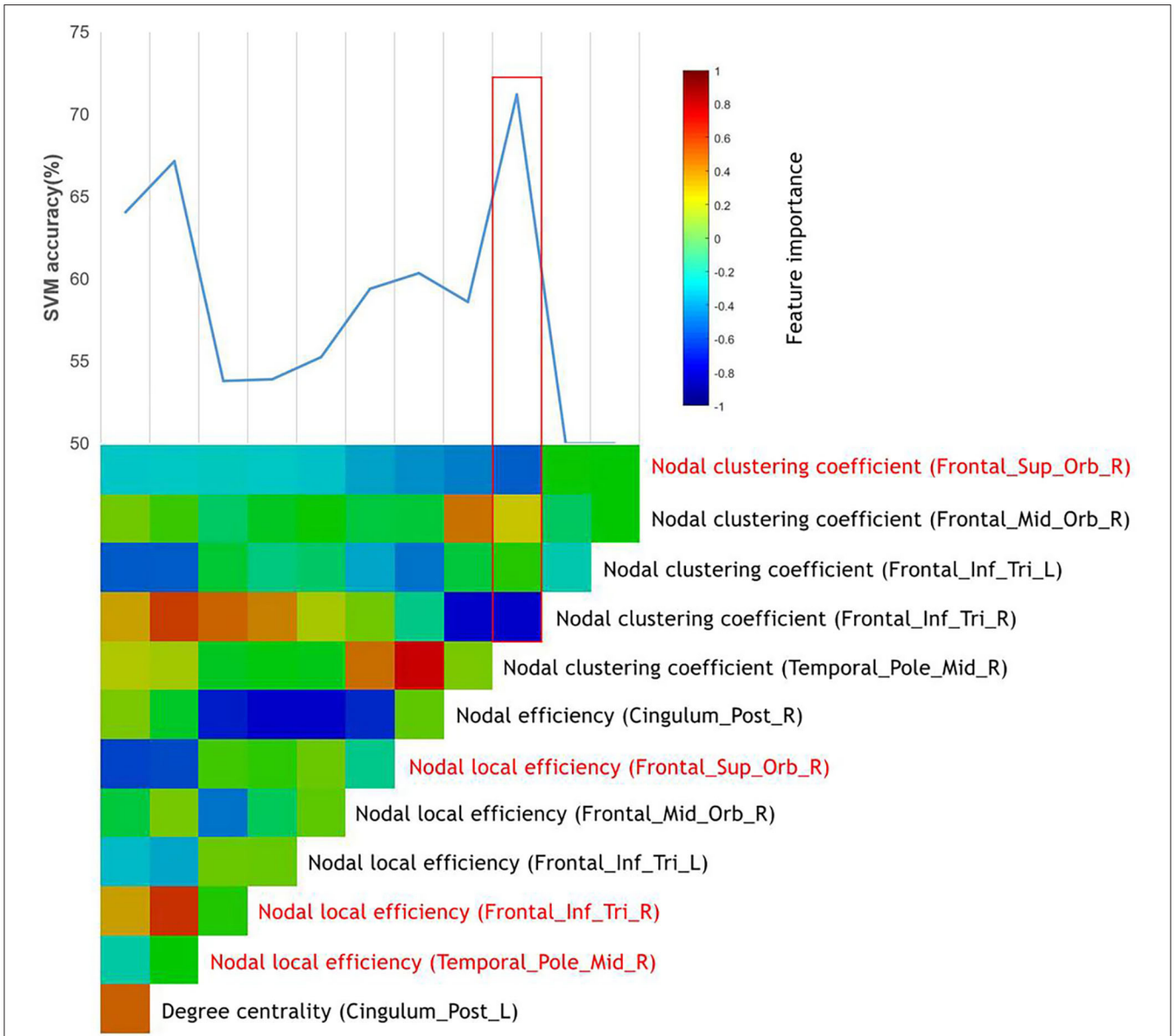

FIGURE 2 | The prediction of responders of MA-dependent patients to abstinence treatment using graph metrics. The vertical color bar represents the relative feature importance of all top features when used together in training an SVM to differentiate the responders of MA-dependent patients. The stepwise removal of the least influential top feature was repeated until a single top feature remained. At each stage of the feature removal, the accuracy of the retrained SVMs was recorded above the color bars. The most influential feature combination was outlined in red, which included the top features highlighted in red.

efficiency (IFGtriang.R), and nodal local efficiency (TPOmid.R) performed best. The nodal clustering coefficient of a given node describes the range or amount of potential connections that the closest neighbors of the node actually have and measures the likelihood that its neighborhoods are connected to each other (51, 52). In addition, the nodal local efficiency is the global efficiency computed on the neighborhood of the node and measures how efficient the communication is among the first neighbors of this node when it is removed (43). Both metrics describe the importance of nodes in brain networks, and changes of these, usually in mean disordered network functions. The activation of the orbitofrontal cortex and the temporal poles is correlated to empathy ability. Kim and colleagues (53) reported hypoactivation in the orbitofrontal cortex and temporal poles in MA abusers relative to healthy subjects during empathy progression. This may be correlated to the lower levels of dopamine D_2 receptor availability and decreased dopamine transporter density in the orbitofrontal cortex and temporal poles in the MA abusers (54, 55). During the social interaction, empathy plays a pivotal role that enables

the understanding of others' thoughts well and predicts their future actions, allowing for better social communication. Disordered empathy in MA abusers could reduce impairments in social interactions that contribute to stress, which may increase the negative mood states and the risk of relapse. In addition, Paulus et al. (56) suggested that the integrity of the orbitofrontal cortex is important for decision-making when the results are uncertain. Similarly, the inferior temporal cortex has been considered to be involved in the brain networks of decision-making (57). Using a two-choice prediction task with fixed error rates, Stewart et al. (58) reported the reduced activation in the inferior temporal cortex, which is suggestive of reduced attentional resources allocated to decision making. These provide insight into the nature that MA abusers, who usually ignore the risk factors of drug use, exhibit compulsive drug use behavior. Taken together, these present results suggest that better treatment may improve the impaired network function of the orbitofrontal cortex, temporal poles, and the inferior temporal cortex, rehabilitating patient's ability of empathy and decisions making, to some extent. Improved interpersonal communication could further improve the patient's negative mood status, making MA withdrawal smoother. The restoration of decision-making ability also enables patients to make choices that are better for their own health. The present study also provides a starting point for studying the recovery mechanism of brain regions damaged by MA and a new thought about therapeutic targets from the perspective of treatment prediction.

