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Community series in neurobiological biomarkers for developing novel treatments of substance and non-substance addiction, volume II

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Community series in neurobiological biomarkers for developing novel treatments of substance and non-substance addiction, volume II

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Editorial: Community series in neurobiological biomarkers for developing novel treatments of substance and non-substance addiction, volume II

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Editorial on the Research Topic
Community series in neurobiological biomarkers for developing novel treatments of substance and non-substance addiction, volume II

In 2021, we published the first special issue on "*Neurobiological Biomarkers for Developing Novel Treatments of Substance and Non-substance Addiction*" in *Frontiers in Psychiatry* that covered a wide breadth of research, such as animal studies, clinical studies, cross-section studies, and meta-analyses (1). These studies highlighted neural mechanisms, detecting biomarkers, diagnosis, and treatment responses of substance and non-substance addiction. In parallel with significant breakthroughs in neuroscience studies, recent years have seen tremendous advances in several frontiers in substance and non-substance addiction. Therefore, it is time for us to summarize the recent progress in this second special issue. A total of 13 original studies, a systematic review, and a clinical trial are included in this issue.

In the studies on nicotine addiction, Wen et al. focused on the local spontaneous brain activity of cigarette smoking and used resting-state fMRI, mainly the regional homogeneity (ReHo) to investigate the sex-related effects on the brain structural and functional changes. They found some sex-dependent differences in spontaneous brain activity, e.g., the ReHo within the left cerebellum crus1 was negatively related to craving scores in male smokers but not in female smokers. These findings further our understanding of the neuropathological (from the neuroimaging perspective) sex-specific effects of nicotine addiction. In addition, Li et al. group conducted an ecological momentary assessment study to investigate the different effects of two strategies of smoking cessation, immediate reduction and gradual reduction, on cravings. In contrast with the gradual nicotine reduction group, the immediate nicotine reduction group showed significantly lower cravings, which adds to the evidence base for reduced nicotine content in cigarettes.

Alcohol use disorder is one of the common substance use disorders in the general population (2, 3). To explore the prevalence of alcohol use, especially among elderly people, Qiu, Lv, et al. conducted a national community-based survey to derive the prevalence and correlates of risky drinking in Chinese elderly people aged 80 and over. They proposed that more attention should be given to risky drinking among very elderly people. The consensus on alcohol dependence is that both genetic and environmental determinants are involved in this chronic mental disease (4). As a result, a high prevalence of alcohol dependence is often observed in offspring who have parents with alcohol dependence. Among the offspring of parents with alcohol dependence (OPAD), Qiu, Wang, et al. investigated the personality traits and P300 component in those currently risky drinking and not risky drinking. They found P300 component was differentiated between OPAD with and without risky drinking, which indicated that P300 may be used as an early detection index of vulnerable OPAD. Indeed, given the serious dangers of alcohol dependence and smoking, numerous studies have focused on interventions and treatments for smoking cessation and alcohol withdrawal (5, 6). High-frequency repetitive transcranial magnetic stimulation (rTMS) is one of the popular non-invasive interventions and recently it has been applied in the field of addiction (7, 8). Feng et al. investigated the effect of high-frequency rTMS on the attention bias of alcohol-related cues in male patients with alcohol use disorder and obtained a positive result.

The prevalence of methamphetamine use and methamphetamine use disorder (MAUD) is a serious public health issue (9). A high level of childhood trauma and aggression is found to be associated with MAUD. Therefore, Liu et al. conducted a cross-sectional study to examine the relationship between methamphetamine-use characteristics and childhood trauma with aggression in men with MAUD. The result showed a positive correlation between high levels of childhood trauma and aggression in the MAUD population. Relapse occurs during abstinence from methamphetamine. Wang et al. team investigated electroencephalography (EEG) microstate changes in methamphetamine-dependent patients under exposure to drug-related cues in virtual reality environments.

The use of opioids is a double-edged sword. Currently, it is reported that the medicinal use of opioids in Chinese medical institutions may be quite conservative (Fang et al.). As shown in Fang et al. study, the overall level of consumption of opioids in the Second Xiangya Hospital remained relatively low, indicating the urgent necessity for increasing the availability of opioids. However, overprescribing opioids can lead to the development of iatrogenic addiction and finally become an opioid use disorder. Opioid use disorder is a chronic relapsing disorder. Liu et al. and Wang et al. group investigated the peripheral mechanism of opioid use disorder by conducting a whole transcriptome sequencing of peripheral blood and found that GnRH/PI3K/Akt signaling is associated with opioid use disorder.

As for betel quid dependence (BQD), Chen et al. research team also focused on the brain's spontaneous neural activity. In this study, they investigated changes in BQD chewers using a new method called the percent amplitude of fluctuation (PerAF). Brain regions such as the right anterior cingulate cortex (ACC), right middle frontal gyrus (MFG), right insula, right precuneus, left putamen, left supramarginal gyrus (SMG), and left cerebellum were found to have decreased PerAF, and the right orbitofrontal and left superior temporal gyrus (STG) were found to have increased PerAF in BQD chewers. It will be of great interest to test PerAF as a potential sensitive biomarker for identifying spontaneous brain activity changes in other addiction disorders.

Amphetamine shows severe abuse potential and amphetamine withdrawal is a thorny issue. To investigate the cognition improvement differences between d-amphetamine and its prodrug lisdexamfetamine, Chen et al. compared the effects of d-amphetamine (i.p) and lisdexamfetamine (p.o) at equimolar doses by observing rat's behaviors on locomotion, spatial working memory, and recognition memory. The results showed that, in the medial prefrontal cortex (mPFC), lisdexamfetamine leads to a steady and lasting dopamine release pattern and thus shows more effectiveness than d-amphetamine in improving spatial cognitive performance.

Non-substance addiction, especially gaming disorder (GD) has been recognized as an official diagnostic entity in the latest revision of the International Classification of Diseases (ICD-11) (10), but a suitable localized diagnostic tool in Chinese is still missing. Zhang et al. team developed a new tool (the ICD-11 Gaming Disorder Symptom Questionnaire) and examined the effectiveness of identifying individuals at risk of GD both in medical and non-medical settings. Effective screening and detection as well as early intervention are vital for preventing GD. Li et al. investigated escapism-based motivation (EBM) using the eye-tracking analysis in high-risk internet GD (HIGD) students and found EBM may be a potential negative indicator in the HIGD participants as for its significant association with impulsivity, self-emotion management ability, and response inhibition. As for the treatment of non-substance addition, Dai et al. investigated the use of electroacupuncture (EA) and psychotherapy (PT) and observed the effective results in patients with pathological internet use. Furthermore, they found that monoamine oxidase type A (MAOA) may serve as the underlying mechanism of psychotherapy for internet addiction.

The relationship between obesity and gray matter volume (GMV) alterations is still a point of concern. By conducting a meta-analysis of voxel-based GMV, Wang et al. group compared the GMV changes in overweight and obese subjects and lean controls. Compared with lean controls, lower GMV in the left putamen and right precentral gyrus was observed in individuals with overweight and obesity. These results suggested that the structural basis for reward processing and sensorimotor processing may be dysregulated in overweight and obese subjects.

In summary, the second special issue presented here will further our understanding of substance and non-substance addiction. Our ultimate goal was to understand addiction better and to develop more efficacious and safe treatments for addiction.

Author contributions

YF and YLiu contributed to the conception and design of the editorial. YLiao and LL contributed considerably to the writing. DG, JC, KO, and WG contributed to the revision of the

editorial. All authors contributed to the article and approved the submitted version.

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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Escapism-Based Motivation Affected the Psychological Performances of High-Risk Internet Gaming Disorder Individuals

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Background: Escapism-based motivation (EBM) is considered as one of the diagnostic criteria for internet gaming disorder (IGD). However, how EBM affects the high risk of IGD (HIGD) population remains unclear.

Methods: An initial number of 789 college students participated in the general, internet gaming behavior, and motivation surveys. After multiple evaluations, 57 individuals were identified as HIGD (25 with EBM, H-EBM; 32 with non-EBM, H-nEBM). In addition, 51 no-gaming individuals were included as the control group (CONTR). The cohorts completed the psychological assessments and eye-tracking tests, and analyses of group differences, correlations, and influencing factors of the indicators were performed.

Results: The Barratt impulsiveness score of H-nEBM and H-EBM was significantly higher than that of CONTR (MD = 3.605, P = 0.017; MD = 3.744, P = 0.022). In addition, emotional intelligence self-emotion management ability was significantly lower in the H-EBM than in CONTR (MD = –2.038, P = 0.004). Correct rates and reaction times in the anti-saccade task differed significantly between the three groups (F = 3.525, P = 0.033; F = 4.459, P = 0.014). However, no differences were found in the comparison of the digital span test (DST), trail making test (TMT), animal verbal fluency test, Stroop test, and mental rotation test results. The anti-saccade test indicators were positively correlated with the DST results but negatively correlated with the Stroop test results ($P < 0.05$). Correct rates in the mental rotation test were negatively correlated with the TMT results but positively correlated with the DST results ($P < 0.05$). The participants with high Stroop test scores and no lover experience and who were raised by their grandparents were likely to develop EBM to engage in high risk of internet gaming disorder ($P < 0.05$).

Conclusion: EBM has a significantly negative effect on impulsivity, self-emotion management ability, and response inhibition in the HIGD participants.

Keywords: escapism-based motivation, high risk of internet gaming disorder, psychological assessment, eye-tracking test, addiction – computational neuroscience

INTRODUCTION

Currently, the number of internet gamers worldwide is estimated at one billion, with the farthest internet gaming reach observed in the population of China, South Korea, and Japan. Internet gaming audiences are projected to surpass 1.3 billion by 2025 (1). Internet gaming emerged as a popular but controversial online activity for individuals, from minors and seniors, owing to its potential effects on psychological health and wellbeing (2). The increasing popularity of internet gaming can be attributed mainly to severe social environments and the growing diversity of video games, which evolved rapidly from simple point-and-click to highly enticing roleplaying games in virtual worlds (3). Gamers can create representative characters or avatars in games to face situations they are unable to deal with in real life and further escape from their unsatisfactory lives, such as excessive pressure at school or work, strained interpersonal relationships, and so on (4). Whether internet games are beneficial to individual development is a controversial topic. Researchers believe that internet games can improve players' spatial capabilities and responsiveness and reduce stress (5).

However, the most unfavorable effect of internet gaming is players' unknowing progression toward a disease state known as internet gaming disorder (IGD). IGD was included in the third section of the latest (fifth) edition of the Diagnostic and Statistical Manual for Mental Disorders (DSM-5) and 11th revision of the International Classification of Diseases. This inclusion dramatically increased academic discussions and public concern about the importance of examining IGD. This gaming disorder can be observed in 3.05 and 5.0% of the population worldwide and in China, respectively (6). Individuals with IGD typically show symptoms related to a number of psychological and health problems, including depression, social anxiety, fatigue, loneliness, low self-esteem, and impulsivity. IGD patients exhibit high error rates in the case of game-related images in anti-saccade tasks, thereby revealing a dysfunctional attentional bias (7). Furthermore, IGD co-occurs with various psychiatric conditions and can lead to a range of negative outcomes.

Internet gaming disorder is a continuous development process, from not playing games to occasional to high-frequency playing and finally, out-of-control behavior (8). Therefore, the diagnosis for individuals who exhibit some symptoms but do not fully meet the IGD diagnostic criteria must not only judge whether the disorder reached the disease state but also establish awareness of early recognition and intervention (9). Such individuals are seen as high risk of IGD (HIGD). Although there is no unified definition of HIGD till now, some scales were successfully used in the screening of HIGD, such as the nine-item Internet Gaming Disorder Scale-Short Form (IGDS9-SF) (10), Internet Gaming Disorder Questionnaire (IGDQ) (11), and Chen Internet Addiction Scale (CIAS) (12). According to epidemiological surveys in various countries, the proportion of the HIGD population in the total population is approximately 9.0–11.5%, which is significantly higher than that of IGD sufferers (13, 14). Role-playing and other types of games can significantly increase the risk of HIGD (14). Ji et al. (15) observed the instantaneous frequency distribution of respiratory signals in 19 HIGD cases and believed that abdominal breathing training can help reduce this index, thereby reflecting the dynamic change process of sufferers' psychophysiology. However, whether HIGD shares the aforementioned potential with IGD cognitive neural mechanisms and whether the degree of damage is reduced remain unclear.

The attraction of internet games appears to lie in their potential to satisfy different psychological needs, which can be conceptualized as motivation for gaming (16). Escapism-based motivation (EBM) is one of the most important online gaming motivations and belongs under the immersion component (17). Gamers with EBM use online environments to avoid real-life problems. Psychiatric symptoms in patients with IGD are significantly and strongly associated with EBM (16, 18). In addition, the Brief symptom inventory global severity index indicates that psychiatric distress levels have a significant positive direct effect and significant indirect (mediating) effect on problematic internet gaming and EBM (16). Hence, EBM is among the nine diagnostic criteria for IGD in the DSM-5. However, whether and how EBM affects HIGD individuals have yet to be fully understood.

Internet gaming is popular among college students in China, most of whom have not reached the disease state but can be considered as HIGD. Effective screening and detection as well as early intervention of HIGD, will help prevent them from developing into an addictive state. However, there is few studies to investigate the characteristics of HIGD, especially the role that motivation plays in the development of disease state. Hence, recognizing the psychological characteristics of HIGD students and the role of EBM is important step to detection HIGD individual. This study conducted a psychological assessment and eye-tracking analysis on HIGD students to verify the effects of EBM and examine the characteristics of this cohort.

MATERIALS AND METHODS

Participants, General Information, and Internet Gaming Behavior Assessment

A large-scale online survey was conduct to recruit internet gaming college students (between the ages of 18 and 25 years) and controls through an online questionnaire system (Wen Juan Xing[1]). Inclusion criteria were unlimited. However, the exclusion criteria were set as: (1) serious psychological problems, such as depression and anxiety; and (2) definitized mental disorder or its family history, such as schizophrenia, bipolar disorder, substance addiction, and so on. A total of 789 individuals met these criteria and answered all the survey questions completely, including those about general information and internet gaming behavior characteristics. The mean age of the sample was 20.78 ± 3.72 years, and 276 identified as male, whereas 513 identified as female. The participants' flow is detailed in **Figure 1**.

[1]www.wjx.cn

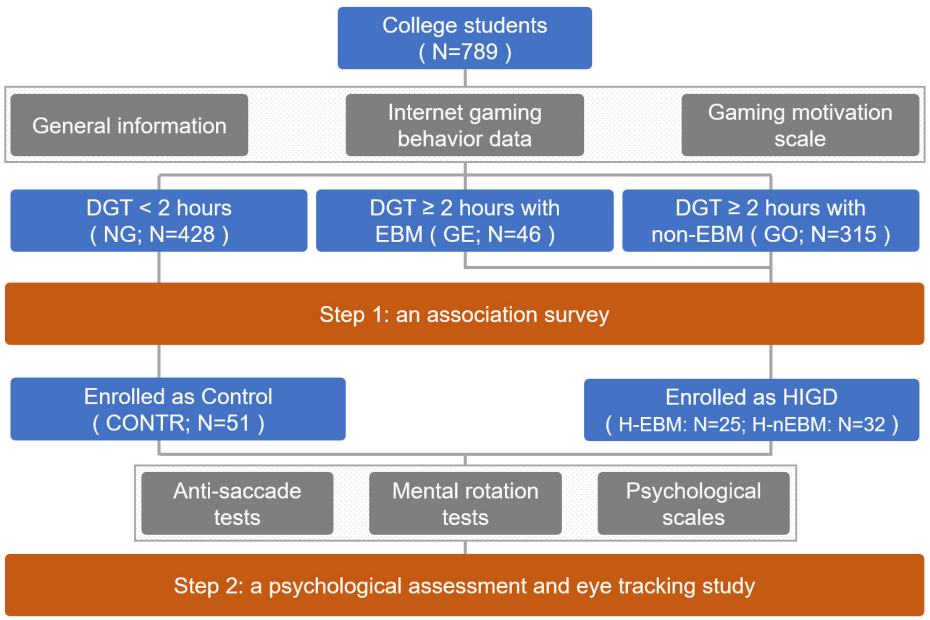

FIGURE 1 | Participants' flow.

The participants' general information, including demographics, academic performance, upbringing, residence, and love experience, was collected with a self-administered questionnaire. Information on internet gaming behavior, including daily gaming time (DGT), gaming motivation, main gaming type, gaming history, and gaming equipment, was also collected. The individuals with an average DGT of ≥2 h in the past 3 months were classified as long-time players (19). Hence, in this study, the participants were divided into long-time players (DGT of ≥2 h) and short-time players (DGT of < 2 h, including the no-gaming individuals). Only the long-time players were asked to answer the remaining questions on internet gaming behavior. Motivation was evaluated using the escape subscale of the Online Game Motivation Scale (17), which had a Cronbach's α of 0.85 in the Chinese college student population. The escape subscale included three items, with a total score of 15 points. The participants with a score over 8 were considered as gamers with EBM (20), and the participants with an IGDQ (11) score of ≥5 and a CIAS (12) score of ≥64 were considered as HIGD.

A total of 428 participants were identified as short-time players and set as the NG group. Among the 361 participants with a DGT of ≥2 h, the proportion with EBM to play internet games was 12.74% (N = 46; set as the GE group), and 315 participants demonstrated no significant EBM (set as the GO group). After IGDQ and CIAS evolutions, the 25 participants in the GE and 32 participants in the GO groups were considered as HIGD individuals and then set as HIGD-EBM group (H-EBM) and HIGD-non-EBM group (H-nEBM), respectively. In addition, 51 no-gaming individuals in this cohort voluntarily participated as the Control group (CONTR). Only individuals in H-EBM, H-nEBM, and CONTR groups needed to finish the following psychology assessments and eye-tracking trails.

Psychological Assessments

The 108 participants were assessed using the trail making test (TMT), digital span test (DST), animal verbal fluency test (AFT), Stroop color and word test (Stroop), Barratt impulsiveness scale in the 11th (BIS) with 0.76 of Cronbach's α and 0.85 of Kappa coefficient (21), and the Wong and Law Emotional Intelligence Scale (WLEIS) with 0.86 of Cronbach's α and 0.78 of Kappa coefficient (22) to measure and screen their changed characteristics in recognition, affection, and behavior.

Eye-Tracking Tests

The EyeLink 1000 system (SR Research Ltd., Canada) was used for the video-based eye-tracking tests. Dominant eye movements were recorded at a sampling rate of 1,000 Hz. With the Experiment Builder software (SR Research Ltd., Canada), the anti-saccade and mental rotation tasks were presented on a computer monitor (1,024 × 768 resolution, 60 Hz refresh rate) at a distance of 50 cm from the participants' eyes.

Anti-saccade Task

The task design included full pro-saccade and anti-saccade trials (**Figure 2**). At the start of each trial, the 108 participants were asked to fixate on a neutral stimulus (a white square with a visual angle of approximately 2°) that appeared at the center of the screen. The neutral fixation stimulus changed to an instructional cue indicating a pro-saccade or an anti-saccade when the participants maintained central fixation for 1,600 ms without generating a saccade. The instructional cues were colored pictograms of the same size as the neutral stimulus. A green square indicated that a pro-saccade should be made, whereas a red square indicated that an anti-saccade was required. At the same time, a peripheral target (a white square with a visual

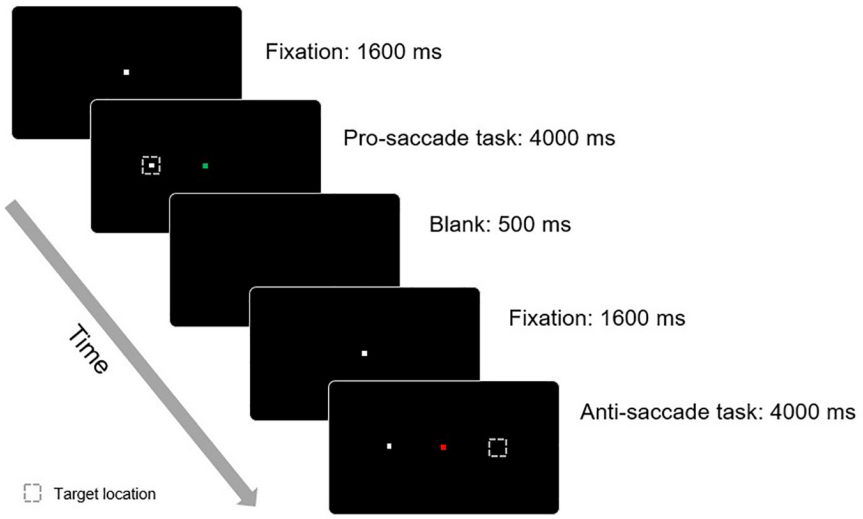

FIGURE 2 | Design of anti-saccade task.

angle of approximately 2°) appeared at the left or right side of the screen at a distance of 5 or 10 cm from the instructional cue. The participants had 4,000 ms to execute the appropriate pro-saccade (look toward the target location) or anti-saccade (look away from the target in the equal but opposite direction) based on the instructional cue presented in the trial. When a trial was completed, a blank (without a fixation, cue, or target) appeared for 500 ms. A total of 64 trials (1:1 for pro- and anti-saccades, randomly presented) were designed for the execution components. The direction correct rate was set as the primary measure for this task performance, and the correct reaction time, fixation count, and saccade count were set as the secondary outcomes.

Mental Rotation Task

The mental rotation task consisted of 20 unique block pairs resulting in a 50% "same" and 50% "different" result. The stimuli were presented pairwise in two different angular disparities of 45° and 90°, and each figure had a dimension of 400 × 400 pixels. A fixation stimulus (a white cross) appeared at the center of the screen at the start of each trial. The neutral fixation stimulus changed to a task trial when the participants maintained central fixation for 1,600 ms. Within 10,000 ms in each trial, the participants must determine the final judgment and press "Y" for "same" and "N" for "different." When a trial was completed, a blank appeared for 500 ms. The task design is presented in **Figure 3**. The reaction correct rate was set as the primary measure for this task performance, and the correct reaction time, fixation count, and saccade count were set as the secondary outcomes.

Data and Statistical Analyses

The eye-tracking test data were extracted using DataViewer (SR Research Ltd., Canada). The general information and internet gaming behavior assessment and psychological assessment data were manually extracted from the original questionnaires. All the statistical analyses were performed using SPSS 22.0 (IBM Co., United States). To examine the differences in the DGT and motivation catastrophizing, demographic and internet gaming behavioral characteristic, and questionnaire data between the groups, X^2 and t-tests were used for the categorical and continuous variables, respectively. To examine the differences in the anti-saccade correct rates and reaction times, a series of repeated analysis of variance (ANOVA) measures was performed, with trial type (anti-saccade vs. pro-saccade), direction (left vs. right), and amplitude (5 cm vs. 10 cm) as the within-group factors and group (H-EBM vs. H-nEBM vs. CONTR) as the between-group variable. To quantify the effect size of the observed results, the partial eta-squared ${\eta_p}^2$ was calculated. Logistic regression was performed to further obtain the associated factors in the HIGD participants with EBM, and $P < 0.05$ results were considered as statistically significant.

RESULTS

Differences in General Characteristics

As shown in **Table 1**, gender, upbringing, and residence distributions differed significantly between the GE, GO, and NG groups ($X^2 = 68.921$, $P < 0.001$; $X^2 = 12.222$, $P = 0.016$; $X^2 = 8.052$, $P = 0.018$). The proportion of the male participants was significantly higher in the GE group than in the NG group ($X^2 = 28.950$, $P < 0.001$). The proportion of the participants raised by their grandparents was significantly higher in the GE group than in the GO group ($X^2 = 8.811$, $P = 0.012$). Furthermore, the proportion of urban residents was significantly higher in the GO group than in the NG group ($X^2 = 8.020$, $P = 0.005$). No significant differences were observed in age, main gaming type, gaming history, and gaming equipment between the three groups or between the GE and GO groups (all $P > 0.05$).

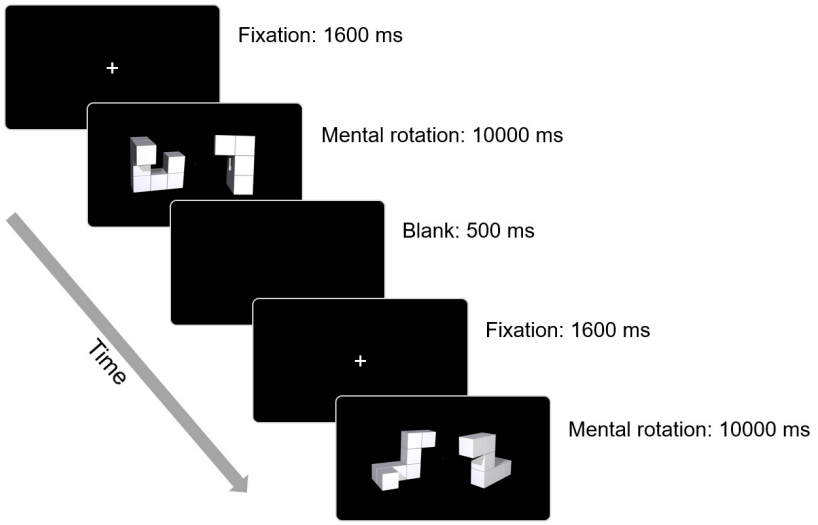

FIGURE 3 | Design of mental rotation task.

TABLE 1 | Association of the general characteristics with gaming experiences and motivations.