Several considerations need to be taken into account with respect to the interpretation of these present findings. First, the sample size of the present study was limited because of the inherent challenges in recruitment of MA dependents. Usually, the machine learning method needs a large sample size to obtain good classification results. Hence, the smaller sample size in the present study may have had an impact the classification accuracy. A larger sample replication would strengthen the generalizability of the present methods and allow for the full exploration of the parameter and feature space. Moreover, because of the limited sample size in different treatment groups, we were not able to distinguish between different treatment groups. Second, The MA and HC groups differed by not only MA use but also education, nicotine, and alcohol use. Although we have removed them as covariables when we performed general linear model between these two groups, it would be difficult to claim that the classifier is only differentiating based on MA use. Third, future studies may go beyond defining responders to treatment by those who showed 50% reduction in craving and, instead, use abstinence criteria. This was not possible in our study because of the strict abstinence policy of the rehabilitation centers from which we drew our participants. Finally, the individual identification of MA-dependent patients was merely based on a sample that included MA-dependent patients and HCs. Hence, patients should be collected in future studies who experience first-episode psychosis in association with conditions other than MA, such as heroin or cocaine.

CONCLUSION

The present study identified the most relevant features of MA addiction and treatment based on SVMs and the features extracted from the graph metrics and provided possible biomarkers to differentiate and predict the treatment response for MA-dependent patients. Brain regions involved in the best combinations need to be given close attention during the treatment of MA. The present study provides a step toward the individualized identification and treatment response prediction of MA-dependent patients, laying a basis for precision medicine approaches.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Second Xiangya Hospital of Central South University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

JLiu conceived of the study. RY contributed to study design and manuscript modification. CY and XY performed analysis and drafted the manuscript. WY, JLuo, and FT performed data collection. SH performed data processing and statistical analysis. All authors revised the manuscript and have read and approved the final version of the manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Associations Between Internet Addiction and Gender, Anxiety, Coping Styles and Acceptance in University Freshmen in South China

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Objective: Internet addiction (IA) has become a global public health issue. Although previous studies revealed several risk factors related to IA, most of them focused on the western societies. The present study assesses the relationships between gender and other factors with IA in university freshmen in the South China.

Methods: A total of 3,380 first-year college students (1,995 males and 1,385 females) participated in an evaluation of their experiences surfing on the Internet. We investigated the severity of IA in the participants by considering their psychological characteristics, such as acceptance, anxiety levels, and coping styles. Then, we compared the results between males and females and between those in addiction group (Chinese Internet Addiction Scale, CIAS, scores≥64) and non-addiction group (CIAS scores ≤27). We also conducted a logistic regression analysis to detect the relationships between severity of IA and psychological characteristics and gender differences.

Results: We observed that males showed significantly higher scores in CIAS than females. The addiction group exhibited significantly higher state anxiety and trait anxiety, and experienced less acceptance of self and others and acceptance by others, and adopted less positive coping style and preferred negative coping style than non-addiction group. The logistic regression analysis revealed that three factors (negative coping styles, acceptance of self and others, state anxiety levels) had a significant association with more severe IA.

Conclusion: Gender differences affect the severity of IA in the first-year students in South China. Males with state anxiety and negative coping styles deserve attention because they are likely to be addicted to the Internet. Thus, health practitioners should perform efficient strategies while considering gender differences to precaution first-year college students with the risk factors for IA.

Keywords: internet addiction, students, prevention, gender differences, anxiety, coping style, acceptance

INTRODUCTION

The number of Internet users increases rapidly worldwide given that the Internet altered people's lifestyles through its advantages in communication, socialization, and even online education (1). However, Internet addiction (IA), as a fast-growing addictive behavior, has become a significantly global health problem (2). The concept of IA, also termed as pathological Internet use, Internet overuse, and problematic Internet use by various researchers, is an interpretation for uncontrollable, disruptive use of the Internet with psychological dependence. People manifest this behavior as significant/overuse, withdrawal, tolerance, negative influence/conflict, desire, and emotional regulation (3, 4). It may cause difficulties in maintaining real-life relationships, impair daily activities, develop poor dietary habits, worsen school performance, and interfere with professional functioning (5, 6). And IA could increase the risk of psychological distress, such as depression, anxiety, low self-esteem (7–9). Others showed its relations with behavior problems, such as aggression, self-hurt behaviors, and even suicide (7, 10).

Education institutions have efficiently used Internet instruments to train students, especially in academic lectures, interaction with teachers, classroom discussion, homework, and sharing of learning resources (11). However, a recent meta-analysis showed that the IA overall prevalence rate in Chinese college students was 11%, which was higher than the other countries, such as Japan (3.7%) and Italy (4.3%). Male students (16%) had a higher rate of IA than their female counterparts (8%) in China (12). Chen et al. showed that the prevalence of IA in grades 3, 5, and 8 students from primary to middle school (aged < 18) was above 10% in Taiwan (13). The prevalence rate for IA in adolescents in Japan was ranging from 2.8 to 9.9% (14). Longitudinal studies have revealed that the incidence rate for junior high school students was 2.5–3.6% after a 2-year follow up in China (15). A cross-cultural epidemiological study showed that Asian adolescents have significantly higher rates of IA than their counterparts in the Western countries (16).

Regarding the gender influence on IA, previous studies produced inconsistent results (17–19). Alamgir et al. found that IA has no relation to gender but has a negative association with physical activity (19). Marzilli et al. concluded similar results (20). However, a recent meta-analysis showed that male Chinese college students (16%) have a higher rate of IA than their female counterparts (8%) (12). Shek et al. revealed that gender difference remains a factor for high Internet addictive behavior among high school teenagers in Hong Kong (21). By contrast, Chiu et al. found that female college students in Taiwan are more likely to develop IA through their smartphones than male students (22). A variety of factors, such as personal habits, access to the Internet, cultural values, and institutional policies, may have an attribute to different results regarding gender and IA. Moreover, the incidence of Internet related mental health problems is significantly higher in only children than in those with siblings. IA has more effect on families with only children and single-parent families (23). Accordingly, studies should consider family factors when evaluating IA.