Characteristics	GE (*N* = 46)	GO (*N* = 315)	NG (*N* = 428)	F/X^2	*P*
Age	20.55 ± 4.29	20.81 ± 3.51	20.77 ± 3.68	0.319	0.652
Gender (Male/Female, *N*)	27/19	154/161	95/333#	68.921	**<0.001**
Academic performance (Excellent/Fair/Poor, *N*)	6/32/8	40/247/28	57/342/29#	6.575	0.160
Upbringing (Parents/Grandparents/Other, *N*)	39/6/1	301/11/3#	386/29/13	12.222	**0.016**
Residence (Urban/Rural, *N*)	21/25	167/148	182/246※	8.052	**0.018**
Love experience (Yes/No, *N*)	24/22	174/141	204/224	4.195	0.123
Main gaming type (Role play/Other, *N*)*	24/22	160/155	–	0.031	0.861
Gaming history (<2y/ ≥2y, *N*)*	16/30	115/200	–	0.052	0.820
Gaming equipment (Phone/Computer/Pad, *N*)*	34/11/1	241/63/11	–	0.545	0.761

**Participants of daily gaming time ≥ 2 h. #Compared with GE, the difference was statistically significant. ※Compared with GO, the difference was statistically significant. Bold values mean P < 0.05.*

Differences in Psychological Results

A total of 57 HIGD and 51 control respondents (H-EBM = 25, H-nEBM = 32, CONTR = 51) participated in the psychological assessments. No significant differences were found between the three groups in the comparison of the TMT, DST, AFT, and Stroop test results (all $P > 0.05$; **Figure 4**). The BIS index was significantly higher in the H-nEBM and H-EBM than in the CONTR ($MD = 3.605$, $P = 0.017$; $MD = 3.744$, $P = 0.022$; **Figure 4**). Furthermore, self-emotion management in the WLEIS was significantly lower in the H-EBM individuals than in the CONTR ($MD = -2.038$, $P = 0.004$; **Figure 4**).

Differences in Eye-Tracking Results

A total of 57 HIGD and 51 control respondents (H-EBM = 25, H-nEBM = 32, CONTR = 51) participated in the eye-tracking tests. As shown in **Table 2**, the correct rates and reaction times in the anti-saccade task differed significantly between the three groups ($F = 3.525$, $P = 0.033$; $F = 4.459$, $P = 0.014$). The H-nEBM had significantly lower correct rates ($MD = -0.126$, $P = 0.010$), longer reaction times ($MD = 176.398$, $P = 0.007$), and more fixation ($MD = 1.776$, $P = 0.033$) and saccade counts ($MD = 1.825$, $P = 0.028$) than the CONTR. Furthermore, the H-EBM had significantly shorter reaction times than the H-nEBM ($MD = -187.01$, $P = 0.016$). However, no significant differences were observed in the correct rates, reaction times, and fixation and saccade count in the mental rotation task between the three groups (all $P > 0.05$).

For the anti-saccade task, a 3 (group: H-EBM vs. H-nEBM vs. CONTR) × 2 (trial: pro-saccade vs. anti-saccade or direction: left vs. right or amplitude: 5 cm vs. 10 cm) ANOVA was performed. As shown in **Figure 5**, an amplitude of 5 cm, compared with an amplitude of 10 cm, was associated with higher correct rates [$F(1,108) = 15.862$, $P < 0.001$, $\eta_p^2 = 0.003$], thereby revealing a significant main effect for amplitude. The trial type and direction were not significant for this parameter [trial: $F(1,108) = 3.485$, $P = 0.062$, $\eta_p^2 = 0.001$; direction: $F(1,108) = 0.143$, $P = 0.705$, $\eta_p^2 < 0.001$]. No interaction was found in the correct rates (all $P > 0.05$). As depicted in **Figure 6**, the anti-saccade trial (compared with the prosaccade trial) and an amplitude of 10 cm (compared with an amplitude of 5 cm) were

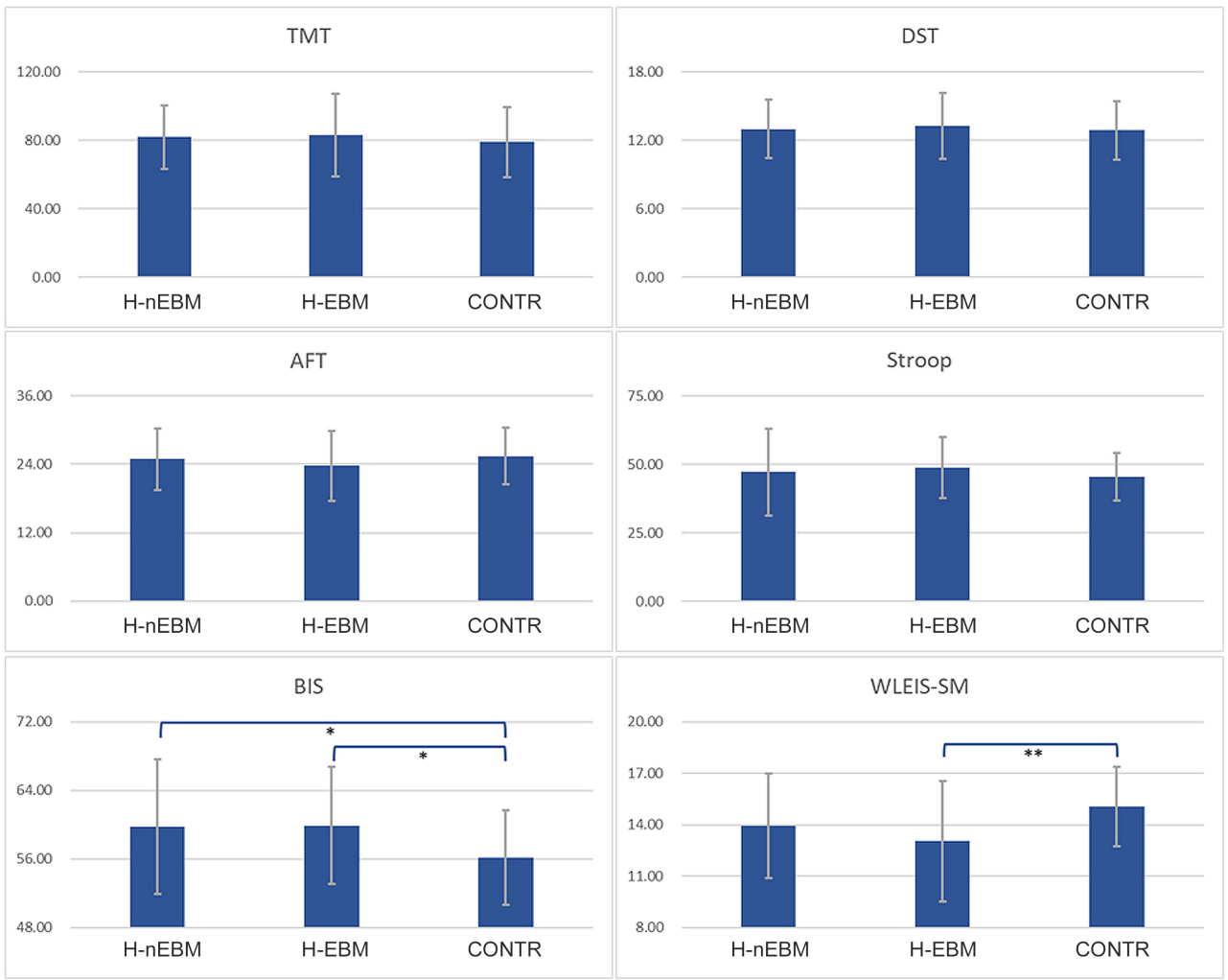

FIGURE 4 | Psychological assessment results of 108 participants. *$P < 0.05$ and **$P < 0.001$.

TABLE 2 | The results of eye-tracking tests.

Indicators	H-EBM ($N = 25$)	H-nEBM ($N = 32$)	CONTR ($N = 51$)	F	P
Anti-saccade task					
Correct rate (%)	0.37 ± 0.22	0.31 ± 0.17	0.43 ± 0.23246※	3.525	**0.033**
Reaction time (ms)	2044.27 ± 271.32	2231.28 ± 315.14#	2054.88 ± 274.12246※	4.459	**0.014**
Fixation count (times)	4.52 ± 2.08	5.62 ± 6.38	3.84 ± 0.67246※	2.347	0.101
Saccade count (times)	3.59 ± 2.11	4.73 ± 6.37	2.90 ± 0.70246※	2.480	0.089
Mental rotation task					
Correct rate (%)	0.80 ± 0.19	0.81 ± 0.16	0.76 ± 0.17	0.910	0.406
Reaction time (ms)	4431.76 ± 121.20	5110.43 ± 159.60	4804.66 ± 139.07	1.609	0.205
Fixation count (times)	17.07 ± 5.00	18.94 ± 5.36	18.02 ± 4.36	1.054	0.352
Saccade count (times)	16.35 ± 5.02	18.24 ± 5.40	17.27 ± 4.37	1.080	0.343

#*Compared with H-EBM, the difference was statistically significant.*
※*Compared with DGT ≥ 2 h with H-nEBM, the difference was statistically significant. Bold values mean $P < 0.05$.*

associated with longer reaction times [trial: $F(1,108) = 19.139$, $P < 0.001$, $\eta_p^2 = 0.003$; amplitude: $F(1,108) = 14.042$, $P < 0.001$, $\eta_p^2 = 0.002$], thereby revealing a significant main effect for trial type and amplitude. Direction was insignificant for this parameter [$F(1,108) = 0.508$, $P = 0.476$, $\eta_p^2 < 0.001$]. No interaction was found in the reaction times (all $P > 0.05$).

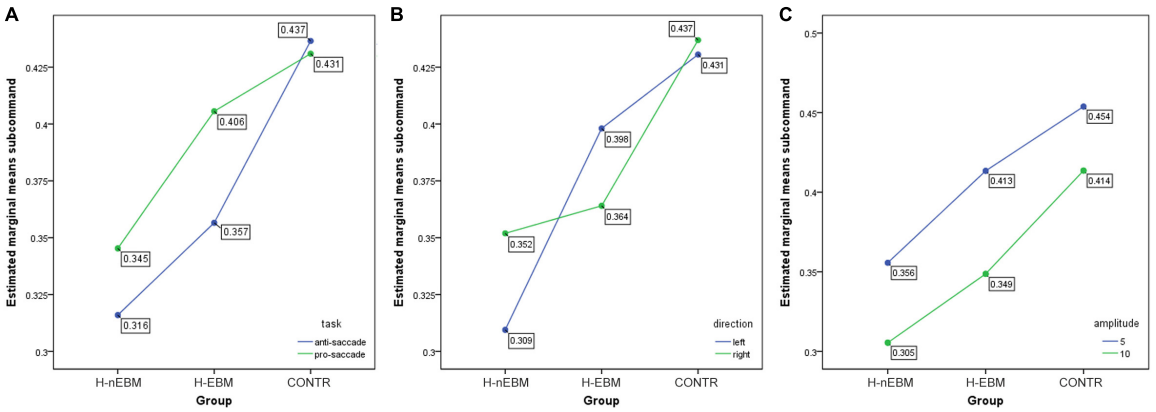

FIGURE 5 | Differences in correct rates in anti-saccade task between H-EBM, H-nEBM, and CONTR groups in **(A)** pro-saccade and anti-saccade trials; **(B)** left and right directions; **(C)** 5 and 10 cm amplitudes.

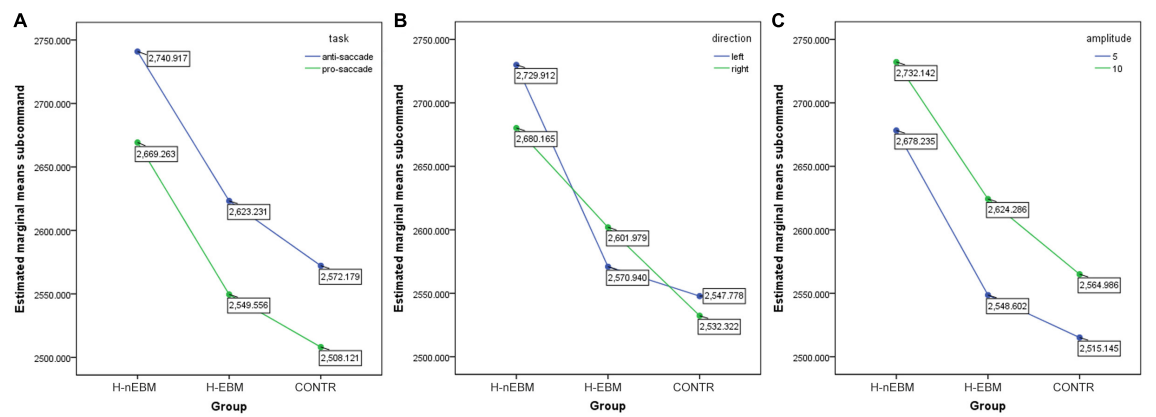

FIGURE 6 | Differences in reaction times in anti-saccade task between H-EBM, H-nEBM, and CONTR groups in **(A)** pro-saccade and anti-saccade trials; **(B)** left and right directions; **(C)** 5 cm and 10 cm amplitudes.

Correlation Analysis in Escapism-Based Motivation

Fixation and saccade count in the anti-saccade test were positively correlated with the DST results (r = 0.543, P = 0.005; r = 0.541, P = 0.005). Reaction times, fixation counts, and saccade counts in the anti-saccade test were negatively correlated with the Stroop test results (r = –0.420, P = 0.037; r = –0.475, P = 0.016; r = –0.474, P = 0.017). Correct rates in the mental rotation test were negatively correlated with the TMT results (r = –0.514, P = 0.009) but positively correlated with the DST results (r = 0.403, P = 0.046).

Logistic Regression Analysis

The participants with high Stroop test scores were 1.088 times more likely to be HIGD with EBM than their counterparts (*Wald* = 4.776, P = 0.029, 95% CI: 1.009–1.173). The participants with satisfactory academic performance were 0.064 times more likely to engage in gaming behavior than those with poor academic performance (*Wald* = 5.517, P = 0.019, 95% CI: 0.006–0.635). The participants raised by their parents had a significantly lower probability of engaging in gaming behavior than those raised by other family members (*Wald* = 132.042, P < 0.001, 95% CI: 0.000–0.000). Furthermore, the participants with no lover experience were 11.157 times more likely to engage in gaming behavior than those with lover experience (*Wald* = 7.282, P = 0.007, 95% CI: 1.935–64.330). The detailed results are presented in **Table 3**.

DISCUSSION

In this study, we observed the pleiotropic effects of EBM on HIGD college students by conducting psychological assessments and eye-tracking tests. The participants with long-time internet gaming experience and EBM were mostly male, raised by their grandparents, and demonstrated higher impulsivity and lower self-emotion management ability than the healthy controls or the participants with long-time internet gaming experience with non-EBM. In an online survey of the Korean

TABLE 3 | Logistic regression of occurring HIGD with EBM.

Variables	*SE*	*Wald*	*P*	*OR (95%CI)*
Age	0.305	1.908	0.167	0.657 (0.361∼1.193)
TMT	0.019	1.814	0.178	1.026 (0.988∼1.066)
DST	0.138	2.273	0.132	1.231 (0.940∼1.612)
AFP	0.074	0.923	0.337	0.931 (0.805∼1.077)
Stroop	**0.038**	**4.776**	**0.029**	**1.088 (1.009∼1.173)**
BIS	0.066	2.418	0.120	1.107 (0.974∼1.259)
WLEIS	0.062	2.558	0.110	1.105 (0.978∼1.248)
Gender				
Female	Ref.			
Male	0.758	0.287	0.592	0.666 (0.151∼2.941)
Academic performance				
Poor	Ref.			
Excellent	9214.551	0.000	0.998	0.000 (0.000∼0.000)
Fair	**1.170**	**5.517**	**0.019**	**0.064 (0.006∼0.635)**
Upbringing				
Other	Ref.			
Parents	**2.141**	**132.042**	**<0.001**	**0.000 (0.000∼0.000)**
Residence				
Rural	Ref.			
Urban	0.777	0.018	0.892	1.111 (0.243∼5.091)
Love experience				
Yes	Ref.			
No	**0.894**	**7.282**	**0.007**	**11.157 (1.935∼64.330)**

Bold values mean P < 0.05.

population, the individuals with IGD were mostly male and exhibited dysfunctional impulsivity (23). The male gender and impulsiveness are considered as risk factors for IGD (24, 25). EBM is a predictor of IGD (26). However, in long-time but not addiction internet gamers, the factors inducing the association between EBM and the male gender and impulsiveness and the role of long-time internet gaming behavior in the relationship between the two factors remain unknown. Grandparents raising their grandchildren is common in China; thus, the monitoring of children's behavioral and emotional difficulties is generally lacking (27, 28). When children become accustomed to playing online games without restriction, they may suffer from online gaming addiction, which is indicated by their tendency to continue playing and ignore social obligations and reality (29). Thus, the result of the considerable proportion of the long-time internet gamers, especially those with EBM, being raised by their grandparents can be explained easily. In addition, the logistic regression analysis revealed that being raised by parents and having satisfactory academic performance were low-risk factors, whereas lack of lover experience was a high-risk factor for HIGD and EBM.

The anti-saccade task is among the methods used to measure response inhibition, which is an important executive control function component (30). Patients with attention-deficit/hyperactivity disorder (31), schizophrenia (32), or IGD (33) show significant deficits in response inhibition and abnormal performance in anti-saccade and/or Stroop tasks. In a previous eye-tracking study with 23 IGD patients and 27 healthy controls, in the anti-saccade task, the IGD group exhibited higher error rates in the case of game-related images compared with neutral or scrambled images (7). An independent component analysis of a probability discounting task showed that IGD patients preferred risky over fixed options and demonstrated shorter reaction times in their behavioral results and less engagement in the executive control network compared with healthy controls, thereby suggesting deficits in the executive control function of IGD patients (34). In the present study, the HIGD participants with EBM, and especially those with non-EBM, had low correct rates in the anti-saccade task. Furthermore, the latter participants had longer reaction times than the healthy controls. However, both groups exhibited improved performance in the short-amplitude and prosaccade trials. These results suggested that non-EBM may induce a response inhibition deficit in the HIGD participants. Therefore, the executive control function of the HIGD individuals may have been impaired before they developed an internet gaming addiction. However, whether different degrees of executive control function impairment exist between IGD and HIGD individuals has yet to be reported.

In the correlation analysis, the anti-saccade results were mostly negatively correlated with the Stroop test results and positively correlated with the DST results. As stated previously, the Stroop effect describes the delay in the reaction time between congruent and incongruent stimuli. The Stroop test is widely used in clinical practice and investigations to measure individuals' selective attention capacity and skills as well as their executive control function (35). In Stroop test results, the higher the score, the stronger the executive control function. However, in the anti-saccade task, the shorter the reaction time, and the less the fixation count or saccade count, the stronger the executive control function. The DST is a simple behavioral measure of working memory capacity, which is the cognitive ability to store and manage information on a transient basis (36). Working memory capacity and executive functions share a common underlying executive attention component that is strongly predictive of high-level cognition (37). Hence, working memory capacity may be associated with executive functions. The positive correlation between the two factors measured by the anti-saccade task and DST in the present study can be explained easily.

Mental rotation is defined as the ability to rotate mental representations of two-dimensional and three-dimensional objects and related to the visual representation of such rotations in the human mind (38). A relationship exists between spatial processing cognitive rates, general intelligence, and mental rotation (39). A previous experiment showed that children's initial low mental rotation performance improves after playing computer games requiring mental rotation skills (40). A subsequent investigation also demonstrated that playing computer games improves mental rotation scores in general, and women's gains are significantly greater than those of men, and the most significant gains are accomplished when practice is accumulated (41). These findings implied that computer-based games could be used in schools to enhance children's spatial abilities. However, in the present study, the mental rotation performance of the three groups demonstrated no significant differences, thereby suggesting that EBM had no effect on the spatial abilities of the HIGD participants. The inconsistent conclusions above may be attributed to different research populations and game types.

Several limitations of this study should be addressed. The sample for step 2 was selected from the sample in step 1 based on the respondents' willingness to participate in the subsequent tests; thus, selection bias may exist. In addition, other motivations collectively referred to as non-EBM in this study may be potential confounding factors affecting the accuracy of the results. Despite such limitations, EBM showed a significantly negative effect on impulsivity, self-emotion management ability, and response inhibition in the HIGD college students. Finally, lover experience and parental upbringing may help college students avoid developing EBM.

CONCLUSION

Escapism-based motivation has a significantly negative effect on impulsivity, self-emotion management ability, and response inhibition in the HIGD participants. These results will help us to understand the psychological characteristics of HIGD and early identify this population, which would help for prevention of IGD.

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

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

AUTHOR CONTRIBUTIONS

All authors contributed to the data analysis, drafting, revision of this manuscript, agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

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Gaming Disorder Symptom Questionnaire: The Development and Validation of a Screening Tool for ICD-11 Gaming Disorder in Adolescents

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Background: Gaming disorder (GD) has been recognized as an official diagnostic entity in the latest revision of the International Classification of Diseases (ICD-11). However, the majority of previous studies used different instruments, which are not fully consistent with the concept of GD in ICD-11. The development of a screening assessment instrument based on ICD-11 for this new disease entity is very urgent and important.

Methods: The ICD-11 Gaming Disorder Symptom Questionnaire (GDSQ), based on the ICD-11 diagnostic guidelines for GD, was developed by a team of GD experts. A total of 7,790 adolescents were included in this study. Criterion validity was assessed by GDSQ, Video Gaming Dependency Scale (VGDS), weekly game playing time, weekly game video viewing time, and monthly money spent on games. Item structure was measured by factorial analysis. Discrimination between GD and non-GD was examined based on the receiver characteristic curve (ROC).

Results: The GDSQ was very well described by three symptoms of GD (i.e., impaired control, increasing priority to gaming, and continued use despite the occurrence of negative consequences). The internal consistency was excellent (Cronbach's $\alpha = 0.964$) with good criterion validity and good discriminatory power. The optimal cutoff point for determining the profile of gamers was found to be $\geq$62 points. The GDSQ revealed that the prevalence of GD was 2.27% in this adolescent sample.

Conclusion: The ICD-11–based GDSQ is a successfully validated measurement scale for GD among adolescents. This study provides a new tool (GDSQ) for us to effectively identify individuals with risk of GD in medical and non-medical settings.

Keywords: gaming disorder, the Gaming Disorder Symptom Questionnaire-21, scale development, validation, ICD-11

INTRODUCTION

Gaming disorder (GD) has become a significant public health concern. According to market analysis, there were 2.8 billion online game players worldwide and the global gaming market generated $175.8 billion in revenue in 2021 (1). There were 509 million online game players in China until June 2021 (2). With the increasing population of game users, the psychological and physical harm due to excessive gaming behaviors has caused concerns in psychiatry, public health, education, and administration (3, 4). Until now, the mainstream view considers excessive and uncontrollable gaming behavior as an addictive disorder (namely, GD). Adolescents are particularly vulnerable to GD (5, 6) and often experience a series of negative consequences, including low self-esteem, intense negative mood states (e.g., sadness, irritability, and boredom) (7), relationship conflicts, and problems at work or school (8–11).

Plenty of studies have been conducted to assess the prevalence of GD and gaming related problems. However, the lack of a unified instrument among these studies resulted in widespread inconsistency in the estimation of the prevalence rates. For example, studies revealed the prevalence of GD ranged from 3.5 to 17% in China (12) and from 0.3 to 4.9% in the United States (13). Therefore, it is indispensable to establish a set of more effective diagnostic criteria and screening tools for GD.

In 2013, the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) included Internet Gaming Disorder (IGD) in the "Conditions for Further Study" section and proposed a set of tentative diagnostic criteria for IGD (14): indicated by five or more of the nine items (preoccupation, withdrawal, tolerance, unsuccessful attempts to control, loss of life interests, continuation despite problems, deception, escape, and jeopardizing important life aspects) for 12 months (15). It encouraged researchers to utilize the same standard to recognize people with IGD and develop screening instruments.

Nonetheless, the proposed diagnostic criteria for IGD in DSM-5 have some limitations. First, it is questionable whether certain criteria (e.g., preoccupation, tolerance, withdrawal response, and deception) have sufficient sensitivity and specificity or not (16–20). Second, all diagnostic criteria for IGD are equally weighted when counting how many of them are met, but this approach is flawed because it fails to distinguish well between core and non-core symptoms (21). Besides, the consensus about the diagnostic cutoff value for IGD (five of nine items) seems not established. By diagnosing IGD with the Nine-Item Internet Gaming Disorder Scale-Short Form (IGDS9-SF) test, the cutoff point score of 36 in Pontes' study is higher than the optimal cutoff point of 32 in Qin's study (with a sample of 3,742 from Chinese universities and 131 from Chinese clinical settings) (22, 23).

The 11th revision of the International Classification of Diseases (ICD-11) included GD as a disease entity in 2019. The WHO Working Group has proposed the diagnostic guidelines for GD (24, 25), which define the core symptoms of GD as (1) impaired control over gaming behavior (e.g., onset, frequency, intensity, duration, termination, and context); (2) increasing priority to gaming over other life interests and daily activities; and (3) continuation or escalation of gaming activities despite the occurrence of negative consequences. Because of the short history of ICD-11, until now, few research studies have been conducted based on these criteria. Some findings suggest that the ICD criteria appeared to be more stringent than the DSM criteria in diagnosing GD (26, 27). For instance, Ko et al. analyzed the diagnostic validity and utility of IGD (DSM-5) and GD (ICD-11) through the empirical data, psychiatrists conducted diagnostic interviews with 69 subjects with IGD based on the DSM-5 IGD criteria, and only 44 participants with IGD (63.8%) fulfilled the criteria for GD (26).

Up until now, only a few studies have been involved in the development of standard questionnaires for the assessment of GD based on ICD-11 criteria (28, 29). Therefore, the current study aimed to develop a screening self-assessment instrument named the Gaming Disorder Symptom Questionnaire (GDSQ) to assess GD among adolescents based on ICD-11 criteria. First, we developed the GDSQ with the GD symptoms listed in ICD-11, and we borrowed the setting style of response options from the classic screening tool Alcohol Use Disorders Identification Test (30) to promote the understandability and usability of GDSQ. Then, we validated GDSQ with a large sample of Chinese adolescents aged 12–18 years. Given that all items were based on ICD-11 criteria and developed by psychiatrists and clinical psychologists with expertise in behavioral addiction, we hypothesized that the GDSQ would be a valid and reliable screening tool to assess GD.

METHODS

Study Population and Procedure

A stratified cluster random sampling method was used to select three urban areas in Xinjiang Uygur Autonomous Region, China. These cities included Urumqi, Kashi, and Bole. In each area, two junior middle school and two senior middle school and, in each school, four classes were selected from each grade (grade 1–3 of junior middle school and grades 1–3 of senior middle school). The classes were randomly selected in each school. The students involved in the survey were aged 12–18 years old. The survey was carried out in the classrooms of the recruited classes. After the explanation of the purpose and requirements of the study, the researchers emphasized the voluntariness of the survey and then delivered the informed consent form to the parents of each adolescent. Electronic informed consent was obtained from each student after parental informed consent had been obtained. After that, the questionnaires were distributed to the students who participated in the survey.

The data in the current survey are only a part of a big set of studies that contained multiple questionnaires that need about 30 min to complete. During the filling process, the researchers answered promptly subjects' questions about the survey. The period of the data collection spanned from October 2020 to November 2021. Next, two psychiatrists assessed whether the subjects were in a high-risk GD group based on factors such as impaired control over gaming behavior, the priority of gaming over other interests and daily activities, and the continuation or escalation of gaming activities despite negative consequences.

Ethics

Informed consent was obtained from all target participants and their parents or legal guardians. The ethical approval for this study was obtained from the Ethics Committee of the Second Xiangya Hospital of Center South University (ID: 2019-S454).

Measures

Sociodemographics and Gameplay Habits

Socio-demographics information included age, gender, race, and family structure (being an only child or having siblings). Additional questions about gaming habits included starting age for gameplay, devices used to play games, preferred games, hours of game-related per week (online games, stand-alone games, and game video watching), and the amount of money spent on games per month over the past 12 months.

Video Gaming Dependency Scale

In the current study, the VGDS was used to assist in the development and psychometric validation process of the GDSQ as a concurrent measure of GD. IGD was assessed using the Chinese version of the VGDS (see **Supplementary Table 1**), which is abbreviated as CSAS in the German version "Computerspielabhängigkeitsskala" and was adapted by Rehbein et al. (31) from a previous instrument (KFN-CSAS-II). This instrument is the 18–descriptive item scale, with every two items representing one of the nine DSM-5 IGD criteria. Each item was rated on a four-point Likert scale (1 = strongly disagree, 2 = somewhat disagree, 3 = somewhat agree, and 4 = strongly agree) to evaluate the symptom severity of the subject's gaming behavior within the last 12 months. According to the DSM-5 recommendations for IGD, a criterion was endorsed if at least one of the two items was answered with "strongly agree". The subjects who endorsed five or more of the nine symptom criteria were considered for IGD. The VGDS was validated in Chinese adolescents and young adults (32). In this study, the VGDS presented an excellent internal consistency of Cronbach's α with 0.968.

Gaming Disorder Symptom Questionnaire

The GDSQ was developed based on the diagnostic guidelines of ICD-11 GD. Initially, we had 24 items (see **Table 1**) after consulting with an expert panel to ensure content validity. The panel meeting was composed of psychiatrists and clinical psychologists with expertise in behavioral addiction. Every eight of the 24 items embodied one of the three symptoms of GD (i.e., impaired control, increasing priority to gaming, and continuing playing games despite the negative consequences). The subjects were asked to respond about the frequency of the event or situation described in the items within the last 12 months on a five-point Likert scale (0 = never, 1 = less than monthly, 2 = monthly, 3 = weekly, and 4 = almost daily). For example, "Once I start playing the game, it is hard to stop" was one item in the dimension of impaired control. The Chinese version of GDSQ is shown in **Supplementary Table 2**.

It should be noted that each of the items reflecting "continuing playing games despite the negative consequences" had two-round responses. The first question was asked about the frequency of game playing. If the subjects respond with "0" (i.e., never), then there is no need to enter the second question. However, if the response is "1" (i.e., less than monthly) or more frequent, then the subjects need to respond to the second question about whether they will continue playing games or not. The options for the second question are dichotomous (0 = no, 1 = yes). The score of the item was calculated by multiplying the point of the first question with the point of the second question. For example, the first question of one item for the dimension of "continued use despite the occurrence of negative consequences" is "Because of playing games, I don't have enough time and energy to get the right things done", and the second question is "After the aforementioned situation, I continue to play games whenever there is a chance".

Statistical Analysis

Missing Data

Among all 7,901 participants, 7,790 (98.60%) adolescents answered all items of the questionnaires and included in this study. A total of 111 (1.40%) adolescents were excluded from the analysis due to missing information in GDSQ.

Statistical Procedures

As the sample size for this study was sufficiently large, SPSS 25.0 was used to divide the sample into two separate data files. The first data file contained 3,871 samples and was used for exploratory factor analysis (EFA). The second data file contained 3,919 samples and was used for the confirmatory factor analysis (CFA). The full sample was used for descriptive statistical analysis and reliability estimation. Retest sample: 554 students from the first sample were retested 8 weeks apart. It was analyzed using MPLUS 8.3 for the CFA and SPSS 25.0 for Windows for the remaining analysis. A significance level of 0.05 was adopted for all statistical tests.

The chi-square (χ^2) values were applied to detect the differences between the model's implied variance–covariance matrix and the observed variance–covariance matrix. The comparative fit index (CFI) was used to compare the hypothesized model with the null hypothesis (33–35). The CFI is also one of the most robust indicators (36). The Tucker–Lewis Index (TLI) is a relative goodness of fit indices. In addition to evaluating the model from the perspective of model fitting, the fitting degree of the model can also be evaluated from the size of the residual error, and then, the fitting situation of the model can be evaluated. The standardized root mean square residual (SRMR) is one of the indicators for the direct evaluation of residual error. The model fit was also assessed by the root mean square error of approximation (RMSEA). McDonald and Ho recommended that the model with a RMSEA < 0.08 as an acceptable one and <0.05 as a good lone. Model goodness of fit was assumed according to the following criteria: RMSEA < 0.05 (35), SRMR < 0.08, TLI > 0.95, and CFI > 0.95 (36).

The GDSQ's ability to distinguish between non-disordered and disordered gamers was evaluated using a receiver characteristic curve (ROC) analysis. To achieve this goal,

TABLE 1 | 24 items parameters of the GDSQ, endorsement of single items ($N = 7,790$).[a]

ICD-11 criteria	Item number in pilot version	Item number in final version	Item content	% Endorsing each rating				
				0 (%)	1 (%)	2 (%)	3 (%)	4 (%)
Impaired control	1	1	I will turn on games uncontrollably sometimes.	3,013 (38.7)	1,516 (19.4)	899 (11.5)	2,047 (26.3)	315 (4.0)
	2	2	When I see or call to mind something about game, I can't help playing the game for a while.	3,786 (48.6)	1,558 (20.0)	833 (10.7)	1,327 (17.0)	286 (3.7)
	3	3	When the devices for playing games is in my sight, I would want to turn it on to play.	4,018 (51.6)	1,448 (18.6)	759 (9.7)	1,219 (15.6)	346 (4.4)
	4	4	Once I start playing the game, it is hard to stop.	4,425 (56.8)	1,210 (15.5)	761 (9.8)	1,086 (13.9)	308 (4.0)
	5		Even at inappropriate occasions or times, I still turn on the game and play for a while.	5,749 (73.8)	909 (11.7)	421 (5.4)	579 (7.4)	132 (1.7)
	6		I experience some discomfort (e.g., decreased vision, dizziness, muscle stiffness, or wrist pain) after high-intensity game playing.	5,192 (66.6)	1,283 (15.8)	590 (7.6)	622 (8.0)	148 (1.9)
	7	5	I intend to play games less recently, but actually there is no reduction in playing time.	4,944 (63.5)	1,183 (15.2)	630 (8.1)	859 (11.0)	174 (2.2)
	8	6	I play longer or more often than the upper limits I set for my game playing.	4,987 (64.0)	1,142 (14.6)	660 (8.5)	817 (10.5)	184 (2.4)
Increasing priority	9		If my time is scheduled by myself, I play games first and put other things on the back burner.	4,774 (61.3)	1,253 (16.1)	673 (8.6)	855 (11.0)	235 (3.0)
	10	7	To play games as soon as possible, I am perfunctory in the everyday things that I have to do.	4,930 (63.3)	1,248 (16.0)	649 (8.3)	748 (9.6)	215 (2.8)
	11	8	I miss regular meals or sleep time because of playing games.	5,483 (60.4)	1,006 (13.0)	499 (6.4)	638 (8.2)	164 (2.1)
	12	9	When I play games, I pay no attention to my personal hygiene.	6,161 (79.1)	694 (8.9)	346 (4.4)	450 (5.8)	139 (1.8)
	13	10	If others' demands occupy my game time, I feel upset.	5,014 (64.4)	1,229 (15.8)	626 (8.0)	679 (8.7)	242 (3.1)
	14	11	Although some activities provoke my interest, I refuse to participate in them because they will delay playing games.	6,060 (77.8)	759 (9.7)	372 (4.8)	462 (5.9)	137 (1.8)
	15	12	I feel that the things people are excited to talk about are not as interesting as games.	6,035 (77.5)	783 (10.0)	357 (4.6)	467 (6.0)	148 (1.9)
	16	13	To play games, I cancel or postpone the leisure activities in my plan.	5,974 (76.7)	876 (11.3)	371 (4.8)	434 (5.6)	135 (1.7)
Continued use despite the occurrence of negative consequences	17a	14a	Because of playing games, I don't have enough time and energy to get the right things done.	4,877 (62.6)	1,604 (20.6)	506 (6.5)	650 (8.3)	153 (2.0)
				No	Yes			
	17b	14b	(if 14a score $\geq$ 1)[b] After the aforementioned situation, I continue to play games whenever there is a chance.	6,878 (88.3)	912 (11.7)			
				0 (%)	1 (%)	2 (%)	3 (%)	4 (%)
	18a	15a	Playing games interferes with my work or learning tasks that I should complete.	5,226 (67.1)	1,376 (17.7)	469 (6.0)	579 (7.4)	140 (1.8)
				No	Yes			
	18b	15b	(if 15a score $\geq$ 1)[b] After the aforementioned situation, I continue to play games whenever there is a chance.	6,941 (89.1)	849 (10.9)			

(Continued)

TABLE 1 | Continued

ICD-11 criteria	Item number in pilot version	Item number in final version	Item content	% Endorsing each rating				
				0 (%)	1 (%)	2 (%)	3 (%)	4 (%)
				0 (%)	1 (%)	2 (%)	3 (%)	4 (%)
	19a	16a	Because of playing games, my performance in homework, academics, or work does not match my ability.	5,369 (68.9)	1,211 (15.5)	531 (6.8)	511 (6.6)	168 (2.2)
				No	Yes			
	19b	16b	(if 16a score ≥ 1)[b] After the aforementioned situation, I continue to play games whenever there is a chance.	6,946 (89.2)	844 (10.8)			
				0 (%)	1 (%)	2 (%)	3 (%)	4 (%)
	20a	17a	Because I play games, my family members or friends express their disappointment with me, argues with me, or become distant from me.	5,899 (75.7)	948 (12.2)	346 (4.4)	450 (5.8)	147 (1.9)
				No	Yes			
	20b	17b	(if 17a score ≥ 1)[b] After the aforementioned situation, I continue to play games whenever there is a chance.	7,035 (90.3)	755 (9.7)			
				0 (%)	1 (%)	2 (%)	3 (%)	4 (%)
	21a	18a	I have common topics only with people who play games. I don't know what to say to someone who does not play games.	6,666 (85.6)	457 (5.9)	206 (2.6)	330 (4.2)	131 (1.7)
				No	Yes			
	21b	18b	(if 18a score ≥ 1)[b] After the aforementioned situation, I continue to play games whenever there is a chance.	7,252 (93.1)	538 (6.9)			
				0 (%)	1 (%)	2 (%)	3 (%)	4 (%)
	22a	19a	I am worried about my future because I play games too much.	5,937 (76.2)	810 (10.4)	375 (4.8)	447 (5.7)	221 (2.8)
				No	Yes			
	22b	19b	(if 19a score ≥ 1)[b] After the aforementioned situation, I continue to play games whenever there is a chance.	7,094 (91.1)	696 (8.9)			
				0 (%)	1 (%)	2 (%)	3 (%)	4 (%)
	23a	20a	I have negative feelings after playing games (e.g., guilt or regret).	5,673 (72.8)	1,009 (13.0)	433 (5.6)	479 (6.1)	196 (2.5)
				No	Yes			
	23b	20b	(if 20a score ≥ 1)[b] After the aforementioned situation, I continue to play games whenever there is a chance.	7,083 (90.9)	707 (9.1)			
				0 (%)	1 (%)	2 (%)	3 (%)	4 (%)
	24a	21a	I experience sustained negative impacts from game playing on my health (e.g., weight gain, sleeping problems, neck and shoulder damage).	6,149 (78.9)	812 (10.4)	296 (3.8)	373 (4.8)	160 (2.1)
				No	Yes			
	24b	21b	(if 21a score ≥ 1)[b] After the aforementioned situation, I continue to play games whenever there is a chance.	7,133 (91.6)	657 (8.4)			

GDSQ, Gaming Disorder Symptom Questionnaire; ICD-11, 11th revision of the International Classification of Diseases.
Five-point Likert scale: "0 = never," "1 = less than monthly," "2 = monthly," "3 = weekly," "4 = almost daily".
[a]*Instructions: These question will ask you about your actual gaming-related activity during the past year (i.e., last 12 months).*
[b]*Instructions: The number in parentheses shows the item number in the final version.*

the GDSQ scores were compared against the standard according to ICD-11 related VGDS items. In this study, we also applied to defined 95% confidence intervals (CI). The Youden Index was calculated by sensitivity and specificity. The diagnostic efficacy of GDSQ was measured by the area under the ROC curve (AUC).

TABLE 2 | Socio-demographic and gameplay characteristics of the sample ($N = 7,790$).

Variables		Mean ± SD or *N* (%)
Age, years		14.99 ± 1.65
Gender	Male	3,742 (48)
	Female	4,048 (52)
Middle school stage	Junior middle school	3,467 (44.5)
	Senior middle school	4,323 (55.5)
Ethnicity	Han nationality	5,943 (76.6)
	Other ethnic minority	1,847 (23.4)
Family structure	Being an only child	3,815 (49.0)
	Having siblings	3,975 (51.0)
Weekly gameplay	0 h	2,505 (32.2)
	<2 h	2,601 (33.4)
	Between 2 and 4 h	1,102 (14.1)
	Between 4 and 8 h	770 (9.9)
	Between 8 and 16 h	371 (4.8)
	Between 16 and 32 h	204 (2.6)
	Between 32 and 64 h	111 (1.4)
	Between 64 and 128 h	70 (0.9)
	More than 128 h	56 (0.7)
Gaming device preference	Smartphones	3,803 (48.8)
	Personal computers	445 (5.7)
	Tablets	349 (4.5)
	Portable gaming devices	21 (0.7)
Game genre preference	MOBAs	2,266 (29.1)
	FPSs, CS: GOs	1,231 (15.8)
	Other games	1,917 (24.6)

RESULTS

Sample's Characteristics

The demographic characteristics are shown in **Table 2**. The mean age of first gameplay was 9.48 years (SD: 2.68). With regard to the gaming platforms preference, the majority of the participants ($n = 3,803$, 48.8%) reported playing on smartphones. The most genre played were multiplayer online battle arena (MOBA) games ($n = 2,266$, 29.1%), followed by first-person shooters (FPS) and counter-strike: global offensive (CS: GO) ($n = 1,231$, 15.8%). The average amount of money spent on gaming was 44.92 CNY (Chinese Yuan) per month (SD: 386.51). In addition, a large amount of gamers was reported spending more than 16 h per week on playing online games ($n = 441$, 5.6%), stand-alone games ($n = 211$, 2.7%), and game video watching ($n = 224$, 2.8%), respectively.

Factor Structure

An EFA using the Principal Axis Factoring extraction method with principal oblique rotation on the 24 items of the GDSQ was performed on the whole sample ($n = 7,790$) to examine its factorial structure and construct validity. The three principal components to be extracted were determined by the convergence of the scree plot in combination with the tendency for Kaiser's criterion. The scree plot of the GDSQ-21 by 21-factor analysis ($n = 3,871$) is shown in **Supplementary Figure 1**.

Items with factor loadings >0.45 and/or parallel loadings <0.20 were retained. After the first rotation, three items ["Even at inappropriate occasions or times, I still turn on the game and play for a while," "I experience some discomfort (e.g., decreased vision, dizziness, muscle stiffness, or wrist pain) after high-intensity game playing," and "If my time is scheduled by myself, I play games first and put other things on the back burner"] were removed due to these three items appeared to be a cross-load problem. The appropriateness for conducting the EFA was confirmed by the Kaiser–Meyer–Olkin criterion value (KMO = 0.970) used for the suitability of the date and the Bartlett's test result of sphericity ($\chi^2 = 80,321.74$, $p < 0.001$). As shown in **Table 3**, component loadings for each item ranged between 0.689 (item 18) and 0.846 (item 11). The three factors that were extracted after six iterations explained 76.04% of the total variance. Moreover, the 21 items of the GDSQ were retained in the model for subsequent analyses (i.e., CFA) to ensure optimal construct validity.

The goodness of fit of the unidimensional model of GDSQ in EFA was assessed using the conventional fit indices. The results showed an overall good fit to the data. The χ^2 was 1,219.11 ($p < 0.001$) with CFI of 0.958, TLI of 0.951, RMSEA of 0.038, and SRMR of 0.039, indicating an acceptable fit. As shown in **Figure 1**, the CFA results showed statistically significant factors ($p < 0.05$) for the 21 items of the GDSQ (GDSQ-21).

Criterion-Related Validity and Reliability

The results of the eight multiplications are summed. By summing up the responses to the 21 items, the total score was calculated to obtain a possible maximum score of 84. To further assess the validity and reliability of the GDSQ-21, the total scores obtained by participants on the VGDS and GDSQ-21 were associated ($r = 0.781$, $p < 0.001$). The GDSQ-21 was associated with self-reported weekly gaming time (online, stand-alone, and game video watching) ($r = 0.619$, 0.514, and 0.504, respectively, ps < 0.001). The GDSQ-21 was also associated with the monthly amount of money spent on games (Spearman's $\rho = 0.338$, $p < 0.001$). As shown in **Table 4**, the results obtained suggest that the GDSQ-21 is strongly positively associated with VGDS, and moderately correlated with the self-reported weekly gaming time and the monthly amount of money spent. The Spearman–Brown split-half reliability of GDSQ-21 was 0.98, and the test–retest reliability was 0.71. Cronbach's α of the GDSQ-21 was 0.964 in this analysis, which showed the scale's good internal consistency. The Cronbach's α for the three factors were 0.929, 0.950, and 0.948, respectively.

Cutoff Points of the GDSQ-21 for GD

As shown in **Figure 2**, by the Youden Index, the optimal cutoff for the overall score was 61.5 with the curve (AUC) of 89.6% (95% CI = 86.6–92.7), sensitivity of 83.1%, and specificity of 88.8%. Factor 1 had a cutoff value of 13.5 with the curve (AUC) of 86.6% (95% CI = 83.5–90.0), specificity of 78.3%, and sensitivity of 86.7%. Factor 2 had a cutoff value of 10.5 with the curve (AUC) of 89.2% (95% CI = 86.3–92.1), specificity of 78.7%, and sensitivity of 89.3%. Factor 3 had a cutoff value of 3.5 with the curve (AUC) of 86.0% (95% CI = 82.6–89.4), specificity of 77.3%, and sensitivity

TABLE 3 | Summary of the results from the EFA on the GDSQ-21 items obtained from the sample (n = 3,871).

Item in GDSQ-21	Factor loadings[a,b,c]			Communalities		
	Factor 1	Factor 2	Factor 3	Extraction	Corrected item-total correlation	Cronbach's α if item deleted
1	**0.826**	0.149	0.175	0.735	0.677	0.964
2	**0.838**	0.256	0.218	0.817	0.768	0.962
3	**0.818**	0.297	0.211	0.801	0.775	0.962
4	**0.725**	0.378	0.260	0.737	0.795	0.962
5	**0.665**	0.448	0.288	0.725	0.813	0.961
6	**0.621**	0.500	0.284	0.716	0.815	0.961
7	0.547	**0.590**	0.322	0.751	0.847	0.961
8	0.422	**0.675**	0.324	0.739	0.823	0.961
9	0.297	**0.766**	0.332	0.786	0.806	0.961
10	0.455	**0.656**	0.276	0.713	0.806	0.961
11	0.284	**0.809**	0.332	0.846	0.824	0.961
12	0.293	**0.784**	0.351	0.823	0.824	0.961
13	0.278	**0.798**	0.355	0.841	0.826	0.961
14	0.262	0.360	**0.728**	0.728	0.770	0.962
15	0.238	0.324	**0.780**	0.770	0.764	0.962
16	0.232	0.327	**0.801**	0.802	0.774	0.962
17	0.188	0.312	**0.766**	0.719	0.720	0.963
18	0.138	0.347	**0.747**	0.689	0.697	0.963
19	0.214	0.159	**0.821**	0.746	0.677	0.963
20	0.221	0.152	**0.809**	0.727	0.671	0.963
21	0.203	0.236	**0.812**	0.757	0.710	0.963

Greatest loadings on each factor are bolded; GDSQ-21, Gaming Disorder Symptom Questionnaire-21; EFA, Exploratory Factor Analysis.
GDSQ-21 factor 1 = impaired control, GDSQ-21 factor 2 = increasing priority, and GDSQ-21 factor 3 = continued use despite the occurrence of negative consequences.
[a]*Percentage of variance explained by three factors = 76.04%.*
[b]*After six iterations, it was possible to extract three factors from the EFA.*
[c]*Cronbach's = 0.964.*

of 89.4%. Finally, the cutoff ≥ 14 is applied for factor 1, ≥11 for factor 2, ≥4 for factor 3, and ≥62 for the whole scale. Participants who met each dimension and total score were classified with GD. The prevalence of GD was estimated at 2.27% in the period of the past 12 months. As shown in **Table 5**, there was a significant difference between GD and No-GD adolescents. For instance, the GD adolescents reported more weekly gaming time than the No-GD adolescents.

DISCUSSION

To the best of our knowledge, this study is the first study to introduce a screening tool for assessing the ICD-11 diagnostic guidelines for GD in adolescents in China. GDSQ-21 was successfully validated in a sample of adolescents as an assessment tool with excellent internal consistency and criterion validity. The instrument covers the three symptoms for IGD/GD, and the concrete manifestations of these symptoms are referred to in detail (i.e., impaired control, increasing priority to gaming, and continued use despite the occurrence of negative consequences) and functional impairment. Thus, in addition to exhibiting psychometrically robust properties, it is an easy-to-use screening instrument that can be used by medical and non-medical institutions to distinguish non-disordered and disordered gamers.

In respect of the test reliability and validity, the GDSQ-21 appears to be a reliable and valid measure for assessing GD. The statistically significant positive associations were found among the GDSQ-21, weekly gaming hours (online, stand-alone, and video watching), average monthly gaming charge, and the VGDS test, providing empirical evidence for the validity of the test. Moreover, results from the EFA and CFA support the population cross validity of the GDSQ-21 as it was shown that the three-factor solution found in the EFA (i.e., sample 1) was also replicated and confirmed in the CFA (i.e., sample 2). Furthermore, the instrument was highly reliable in all samples as the Cronbach's α values were very high, suggesting that the GDSQ-21 measurement is reliable and accurate in detecting changes in GD levels.

Game duration and the game genres preference were significantly correlated with GD. GDSQ-21 sum scores were also associated with weekly playtime and average monthly spending on games. Previous studies have reported that spending a lot of time and money is a predictor of IGD (37). As seen in the game devices, more and more players are turning to smartphone games and table games. The current study found that smartphones may be more addictive than other devices for pathological gamers, and this finding is supported by Christian Montag's study (38, 39). In terms of game genres, the characteristics of different types of games attract different gamers, whereas the occurrence,

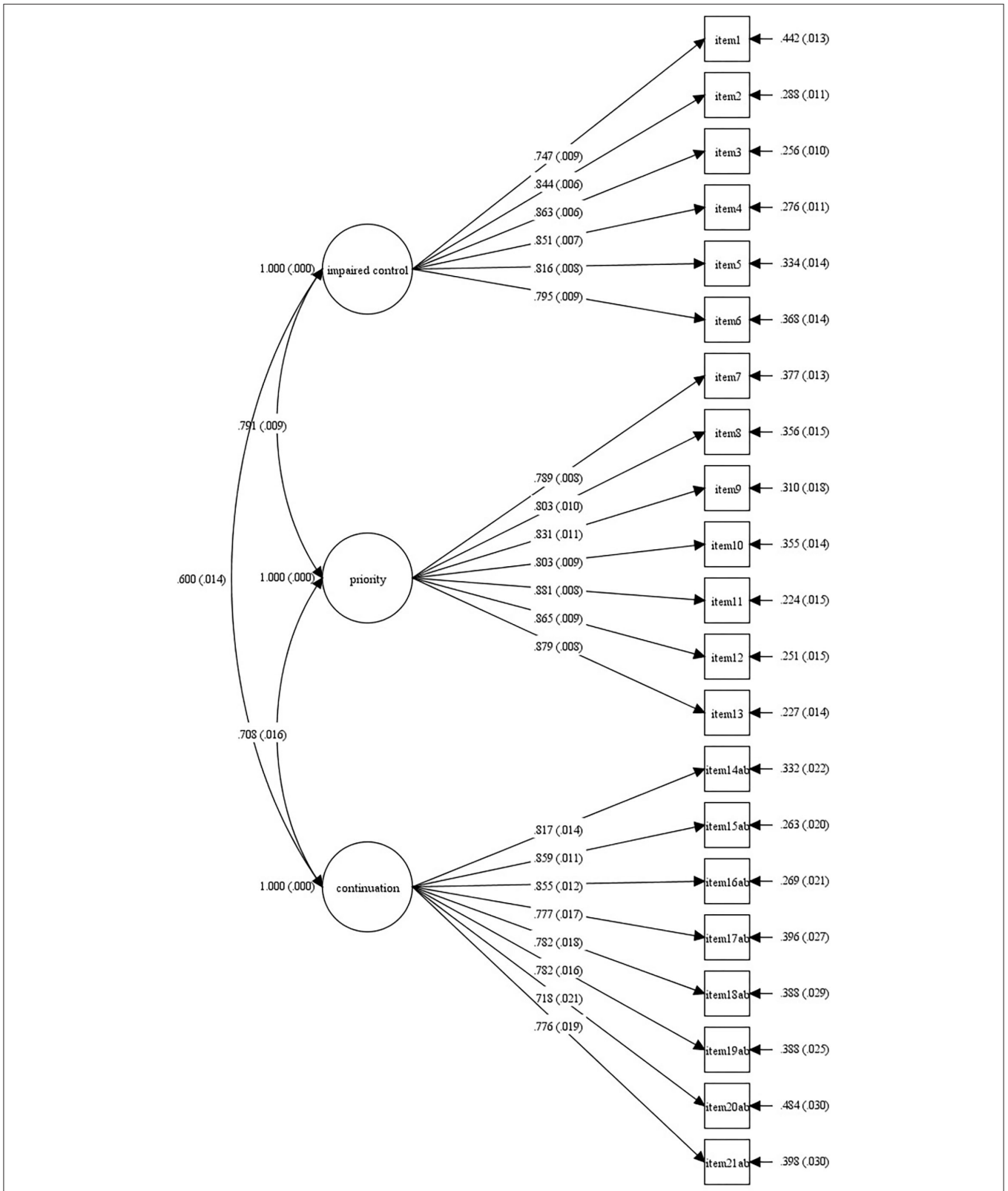

FIGURE 1 | Graphical summary of CFA results obtained from the 21 items of the GDSQ-21 ($N = 3,919$). GDSQ-21, Gaming Disorder Symptom Questionnaire-21; CFA, confirmatory factor analysis.

symptoms, and negative consequences of GD are related to the genres of the game (6, 13). In general, complex, endless, and socially driven game types are more likely to have GD (40). It is worth noting that future studies should focus on different risky situations, as different situations require the adaptation of (early) intervention methods for optimal recovery of GD.