Anxiety is a dangerous element for IA (24, 25) and is one of the personality characteristics of behavioral addiction tendency (26). Cross-sectional studies have shown that IA has a significant relation to anxiety (7, 10, 27). A Meta-analysis also revealed similar results (25). Both symptoms of pathological Internet use and emotional troubles could lead to a vicious cycle that favors the continuation of pathological Internet use (28). In addition, IA studies showed its relations to negative coping styles. Mcnicol et al. found that avoidance coping style has a positive correlation with IA (29). Using less effective stress coping styles might increase the risk of IA after 1 year follow up in college students (30). People who commonly use less effective stress coping strategies might overuse the Internet in order to get rid of or vent stressful events (29, 30). Although previous studies reported the risk factors related to IA, the understanding to it may still be incomplete. Whether the risk factors related to IA are culture-specific remains unclear, as most researches on risk factors have focused on the Western societies. The coping styles, acceptance of self and others, and the relationship between teenagers and their classmates or teachers have a great effect on the psychological status of individuals, which may affect their Internet use. From the perspective of China's development index, the development level of different regions varies greatly, and the Internet resource distribution is uneven. To the best of our knowledge, no study has explored the combined effects of emotional state, coping styles, and acceptance of self and others as leading to IA in first-year college students in the South China. Hence, we think the data in the present study may provide a significant contribution to this field.

Young adult Internet users, particularly college students, are more likely to be addicted to the Internet than older Internet users (31). Psychological and environmental factors in college students' lives might make them easily vulnerable to IA (32). Several reasons might attribute to the situation: (1) universities offer free and unlimited Internet access; (2) college students have considerable free time; (3) college students are out of their parents' control for the first time, with no one monitoring or censoring what they say or do online; (4) young college students have new problems adjusting to college life and making new friends, often seeking friendship through different applications in the Internet; (5) college students are training to use various technological inventions and applications, particularly the Internet (33). This study aims to explore the relationship between IA and gender, coping styles, anxiety, and acceptance in the first-year college students. We expected that these factors could predict the consequences of IA in first-year college students in the South China. We hypothesize that gender differences exist in IA, wherein males may have a higher risk of IA than females. We also hypothesized that higher levels of anxiety, negative coping styles, and less acceptance of self and others might predict a higher risk of IA.

METHODS

Participants

We conducted a cross-sectional study in Changsha, one of the largest cities in the South China, from October 10 to December

20, 2019. Inclusion criteria were as follows: (1) active Internet users for more than 1 year; (2) college freshmen agreed to complete the questionnaire. The exclusion criteria were those who were unwilling to complete the questionnaires or had no access to the Internet. Two master level research assistants spearheaded the recruitment of the participants. They carefully explained the research purposes and procedures and highlighted confidentiality problems to obtain a written informed consent from the participants. A total of 1,995 males and 1,385 females participated in this study. The participants were 16–24 years old. The average age of the participants was 19.8 years old. Then, they were divided to two groups, including the addiction group and non-addiction groups according to the Chinese Internet Addiction Scale (CIAS). Concerning the procedural side, the researchers first explained to the students the purposes of the research. Then, before handing out the questionnaires to the willing participants, the research assistants asked for their informed consent (An additional informed consent was obtained from the parents or guardians for those aged below 18 years old). During the survey, the research assistants instructed the participants to sit separately, stay quiet, and not participate in discussions.

Our study involving human participants obtained approval from the Ethics Committee of the Second Xiangya Hospital of Central South University and the university's institutional review board and executed in line with the Helsinki Declaration.

Measurements

CIAS Measure

CIAS was used to evaluate the severity of IA in the participants. CIAS is one of the most widely applied assessments on IA with a good internal consistency (16). CIAS is a validated evaluation for the main symptoms of IA and physical and social problems with the Internet (34), which has been widely applied as a criterion for IA in Taiwan and mainland China (35–37). This scale includes 26 items on a four-point Likert scale that assesses five dimensions of Internet-related problems, involving compulsive use, tolerance, withdrawal, interpersonal relationships, and time management and health, with a total score ranging from 26 to 104 (34). The reliability and validity of this scale were good (Cronbach's Alpha, 0.79–0.93) (34). The higher the CIAS score, the higher the severity of IA. The cut-off point of screening in this scale, with total scores ranging from 26 to 104, was 57/58 (38). Those with a score of 63/64 or more are considered Internet addicts (39, 40). The Cronbach alpha of this scale in the present study was 0.935.

State-Trait Anxiety Inventory

STAI was used to evaluate the severity of anxiety. It is a 40-item questionnaire on a four-point Likert scale, which comprises two separate subscales that measure the participants' state and trait anxiety levels, respectively, with good reliability and validity ($\alpha = 0.87$ for state anxiety, and $\alpha = 0.85$ for trait anxiety) (41, 42). Higher scores indicate increased state or trait anxiety levels. The subscale consists of 20 items, with scores ranging from 20 to 80. The "state anxiety" used in the present study shows how the participants feel "at the moment," whereas "trait anxiety" shows their "general feelings." The Cronbach alpha of this scale in the present study was 0.91.

Acceptance of Others

AO was compiled by American psychologist Fey (43) and translated and revised by a domestic scholar Xiaodong Fan. This scale, including 20 items on a five-point Likert scale with total scores from 20 to 100, assesses the participants' acceptance of self and others and acceptance by others (43, 44). The higher the two subscale scores, the higher the level of the participants' acceptance of self and others and acceptance by others. The split-half reliabilities for acceptance of self and others and acceptance by others were 0.90 and 0.89, respectively (43). The Cronbach's alpha coefficients for acceptance of self and others and acceptance by others in present study were 0.703 and 0.603, respectively. The split-half reliability in the present study was 0.758.

Simplified Coping Style Questionnaire

SCSQ, including 20 items on a four-point Likert scale with scores on each item ranging from 0 to 3, assesses the participants' positive and negative coping styles (45, 46). The higher the two subscale scores, the higher the level of the participants' positive or negative coping styles. The positive coping subscale includes items 1–12, which reflects the features of individuals' positive coping style in face of pressure. The negative coping subscale includes items 13–20, which reflects the features of individual negative coping style (47). The Cronbach's alpha coefficients for positive and negative coping styles were 0.89 and 0.78, respectively (45). The Cronbach's alpha coefficients for positive and negative coping styles in the present study were 0.637 and 0.626, respectively. The Cronbach's alpha of this scale in the present study was 0.61.