TABLE 4 | Correlations between GDSQ-21 and other related measures.

Construct	*r*/ρ	*p*
VGDS sum score	0.781	<0.001
Weekly online gaming time (h)	0.619	<0.001
Weekly stand-alone gaming time (h)	0.514	<0.001
Weekly game video watching time (h)	0.504	<0.001
Money spent on gaming/month (CNY)	0.338	<0.001

GDSQ-21, Gaming Disorder Symptom Questionnaire-21; r, Pearson's correlation coefficient; ρ, Spearman's correlation; VGDS, Video Gaming Dependency Scale; CNY, Chinese Yuan.

In the ROC analysis, the AUC was 89.6%, indicating strong discriminatory power. With adequate sensitivity and specificity, the GD was effectively distinguished from no GD. However, it is important to further examine whether the cutoff point of 62 (of a total score of 84) can distinguish disordered gamers from non-disordered among different populations, such as adults and individuals from other regions or countries.

Compared to the DSM-5 diagnostic criteria for IGD, the ICD-11 definition of GD may be more concise and research-based, highlighting the most central symptom presentation. Studies have found different rates of GD in screening between the DSM-5–based IGD criteria and the ICD-11 GD guidelines. For instance, the 12-month prevalence of IGD in Chinese

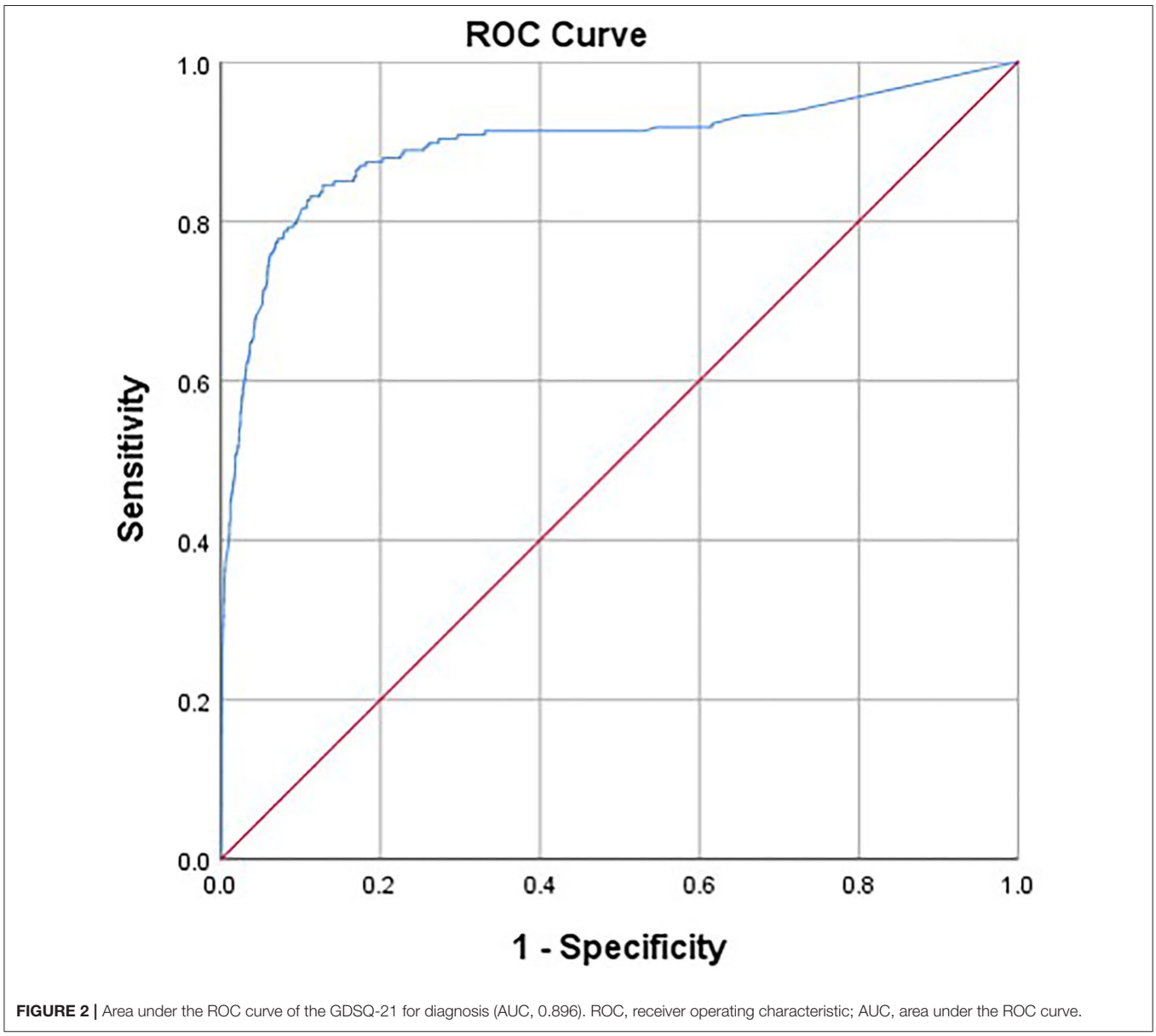

FIGURE 2 | Area under the ROC curve of the GDSQ-21 for diagnosis (AUC, 0.896). ROC, receiver operating characteristic; AUC, area under the ROC curve.

TABLE 5 | Grouping of GD and No GD according to ROC curve cutoffs (mean ± SD).

Variables	GD (n = 177)	No GD (n = 7,673)	t	p
Age, years	15.65 ± 1.73	14.98 ± 1.65	5.19	<0.001
GDSQ-21 factor 1 score	21.07 ± 2.65	5.50 ± 5.94	73.966	<0.001
GDSQ-21 factor 2 score	23.82 ± 3.38	3.27 ± 5.40	78.369	<0.001
GDSQ-21 factor 3 score	24.90 ± 4.43	1.31 ± 3.89	70.158	<0.001
VGDS sum score	59.82 ± 10.32	25.18 ± 10.32	44.155	<0.001
Weekly online gaming time (h)	5.18 ± 2.01	2.38 ± 1.53	18.390	<0.001
Weekly stand-alone gaming time (h)	4.8 ± 2.23	1.84 ± 1.20	17.614	<0.001
Weekly game video watching time (h)	4.74 ± 2.35	1.75 ± 1.19	16.899	<0.001
Money spent on gaming/month (CNY)	406.14 ± 1,296.80	35.34 ± 325.81	3.356	<0.001

GD, Gaming Disorder; No-GD, No Gaming Disorder; ROC, Receiver Operating Characteristic; GDSQ-21, Gaming Disorder Symptom Questionnaire-21; GDSQ-21 factor 1 = impaired control, GDSQ-21 factor 2 = increasing priority, and GDSQ-21 factor 3 = continued use despite the occurrence of negative consequences; VGDS, Video Gaming Dependency Scale; CNY, Chinese Yuan.

adolescents was 2.9% according to DSM-5 criteria (22) and 2.27% in currently study.

Pontes and Mark (41) have also reported various inconsistencies and psychometric weaknesses in IGD instrumentation, but the GDSQ-21 instrument is considerable and has sufficiently sufficient reliability and validity. Its three factors take into account the size and intensity of the range of problematic game performance, and their items are not independent but interrelated. All items were good construct indicators due to all factor loadings being statistically significant and relatively high.

Although the available IGD instruments are still applicable measures based on the DSM-5 framework, the GDSQ-21 is a psychometric instrument developed under the new ICD-11 framework that will yield fundamental clinical and diagnostic differences between GD-based psychometric assessment instruments. The GDSQ-21 scale has excellent reliability and validity, and the theoretical concepts and connotations based on it are consistent with international standards.

Although the present study provides unique information about the ICD-11 criteria for GD, the limitations should be considered. First, given that adolescents are the most vulnerable group of GD, all participants in this study were adolescent students (aged 12–18 years). Thus, the current samples was not fully representative, and findings from current study should be cautiously interpreted in terms of its generalizability. Future studies should include adults and children to further confirm the robustness of the GDSQ-21. Second, the GD was assessed by the self-report questionnaires but not by professional clinical interviews and diagnoses. Future research in the field should compare clinically diagnosed sample with actual GDSQ-21 test scores.

CONCLUSIONS

In conclusion, the value of the GDSQ-21 as a GD screener for adolescents is evidenced by the current findings. The GDSQ-21 has excellent internal consistency reliability and criterion validity in a representative sample of adolescent game players. Furthermore, its three-factor structure supports the ICD-11 new diagnostic concept of GD, regarding persistent gaming behavior, impaired control over gaming, and functional impairment due to gaming for at least 12 months in most instances. Findings from this study recommend the use of the GDSQ-21 as a screening tool to assess GD, which can assist non-medical providers to screen people with GD.

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 authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Second Xiangya Hospital of Center South University (ID: 2019-S454). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

WH, MY, and LZ designed the assessment tools and study protocol. LZ and YC collected the data. LZ, YL, and TL performed the literature review, statistical analysis, and wrote the first draft. YL and TL commented on the manuscript. All authors interpreted the data, critically reviewed the content, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Sex-Dependent Alterations of Regional Homogeneity in Cigarette Smokers

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Biological sex may play a large role in cigarette use and cessation outcomes and neuroimaging studies have demonstrated that cigarette smoking is associated with sex-related differences in brain structure and function. However, less is known about sex-specific alterations in spontaneous brain activity in cigarette smokers. In this study, we investigated the sex-related effects of cigarette smoking on local spontaneous brain activity using regional homogeneity (ReHo) based on resting-state fMRI. Fifty-six smokers (24 females) and sixty-three (25 females) healthy non-smoking controls were recruited. Whole-brain voxelwise 2-way analysis of covariance of ReHo was performed to detect brain regions with sex-dependent alterations on the spontaneous brain activity. Compared to non-smokers, smokers exhibited significant ReHo differences in several brain regions, including the right medial orbitofrontal cortex extended to the ventral striatum/amygdala/parahippocampus, left precuneus, and bilateral cerebellum crus. Smoking and sex interaction analysis revealed that male smokers showed significantly lower ReHo in the right ventral striatum, left cerebellum crus1, and left fusiform gyrus compared to male non-smokers, whereas there are no significant differences between female smokers and non-smokers. Furthermore, the ReHo within the left cerebellum crus1 was negatively correlated with craving scores in male smokers but not in female smokers. Such sex-dependent differences in spontaneous brain activity lays a foundation for further understanding the neural pathophysiology of sex-specific effects of nicotine addiction and promoting more effective health management of quitting smoking.

Keywords: sex, regional homogeneity, resting-state fMRI, craving, smokers

INTRODUCTION

Tobacco smoking, the leading cause of preventable death worldwide, is associated with many serious health problems including cardiovascular disease and lung cancer (1). Despite the growing number of evidence-based treatments for smoking addiction, the efficacy remains moderate, with high relapse rate (2). Biological sex may play a large role in patterns of nicotine use and cessation.

outcomes For instance, higher smoking rates, greater reinforcement of nicotine and better nicotine replacement therapies has been reported among men than women (3). Women are inclined to smoke for stress relief and mood regulation, and difficult to maintain abstinence (4). Therefore, sex differences may be crucial to target the neural pathophysiology of nicotine addiction and develop more effective relapse prevention therapies (5).

Neuroimaging studies showed that cigarette smoking is associated with sex-specific alterations in brain structure and function. Compared to sex-matched non-smokers, male rather than female nicotine-dependent subjects had a larger volume in the left putamen and a smaller volume in the left caudate, whereas female but not male smokers showed a smaller volume in the right amygdala (6). Exposure to smoking cues, direct comparisons between male and female smokers revealed that male smokers showed greater reactivity in reward-related brain regions (7) and greater coupling between the interhemispheric regions within the executive control network (8) than female smokers. In contrast, female smokers had greater connectivity within the limbic and default mode network than male smokers (9, 10). Earlier work of our team analyzed middle-aged chronic heavy smokers and found that the regional homogeneity (ReHo) of the right paracentral lobule of male smokers was significantly different from that of female smokers (11). Another study of our team reported that young male but not young female smokers had decreased resting-state functional connectivity (rsFC) between the right amygdala and right orbitofrontal cortex (OFC) (12). RsFC of the amygdala-OFC circuity was negatively correlated with the craving score in male but not female smokers (12). Despite increasing knowledge of the sex-specific effects of tobacco use on brain structure and connectivity between spatially distinct brain regions, it remains unclear whether cigarette smoking has differential influence on the local spontaneous brain activity between men and women, particularly young adults.

Resting-state fMRI (rs-fMRI) provides a non-invasive method of assessing changes in resting-state brain activity and functional connectivity across the whole-brain (13). ReHo measures the synchronization of intra-regional spontaneous low-frequency BOLD signal fluctuations by calculating Kendall's coefficient of concordance (14), which have high test-retest reliability (15). The ReHo metric has been widely used to investigate abnormal local spontaneous brain activity in psychiatric and neurologic conditions (16). Recent literature review demonstrated differences in adolescent vulnerability to neurotoxicity of drugs of abuse from adult (17). Brain imaging studies showed humans might achieve full adulthood after the ages of 25 and 30 (18, 19). Hence, it is important to study the abnormal changes of brain function in smokers at younger age group. In view of the gender influence of smoking on neural structure and functional networks, we assume that young male and female smokers would show constant change patterns in the reward-related areas [as in our previous article (11)], and display different change patterns in local synchronization of the brain.

To test our hypothesis, we employed ReHo method to depict spontaneous brain activity and then performed a voxel-wise analysis to examine the intrinsic brain activity differences between smokers and non-smokers, and to further explore the sex interaction on smoking dependency. Such sex-dependent differences in spontaneous brain activity lays a foundation for further understanding the neural pathophysiology nicotine addiction and promoting more effective health management of quitting smoking.

MATERIALS AND METHODS

Subjects

Sixty cigarette smokers (25 females) and 67 healthy nonsmoking controls (28 females) aged 18–29 years participated in this study. The Mini International Neuropsychiatric Interview was used to exclude participants who had substance use disorder other than nicotine dependence, current Axis 1 DSM V psychiatric diagnoses, or significant medical conditions. Exclusion criteria for both smokers and non-smokers were a history of head trauma or injury causing loss of consciousness lasting >3 min or associated with skull fracture or intracranial bleeding, or had irremovable magnetically active objects on or within their body. Smokers were defined as those who smoked at least 10 cigarettes/day on any given day during the last year. The severity of nicotine addiction was determined from the Fagerström Test for Nicotine Dependence (FTND) (20), and the measurement of "craving" for cigarettes was evaluated using a brief questionnaire of smoking urges (21). All smokers had no period of smoking abstinence longer than 3 months in the past year. It was about 1 h since last cigarette before smokers were scanned. Non-smokers had not smoked more than five cigarettes in their lifetimes. All participants were administered a set of questionnaires at the beginning of the study, namely, the Self-rating Anxiety Scale (SAS), Self-rating Depression Scale (SDS) and Barratt Impulsiveness Scale (BIS) version 11. The dataset of subjects were described in our previous article (12), but the analyses of this study are different from that in previous reports; here we focus on exploring sex-specific effects of cigarette smoking on spontaneous brain activity.

The current study adhered to the Declaration of Helsinki and was approved by the Research Ethics Committee of Renji Hospital, School of Medicine of Shanghai Jiaotong University. All participants were informed of the measurements and the experimental procedures of the study before their MRI examinations. Each participant provided written informed consent.

Data Acquisition

Images were obtained using a 3.0-T GE Signa HDx (Milwaukee, WI, USA) scanner with a standard 8-channel head coil. Restraining foam pads were used to reduce head motion, and earplugs were used to reduce scanner noise. Before MRI scanning, all subjects were instructed to relax with their eyes closed while refraining from falling asleep and without engaging in any directed, systematic thought. The physiological state of the smokers when they were in the scanner as spontaneous, rather than abstinence or satiety, that was, none of the smokers felt the acute urge to smoke or experience any withdrawal symptoms

confirmed by a self-report completed by each participant immediately after the scan.

Rs-fMRI data were acquired using a gradient-echo echo-planar imaging sequence (TR = 2,000 ms, TE = 24 ms, flip angle = 90°, matrix = 64 × 64, FOV = 230 × 230 mm^2, thickness = 4 mm with no gap, 34 slices). For each participant, the rs-fMRI scan lasted 7 min and 20 s and a total of 220 volumes were acquired. The axial T1-weighted and T2-weighted images were also performed to confirm the absence of structural lesions. All images were evaluated by two experienced neuroradiologists and no participants were excluded on this basis.

Image Preprocessing

Rs-fMRI data were preprocessed using the Data Processing Assistant for Resting-State fMRI (http://rfmri.org/DPARSF). After removing the first 10 volumes for each subject, the remaining 210 volumes were corrected for slice timing and realigned to the first volume for head-motion correction. The Friston 24-parameter model (i.e., six head motion parameters, six head motion parameters one time point before, and 12 corresponding squared items) was performed to regress out the head motion effects. The scrubbing strategy was further carried out to correct the head motion effects. Time points with framewise displacement larger than 0.2 mm were identified and removed along with 1 back and 2 forward neighbors (22). Subjects with <90 time points after scrubbing were not used because too few remaining time points may lead to unreliable results (23). As a result, four smokers and four non-smokers were discarded, and a total of 56 smokers (24 females) and 63 non-smokers (25 females) were ultimately used in the ReHo analysis. The detailed demographic and clinical data of the subjects used in this study are provided in **Table 1**. Smokers and non-smokers were not statistically different in terms of time points removed (28.08 ± 32.07 for smokers, 36.25 ± 32.66 for non-smokers, $p = 0.17$). Afterward, the functional images were normalized to the standard Montreal Neurological Institute (MNI) space and resampled to a 3-mm isotropic voxel. Then, linear and quadratic trends as well as white matter and corticospinal fluid signals were removed. Finally, temporal bandpass filtering (0.01–0.1 Hz) was performed to reduce the effects of high-frequency physiological noise.

ReHo Analysis

Based on the temporally bandpass-filtered data, the ReHo value for each voxel was defined as the KCC of the time series of a given voxel and those of its 26 nearest neighbors. For standardization purposes, each individual ReHo map was divided by its mean ReHo within a brain mask without non-brain tissue (14). Finally, the ReHo maps were smoothed by 6 mm full width at half maximum Gaussian kernel.

Statistical Analysis

A 2-way analysis of variance (ANOVA) was used to identify differences among groups in age, education level, SAS, SDS and BIS scores. A voxelwise 2-way analysis of covariance (ANCOVA) with age and education level as covariates was performed to evaluate the main effects of group (smokers vs. non-smokers) and sex (male vs. female) as well as the interaction between group and sex. A false discovery rate (FDR) corrected p-value of 0.05 was used for multiple comparisons. When the group effects occurred, we further explore the specific smoking effects (i.e., smokers > non-smokers or smokers < non-smokers) on ReHo. We extracted the averaged ReHo from significant clusters showing group effects, and then performed the 1-way ANCOVA controlling for age and education level to determine the specific smoking effects on ReHo. When the group-by-sex interaction effects occurred, the sex-specific effects of smoking on ReHo were investigated. The mean ReHo values from clusters showing group-by-sex interaction effects were extracted, and then the 1-way ANCOVAs controlling for age and education level were conducted to compare male smokers vs. male non-smokers and female smokers vs. female non-smokers separately.

For smokers, Pearson correlation analysis was performed to investigate whether there were correlations between the altered ReHo and the smoking-related variables (i.e., duration of smoking, age at first smoking, FTND and craving scores). A p-value of <0.05 was considered significant.

RESULTS

Demographic and Clinical Measures of Participants

Table 1 lists the demographic and clinical measures of the subjects used in this study. No significant difference was found in gender among the groups. For age, no main effect of group ($p = 0.77$) or group by sex interaction ($p = 0.83$) were found, while there was a significant main effect of sex ($p = 0.002$). For years of education, significant main effect of group ($p < 0.001$) and group by sex interaction ($p < 0.001$) were revealed, while there was no main effect of sex ($p = 0.35$). Specifically, male smokers had higher education level than female smokers ($p = 0.006$), while male non-smokers had lower education level than female non-smokers ($p = 0.005$).

There were no significant differences in duration of smoking ($p = 0.09$), FTND ($p = 0.42$) or craving scores ($p = 0.88$) between male and female smokers, while male smokers had an earlier age at first smoking ($p < 0.001$) than female smokers. Smokers as a whole had higher scores than non-smokers on SAS ($p < 0.001$), SDS ($p < 0.001$) and BIS ($p = 0.003$), which is consistent with prior studies showing that smoking has often been associated with increased depression, anxiety and impulsivity (24, 25). Women had higher scores on SAS ($p = 0.07$), SDS ($p = 0.03$) and BIS ($p = 0.003$) than men. Although the SAS, SDS and BIS scores of smokers were higher than those of non-smokers, they did not meet the criteria for comorbid mood or attention disorders.

ReHo Changes in Smokers

Statistically significant main effects of smoking were observed for ReHo (voxel-level $p < 0.05$ FDR corrected; **Figure 1**). Further 1-way ANCOVA with age and education as covariates demonstrated that compared to non-smokers, smokers had significant greater ReHo in the left precuneus and right cerebellum posterior lobe (crus1/2) while lower

TABLE 1 | Demographic information of the subjects in each group and between-group comparisons.

	Smokers		Non-smokers		Group comparisons[a] (F/*p* values)		
	Male ($n = 32$)	Female ($n = 24$)	Male ($n = 38$)	Female ($n = 25$)	Group	Sex	Group*sex
Age	22.63 ± 2.70	24.50 ± 2.95	22.58 ± 3.12	24.22 ± 3.41	0.09/0.77	9.58/0.002	0.05/0.83
Education level	13.00 ± 2.03	11.29 ± 2.48	13.82 ± 3.31	16.60 ± 4.23	28.04/<0.001	0.87/0.35	15.09/<0.001
Age at first smoking	16.84 ± 2.47	20.00 ± 2.86	–	–	–	–	–
Duration of smoking	5.78 ± 2.84	4.50 ± 2.50	–	–	–	–	–
FTND score	6.53 ± 2.06	6.04 ± 2.48	–	–	–	–	–
Craving score	30.94 ± 11.92	30.38 ± 16.14	–	–	–	–	–
SAS score	45.03 ± 9.39	49.83 ± 10.05	38.95 ± 6.28	40.08 ± 8.78	24.76/<0.001	3.48/0.07	1.33/0.25
SDS score	47.91 ± 9.61	53.42 ± 9.89	42.84 ± 8.67	44.82 ± 9.00	15.71/<0.001	4.68/0.03	1.06/0.31
BIS score	52.72 ± 7.79	60.67 ± 10.81	51.61 ± 6.28	52.68 ± 7.70	9.17/0.003	9.02/0.003	5.23/0.02

[a]*Analysis of variance, df = 1,115.*

Values are expressed as the means ± standard deviations. The age, educational level, age at first cigarette and duration of smoking are displayed in years. FTND, Fagerström Test of Nicotine Dependence; SAS, Self-rating Anxiety Scale; SDS, Self-rating Depression Scale; BIS, Barratt Impulsiveness Scale version 11. Educational level was defined as the number of years of scholarship since primary school.

ReHo in the right medial OFC extending to the ventral striatum/amygdala/parahippocampal gyrus, right lateral OFC, and left cerebellum anterior lobe (crus1) extending to the inferior temporal gyrus/fusiform gyrus (**Table 2**).

At a second stage, we analyzed the simple effects of smoking in the females and males in a separate analysis within the brain regions showing main effects of smoking. The male smokers were compared with male non-smokers and female smokers with female non-smokers. Independent 1-way ANCOVA controlling for age and education revealed that the main effects of smoking mostly came from male smokers except that female smokers had significantly higher ReHo as compared to female non-smokers in the left precuneus (for details, see **Table 2**).

A significant smoking and sex interaction effect on ReHo was identified in the left fusiform gyrus, left cerebellum crus1 extending to the inferior temporal gyrus, and right ventral striatum/putamen (voxel-level $p < 0.05$ FDR corrected, **Table 3**; **Figure 2A**). *Post-hoc* analyses revealed that male smokers had lower ReHo than male non-smokers in these regions, but no differences were observed between female smokers and non-smokers (**Figure 2B**). The ReHo in these regions showing interaction effects between smoking and sex did not significantly differ between male and female non-smokers, whereas male smokers demonstrated less ReHo than female smokers in these regions.

We also found that the lower ReHo in the left cerebellum crus1 exhibited higher craving scores in male smokers ($r = -0.402$; $p = 0.022$), whereas there was no significant correlation between the ReHo and the craving scores in female smokers ($r = 0.110$; $p = 0.608$) (**Figure 3**). The ReHo in the left cerebellum crus1 were more strongly correlated with craving in male smokers than in female smokers ($p = 0.03$).

DISCUSSION

In this study, we investigated sex-specific differences in local spontaneous brain activity between smokers and healthy non-smoking subjects using rs-fMRI and ReHo analysis. Our findings demonstrated that both male and female smokers showed higher ReHo in the left precuneus compared to sex-matched non-smokers while only male smokers had greater ReHo value in the cerebellum crus 1/2. However, male smokers had lower ReHo in the right medial OFC extending to the ventral staiatum/amygdala/parahippocampal gyrus, right lateral OFC, left cerebellum crus 1 extending to the inferior temporal gyrus, and left fusiform gyrus. Moreover, the ReHo in the left cerebellum crus1 was negatively correlated with craving scores in male smokers but not in female smokers. Taken together, these findings suggest that cigarette smoking may have differential effects on the spontaneous brain activity between men and women, especially in regions that are associated with reward and motivation functions, which are known to be affected by nicotine dependence.

One of the main results of this study using smoking by sex interaction, was found to reduce ReHo in several regions, such as the right ventral striatum/putamen, left fusiform gyrus, and left cerebellum crus 1 extending to the inferior temporal gyrus (**Table 3**; **Figure 2**). The ventral striatum is functionally implicated in reward anticipation, reward-related behaviors, and reward outcome of individuals with substance use disorders (26) and it is a reinforcement site (27). Men who smoke for the reinforcing effects of nicotine have significant responses in the ventral striatum. Using the same dataset and functional connectivity analysis, Lin et al. found that smoking has differential effects on the amygdala-OFC circuits between men and women, which is characterized by greater functional connectivity in the male circuits than that in women (12). Therefore, the change of ventral striatum activity may indicate that the reward learning defects of nicotine-addicted male individuals will eventually lead to the impairment of decision-making ability.

There is increasing evidence that cerebellum is involved in substance use disorders (28). Cerebellum was considered as a critical component of the addiction circuit, which is

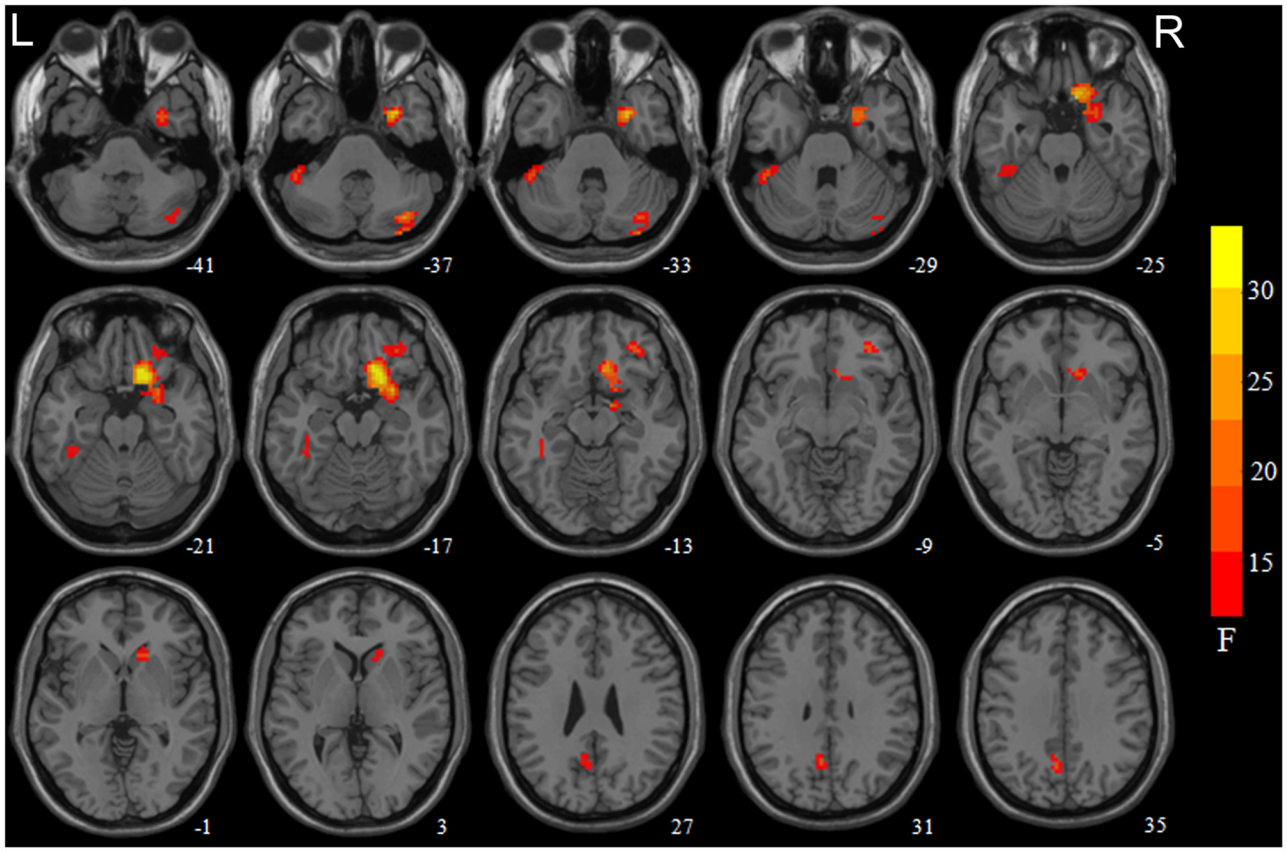

FIGURE 1 | Brain areas with significant main effect of smoking on regional homogeneity (ReHo) revealed by the 2-way analysis of covariance with age and education levels as covariates. Regions in red-yellow are brain areas where ReHo was significantly altered in smokers relative non-smokers. The areas include the right medial orbitofrontal cortex (OFC) extend to the ventral striatum/amygdala/parahippocampa gyrus, right cerebellum crus1/2, left precuneus, left cerebellum crus1 extended to the inferior temporal gyrus/fusiform gyrus, and right lateral OFC. The results were multiple compared at voxel-level $p < 0.05$ (FDR corrected). The left side of the image corresponds to the left hemisphere of the brain.

TABLE 2 | Brain regions showing significant main effect of smoking on regional homogeneity (ReHo) (voxel-level $p < 0.05$ corrected for false discovery rate).

Specific effects	Identified brain regions	Peak coordinates (MNI)			Side	Peak F	Cluster size (voxels)
		X	*Y*	*Z*			
Smokers > non-smokers							
fSM > fNS, mSM > mNS	Precuneus	−6	−63	42	L	19.74	76
mSM>mNS	Cerebellum posterior lobe (Crus1, Crus2)	36	−75	−39	R	24.02	73
Smokers < non-smokers							
mSM < mNS	Medial orbital frontal cortex/Ventral striatum/amygdala/parahippocampal gyrus	15	21	−21	R	33.94	398
mSM < mNS	Cerebellum anterior lobe (Crus1)/inferior temporal gyrus/fusiform gyrus	−48	−42	−36	L	18.68	103
mSM < mNS	Lateral orbital frontal cortex	33	39	−12	R	18.13	61

MNI, Montreal Neurological Institute; fSM, female smokers; fNS, female non-smokers; mSM, male smokers; mNS, male non-smokers; R, right; L, left.

related to coordination of the smoking movement, motivation driving, and inhibition control (28). Male smokers, but not female smokers, have great cerebellar β2-nicotinic acetylcholine receptors (nAChRs) availability (29) and low putamen D2/D3 receptor availability (30), which may affect the up- and down-regulation. A PET studies showed that cigarette smoking increased density of nicotine binding and increased CBF in the cerebellum, which was correlated with plasma nicotine levels (31). Unlike the consensus that the GMV of cerebellum crus 1 in nicotine dependents is less than that in the controls (32–35), the activation of cerebellum varied with the expected degree of drug during exposure to drug-related cues rather than neutral cues (28, 36). In this study, we found decreased ReHo in the cerebellum crus 1/2 in male smokers (**Table 3**; **Figure 2**), while our previous studies on middle-aged heavy smokers reported that the ReHo of cerebellum increased (11). Lower ReHo in the left cerebellum crus 1 was correlated with the higher craving scores ($r = -0.402$; $p = 0.022$) in male smokers. This may be explained by the significant differences in smoking duration (SM 5.78 ± 2.84 years, SW 4.50 ± 2.50 years vs. 25.42 ± 9.10 years), and the complex mechanisms of chronic smoking, such as early dysfunction and late compensation Interestingly, female smokers did not show the same pattern of brain activity as male smokers. This finding may be partially explained by circulations in the menstrual cycle phase that modulates the reward-related regions in females (37, 38). An arterial spin labeling study examined the neural activity in response to video clip of smoking clues in females, differences emerged in the medial OFC in the follicular phase of the cycle (when progesterone levels are low), but no significant differences were observed in the luteal phase (39). Together, one may speculate that altered activity in the ventral striatum and cerebellum may indicate the selective effects of smoking on dopamine receptors and nicotinic receptors in men and women. The effect might be hampered in male smokers because of their greater reliance on nicotine. Female smokers may be exempted from smoking addiction because of their menstrual cycle.

Our team has been focusing on structural and functional MRI to study the neural mechanism underlying substance addictions. For example, Wu et al. (11) revealed that ReHo was significantly reduced in default-mode, frontoparietal attention and inhibition control networks, and regions related to motor planning increased in among the middle-aged chronic smokers (11). Lin et al. (40) and Cai et al. (35) reported the decreased nodal efficiency in default-mode and cerebellum-striatum networks, respectively, among the middle-aged chronic smokers. Qiu et al. comparing the differences of whole-brain ALFF between young adult smokers and controls, it was found that the ALFF of smoking group in the right MPFC/ventral striatum and left temporal gyrus was significantly higher than that of healthy group (41). Similarly, compared to non-smokers, our study observed that significant spontaneous brain activity changes in the right medial OFC extending to the ventral striatum, amygdala and parahippocampal gyrus of young adult smokers (**Table 2**; **Figure 1**), which is consistent with our previous results.

TABLE 3 | Brain regions showing significant smoking-by-sex interaction on regional homogeneity (ReHo) (voxel-level $p < 0.05$ corrected for false discovery rate).

Identified brain regions	Peak coordinates (MNI)			Side	Peak F	Cluster size voxels)
	X	*Y*	*Z*			
Fusiform gyrus	−36	−78	−15	L	30.35	32
Cerebellum Crus 1/Inferior temporal gyrus	−45	−48	−33	L	25.89	68
Ventral stiatum/putamen	21	15	−12	R	24.88	56

MNI, Montreal Neurological Institute; R, right; L, left.

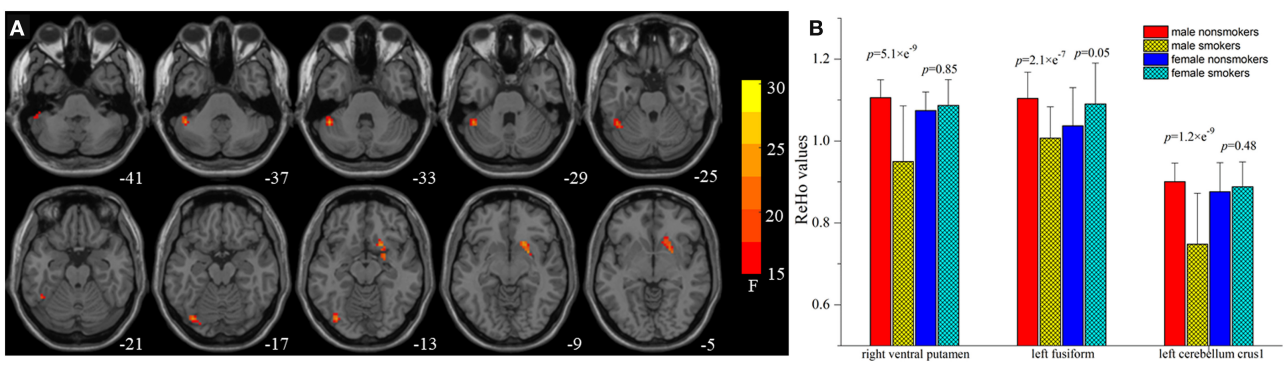

FIGURE 2 | Brain areas with interaction between smoking and sex on regional homogeneity (ReHo) by the 2-way analysis of covariance (ANCOVA) with age and education levels as covariates. **(A)** Brain regions showing smoking-sex interaction on ReHo include the left fusiform gyrus, left cerebellum crus1 extended to the inferior temporal gyrus, and right ventral striatum [voxel-level $p < 0.05$ (FDR corrected]. **(B)** *Post-hoc* 1-way ANCOVA analyses revealed that male smokers had lower ReHo than male non-smokers in the right ventral striatum ($p = 5.1 \times e^{-9}$), left fusiform gyrus ($p = 2.1 \times e^{-7}$), left cerebellum crus1 extended to the inferior temporal gyrus ($p = 1.2 \times e^{-9}$), whereas female smokers showed no but no differences on ReHo values in these regions.

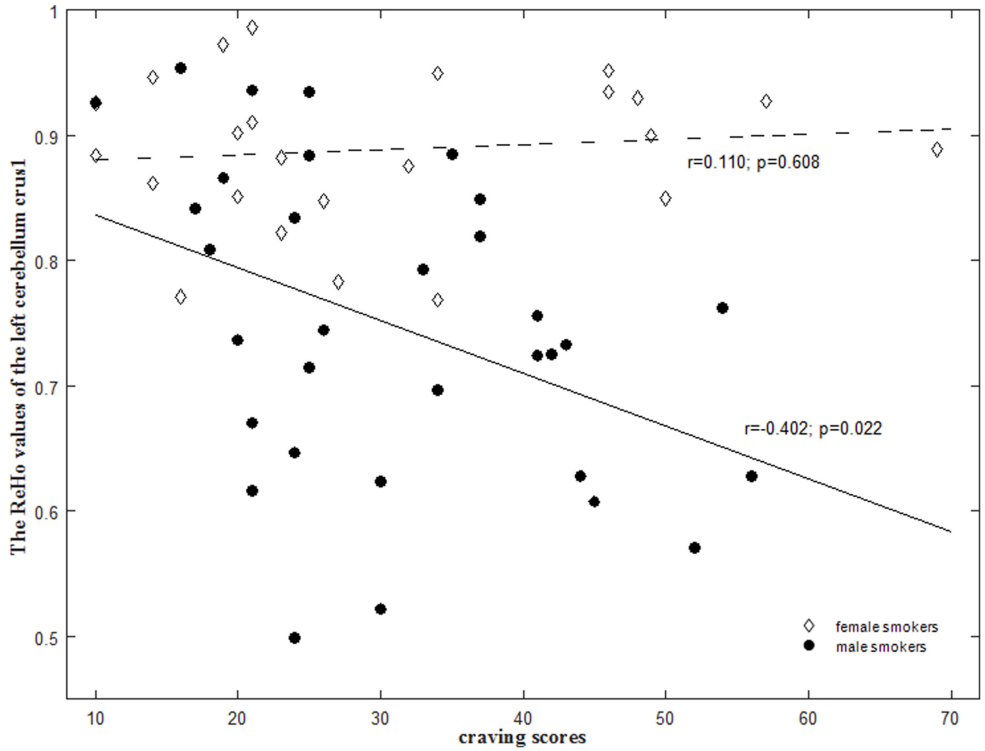

FIGURE 3 | Sex-specific correlations between the regional homogeneity (ReHo) values and smoking-related measures in smokers. There were significant negative correlation of the mean ReHo values in the left cerebellum crus1 with the craving scores in male smokers ($r = -0.402$; $p = 0.022$) but not in female smokers ($r = 0.110$; $p = 0.608$). These correlations between ReHo values and craving scores were significantly different between male and female smokers ($p = 0.03$).

It is well-accepted that addiction produce the long-lasting functional and structural plasticity alterations in the corticostriatal-limbic circuitry (42). The medial OFC, ventral striatum, and amygdala, are core components of the corticostriatal-limbic circuitry related to addiction. The medial OFC is involved in integrating endogenous and exogenous information to make decision, playing an important role in the development and maintenance of addictive behaviors (43). The parahippocampal gyrus and amygdala are brain areas associated with emotional and memory processing (44), which may mediate the relief from negative emotions by smoking (45). The mesocorticolimbic dopamine systems including the amygdala, nucleus accumbens, and prefrontal cortex, are thought to be involved in burst release of dopamine and production of pleasure feelings (46). The finding of dysfunction within the prefrontal-striatal circuits is analogous to previous task-related fMRI studies showing greater activity in the reward regions in response to SCs relative to non-SCs (47–49). Similarly, rs-fMRI studies showed disrupted activity in the medial frontal cortex and precuneus/posterior cingulate cortex (11, 50, 51). Moreover, structural MRI studies exhibited that smokers had lower gray matter volumes or densities in the medial frontal cortex, precuneus, parahippocampal gyrus and temporal lobe (52–55) and reduced cortical thickness in the medial OFC (56). Taken together, our study backed up the idea that the imbalance between cognitive and reward functions in the brain may lead to smoking addiction.

Several limitations in this study must be considered before interpreting the findings. First, the number of participants in each group is relatively small, which may bias the sex-specific effects of cigarette smoking on spontaneous brain activity. Second, age or education level was not well-matched among groups. Although these factors were used as covariates in the statistical analysis, potential effects from these factors cannot be absolutely ruled out. Future studies with matched larger samples should be needed to verify the results. Third, this study is staged, longitudinal study is needed to draw conclusions about whether sex differences are a cause or a consequence of nicotine addiction. Therefore, our findings should be considered as preliminary and further longitudinal studies with larger sample size should be investigated to formulate final conclusions.

In summary, the harm of cigarette smoking is recessive, and to a certain extent it is lagging. It is crucial to investigate the different neural mechanisms underlying male and female in smoking behaviors and nicotine dependence. Our findings indicate that cigarette smoking has differential effects on reward-related activity between men and women.

Our study may provide a methodological framework for education of quitting smoke, intervention treatment, and health management.

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 Ethics Committee of Renji Hospital, School of Medicine of Shanghai Jiaotong University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FL and HL designed and conceptualized the study. XH, YW, WD, YS, and YZ collected the clinical and MRI data. YK organized the data. ZW and FL analyzed the MRI data and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Effects of Lisdexamfetamine, a Prodrug of D-Amphetamine, on Locomotion, Spatial Cognitive Processing and Neurochemical Profiles in Rats: A Comparison With Immediate-Release Amphetamine

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D-amphetamine has been used to enhance cognitive performance over the last few decades. Due to the rapid absorption after administration, d-amphetamine shows narrow effective window and severe abuse potential. Lisdexamfetamine, a prodrug of d-amphetamine, reduces the magnitude of plasma d-amphetamine concentration and prolongs the action duration when compared with immediate-release d-amphetamine at equimolar doses. However, the differences of these two drugs, which produce distinct pharmacokinetic characteristics, in cognition improvement still unclear. In present study, we compared the effects of d-amphetamine (i.p) and lisdexamfetamine (p.o) at equimolar doses (0.2, 0.5, 1.5, 4.5, and 13.5 mg/kg of d-amphetamine base) on locomotion, spatial working memory and recognition memory in rats. Given the crucial involvement of dopamine neurotransmitter system within the medial prefrontal cortex (mPFC) in cognitive processing, microdialysis was conducted to profile the difference in neurochemical characteristics between the two drugs. In our results, d-amphetamine ranges from 0.5 to 1.5 mg/kg significantly increased locomotor activity. However, d-amphetamine ranges from 0.2 to 13.5 mg/kg failed to improve spatial working memory and recognition memory in Y-maze-based spontaneous alternation and two-trial delayed alternation tasks of rats, respectively. In contrast, lisdexamfetamine with 4.5 mg/kg significantly increased the locomotion and improved both spatial working and recognition memory. Further, microdialysis showed that lisdexamfetamine induced lower magnitude and longer duration of extracellular dopamine increase than that of d-amphetamine. These results suggest that lisdexamfetamine was more effective than d-amphetamine in improving spatial cognitive performance, which was attributed to the steady and lasting dopamine release pattern within the mPFC.

Keywords: lisdexamfetamine, d-amphetamine, spatial cognition, pharmacokinetic characteristics, dopamine

INTRODUCTION

Psychostimulants, such as d-amphetamine, modafinil and methylphenidate, have been used to treat attention-deficit/hyperactivity disorder (ADHD) (1, 2), narcolepsy (3) and bipolar disorder (4). In addition, another application of these drugs is to enhance cognition in healthy individuals who are engaging in certain vocations, referred to as cognitive enhancers (5). In the last decades, d-amphetamine has been reported to improve spatial working memory and language production in healthy volunteers (6), as well as increasing vigilance both in boys and adult men (7). However, severe addiction to amphetamine and its analogs (called amphetamine-type stimulants, such as methamphetamine) has become a worldwide public health problem, extensively limiting their applications (8).

Several studies have revealed that both cognitive enhancement and drug addiction are highly associated with pharmacokinetic properties (9, 10). D-amphetamine and methamphetamine enter brain rapidly after administration. They competitively inhibit dopamine transporter (DAT) clearing dopamine (DA), and also release DA via reversing DAT direction, ultimately causing excessive DA accumulation in synaptic cleft ultimately (11). DA acts as an inverted U-shaped pattern to cognitive performance. As is reported, too lower or higher DA level elicited by clinically-inappropriate doses of d-amphetamine impairs cognition (12). In addition, the dramatic increase in DA in the nucleus accumbens (NAc), a key brain region responding to reward (13, 14), is also related to severe abuse and addiction. Thus, it is challenging to change the pharmacokinetic properties of d-amphetamine to increase the effects of cognition improvement, while decrease its potential for abuse.

An alternative strategy is to modify its chemical structure, coupling the active drug with another compound, such as an amino acid, to create a novel prodrug (15). Lisdexamfetamine, the first prodrug approved for ADHD (16, 17) and binge eating disorder (18) treatment, is synthesized by covalently linking d-amphetamine to the amino acid l-lysine (19). Hutson et al. reported that the pharmacodynamic effects of lisdexamfetamine are independent of the route of administration (20), which is enzymatically hydrolyzed by an erythrocyte peptidase (the rate-limiting step) to yield d-amphetamine, the actual pharmacological active metabolite. In comparison with immediate-release d-amphetamine, lisdexamfetamine produced an identical AUC for plasma d -amphetamine, but a 50% lower C_{max} and significantly delayed t_{max} at equimolar doses (21). Such pharmacokinetic profile of lisdexamfetamine shows lower inter- and intra-individual variability in exposure compared with the pharmacokinetic profile of an equivalent dose of immediate-release d-amphetamine (22). Thus, it is reasonable to believe lisdexamfetamine may exhibit wider effective window than d-amphetamine in cognition improvement. Dolder et al. compared the effects of d-amphetamine and lisdexamfetamine on several cognitive tasks in healthy non-sleep-deprived subjects. They just vaguely concluded single, high, equimolar doses of d-amphetamine and lisdexamfetamine enhanced certain aspects of cognitive performance in healthy non-sleep-deprived subjects (14). The exact difference in pharmacological action and mechanisms between d-amphetamine and lisdexamfetamine should be further illumination.

In preclinical study, using appropriate cognitive paradigms in rodents are effective for pharmacological action and the underlying mechanisms exploration, as well as side effects anticipation (23). In fact, several researches have employed various translational rodents paradigms to reflect attention (24), visual discrimination (25) and inhibitory control (26).

Among numerous cognitive domains, working memory serves as the basis of other higher order cognitive processes including but not limited to recognition memory (27–29), which is mediated by mPFC function. In order to compare the effects of d-amphetamine and lisdexamfetamine on mPFC-associating cognition, we focused on the two types of memory: spatial working memory and spatial recognition memory using Y-maze-based spontaneous alternation task (30, 31) and two-trial delayed alteration (32) task respectively. Given that DA plays a vital role in mediating cognitive performance, microdialysis was performed to assess DA, as well as the corresponding metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), within the medial prefrontal cortex (mPFC) in freely moving rats to explore the neurochemical profiles of distinct pharmacokinetics induced by d-amphetamine and lisdexamfetamine.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley (SD) rats weighting 220-240 g were purchased from SPF (Beijing) Biotechnology Co., Ltd. (SCXK (Jing) 2019-0010). All rats were housed under a regular light-dark cycle (lights on from 7:00 am to 7:00 pm) at a constant temperature of 22 ± 2°C and relative humidity of 40-60%. The rats were given free access to food and water. The animal protocol was strictly in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

Drugs and Reagents

D-amphetamine hydrochloride and lisdexamfetamine dimesylate were provided by Beijing Institute of Pharmacology and Toxicology. All drugs were dissolved in sterilized 0.9% saline. D-amphetamine were injected at a volume of 1 ml/kg (intraperitoneal [i.p.]), and lisdexamfetamine was infused at a volume of 2 ml/kg (*per os* [p.o.]).

Animal Groups and Drug Treatments

SD rats were divided into control, d-amphetamine and lisdexamfetamine treatment groups. Drugs doses were calculated based on free amfetamine base (0.2, 0.5, 1.5, 4.5, 13.5 mg/kg) and transformed to μmol/kg as followed: d-amphetamine: 1.17, 2.91, 8.74, 26.21, 78.64 μmol/kg; lisdexamfetamine: 1.48, 3.70, 11.10, 33.29, 99.86 μmol/kg. For locomotor activity measurement, rats were tested immediately after drug treatment. For the Y-maze spontaneous alternation task, d-amphetamine (i.p.) and lisdexamfetamine (p.o.) were treated 30 and 60 min before the test, respectively. For the two-trial Y-maze delayed alternation

task, d-amphetamine (i.p.) and lisdexamfetamine (p.o.) were treated 30 min and 60 min before the first phase (memory acquisition), respectively.