Statistical Analyses

All participants completed the four inventories after receiving instructions from the researchers. We used chi-square to analyze the difference in gender and family factors, including single-child family, adoptive parent condition and parents being alive between male and female groups. Then, we compared gender differences in age, CIAS, STAI, AO, and SCSQ scores by two-sample *t*-tests. Previous studies showed the cut-off point of 63/64 or more in CIAS has good accuracy, sensitivity and specificity for diagnosing IA (39, 40). To study the relationship between IA and other factors, including anxiety, acceptance, and coping styles, the subjects were divided into two groups, including the addiction group (CIAS scores ≥ 64) and the non-addiction group (CIAS scores ≤ 27). The matching non-addiction participants were selected from the lowest scores of CIAS. We also compared the differences in the STAI, AO, SCSQ, and CIAS scores between addiction group and non-addiction group by two-sample *t*-tests.

We analyzed the correlations between the severity of IA and STAI, AO, and SCSQ scores in male and female groups by using partial correlation analysis while correcting the confounding factor of age.

We further used a logistic regression to evaluate the relationship of IA in the predictive model between gender differences and other variables, including STAI, AO, and SCSQ,

while controlling the effects of age. A *P*-value of <0.05 was statistically significant.

RESULTS

Demographic and Clinical Psychological Characteristics

A total of 1,995 males and 1,385 females participated in this study. Among them, 33 lacked a record of their age who were not removed from the study, and 98 individuals lacked a record of whether they were a single child. Moreover, 16 participants lacked a record of whether they had stepparents; 109 participants lacked a record of whether their father is alive; 93 participants lacked a record of whether their mother is alive; 17 participants lacked a record of their satisfaction degree for the admission. The mean age of male and female participants was 19.91 ± 0.86 and 19.73 ±0.73, respectively ($P < 0.05$). For the family factor, only a small percentage of participants lost their father or mother, and no significant differences were found in the parents' alive condition between the male and female groups. Significant differences were evident in single-child families between the male and female groups (P <0.05), more participants in the male group were not single-child (male group: 1,141 for not single-child; and 785 for single-child). By contrast, no substantial differences were evident in the adoptive parent condition between the two groups. Most participants had no adoptive parents (male: 2.67/97.33%; and female: 3.56/97.44%). The details were offered in **Supplementary Table 1**. The following reasons may account for the unrecorded familial data: first, some participants might fail to complete the familial data; second, some participants were unwilling to finish them because family data were some personal privacy. The satisfaction degree of admission is used to measure the degree that the college freshmen were satisfactory with the admission of the current college. The survey of satisfaction is not only a rational evaluation of the students' perceptions and expectations, but also the psychological representation of college students' learning and attitudes, feelings and experiences. The satisfaction degree of admission may influence the emotion and behavior of college students. And it plays an important role in improving the overall quality of education and promoting education reform. For the satisfaction degree of the admission, 724 participants were very satisfactory for the admission; 1,462 participants were fairly satisfactory; 640 participants felt general; 404 participants were not quite satisfactory; 49 participants did not care for it; and 84 participants were very unsatisfactory.

Psychological Characteristics of College Freshmen by Gender

Males showed significantly higher scores in CIAS than females ($P < 0.05$), which indicated that the former had a higher risk factor of IA than the latter. Females experienced more acceptance of self and others ($P < 0.05$) and acceptance by others ($P = 0.003$) and adopted more negative coping styles ($P = 0.004$) than males. No gender differences were evident in state and trait anxiety levels and positive coping styles (Ps > 0.05). The details were offered in **Supplementary Table 2**. We have also conducted the comparison of scale variables between male and female groups using Wilcoxon tests of two independent samples. The results were offered in **Supplementary Table 3**.

Correlation Analysis

The CIAS scores in the male and female groups had significant correlations with acceptance of self and others (male: $P < 0.05$; female: $P < 0.05$), acceptance by others (male: $P < 0.05$; female: $P = 0.003$), positive coping styles (male: $P = 0.0007$; female: $P = 0.019$), negative coping styles (male: $P < 0.05$; female: $P < 0.05$), and state and trait anxiety levels (male: $P < 0.05$; female: $P < 0.05$) after controlling the confounding effects of age (**Figures 1**, **2**). The *p*-values were significant while correlation coefficients were very weak ($r < 0.20$) because of the high number of participants in our study. The details of correlation analysis result were offered in the **Table 1**. In addition, simple correlation coefficients among all variables in the study have been calculated and the results were offered in the **Supplementary Table 4**.

Comparisons Between Addiction Group and Non-addiction Group

We divided the participants into two groups, including addiction group (CIAS scores ≥ 64, about 477 persons) and non-addiction group (CIAS score ≤ 27, about 572 persons) based on the cut-off point of 64 for diagnosing IA (39, 40). The addiction group exhibited significantly higher state anxiety and trait anxiety ($P < 0.05$), and experienced less acceptance of self and others and acceptance by others ($P < 0.05$), and adopted less positive coping style ($P < 0.05$) and preferred negative coping style ($P < 0.05$) than non-addiction group, indicating that IA was associated with emotional and behavior disorders (**Table 2**).

Factors Related to IA

We used a logistic regression analysis (both unadjusted and adjusted) to investigate the predictors in the addiction and non-addiction groups while controlling the effects of age (1 = the addiction group, 0 = the non-addiction group). We found significant relationships between IA and negative coping styles ($P < 0.05$), acceptance of self and others ($P = 0.001$), and state anxiety levels ($P = 0.003$) (**Table 3**) in adjusted results indicating that these three factors had a significant association with more severe IA. In addition, we also conducted the regression analysis using a backward method and obtained results (**Supplementary Table 5**).

DISCUSSION

Our study focuses on exploring the relationship between IA and gender, coping styles, anxiety, and acceptance in the first-year college students in the South China. The results disclosed that males had higher scores in CIAS than females indicating that male might be a risk factor for IA, which is consistent with previous findings (12, 21). Among the participants, male first-year college students experienced less acceptance of self and others and acceptance by others and adopted less negative coping styles than their female counterparts.