Locomotor Activity Measurement in Rats

The locomotor activity box was 46 cm × 46 cm × 46 cm and made of black plastic. A camera was fixed to the top of the box. After drug treatment, rats were placed in the box immediately, and locomotion was recorded for 180 min in 15 min interval.

Y-Maze-Based Spontaneous Alternation in Rats

The apparatus consisted of a Y-shaped maze with three arms (30 cm × 8 cm × 15 cm of each arm, 120° between arms) defined as A, B, and C. Three distinct cues were placed outside the arms to help rats distinguish spatial location. Above the center of the apparatus, a yellow light lamp (1 W) was used to induce a dim environment. All experiments were performed from 8:00 am to 12:00 pm. The procedure was performed as described by Kraeuter et al. (33). Briefly, rats were randomly placed in one of the three arms and allowed to explore freely for 5 min. The maximum alternation is defined as the number of consecutive entries into three different arms (e.g., ABCABC was regarded as four times alteration). Spatial working memory was assessed by the percentage of alterations within 5 min, which can be calculated according to the following formula: number of maximum alternations/(total number of arm entries- 2) × 100. Each rat was placed in a different starting arm.

Two-Trial Y-Maze-Based Delayed Alternation in Rats

The operation was performed according to the method described by Fu et al. (33, 34). This task consisted of two phases: memory acquisition and memory retrieval. In the first stage, we randomly closed one of the three arms and allowed the rats to freely explore the other two arms for 10 min. After an inter-trial-interval (ITI) of 60 min, the closed arm was opened and the same rat was placed from the same arm as in the first stage and allowed to explore for 5 min. The percentage of novel arm visits (number of novel arm visits/total arm visits × 100) and percentage of novel arm retention (total time in novel arm retention/total time in three arms × 100) within 5 min were calculated to reflect the spatial recognition memory of rats.

Surgery

Briefly, rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). The head was placed in a stereotaxic apparatus. The upper incisor bar was set at 3.3 mm below the interaural line so that the skull surface between the bregma and lambda was horizontal according to Paxinos and Watson (35). A microdialysis guide cannula (CMA, United Kingdom) was implanted at the following coordinates: AP, 3.0 mm; ML, 0.6 mm relative to bregma; and V, 2.5 mm relative to skull surface. In addition, two additional burr holes were made for skull screws (stainless steel) and secured using dental cement. After surgery, rats were injected with benzylpenicillin sodium (300,000 IU/kg, i.p.) to prevent infection.

Microdialysis and High-Performance Liquid Chromatography-Electrochemical Detection (HPLC-ECD) Analysis

Following 5-7 days of recovery after surgery, the microdialysis experiment was conducted. A microdialysis probe (EICOM A-I: 0.22-mm OD, 4-mm membrane length with 50 kDa cutoff) was inserted into the guide cannula of awake rats. The rats were then perfused with artificial cerebrospinal fluid (aCSF; containing 148 mM NaCl, 4 mM KCl, 0.8 mM $MgCl_2$, 1.4 mM $CaCl_2$, 1.2 mM Na_2HPO_4, 0.3 mM NaH_2PO_4, pH 7.2) at a low speed (0.5 μl/min) overnight. On the next day, the perfusion speed was increased to 1.0 μl/min for 2 h. The dialysate samples were collected at 30-min intervals from 90 min before drug administration to 3 h after drug administration. The collection vials contained 7.5 μl of 0.5 M perchloric acid to prevent oxidation of catecholamines.

Reverse-phase, ion-pair HPLC (Waters 2695, MA, United States) coupled with ECD (Antect Leyden, Zoeterwoude, and Holland) was used to analyze DA and the corresponding metabolites DOPAC and HVA. The collected samples were immediately analyzed. Samples (30 μl) were separated using a 250 × 4.5-mm IDT3 analytical column (Waters) at 1.0 ml/min. The mobile phase consisted of 100 mM phosphate buffer, 0.74 mM sodium 1-octanesulfonate, 0.027 mM EDTA·Na_2 at pH 3.0, 8% (v/v) methanol, and 8% (v/v) acetonitrile; an Antec Intro ECD was used with a high-density, glassy carbon electrode (+ 0.72 V) combined with an Ag/AgCl reference electrode.

Nissl Staining

After dialysis, brain tissues were stained with cresyl violet to detect the surgery position. Briefly, rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and perfused with 0.9% saline and 4% paraformaldehyde to fix the brain tissue. After that, the brain was immersed in 4% paraformaldehyde for 24 h, and dehydrated with 30% sucrose for 24 h. Brain tissue was embedded in O.C.T compound (SAKURA) and sectioned into 30 μm-thick sections using a freezing microtome (Leica, Germany). The Nissl staining protocol was performed according to the manufacturer's instructions. Images were captured using a NanoZoomer Digital Pathology microscope (Hamamatsu Photonics, Japan). The injection site of the guide cannula, which was not in the mPFC, was deleted.

Statistical Analysis

Levene's homogeneous variance test and Shapiro-Wilk normal test were used to analysis the homogeneous variance and normal distribution of (1) total distance traveled over 180 min, (2) percentage of alterations within 5 min in the Y-maze-based spontaneous alternation, and (3) percentage of novel arm visits and retention in the two-trial Y-maze-based delayed alternation. Data was presented as mean ± SEM when conform to homogeneous variance and normal distribution, and one-way analysis of variance (ANOVA) followed by Dunnett's t-test was used for analysis. Otherwise, data were presented as

median ± interquartile and Kruskal-Wallis test was employed for analysis. For the 15 min interval distances within 180 min in locomotor activity experiment and the percentage of each arm visits and retention in two-trial Y-maze-based delayed alternation experiment (treatment as between-group factor, arm differences as within-group factor), data were presented as mean ± SEM and two-way ANOVA with one repeated measurement followed by Bonferroni test was performed. All statistical analyses were performed using SPSS software (version 26.0). Statistical significance was set at $P < 0.05$.

RESULTS

Effects of D-Amphetamine and Lisdexamfetamine on Locomotor Activity in Rats

Generally, d-amphetamine increased locomotor activity of rats at dosage of 0.2-1.5 mg/kg, whereas decreased locomotor activity at dosage of 4.5-13.5 mg/kg. In comparison with the control group, 0.5 and 1.5 mg/kg d-amphetamine significantly enhanced locomotor activity over a total of 180 min (Kruskal-Wallis test: $P = 0.018$ for 0.5 mg/kg and $P < 0.001$ for 1.5 mg/kg *vs.* control, **Figure 1A**). The results for the 15-min interval distances showed that 1.5 mg/kg produced the lasting increased locomotion from 60 to 180 min after administration (two-way ANOVA with one repeated measurement: main effect of time: $F_{(11,38)} = 25.818$, $P < 0.001$; main effect of treatment: $F_{(5,48)} = 10.391$, $P < 0.001$; time × treatment interaction: $F_{(55,210)} = 1.519$, $P = 0.019$; Bonferroni test for *post hoc* test: $P < 0.05$ from 60 min to 180 min *vs.* control, **Figure 1B**).

Lisdexamfetamine exhibited an inverted-U-shaped dose-response relationship with locomotion. Specifically, 4.5 mg/kg lisdexamfetamine significantly increased locomotion within 180 min (Kruskal-Wallis test: $P = 0.016$ *vs.* control, **Figure 1C**). The results for the 15-min interval distances showed that 4.5 mg/kg lisdexamfetamine significantly increased locomotor activity from 75 min to 180 min, while a dose of 13.5 mg/kg significantly increased activity at 60 min after administration (two-way ANOVA with one repeated measurement: main effect of time: $F_{(11,38)} = 21.488$, $P < 0.001$ main effect of treatment:

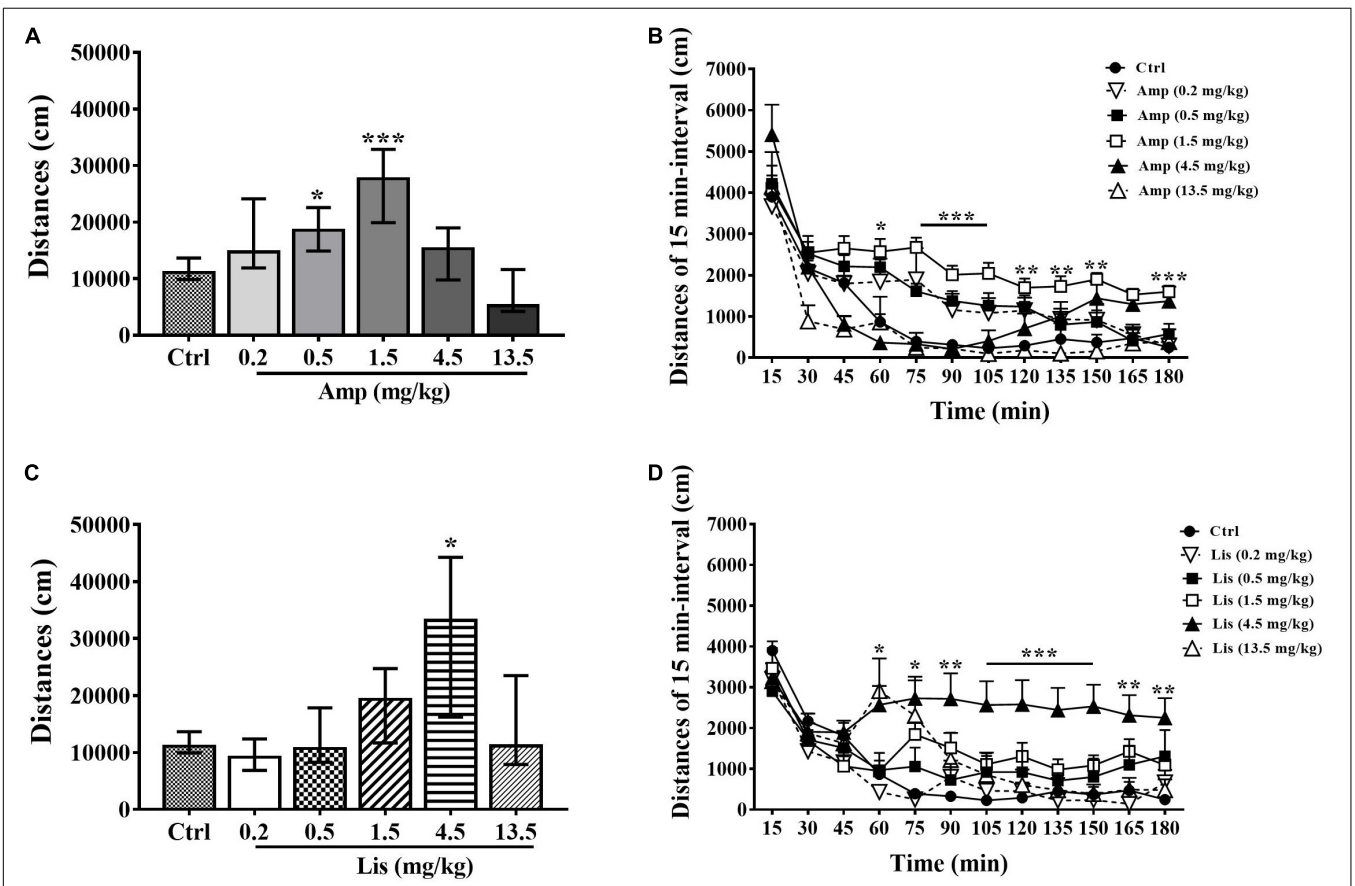

FIGURE 1 | The effects of d-amphetamine and lisdexamfetamine on locomotor activity. **(A)** Total distances induced by d-amphetamine within 180 min (median ± interquartile, Kruskal-Wallis test). **(B)** Distances induced by d-amphetamine with 15-min interval (mean ± SEM, Repeated measure ANOVA followed by Bonferroni test). **(C)** Total distances induced by lisdexamfetamine within 180 min (median ± interquartile, Kruskal-Wallis test). **(D)** Distances induced by lisdexamfetamine with 15-min interval (mean ± SEM, Repeated measure ANOVA followed by Bonferroni test). Ctrl: control; Amp: d-amphetamine; Lis: lisdexamfetamine. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs*. Ctrl, $n = 9$ in each group.

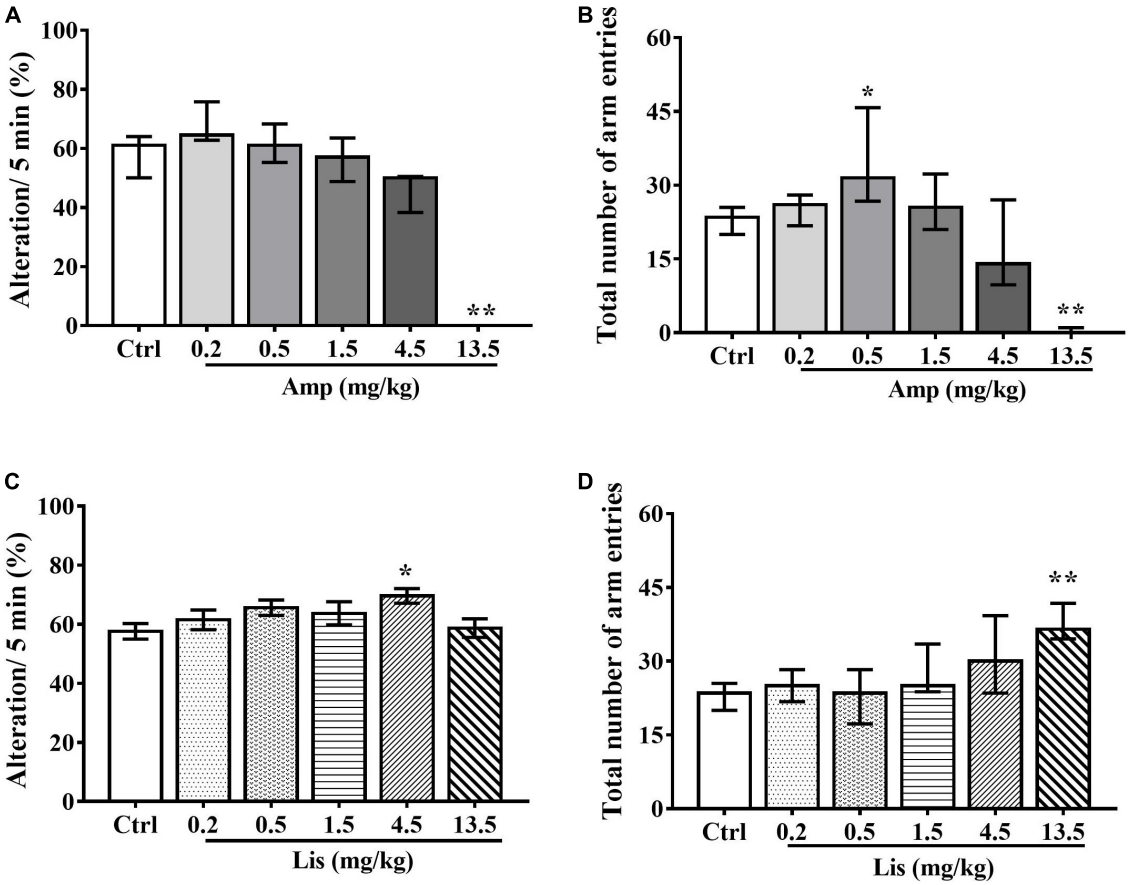

FIGURE 2 | The effects of d-amphetamine and lisdexamfetamine on Y-maze-based spontaneous alternation. **(A)** The effect of d-amphetamine on percentage of Alteration/5min (median ± interquartile, Kruskal-Wallis test). **(B)** The effect of d-amphetamine on transform times within 5 min (median ± interquartile, Kruskal-Wallis test). **(C)** The effect of lisdexamfetamine on percentage of Alteration/5 min (mean ± SEM, One-way ANOVA, followed by Dunnett-*t* test). **(D)** The effect of lisdexamfetamine on transform times within 5 min (median ± interquartile, Kruskal-Wallis test). Ctrl: control; Amp: d-amphetamine; Lis: lisdexamfetamine. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. Ctrl, $n = 10$ in each group.

$F_{(5,48)} = 4.899$, $P = 0.001$; time × treatment interaction: $F_{(55,210)} = 1.594$, $P = 0.01$; Bonferroni test for *post hoc* test: 4.5 mg/kg: $P < 0.05$ from 75 to 180 min, 13.5 mg/kg: $P = 0.027$ at 60 min *vs.* control, **Figure 1D**].

Effects of D-Amphetamine and Lisdexamfetamine on Y-Maze-Based Spontaneous Alternation in Rats

In rats, as d-amphetamine dose increased ranging from 0.2-13.5 mg/kg, the percentage of spontaneous alterations within 5 min was gradually decreased. Compared with the control group, 13.5 mg/kg d-amphetamine significantly reduced spontaneous alternations (Kruskal-Wallis test: $P = 0.004$ *vs.* control, **Figure 2A**). In total number of arm entries, d-amphetamine at dosage of 0.5 mg/kg significantly increased, whereas 13.5 mg/kg d-amphetamine significantly reduced arm visiting times (Kruskal-Wallis test: $P = 0.048$ for 0.5 mg/kg and $P = 0.001$ for 13.5 mg/kg *vs.* control, **Figure 2B**).

Compared with control group, lisdexamfetamine at dosage of 4.5 mg/kg significantly increased the percentage of spontaneous alterations within 5 min in rats (One-way ANOVA followed by Dunnett's *t*-test: $P = 0.032$ *vs.* control, **Figure 2C**). Total number of arm entries were significantly increased by 13.5 mg/kg lisdexamfetamine (Kruskal-Wallis test: $P < 0.001$ *vs.* control, **Figure 2D**).

Effects of D-Amphetamine and Lisdexamfetamine on Y-Maze-Based Delayed Alternation in Rats

Generally, d-amphetamine ranges from 0.2 to 13.5 mg/kg increased both the percentage of novel arm visits and retention at lower doses and then decreased at higher doses in rats. Specifically, 13.5 mg/kg led to a significant reduction in the percentage of retention in the novel arm compared with the control group (Kruskal-Wallis test: $P = 0.001$ in 13.5 mg/kg *vs.* control, **Figures 3A,C**). Two-way ANOVA with one repeated measurement revealed that 4.5 mg/kg d-amphetamine produced more novel arm visits than start arm visits, while 13.5 mg/kg caused fewer novel arm visits than start and other arm visits (**arm visits:** main effect of treatment: $F_{(5,54)} = 8.995$, $P < 0.001$;

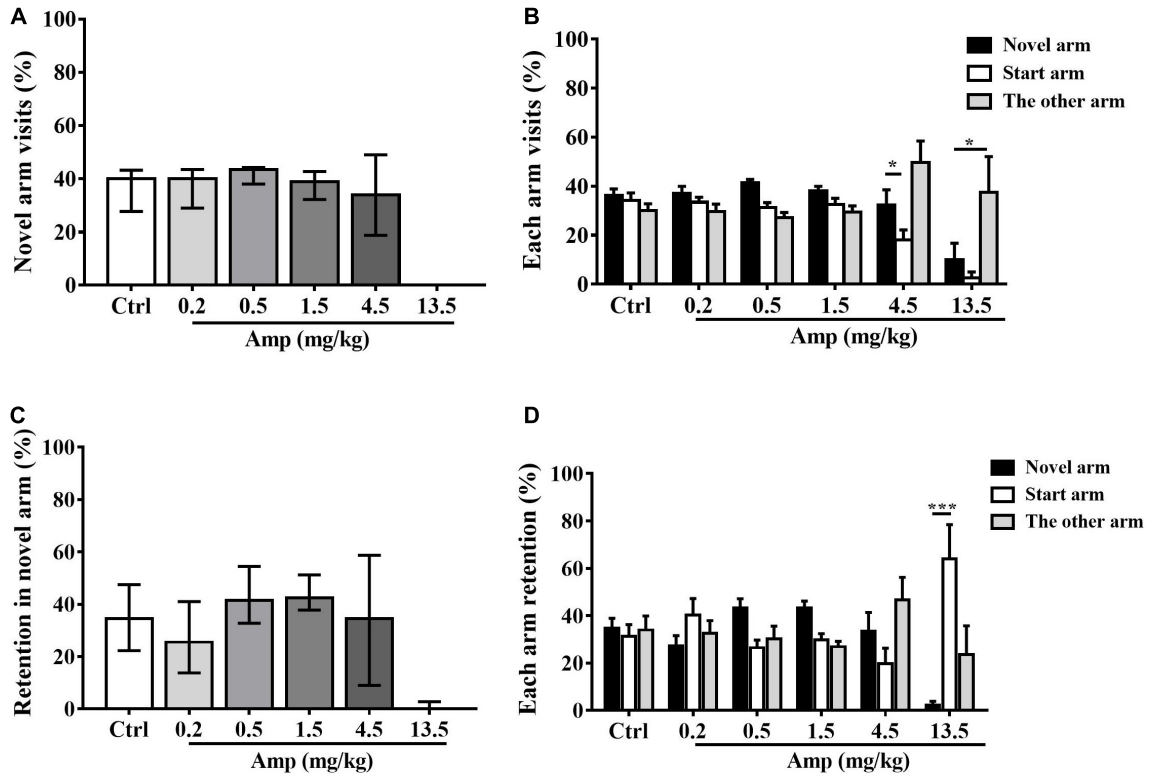

FIGURE 3 | The effects of d-amphetamine on Tow-trial Y-maze based delayed alternation. **(A)** The effect of d-amphetamine on percentage of novel arm visit times (median ± interquartile, Kruskal-Wallis test). **(B)** The percentage of each arm visits (mean ± SEM, Repeated measure ANOVA followed by Bonferroni test). **(C)** The effect of d-amphetamine on percentage of novel arm retention (median ± interquartile, Kruskal-Wallis test). **(D)** The percentage of each arm retention (mean ± SEM, Repeated measure ANOVA followed by Bonferroni test). Ctrl: control; Amp: d-amphetamine. **(A,C)** $^{*}P < 0.05$, $^{***}P < 0.001$ vs. Ctrl; **(B,D)** $^{*}P < 0.05$, $^{***}P < 0.001$ vs. Novel arm, $n = 10$ in each group.

main effect of arm: $F_{(2,53)} = 9.489$, $P < 0.001$, treatment × arm interaction: $F_{(10,108)} = 2.543$, $P = 0.011$; Bonferroni test for *post hoc* test: 4.5 mg/kg: $P = 0.046$ in novel arm *vs.* start arm, 13.5 mg/kg: $P = 0.017$ in novel arm *vs.* the other arm. **arm retention:** main effect of treatment: $F_{(5,54)} = 0.991$, $P = 0.432$; main effect of arm: $F_{(2,53)} = 0.714$, $P = 0.494$; treatment × arm interaction: $F_{(10,108)} = 4.347$, $P < 0.001$, **Figures 3B,D**).

In comparison with the control group, 4.5 mg/kg lisdexamfetamine significantly increased both the percentage of novel arm visits (one-way ANOVA followed by Dunnett's t-test: $P = 0.047$ *vs.* control, **Figure 4A**) and the percentage of retention in the novel arm (one-way ANOVA followed by Dunnett's t-test: $P = 0.043$ *vs.* control, **Figure 4C**). Two-way ANOVA with one repeated measurement revealed that 0.5 and 4.5 mg/kg lisdexamfetamine significantly increased the number of visits and retention in the novel arm relative to those in the start and other arm (**arm visits:** treatment main effect: $F_{(5,54)} = 1.413$, $P = 0.234$, arm main effect: $F_{(2,53)} = 13.995$, $P < 0.001$, treatment × arm interaction: $F_{(10,108)} = 1.489$, $P = 0.153$, Bonferroni test for *post hoc* test: 0.5 mg/kg: $P = 0.032$ in novel arm *vs.* the other arm, 4.5 mg/kg: $P < 0.001$ in novel arm *vs.* start arm and other arm; **arm retention:** treatment main effect: $F_{(6,63)} = 0.849$, $P = 0.537$; arm main effect: $F_{(2,53)} = 16.257$, $P < 0.001$; treatment × arm: $F_{(10,108)} = 1.421$, $P = 0.181$; Bonferroni test for *post hoc* test: 0.5 mg/kg: $P = 0.013$ in novel arm *vs.* the other arm, 4.5 mg/kg: $P < 0.001$ in novel arm *vs.* start arm and the other arm, **Figures 4B,D**).