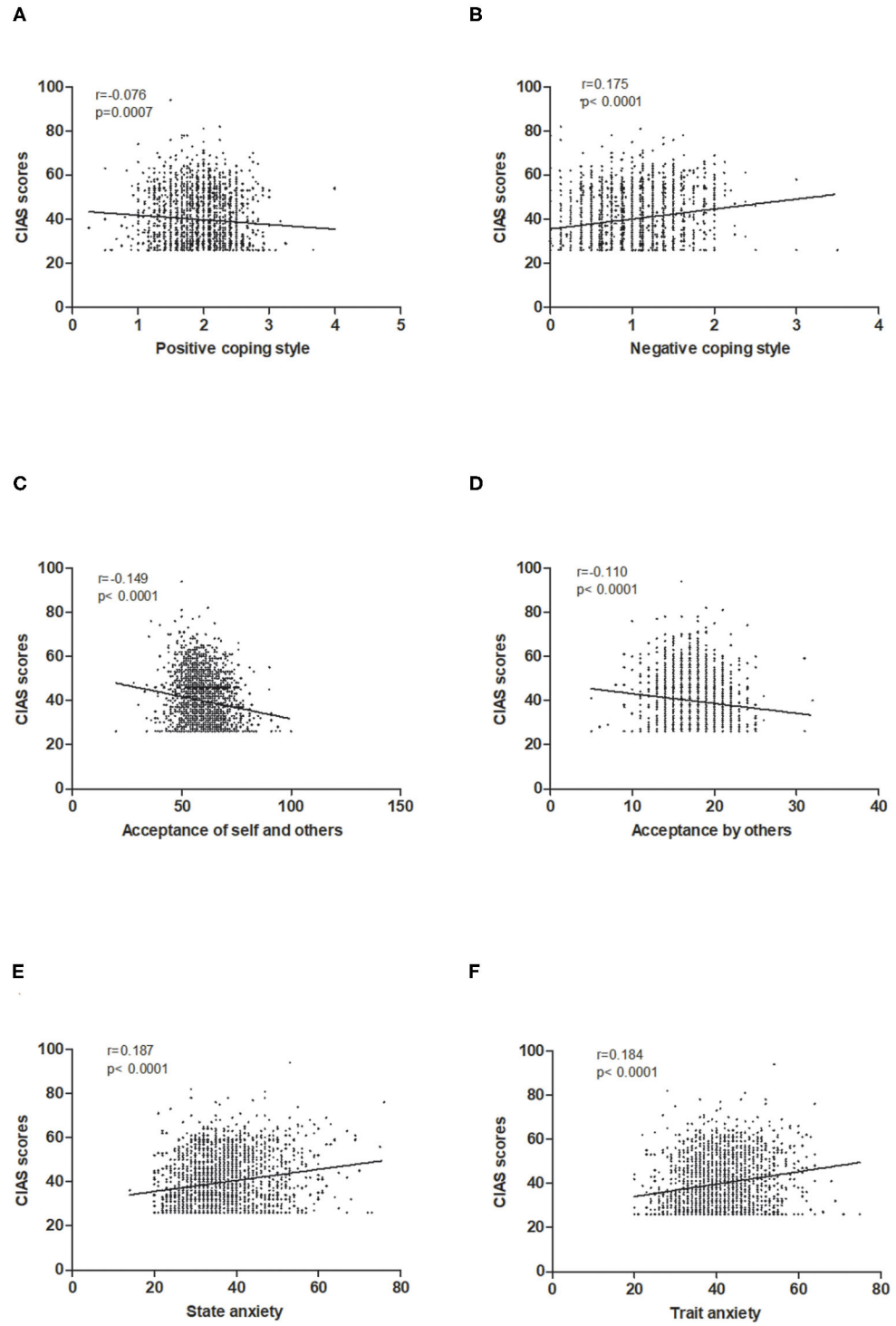

FIGURE 1 | Correlations between the severity of Internet addiction and psychological characteristics in the male group. **(A,C,D)** Negative correlations were observed between CIAS scores and acceptance of self and others, acceptance by others and positive coping style. **(B,E,F)** Positive correlations were observed between the CIAS scores and negative coping style, state anxiety and trait anxiety. CIAS, Chinese Internet Addiction Scale.

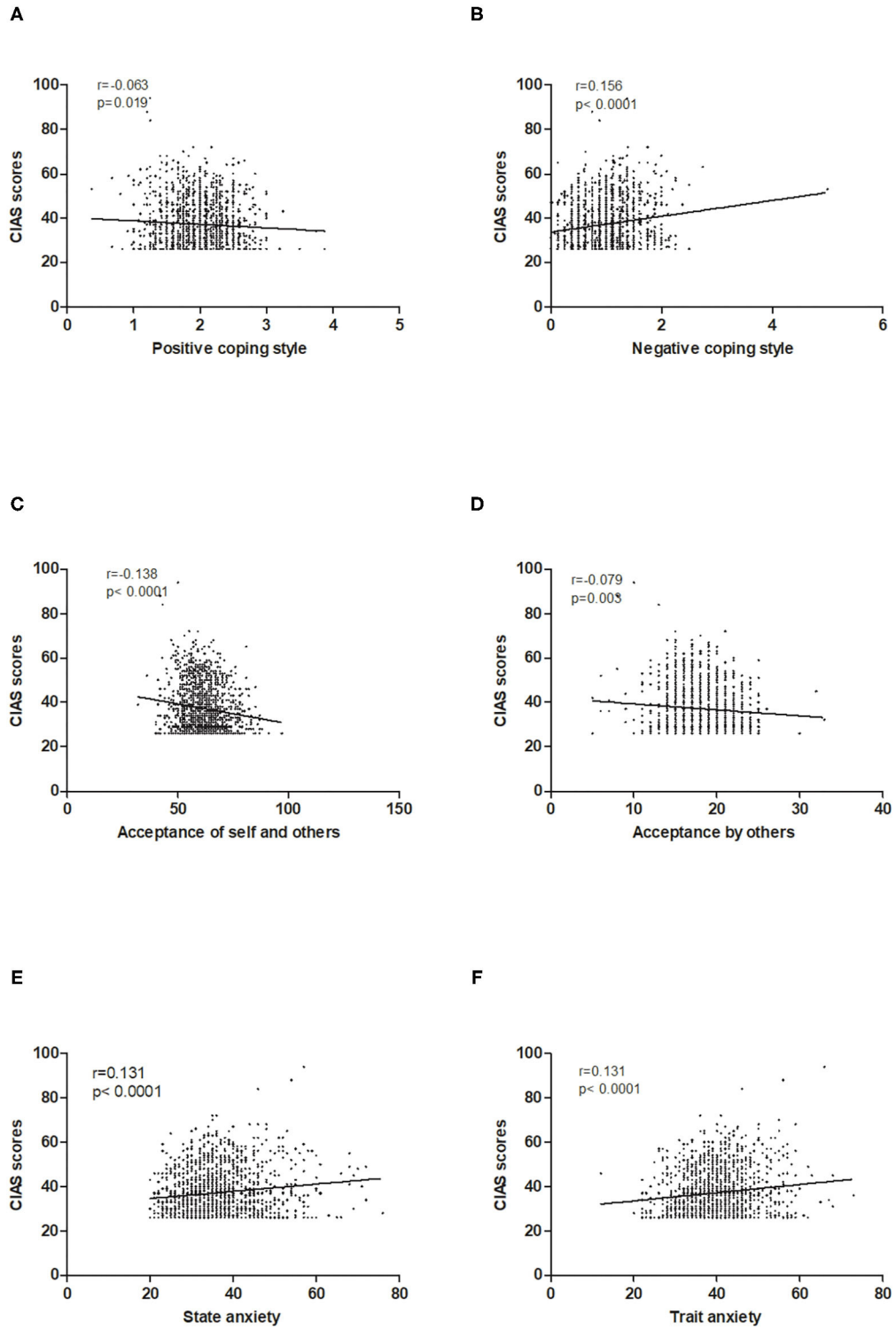

FIGURE 2 | Correlations between the severity of Internet addiction and psychological characteristics in the female group. **(A,C,D)** Negative correlations were observed between CIAS scores and acceptance of self and others, acceptance by others and positive coping style. **(B,E,F)** Positive correlations were observed between the CIAS scores and negative coping style, state anxiety and trait anxiety. CIAS, Chinese Internet Addiction Scale.

TABLE 1 | The correlations between CIAS scores and related factors in the male and female groups.

	Group	*R*	*P*
Acceptance of self and others	Male	−0.149	<0.0001
	Female	−0.138	<0.0001
Acceptance by others	Male	−0.110	<0.0001
	Female	−0.079	0.003
Positive coping styles	Male	−0.076	0.0007
	Female	−0.063	0.019
Negative coping styles	Male	0.175	<0.0001
	Female	0.156	<0.0001
State anxiety levels	Male	0.187	<0.0001
	Female	0.131	<0.0001
Trait anxiety levels	Male	0.184	<0.0001
	Female	0.131	<0.0001

CIAS, Chinese Internet Addiction Scale.

TABLE 2 | Comparisons between addiction group and non-addiction group.

Measure	Addiction group ($n = 477$)	Non-addiction ($n = 572$)	*p*
State anxiety	39.61 ± 9.11	35.29 ± 8.43	<0.001
Trait anxiety	42.72 ± 7.76	39.42 ± 7.89	<0.001
Acceptance of self and others	57.82 ± 7.40	61.21 ± 9.33	<0.001
Acceptance by others	16.93 ± 2.78	17.70 ± 3.15	<0.001
Positive coping style	1.89 ± 0.44	1.99 ± 0.44	<0.001
Negative coping style	1.10 ± 0.52	0.87 ± 0.47	<0.001
Gender (male/female)	70.44/29.56% (336/141)	66.96/33.04% (383/189)	0.227

P-values were obtained by two-sample t-tests except for gender (a Chi-square test).
CIAS, Chinese Internet Addiction Scale.

TABLE 3 | Factors related to Internet addiction ($n = 1,049$).

Regression factors	B		Wald test		*P*		Exp (B)		95%CI	
	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted
State anxiety	0.032	0.055	8.655	52.191	0.003	<0.001	1.032	1.056	1.011–1.054	1.041–1.072
Trait anxiety	0.004	0.053	0.089	40.105	0.766	<0.001	1.004	1.054	0.980–1.028	1.037–1.071
Acceptance of self and others	−0.029	−0.047	10.561	36.970	0.001	<0.001	0.972	0.954	0.955–0.989	0.939–0.968
Acceptance by others	−0.003	−0.086	0.013	16.311	0.909	<0.001	0.997	0.917	0.948–1.048	0.879–0.956
Positive coping style	−0.230	−0.555	1.967	14.883	0.161	<0.001	0.794	0.574	0.576–1.096	0.433–0.761
Negative coping style	0.750	0.953	26.615	48.245	<0.001	<0.001	2.116	2.594	1.592–2.814	1.982–3.395
Gender	0.217	0.164	2.305	1.493	0.129	0.222	1.242	1.178	0.939–1.643	0.906–1.533

Some possibilities might explain these differences. First, we found that males had a higher risk of developing IA than females. Previous studies revealed that males are more likely keen on the Internet for games, cybersex, and gambling to pursue feelings of achievement than females (48, 49). In addition to academic purposes, males tend to use the Internet for online games and information searching (50). By contrast, females tend to use the Internet for writing blogs, chatting, updating personal homepage, sending messages, and searching for information (51). Tateno et al. demonstrated that more males use the Internet for games, whereas more females use the Internet for social networking services, including Facebook and Twitter (52). Moon et al.

revealed that males are more likely than females to engage in drug use to improve social bonding and self-esteem (53). Although previous studies produced inconsistent results regarding the gender effects on IA, most studies in China reported that IA is significantly higher in males than in females which is consistent with our result (12). Shek and Yu revealed that gender difference remains a factor for a high Internet addictive behavior in high school teenagers in Hong Kong (21). IA studies also showed that males have a general loss of control over their ability to limit their usage and engaged themselves in online gaming and Internet relay chats (50, 54, 55). Such activities could increase self-image by providing opportunities to interact with people with similar interests and pursue strong and intelligent self-identification, both of which augment the additive risk for males (39). Males also tend to be unwilling to communicate with others and seek help, resulting in a low exploitation rate of social support (56). Second, female college freshmen tend to have more family supervision than their male counterparts, which can limit the time for females spending online. Females are more likely to treasure off-line interpersonal relationships and are usually more cautious to new relationships online (57, 58). Some females may also make boyfriends online. Nevertheless, it may not conduce to IA because they would like to spend more time nose to nose (51). Females are often taught to build a considerable, passive, and graceful self-image since childhood (55, 59). Thus, they often represent acceptance of self and others and acceptance by others and practice negative coping styles in daily life.