Effects of D-Amphetamine and Lisdexamfetamine on mPFC DA, DOPAC, and HVA Levels in Rats

Figure 5A showed the implantation location of the microdialysis guide cannula. For DA, d-amphetamine dramatically increased DA efflux (% of baseline: 222.49 ± 84.42) at 30 min after administration, whereas lisdexamfetamine induced extracellular DA elevation and peaked (% of baseline: 177.35 ± 37.94) at 60 min after administration. As time passed, the increased DA levels induced by d-amphetamine gradually returned to baseline from 90 min after administration (% of baseline: 90 min: 108.25 ± 16.28, 120 min: 112.36 ± 17.36, 150 min: 100.31 ± 21.41, 180 min: 104.02 ± 24.13). However, lisdexamfetamine had a lower magnitude effect and longer duration of DA efflux from 90 to 180 min (% of baseline: 90 min: 148.07 ± 7.85, 120 min: 136.88 ± 4.98, 150 min: 149.73 ± 8.49, 180 min: 151.31 ± 13.27, **Figure 5B**). For DOPAC, there were obvious differences between d-amphetamine and lisdexamfetamine. D-amphetamine led to a gradual decrease in

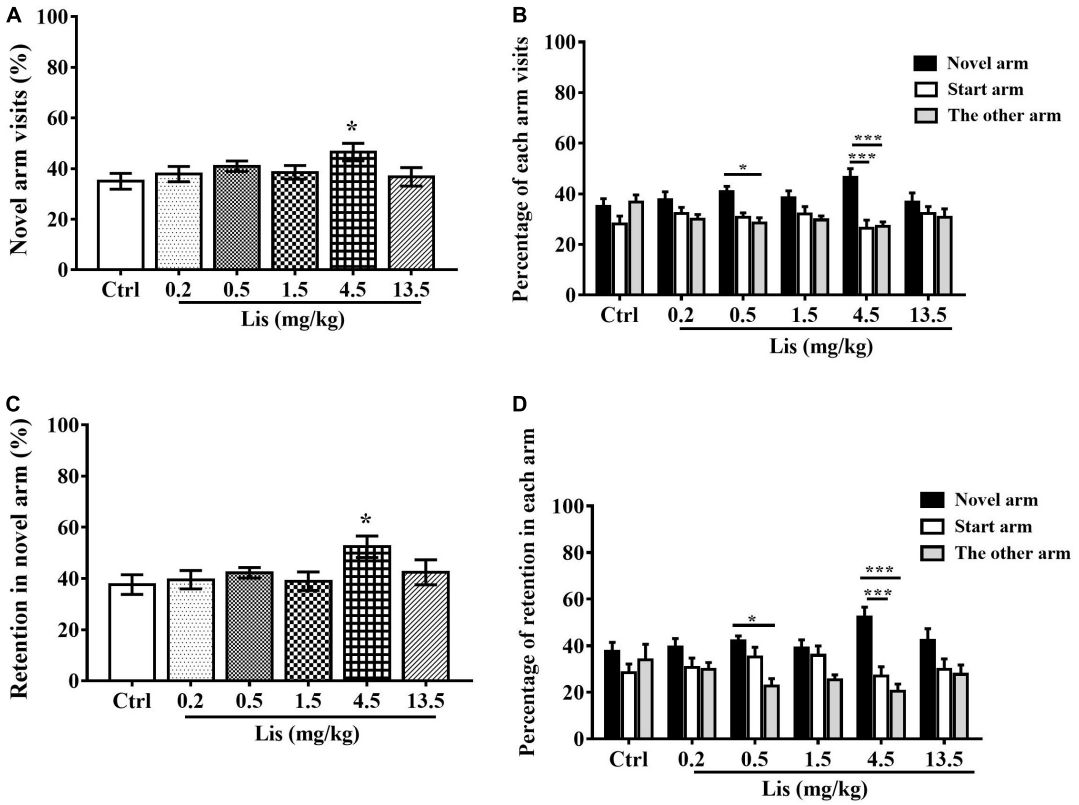

FIGURE 4 | The effects of lisdexamfetamine on Tow-trial Y-maze based delayed alternation. **(A)** The effect of lisdexamfetamine on percentage of novel arm visit times (mean ± SEM, One-way ANOVA, followed by Dunnett-*t* test). **(B)** The percentage of each arm visits (mean ± SEM, Repeated measure ANOVA followed by Bonferroni test). **(C)** The effect of lisdexamfetamine on percentage of novel arm retention (mean ± SEM, One-way ANOVA, followed by Dunnett-*t* test). **(D)** The percentage of each arm retention (mean ± SEM, Repeated measure ANOVA followed by Bonferroni test). Ctrl: control; Caf: caffeine; Lis: lisdexamfetamine. **(A,C)** $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs*. Ctrl; **(B,D)** $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs*. novel arm, $n = 10$ in each group.

DOPAC concentration from 30 to 120 min, reaching its lowest (% baseline: 87.52 ± 12.04) at 120 min after administration, and then returning to approximately the baseline level. However, lisdexamfetamine led to a constant decrease in DOPAC concentration, reaching the lowest levels at 180 min (% baseline: 68.95 ± 16.61, **Figure 5C**). For HVA, there was no difference between d-amphetamine and lisdexamfetamine, which both led to a gradual decrease in HVA concentration (**Figure 5D**).

DISCUSSION

D-amphetamine, due to the immediate-released pharmacokinetic characteristics, shows narrow effective window in cognition improvement. Lisdexamfetamine, based on the sustained-released pharmacokinetic profile with lower magnitude of plasma d-amphetamine concentration, is reasonably believed to produce wider efficient and less individual variability to enhance cognitive performance. The present study found that lisdexamfetamine was more effective than immediate-released d-amphetamine in improving spatial cognitive performance in rats, which was attributed to its inducing the steady and lasting dopamine release pattern within the mPFC.

Locomotor activity measurement is a method used to evaluate the behavioral stimulant properties of drugs (36). To further study the pharmacological actions on cognitive performance, it is necessary to firstly explore the neuronal excitatory effects of lisdexamfetamine and d-amphetamine. In our results, 0.5 and 1.5 mg/kg d-amphetamine significantly increased locomotor activity after i.p. administration, revealing neuronal stimulation caused by this dose. Further increased dose to 13.5 mg/kg, d-amphetamine significantly decreased locomotion 30 min after administration, which is similar to findings in other studies. Namara et al. found 0.2 mg/kg d-amphetamine (i.p.) failed to increase locomotor activity, while doses of 5 and 10 mg/kg d-amphetamine caused prominent stereotypy (37). In addition, Antoniou et al. concluded that amphetamine had a complex effect on locomotion, characterized mainly by motor activation at lower doses and stereotypy at high doses (36). Unlike d-amphetamine, lisdexamfetamine from 4.5 to 13.5 mg/kg increased locomotor activity from 60 min to 90 min after administration (p.o.). These results suggest that lisdexamfetamine produced substantially less locomotor activation than d-amphetamine due to its sustained and lower magnitude pharmacokinetic profile. Rowley et al. (21) also reported that lisdexamfetamine at 1.5 mg/kg produced less locomotor activation than that of equivalent dose of

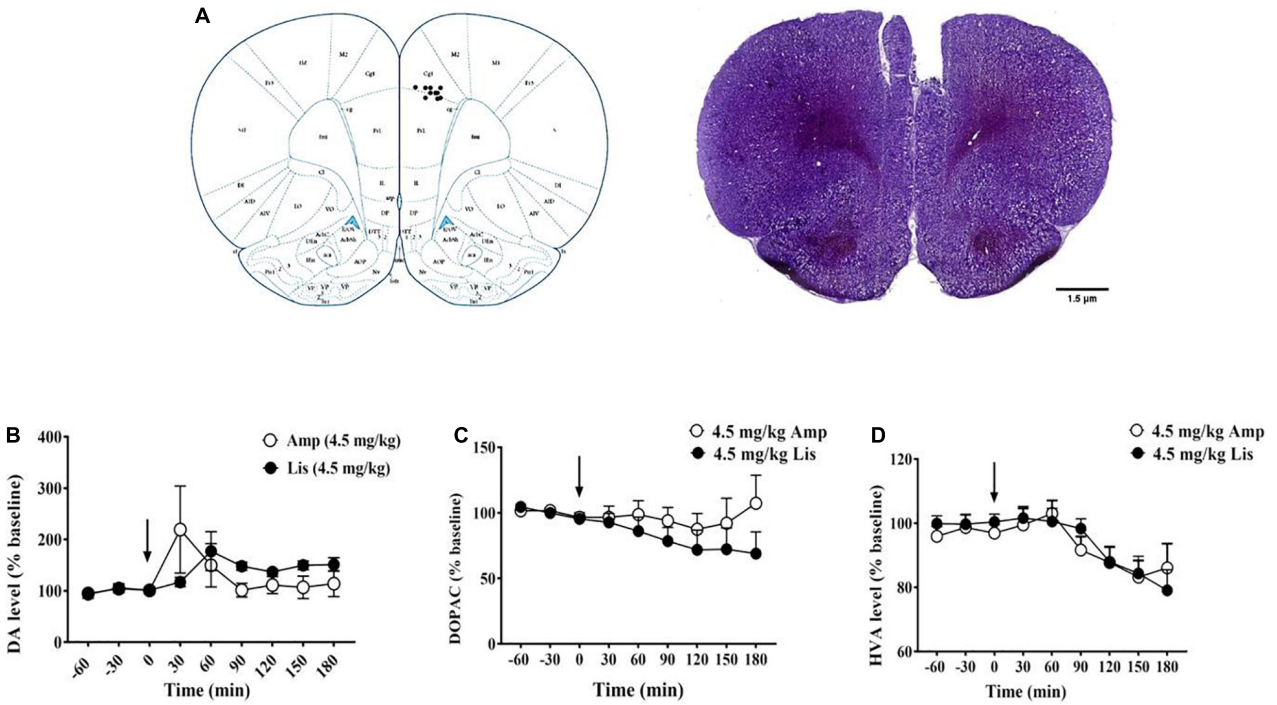

FIGURE 5 | The effects of d-amphetamine and lisdexamfetamine on catecholamine neurotransmitters and the metabolites in mPFC. **(A)** The implantation location of the microdialysis guide cannula. **(B)** The effects of d-amphetamine and lisdexamfetamine on DA efflux. **(C)** The effects of d-amphetamine and lisdexamfetamine on DOPAC efflux. **(D)** The effects of d-amphetamine and lisdexamfetamine on HVA efflux. Amp: d-amphetamine; Lis: lisdexamfetamine. Data are presented by mean ± SEM, $n = 5$ in each group.

d-amphetamine, which is similar to our results. Due to the unique pharmacokinetics, lisdexamfetamine shows a lower reward property and larger therapeutic window than d-amphetamine (21, 38).

Increasing neuronal excitability appropriately with a certain dose of stimulant drugs is beneficial for enhancing cognitive activity. Based on the locomotor activity measure, we compared the stimulant properties of lisdexamfetamine and d-amphetamine, and found that lisdexamfetamine was less stimulating than d-amphetamine. However, the effects of both drugs on cognitive performance remain unknown. As doses increased to levels that stimulated locomotion (0.5-1.5 mg/kg), d-amphetamine failed to improve working memory; further increases to 13.5 mg/kg significantly reduced spatial working memory, which may associate with stereotype behavior. In fact, psychostimulants (i.e., d-amphetamine) action on the DA system exerts bidirectional effects, improving or decreasing working memory performance depending on the dosage (inverted U-shaped curve) (39, 40). It is widely accepted that clinically relevant doses improve and supra-clinical doses impair working memory (41). This standpoint has also been confirmed by other studies. Brut et al. found that acute treatment with low doses of d-amphetamine (1.0 mg/kg) eliminated the alternation tendency, while higher doses (5.0- 7.0 mg/kg) also produced marked stimulus perseveration in a radial maze (42). In addition, several lines of evidence have shown that d-amphetamine causes significantly perseverative patterns (i.e., repetition of location rather than direction) in exploration in the Y-maze (43, 44). Except for dosage, baseline working memory capacity is another key factor affecting cognitive performance. D-amphetamine selectively improved working memory in poor and intermediate performers at low doses, whereas it was impaired good performers at a higher dose (14, 45). In our animal results and other studies of healthy non-sleep-deprived individuals, d-amphetamine failed to improve spatial working memory (46). D-amphetamine exhibits a narrow effective window for cognitive improvement. Unlike d-amphetamine, lisdexamfetamine (4.5 mg/kg) significantly improved working memory. As the dose increased to 13.5 mg/kg, lisdexamfetamine did not significantly impair performance. To our knowledge, only one study has reported that chronic lisdexamfetamine treatment effectively enhanced spatial working memory in the Morris water maze (15), which is consistence to our results. In a word, our results suggested the reduced rate of appearance and magnitude of d-amphetamine in plasma by lisdexamfetamine may be more beneficial in working memory improvement.

The two-trial Y-maze delayed alternation task is a specific and sensitive test of spatial recognition memory in rodents (47, 48). This paradigm requires rats to explore and remember two arms first (memory acquisition). After a 60-min ITI, rats spend more time in the novel arm because of their natural exploration tendency (memory expression). Chronic administration and withdrawal of amphetamine and morphine have been shown to cause severe spatial recognition memory and

executive impairment in rodents and humans (49–51). However, whether acute administration of amphetamine influences this cognitive domain and whether there is a difference between amphetamine and lisdexamfetamine remain unknown. In our study, d-amphetamine was administered before the first training phase, affecting memory acquisition. Our results showed that acute treatment with 4.5 and 13.5 mg/kg d-amphetamine significantly damaged recognition memory acquisition and showed similar effects on spatial working memory. Exploration between two given arms is crucial to guide free exploration after a 60-min ITI. Thus, larger doses of d-amphetamine may cause perseverative behavior in the first exploration, causing rats to fail to remember and distinguish which arm is the novel one. Compared to d-amphetamine, lisdexamfetamine (4.5 mg/kg significantly improved spatial recognition memory acquisition, upon further increasing the dose to 13.5 mg/kg, lisdexamfetamine did not produce a significant impairment relative to that of d-amphetamine. The results here were consistent to that of Y-maze-based spontaneous alternation.

DA within the mPFC plays a crucial role in mediating several cognitive domains, such as working memory (52) and attention (53). We demonstrated that lisdexamfetamine (4.5 mg/kg d-amphetamine base) significantly enhanced spatial working memory and spatial recognition memory in comparison to equivalent doses of d-amphetamine. Thus, we speculated that the neurochemical profiles of lisdexamfetamine and d-amphetamine are different, so that the release pattern elicited by lisdexamfetamine may be more beneficial to improving cognitive performance. Here, we found that lisdexamfetamine evoked a smaller magnitude but sustained increase in DA levels within the mPFC compared to that with d-amphetamine (222% of baseline for d-amphetamine at 30 min and 177% for lisdexamfetamine at 60 min after administration), which is similar to the case in the striatum and the pharmacokinetic characteristics reported by Rowley et al. (21). Except for DA, we also observed that both stimulants decreased DOPAC and HVA levels. D-amphetamine produced a transient decrease in extracellular DOPAC levels, which is consistent with several other results (54, 55). DOPAC is a major metabolite of DA, catalyzed by monoamine oxidase (MAO), and further forms HVA under the action of catechol-o-methyl-transferase (COMT). D-amphetamine has been proven to decrease MAO activity in striatal tissue (56), contributing to a reduction in DOPAC and final metabolite HVA reduction.

Taken together, as a prodrug of d-amphetamine, lisdexamfetamine displays pharmacokinetical sustained and lower magnitude plasma amphetamine base concentration, as well as its eliciting DA level in mPFC. This unique characteristic may be more benefit to improve cognition than immediate-release d-amphetamine.

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 National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

AUTHOR CONTRIBUTIONS

CJ-M and WS-X performed the behavioral experiments. CJ-M and WZ-Y analyzed the data. SR conducted *in vivo* microdialysis experiment. CJ-M wrote the manuscript. WN and LJ designed the experiments and modified the manuscript. All authors contributed to the article and approved the submitted version.

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Effect of High-Frequency Repetitive Transcranial Magnetic Stimulation on Visual Selective Attention in Male Patients With Alcohol Use Disorder After the Acute Withdrawal

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Objective: To investigate the effect of high-frequency repetitive transcranial magnetic stimulation (rTMS) on attention cue reactivity in male patients with alcohol use disorder (AUD) after acute withdrawal.

Methods: A total of 90 male patients with AUD who were hospitalized were enrolled and divided into study and waiting groups by a random number table. During the study, 18 patients dropped out. After the alcohol withdrawal symptoms were eliminated, the study group received high-frequency rTMS at 10 Hz for 14 consecutive days, and the waiting group was administrated by sham rTMS. All subjects were evaluated for attention cue reactivity, impulsiveness, cognitive function by oddball paradigm, Barratt Impulsiveness Scale version II (BIS-II), and the Montreal Cognitive Assessment (MoCA) at baseline and after true or sham rTMS.

Results: 1. There was no significant difference between the study and the waiting groups regarding the drinking level, cognition level, and demographic data at baseline. 2. In the oddball paradigm, both for alcohol-related and non-alcohol-related cues, the response times were significantly shorter in the study group after rTMS treatment than in the waiting-for-treatment group, either between the two groups or within the study group. There was no significant difference in the accuracy rate for alcohol-related and non-alcohol-related cues between the two groups or within the study group after rTMS intervention. 3. The total score of MoCA was significantly increased, and the total score of BIS-II was significantly decreased in the study group after rTMS treatment, either between the two groups or within the study group.

Conclusion: The results suggested that high-frequency rTMS could improve the attention bias of alcohol-related cues and impulsivity for patients with AUD.

Keywords: alcohol use disorder, cognitive dysfunction, transcranial magnetic stimulation, oddball paradigm, attention bias

INTRODUCTION

Alcohol use disorder (AUD) is clinically defined as a loss of control over alcohol intake and risky alcohol intake, maintaining cues despite negative consequences, social impairment, and pharmacological dependence. It is the second most common substance use disorder in the general population after tobacco use disorder (1), with a 12-month and lifetime prevalence in the total population of 13.9 and 29.1%, respectively (2). Craving, defined as a strong and uncontrollable desire to use a substance (3), is one of the fundamental aspects of substance dependency and has been demonstrated to be one of the most critical variables contributing to AUD relapse (4).

In the cognitive theories of alcohol attention bias (AAB) (5), it is hypothesized that when the dopamine system is repeatedly exposed to the rewarding effects of alcohol, it develops to correlate "wanting" with alcohol-related information, resulting in cravings and "loss of control." Alcohol messages are motivated and given preferred attention automatically and unconsciously, once this relationship is established. Noël et al. (6) suggested that addiction-related behaviors could be gradually controlled by addiction-related information that acquires the property of automatically producing drinking-related behaviors and cravings through Pavlovian and instrumental learning mechanisms (7). At the cognitive processing level, continued drinking leads to implicit "wanting" motivation-related enhancement of associative memory (8), and addiction-related cues are marked as salient cues that capture the addict's attention (9), generating automatic approach tendencies. Cue-induced craving gradually increases in the early stages of abstinence and remains high for a more extended period (10, 11). A quick burst of acute craving enters the consciousness after exposure to the cue, followed by a restart of drinking. In well-controlled laboratory and clinical settings, cue-induced craving could reliably predict relapse across environmental settings and different types of addiction (10, 12). The classic oddball paradigm (13–15) reflects subjects' bias toward alcohol-related cue attention and has important implications for studying alcohol-related cue attention in patients with AUD.

Repetitive transcranial magnetic stimulation (rTMS) has emerged as a promising treatment for substance dependence due to its potential to suppress cravings (16). Studies suggested that excitatory rTMS in the dorsolateral prefrontal cortex (DLPFC) reduced craving in patients with substance dependence (17). However, there are few studies on the effect of rTMS treatment on the spontaneous attentional bias of alcohol cues and impulse processing in patients with AUD. Therefore, this study investigated the effect of rTMS on attention cue reactivity, impulsiveness, and cognitive function in patients with AUD. We hypothesized that consecutive rTMS could improve the attentional bias, impulsiveness, and cognitive function in AUD.

OBJECTS AND METHODS

Objects

A cohort of 90 male subjects with AUD was recruited from the Second Affiliated Hospital of Kunming Medical University and Mental Health Hospital of Yunnan Province. All subjects met the criteria for the Diagnosis and Statistics of Mental Disorder 5th edition (DSM-5) for AUD, with normal vision and hearing or within the normal range after correction and were right-handed. To rule out the influence of acute withdrawal, it was required no alcohol was consumed in the 72 h before the experiment.

The exclusion criteria were (1) Clinical Institute Withdrawal Assessment Alcohol Scale-Revised (CIWA-Ar) (18) score >9 points in acute alcohol withdrawal reaction stage; (2) have experienced a traumatic brain injury or other brain tissue damage; (3) have severe neurological or psychiatric disorders caused by diseases other than chronic alcohol dependence; (4) contraindications to the use of TMS, such as pacemakers, hearing aids, and intracranial metal implants, and a history of epilepsy; (5) diagnosis of other substance use disorders; (6) history of serious physical diseases, including cardiovascular disease and neurological diseases; and (7) have depressed mood and anxiety symptoms rated by Chinese versions of the 9-item Patient Health Questionnaire (PHQ-9) (19) for depressive symptoms (PHQ-9 >5 points), the 7-item Generalized Anxiety Disorder scale (GAD-7) for generalized anxiety symptoms (GAD-7 >5 points) (20).

This study was approved by the Ethics Committee of the Second Affiliated Hospital of Kunming Medical University. All participants provided written informed consent and participated in the study voluntarily. The registration number of this study is NTC 03910686.

Methods

Measures

Self-Designed General Information Checklist

The checklist included age, education level, daily alcohol consumption, drinking year, and alcohol consumption variety.

Alcohol Dependence Scale

The Alcohol Dependence Scale (ADS) consists of 25 questions, and the scores were recorded as 0, 1, or 2 in the order of each question, and the total score of alcohol dependence (0–47) was obtained by adding up the scores of each question. The score of 0 indicates no alcohol dependence; the score of 1–13 indicates low alcohol dependence; the score of 14–21 indicates moderate alcohol dependence; the score of 22–30 indicates severe alcohol dependence; and the score of 31–47 indicates severe alcohol dependence (21).

Montreal Cognitive Assessment

Montreal Cognitive Assessment (MoCA) (22) was used for the screening of cognitive function in patients with AUD. There are six dimensions, namely, visuospatial/executive, naming, attention, language, abstraction, memory, and orientation. The optimal cutoff point of the MoCA to detect cognitive impairment in the general population is a score of <26 (23).

Barratt Impulsiveness Scale

Barratt Impulsiveness Scale II (BIS-II) is a self-report measure for assessing individual impulsive personality traits (24). The Chinese version of BIS-II has 26 items

and shows good reliability and validity (25). Each subscale uses a 4-point scale: "never" is rated as 1, "occasionally" is rated as 2, "often" is rated as 3, and "always" is rated as 4. The higher the score, the stronger the impulsivity.

Attention Cue Response Task

As shown in **Figure 1**, a visual oddball paradigm was used to evaluate the attention cue response. The task and its operation were explained to the subjects before starting the formal experiment. The experiment was not started until the patient passed the exercise unit.

Subjects were presented with the visual oddball paradigm containing repeated high-frequency stimuli (e.g., pictures of a bottle of neutral mineral water) and low-frequency-biased stimuli, including pictures of a bottle of alcohol-related drinks (e.g., beer, liquor, or wine) and non-alcohol-related drinks (e.g., Sprite, Coke, or Fanta). The total number of stimuli was 840. In each block, 75% were high-frequency stimuli ($n = 120$) and 25% were low-frequency stimuli ($n = 30$). Among the low-frequency stimuli, alcohol-related-biased stimuli and non-alcohol-related-biased stimuli, each appeared 15 times. The "+" symbol appeared in the center of the screen for 100 ms before the pictures were presented, attracting the subjects to focus their attention. Each picture was then displayed for 800 ms, with a black screen randomly displayed for 600–1,000 ms between the two pictures. Subjects were given at least 1,400 ms from the onset of the stimulus to respond and were asked to indicate the onset of any low-frequency stimulus by tapping the space bar with their right finger as quickly and accurately as possible. The reaction time and accuracy rate of subjects were recorded. The attention cue response task was reevaluated after the 14 rTMS interventions.

Procedure

As shown in **Figure 2**, after CIWA-Ar score <9 points, demonstrating that the subjects were not in an acute alcohol withdrawal stage, a total of 90 male patients with AUD were treated with the same drug regimen: methylcobalamin 0.5 mg *p.o* Tid, vitamin B_1 100 mg *i.m* Qd, and vitamin B_6 100 mg *ivgtt* Qd.

A random number table was used to divide the subjects into 45 in the study group (i.e., actual stimulation group) and 45 in the waiting-for-treatment group (i.e., pseudo-stimulation group). Except for the operator, the subjects were blind to true or false stimulation in the rTMS trial. The wait-for-treatment group would continue to receive actual stimulation for 2 weeks after the 2-week study is completed, but the data would not include in this study. All subjects were evaluated by using the visual oddball paradigm, MoCA, and BIS-II at baseline and after rTMS.

There were 18 dropouts during the study. Reasons for dropping out were as follows. There were 11 patients unwilling to complete the follow-up evaluation, 2 patients unwilling to conduct rTMS intervention, 1 patient discontinued rTMS due to side effects, and 4 patients discharged from the hospital to terminate the experiment. Finally, 36 subjects in the study group and 36 subjects in the wait-for-treatment group completed the experiment.

rTMS Intervention

Repetitive transcranial magnetic stimulation was administered using a CCY-1 TMS stimulator (Eride Inc, Wuhan, China) equipped with 8 coils. Patients in the study group received rTMS treatment for 14 consecutive days once a day. The stimulation site was selected as left DLPFC (17), and the international 10–20 EEG system (F3) was used for localization. The motion thresholds of individual magnetic stimuli were measured. Treatment parameters were set as shown in **Figure 3**: the stimulation intensity: 110% of the resting motor threshold; the stimulation frequency: 10 Hz; the stimulation interval: 20 s; the number of pulses per treatment: 1,530; the duration of one treatment: 12 min and 33 s; and the total number of pulses for 14 treatments: 21,420. For sham stimulation, the stimulation coil was tilted at a 90°angle to the scalp, and the coil was spaced approximately 3 cm apart from the scalp to reduce the effect of the magnetic field on the brain (17). After rTMS treatment, patients were invited to report adverse reactions.

Statistical Processing

Statistical analyses were performed using SPSS 21.0 (Statistical Package for Social Sciences, IBM, Armonk, NY). Continuous data were presented as mean ± standard deviation (SD) or median [interquartile range]. Categorical data were presented as absolute numbers and percentages. The demographic and clinical characteristics of the two groups were compared by using the one-way ANOVA, Kruskal-Wallis H-test, or Fisher's exact test. The pre-post results of attention cue response after rTMS were compared using a paired-sample *t*-test. The correlation between BIS-II scores and results of the oddball paradigm, MoCA after rTMS in the study group were analyzed using the Spearman correlation analysis. We used a two-sided α of 0.05 for statistical significance.

RESULTS

Demographic and Clinical Characteristics Between the Two Groups

As shown in **Table 1**, for the mean age, education, type of alcohol consumption, daily alcohol consumption, drinking year, and scores of ADS, PHQ-9, GAD-7, MoCA, and BIS-II, there were no significant differences between the study group and the waiting-for-treatment group at baseline ($P > 0.05$). After rTMS, for the scores of PHQ-9 and GAD-7, there were no significant differences between the two groups.

Pre-post Comparison of Attention cue Response After rTMS

Before rTMS intervention, there were no significant differences in response time and accuracy rate between the two groups, neither in alcohol-related cues nor in non-alcohol-related cues.

As shown in **Table 2**, after 14 rTMS interventions, both for alcohol-related cues and non-alcohol-related cues, the response times were significantly shorter in the study group compared with those in the waiting-for-treatment group. Within the study group, the response times for two kinds of cues after rTMS were shorter than baseline.