Regarding psychological characteristics, the correlation analysis and logistic regression results in the present study showed that IA has a significant association with negative coping style, acceptance of self and others, and anxiety levels. This finding is consistent with previous studies that IA has a significant relation to emotional and behavior disorders, including anxiety, depression, obsessive-compulsive, aggressive, and avoidance behaviors (7, 27). The symptoms of pathological Internet use and emotional troubles could lead to a vicious cycle that favors the continuation of pathological Internet use (28). Ko et al. identified social phobia, depression, and emotional troubles as predictors of IA (60). Kitazawa et al. also revealed that anxiety and depression might predict IA in young adults (42). A longitudinal study revealed a bidirectional relationship between psychiatric symptoms and IA in college students after one-year follow up (37). The addiction group exhibited more negative emotions, including state and trait anxiety levels compared with the non-addiction group in the present study, indicating that those individuals with IA have a high risk of anxious in daily life, which is consistent with previous studies (24, 25, 27). Individuals with long-term IA have a low level of mental health, given that their condition leads to a lack of interpersonal interaction, which is a dangerous element for mental illness (56). As for anxiety, more Internet use has an association with an individual's shrinking social circle, low self-esteem, loneliness, and low life satisfaction (55). As the level of social anxiety increases, adults will spend less time in their interpersonal relationships and invest more time in the Internet, implying a great risk of developing IA (54).

The addiction group experienced little acceptance of self and others and acceptance by others. These individuals adopt negative coping styles rather than positive coping styles when they encounter some obstacles. Wei-Po showed that using less effective stress coping styles might induce high risk of IA, significant depression, and suicide attempts in college students after 1 year follow up (30). Individuals with negative coping styles, such as avoidance coping style, have a positive association with IA (29). Tonioni et al. and Chou et al. have reported relationship between inappropriate coping strategies and IA (61, 62). Less effective coping styles might lead to difficulties in the real world, and worsen the emotional state in young people (30). The risk of suicide might augment in a vicious cycle of ineffective coping styles and negative emotions (30). Of note, correlation coefficients were very weak ($rs < 0.20$) although the p-values were significant for correlation analysis because of the high number of participants in our study. Usually correlation coefficients reflect that $r < 0.20$ is very weak, and 0.20–0.40 is weak correlation. Our results show significant relationships between IA and negative coping style, acceptance of self and others, and state anxiety by using a logistic regression. Thus, health practitioners and scholars should pay attention to these three factors when consulting with Internet addicts or doing some researches on IA.

The Internet provides many benefits to its users, which tempts an increasing number of college students to become immersed in it. Previous studies have revealed that parental guidance of Internet behavior is associated with IA, especially when parents rarely communicate with regards to Internet use, lack of rules regarding Internet time (63) and lack of rules regarding Internet use (64). Other family variables, including parental relationship (65), perceived parental monitoring (66), and family conflict (66, 67), were also related to IA. Our results have the following suggestions to address such a problem. First, parents should guide their children to ideal Internet use and effectively monitor their children's Internet use based on their educational knowledge of the Internet (51). Regardless of people's perspectives of it, the Internet has entered most people's lives and becomes an important part of their lifestyles. Parents should encourage their children to use the Internet reasonably and avoid the negative points of the Internet if possible. Second, young children should form a good habit of using the Internet "positively" under the supervision of their parents. Parents were required to establish an Internet use plan for their children to let them know the severity of IA (68). Third, parents should also communicate with their children to understand their needs and invest more time to help relieve their inner troubles (69). Finally, Internet addicts should consult with psychologists and psychiatrists and jump the traces of the Internet as early as possible.

Several limitations in the present study should be considered. First, the cross-sectional design in the present study could not allow inferring cause–effect relationship between IA and its related factors. Second, we collected data from a single college, and the findings might not generalize to all China or all university students. Third, this study did not explore the details of the participants' Internet use. We were unclear which games, social

networking services, or other scanning habits might significantly change Internet habits or dependence in the freshmen. Fourth, although the CIAS involves an assessment of time management to the Internet, we did not measure how much time the subjects spent in the Internet, and the usage of media device, such as game, smartphone, and laptop was not recorded in the present study. Fifth, the assessments are self-rating scales, which could easily generate potential information deviation. Sixth, previous studies have revealed that the acceptable alpha values range from 0.70 to 0.95 (59, 60). The Cronbach's alphas for SCSQ have a low internal consistency reliability in the present study (<0.7), suggesting that some items may need to be revised or deleted. Hence, the result should be interpreted with caution.

CONCLUSION

The present study revealed that IA is more common in male first-year college students than in their female counterparts, and males have a greater risk of developing IA than females. Although some studies have shown opposite results or no difference, research evidence (12, 21) has revealed that men tend to have a higher risk of IA. Our results are in line with the earlier and more recent findings that IA is high in men. State and trait anxiety levels, negative coping styles, little acceptance by others, little acceptance of self and others, and non-adoption of positive coping styles have an association with more severe IA in college freshmen. Thus, scholars and health practitioners should consider gender differences when formulating strategies to prevent IA, so that it could offer significative guidance for clinicians, parents and educators. Except for parental intervention and supervision, schools should encourage students to take part in more social practice activities and instruct students to use the Internet with a good habit. We should focus on the social education of IA in future research. In addition, future studies with more objective methods and lager sample are required to evaluate IA and related risk factors in high-risk populations.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

The study involving human participants was reviewed and approved by the Ethics Committee of the Second Xiangya Hospital of Central South University, and was also approved by the university's institutional review board and executed in line with the Helsinki Declaration. All the participants provided informed consent. An additional informed consent was obtained from the parents or guardians for those aged below 18 years old.

AUTHOR CONTRIBUTIONS

XS is mainly responsible for essay writing and conducting the study. YO, YD, and HY managed and analyzed the data. WG, JC, and JZ designed the study. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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GENERAL COMMENTARY
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Commentary: Craving in Opioid Use Disorder: From Neurobiology to Clinical Practice

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INTRODUCTION

We read with great interest the review article by Kakko et al. (1), which focused on the opioid use disorder-related craving. A recent review also discussed underlying neurobiological changes in opioid use disorder (OUD) that likely contribute to drug craving (2). However, how to assess the craving in clinical practice is challenging. Here, we would like to present the recent developments and briefly introduce our work on objectively assessing craving.

Current Practice of Evaluating Drug Addiction Cravings and the Limitations

Craving is the central concept of almost all major drug dependence models (3–7). In the International Classification of Diseases [ICD-11 (8)], craving is listed as one of the six characteristics of psychoactive substance dependence. In DSM-5 (9, 10), craving has once again become the recommended standard for diagnosing substance use disorder (SUD).

Current craving assessments are often accomplished through self-reports and observations of cognitive performance (11).

Despite the development of several psychometrically validated multi-item craving inventory tools (12, 13), self-reported cravings are still most commonly evaluated using a single item craving inventory (11). Since the intensity, latency, frequency, and significance of craving episodes may vary (14), self-reported assessments may have difficulty in reflecting true differences of cravings among patients (15). In daily clinical experiences, denial is one of the typical responses when we ask patients with alcohol and drug problems for their addictive behaviors, and in certain situations, for instance, in order to gain acceptance from family members, or gain enough trust to seek drugs privately, patients may conceal the true level of cravings.

Cognitive performance tasks are used to implicitly assess cravings (6, 16), based on the assumption that craving leads to the redistribution of cognitive processing resources and shifts that to drug-related cues (6). Therefore, facing the drug-related cues at a certain intensity, people with different intensities of drug addiction cravings would show different cognitive performances. Through recording cognitive performance parameters (e.g., the reaction time), it could reflect the situations of redistribution of cognitive processing resources, and therefore indirectly reflect the

related craving. This indirect assessment method may avoid subjective bias (15) and may help to understand how craving changes perceptions and decisions. However, it is very challenging to implement in clinical practice (11, 12).

Kakko et al. (1) also mentioned using ecological momentary assessments (EMA) to assess cravings (17, 18). EMA uses mobile technology to record a real-time (including daily, random, or event-triggered) cravings (17–20). However, EMA only improves the understanding of craving-related time fluctuations (17, 18), but fail to improve craving assessment itself.

Current Approach of the Assessment of Induced Cravings and the Limitations

In addition to Background cravings (7), cravings can also be induced through specific drug-related prompts or stressful life events (7). Most craving-inducing studies use the cue-reactivity paradigm (7), in which drug users respond differently to drug-related cues, in contrast with neutral cues, resulting in changes in brain activity related to the degree of craving (7).

The way to induce cravings at present depends mainly on the contents of pictures or videos. Although related to drug-seeking behavior and successfully induced cravings, they may not trigger reactions representing the real social or environmental cues (21), of which studies have shown that the treatment requires intervention (21).

New Approach to Evaluating Drug Addiction Cravings With New Technology

Our research team has designed the VR paradigm (22, 23) to improve the way to induce cravings, which can simulate the real world with the implicit cue, rebuild specific environments and characters, and allow certain social immersion and interaction, as well as the balanced drug-related cue and neutral stimuli. By technically arranging the sequence of stimuli and social interactions in the scenes, an effective evaluation experimental paradigm is formed.

Our results showed that the effects of different cues are related to the types of drug dependence, and VR stimulation could cause a higher level of cravings than the traditional picture and video stimulations, consistent with previous findings (14, 24). Through different types of induced stimuli, specific incentives for craving can provide a valuable therapeutic reference for clinical consultation and treatment.

During VR stimulation, we recorded several physiological parameters, including electroencephalogram (EEG), galvanic skin response, and heart rate (22, 23). We employed machine learning methods to analyze these physiological data to distinguish drug-dependent individuals from normal controls (22, 23).

We used machine learning algorithms, including random forest, AdaBoost, logistic regression, and ensemble model to build classification models (22, 23). The most striking findings were that the highest classification accuracy of 86% was obtained by the AdaBoost algorithm, with 84% precision, 83% recall rate, and 83% f1 score (23). Our results show that compared with traditional statistical methods, machine learning has advantages in processing massive and complex physiological data, which helps identify features that can distinguish different populations.

DISCUSSION

The level of craving is unstable and affected by multiple factors and changes along with the internal and external environments over time, while the patient's dependent state is relatively stable. Thus, it is possible to find stable biomarkers reflecting a patient's status related to dependence rather than reflecting the real-time craving level. This is urgently needed for clinical practice, not only for objective diagnosis and severity of dependence but also to be used as an early sign for relapse.

Because of the complexity of mental illnesses such as addiction, the traditional research methods based on reductionism were limited and overwhelmed (25). The emergence of new technologies, such as machine learning, has made holistic research possible in this field. The previous reductionist studies are hypothesis-driven; that is, they explore the regularity based on a relatively clear hypothesis. Such studies often focus on specific mechanisms, and the results are highly explanatory, while machine learning studies are driven by holistic data. Through in-depth mining of overall data, machine learning studies could provide more detailed results and new directions, although the interpretability is sometimes poor.

The advantage of the holism method should be considered with the help of multidisciplinary forces and high-level technology, such as biophysics, non-linear computing, and machine learning. Using holism methods to find the regularity first and then exploring the specific mechanism may be a promising solution. We developed a model that could accurately distinguish drug-dependent individuals and normal controls by using machine learning algorithms, obtained potential key biomarkers of the dependent state, and achieved some promising results (26).

At the same time, we explored the correlation between the probability results of machine learning algorithms and some other clinical indicators, including not only the subjective craving inventory score but also drug use-related indicators, such as drug use duration, dosage, and frequency. Since the correlation could be intricate, we track and analyze most of the relevant indicators. Even though the research may not yield satisfying results in the short term, using machine learning to classify the population is meaningful in itself and provides a direction for further exploration of the biological nature of dependence and addiction.

AUTHOR CONTRIBUTIONS

WH initiated the project and designed the work. RZ and LH were approached to write the paper, structured it, and wrote sections Introduction and Current Practice of Evaluating Drug Addiction Cravings and the Limitations. YL and TZ wrote sections Current Approach of the Assessment of Induced cravings and the Limitations and New Approach to Evaluating

Drug Addiction Cravings With New Technology. DB and LZ wrote section Discussion. RZ, LH, DL, and WH revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: RZ, YL, TZ, DB, LZ, and DL were employed by the company Adai Technology (Beijing) Ltd., Co.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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