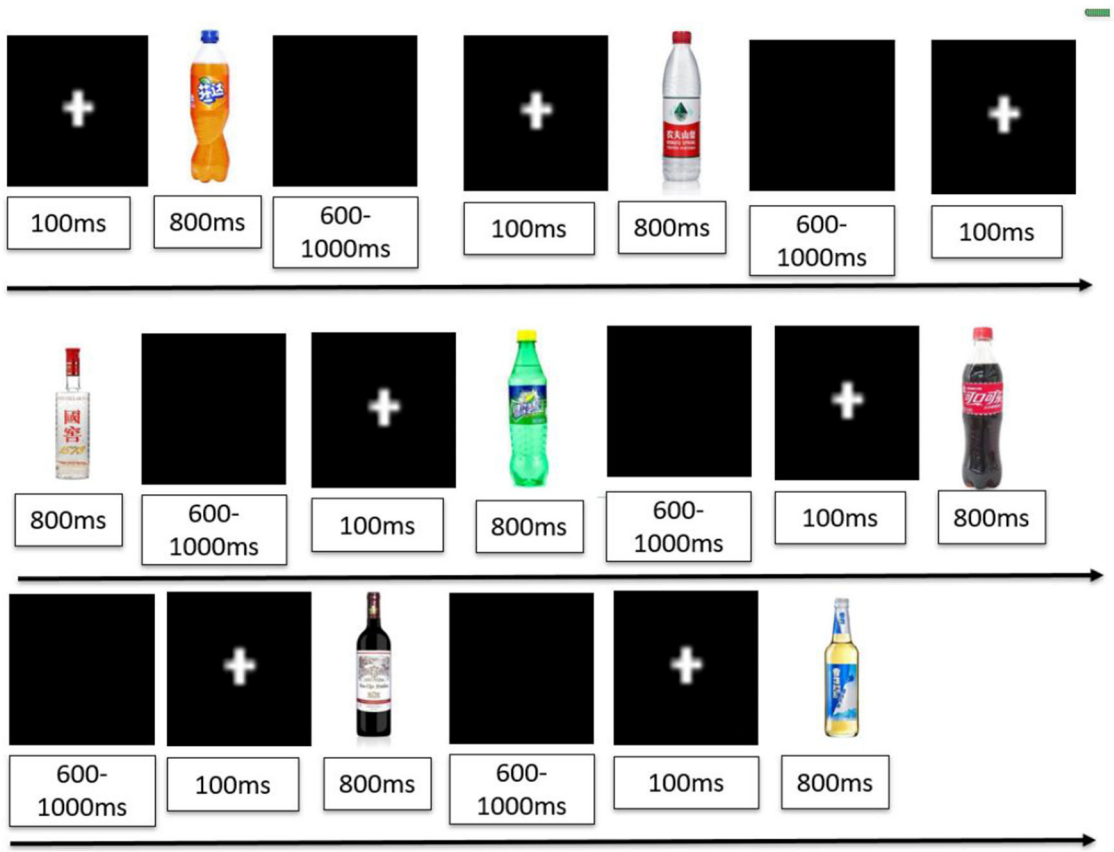

FIGURE 1 | Cue response task-visual oddball paradigm.

There was no significant difference in accuracy rates for both alcohol-related and non-alcohol-related cues between the two groups after rTMS intervention. Within the study group or waiting-for-treatment group itself, there was also no significant difference in accuracy rates for two kinds of cues after rTMS.

Pre-post Comparison of BIS-II and MoCA After rTMS

As shown in **Table 3**, the total score of MoCA was significantly increased, and the total score of BIS-II was significantly decreased in the study group after rTMS treatment, either between the two groups or within the study group itself. For the waiting-for-treatment group, there was no significant difference in the total scores of MoCA and BIS-II after sham rTMS.

The Correlation Between BIS-II Scores and Results of the Oddball Paradigm, MoCA After rTMS in the Study Group

There was no significant correlation between the BIS-II score and MoCA scores after rTMS. However, as shown in **Table 4**, there was a significantly negative correlation between change in BIS-II and change in response time of alcohol-related cues or change in response time of non-alcohol-related cues after rTMS.

There was a significantly positive correlation between the change in MoCA and response time of alcohol-related cues or the change in response time of non-alcohol-related cues after rTMS. Spearman's correlation analysis showed further a significant positive association between the change in attention score in MoCA and the change in response time of alcohol-related cues ($F = 7.838$, $P = 0.001$), or the change in response time of non-alcohol-related cues ($F = 7.016$, $P = 0.009$) after rTMS. Another significant positive association between the change in Memory and Orientation score in MoCA and the change in response time of alcohol-related cues ($F = 8.252$, $P < 0.001$) or the change in response time of non-alcohol-related cues ($F = 9.067$, $P < 0.001$) was found after rTMS.

There were no significant correlations found between the score of BIS-II and MoCA and the change in the accuracy rate of alcohol-related cues or non-alcohol-related cues.

Side Effects During rTMS Stimulation

As shown in **Table 5**, there were some side effects during rTMS stimulation, especially in the actual rTMS group. These side effects included headache, tinnitus, dizziness, and eye discomfort, most of which were mild degrees and relieved after stopping rTMS stimulation. Only one patient discontinued rTMS due to a headache.

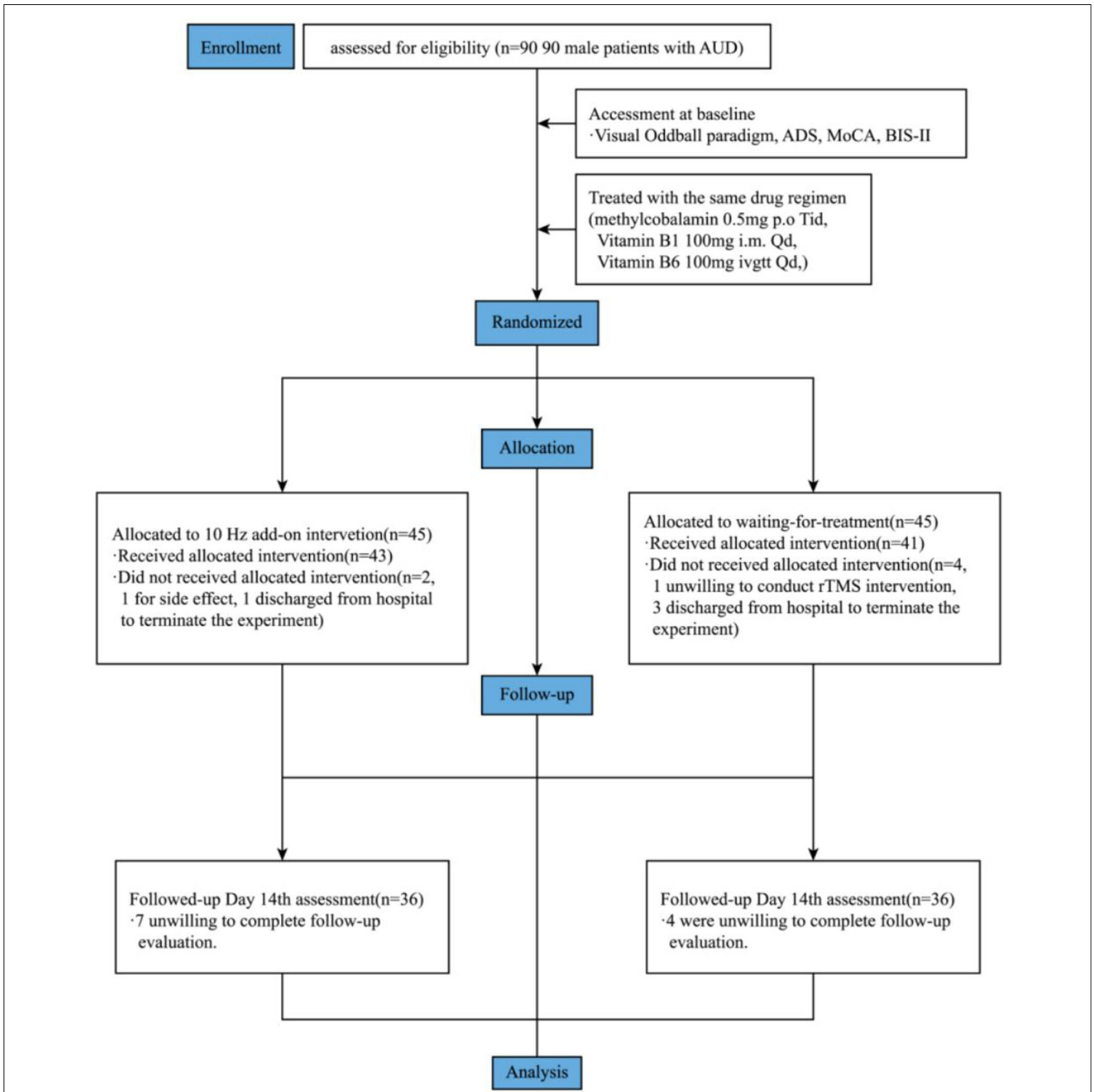

FIGURE 2 | Flowchart of study. ADS, Alcohol dependence scale. MoCA, Montreal Cognitive Assessment; BIS-II, Barratt impulsiveness scale.

DISCUSSION

The evidence suggested that the orbitofrontal cortex of patients with AUD could be strongly activated by alcohol-related cues (26) and that activation of this region was associated with craving and relapse into drinking (27). Patients with AUD with impaired executive function were more inclined to drinking due to impulse processing, thus creating a vicious circle (28).

In this study, there were no statistical differences in the mean age, education, daily drinking, year of drinking, level of alcohol dependence, cognitive function, impulsivity, response times, and accuracy rate for the two types of cue stimuli (e.g., alcohol-related cue and non-alcohol-related cue) between the two groups of patients at the baseline, suggesting that the subjects in two groups were at the same baseline level in terms of demographic profile, drinking and severity, and cognitive level.

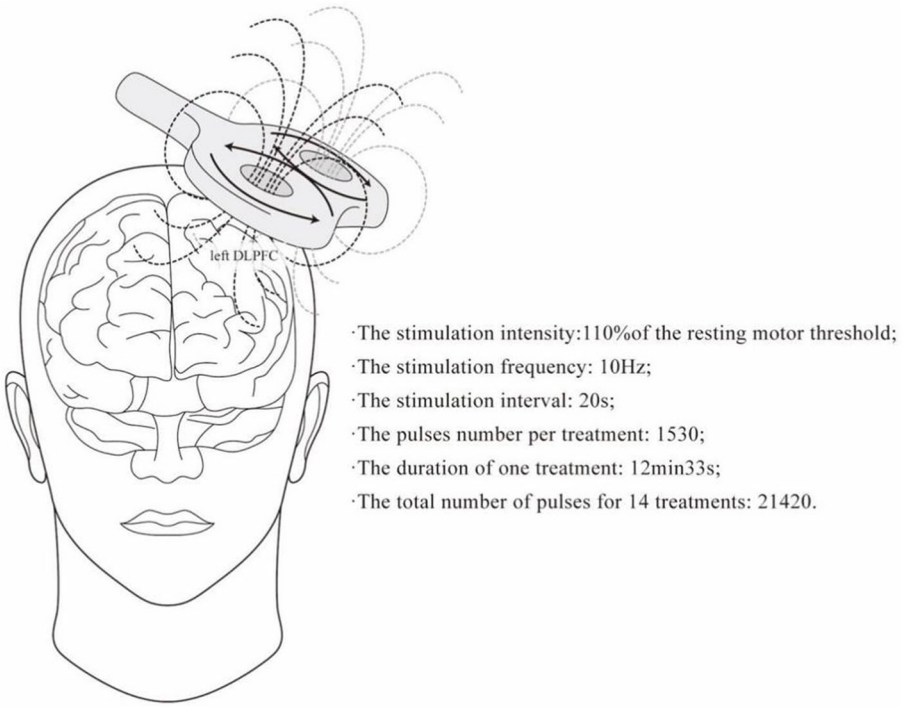

FIGURE 3 | Protocol and parameters of rTMS intervention.

TABLE 1 | Comparison of basic demographic information between the study group and the waiting-for- treatment group [x ± s/*n* (%)].

	Study group (*n* = 45)	**Waiting-for-treatment group (*n* = 45)**	***t* / χ^2/ z**	***P***
Age	37.74 ± 6.42	38.11 ± 5.33	−0.273	0.785
Drinking year	17.39 ± 6.51	18.43 ± 6.01	−0.704	0.484
Educational level				
Primary	7 (22.22)	2 (5.56)	−0.476	0.634
Junior secondary school	7 (19.44)	8 (22.22)		
High school	12 (33.33)	19 (52.78)		
University	10 (25)	7 (19.44)		
Type of alcohol consumption			−1.381	0.167
White Wine	32 (88.89)	35 (97.22)		
Beer	4 (11.11)	1 (2.78)		
Daily alcohol consumption(g)	141.43 ± 29.46	132.54 ± 39.89	1.076	0.286
ADS	30.26 ± 5.16	31.78 ± 4.57	−1.323	0.190
GAD-7	3.07 ± 0.88	3.15 ± 0.76	−0.413	0.681
PHQ-9	2.89 ± 0.82	3.11 ± 0.71	−1.175	0.244
MoCA	22.91 ± 1.98	23.04 ± 1.74	−0.296	0.768
BIS-II	62.30 ± 9.92	66.56 ± 11.26	−1.703	0.093

After high-frequency rTMS treatment at 10 Hz for a continued 14 days, the reaction time of an alcohol-related cue and non-alcohol-related cue in the study group were both shorter when compared with the baseline or the waiting-for-treatment group. Psychomotor vigilance and sustained and selective attention are reflected by shorter reaction times (29). Recently, in a systemic review, it was found that rTMS could influence the attentional networks in alcohol-dependent and other addicted patients (30). These results indicated that high-frequency rTMS acting on the left DLPFC could help to improve attentional drift and bias in patients with AUD after acute detoxification. The total score of MoCA was significantly increased, and the total score of BIS-II

TABLE 2 | Pre-post comparison of response time (ms) and accuracy rate (%) after rTMS in the oddball paradigm.

	Study group (*N* = 36)	Waiting-for-treatment (*N* = 36)	t	*P*
Response time of alcohol–related cues				
Pre- rTMS	530.44 ± 71.31	531.50 ± 80.58	−0.059	0.953
Post- rTMS	499.31 ± 62.97	530.69 ± 58.15	−2.197	0.031*
t	2.306	0.051		
P	0.027*	0.959		
Response time of non-alcohol-related cues				
Pre- rTMS	531.64 ± 70.31	530.75 ± 71.87	0.053	0.958
Post- rTMS	493.14 ± 64.79	526.47 ± 60.77	−2.251	0.027 *
t	2.504	0.235		
P	0.017 *	0.815		
Accuracy rate of alcohol-related cues				
Pre- rTMS	0.97 ± 0.05	0.98 ± 0.04	−0.583	0.562
Post- rTMS	0.98 ± 0.05	0.98 ± 0.05	−0.189	0.851
t	0.594	0.271		
P	0.557	0.788		
Accuracy rate of non-alcohol-related cues				
Pre- rTMS	0.99 ± 0.06	0.97 ± 0.04	0.93	0.355
Post- rTMS	0.99 ± 0.04	0.98 ± 0.05	0.69	0.492
t	0.368	0.713		
P	0.715	0.480		

*$P < 0.05$.

TABLE 3 | Pre-post comparison of BIS-II, MoCA after rTMS.

	Study group (*N* = 36)	Waiting–for–treatment (*N* = 36)	t	*P*
Total score of BIS-II				
Pre- rTMS	65.86 ± 10.89	66.75 ± 10.23	−1.877	0.148
Post- rTMS	55.10 ± 7.75	65.41 ± 10.49	−19.197	<0.001
t	21.54	0.981		
P	<0.001	0.959		
Total score of MoCA				
Pre- rTMS	20.04 ± 3.83	20.86 ± 4.07	0.071	0.548
Post- rTMS	23.43 ± 4.09	21.03 ± 4.18	3.253	0.029*
t	−10.681	0.935		
P	<0.001	0.725		

was significantly decreased in the study group, suggesting that rTMS improved cognitive function and decreased impulsivity for patients with AUD. Spearman's correlation analysis further demonstrated that the level of BIS-II negatively correlated with the improvement of response time in two kinds of attention cue response, and the improvement of MoCA and reaction time was mutually reinforcing, suggesting that decreased impulsivity and improved cognitive function, especially the improvement of attention, memory, and orientation, could also be helpful for the improvement of attentional bias after rTMS. These results were consistent with previous studies that rTMS treatment could improve alcohol craving, cognitive function, and heavy drinking (31–33) and affect the dorsal anterior cingulate cortex (34). Alcohol-attentional bias and impulsive decision-making are vulnerability markers for maintaining addiction-like behaviors (29). A potential candidate mechanism of rTMS acting on the left DLPFC could be that rTMS modulates the attention bias to alcohol-related cues and impulsivity.

However, after 14 days of treatment, there was no significant difference in the accuracy rate for alcohol-related cues and non-alcohol-related cues between the two groups after rTMS intervention. Although high-frequency rTMS treatment significantly improved the reaction time, patients with AUD generally had deficits of inhibition ability resistance

TABLE 4 | The correlation between BIC-II scores, results of the Oddball paradigm, and MoCA after rTMS in the study group ($n = 36$).

	Change of BIS-II (r, *P*)	Change of MoCA (r, *P*)
Change of response time of alcohol-related cues	−0.419, 0.011	0.515, 0.001
Change of response time of non-alcohol-related cues	−0.477, 0.003	0.639, <0.001
Change of accuracy rate of alcohol-related cues	−0.207, 0.226	0.113, 0.511
Change of accuracy rate of non-alcohol-related cues	−0.269, 0.113	0.067, 0.700

TABLE 5 | Side effects during rTMS stimulation.

Side effects	Study group ($n = 36$)	Waiting-for-treatment group ($n = 36$)
Headache	2	0
Tinnitus	3	1
Dizzy	2	0
Eye discomfort	1	0

to interference ability reflected by lower commission error number and accuracy rates (35). The damage by alcohol to cognitive function would persist longer. Previous studies demonstrated that the cognitive dysfunction in AUD did not completely recover after prolonged abstinence and remained lower when compared with controls (36). In this study, 14 days of rTMS intervention was only short-term treatment. It might be that a more extended treatment period or more comprehensive treatment approaches are needed to improve cognitive deficits further.

In this study, some side effects mainly happened in the actual rTMS group, most of which were to mild degrees and relieved after stopping rTMS stimulation. The safety of rTMS continued to be supported by meta-analyses, or evidence-based guidelines (37, 38), demonstrating that rTMS is a promising non-invasive treatment for various neuropsychiatric conditions, including AUD.

There were several limitations of this study. First, the standard 10–20 EEG partitions were utilized for brain area localization during the rTMS intervention in this study, and the accuracy might be enhanced by utilizing more EEG recorder leads. Second, due to 18 dropouts, the number of enrolled subjects decreased. Therefore, the results needed more replication in a larger sample. Although the comparison between the two groups or pre-post rTMS was made, the healthy control group is needed in future research, especially for the results of the oddball paradigm at baseline. We used the classic oddball task for the oddball paradigm, which required subjects to respond to the target stimuli (low-frequency stimuli) but not to the standard stimuli (high-frequency stimuli), so we could not record the reaction time and accuracy rate of unattended stimuli. In the later research, a two-choice oddball task (39) should be used to reflect the ability of behavioral inhibitory control, and both reaction time and accuracy rate in standard stimuli could be recorded. In addition, this study was conducted using a single-blind method, and the results are yet to be confirmed using a double-blind methodology. Furthermore, clinical evaluations such as MoCA and BIS-II were assessed using self-rating scales by patients themselves. All these evaluations, including the visual oddball paradigm, were repeatedly assessed after 2 weeks; thus, some subjective bias or subjective bias recall error might have occurred. In addition, more extended follow-up observation is needed to demonstrate how long the change of clinical evaluations would be persistent after discontinuation of rTMS. Finally, combined application of event-related potentials and eye-tracking could be used to evaluate how brain functions are enhanced in the future.

CONCLUSION

This study revealed that high-frequency rTMS treatment at 10 Hz for continuous 14 days could improve the attention bias of alcohol-related cues and impulsivity in patients with AUD.

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 authors.

ETHICS STATEMENT

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

AUTHOR CONTRIBUTIONS

ZF performed data collection, data analysis, data interpretation, and manuscript writing. QW and LW conducted data collection, statistical analysis and data interpretation, and manuscript preparation. QW wrote the manuscript of the Chinese version. TZ, JYu, and XW collected the data. JYa and CK were responsible for project design, data analysis, and manuscript writing and modification. All authors contributed to the article and approved the submitted version.

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Altered Spontaneous Brain Activity in Betel Quid Dependence Chewers: A Resting-State Functional MRI Study With Percent Amplitude of Fluctuation

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Objective: This study aimed to investigate brain spontaneous neural activity changes in betel quid dependence (BQD) chewers using the percent amplitude of fluctuation (PerAF) method.

Methods: This study included 48 BQD chewers. The healthy control (HC) group comprised 35 volunteers who were matched with BQD chewers in age, gender, and educational status. All subjects underwent resting-state functional magnetic resonance imaging (rs-fMRI) and neuropsychological tests. The PerAF method was used to identify BQD-related regional brain activity changes. An independent samples *t*-test was used to evaluate the PerAF difference across two groups. The association between PerAF changes and clinical features such as BQD scores, duration of BQD, Hamilton Depression Rating Scale-24 item (HAMD-24), and Hamilton Anxiety Rating Scale-14 item (HAMA-14) was evaluated by using Spearman's correlation analysis. It assessed the ability of the PerAF method to distinguish between BQD chewers and HCs using a receiver operating characteristic (ROC) curve.

Results: Compared to the control group, BQD chewers showed decreased PerAF in right anterior cingulate cortex (ACC), right middle frontal gyrus (MFG), right insula, right precuneus, left putamen, left supramarginal gyrus (SMG), and left cerebellum and increased PerAF in right orbitofrontal and left superior temporal gyrus (STG) [$P < 0.05$, Gaussian random field (GRF) corrected]. PerAF values of the right MFG and right ACC had a significant negative relationship with the duration of BQD ($P < 0.05$). The average values of PerAF in the left putamen, left cerebellum, and left STG showed significant discriminatory power in distinguishing BQD chewers from HCs, with relatively prime area under the curve (AUC) values.

Conclusion: Our findings suggested that betel quid chewing is associated with spontaneous neural activity alterations in the impulsivity areas (MFG and ACC), cognitive (MFG, ACC, precuneus, and the cerebellum), and reward (orbitofrontal, putamen, and

insula) systems, which may be correlated with neuropathological mechanisms of BQD. Also, PerAF may be useful as a potential sensitive biomarker for identifying spontaneous brain activity changes in BQD chewers.

Keywords: betel quid, betel quid dependent, drug dependence, resting-state fMRI, percent amplitude of fluctuation

INTRODUCTION

Betel quid (BQ) is a psychoactive stimulant that, after ethanol, nicotine, and caffeine, is the most commonly used addictive substance (1). There are over 600 million BQ consumers globally, most of whom they concentrated in Asia and Pacific islands (2). The term "betel quid" refers to a mixture of ingredients that includes areca nut (AN), piper betel leaf (a common vine), and slaked lime (calcium hydroxide), but the composition of quid varies among different populations and areas (3, 4). The parasympathomimetic characteristics of BQ stimulate nicotine and muscarinic receptors. Therefore, chewing BQ usually leads to a reliance syndrome, which is featured with increased attention, mild pleasure, comfort, and postprandial satisfaction, as well as a withdrawal syndrome that includes insomnia, mood swings, anxiety, and irritability (5). BQ chewing is a public health concern since that can lead to a range of health problems, such as oral cavity cancer and many precancerous lesions associated with leukoplakia and submucosal fibrosis of the oral cavity (6). The International Agency for Research on Cancer has classified BQ as a human carcinogen (3, 7). However, at present, the mechanism behind betel quid dependence (BQD) has remained unclear. Most studies have been restricted to either epidemiological or biological studies (8, 9). Only a few studies have been conducted to determine the behavioral and psychological characteristics associated with individuals starting and/or maintaining BQ use.

Due to the advantages of being non-invasive, easy to get signals, pretty high space-time resolution, and requirement of a minimum workload from patients, the resting-state functional magnetic resonance imaging (rs-fMRI) has been widely used to reveal abnormal spontaneous brain activity. The amplitude of low-frequency fluctuation (ALFF), fractional ALFF (fALFF), and Regional Homogeneity (ReHo) are the three common parameters to address regional brain alterations (10–16). However, due to the arbitrary unit of blood oxygenation level-dependent (BOLD) signals, ALFF cannot be immediately applied to further statistical analysis at the group level. Besides that, ReHo cannot reflect accurately the activity of neurons in a specific integrin because it is based on time consistency.

A new method, the percent amplitude of fluctuation (PerAF), has been proposed, which is based on the percentage of signal alterations in the task functional magnetic resonance imaging (fMRI) (17). Even though there is no explicit task vs. control design in rs-fMRI, an index similar to the percentage of signal alterations can be developed by calculating the percentage of BOLD fluctuations compared to mean BOLD signal changes for every point in time, then measuring the mean value across the entire time sequence, that is namely the "percent amplitude of fluctuation" or PerAF (18). To further standardize the data, the PerAF of each voxel can be divided by the globally averaged PerAF of each individual, resulting in mPerAF. When compared to ALFF, PerAF and mPerAF can perform group-level data analysis directly. In fractional ALFF, PerAF can also eliminate the perplexing combination of voxel-specific fluctuation amplitude (18). PerAF has been proven in several studies to be more reliable than ALFF, ReHo, and degree centrality (17, 19). Therefore, PerAF is a relatively reliable, effective, and direct index for voxel-level-based rs-fMRI research and thus a promising index. The increase of PerAF means the enhancement of spontaneous neural activity, while the decrease of PerAF means the diminution of that.

Previous studies have linked BQ chewing and dependence to spontaneous brain activities. BQD patients had significantly slowed ALFF and ReHo values in the prefrontal gyrus and left fusiform and significantly higher ALFF and ReHo values in the primary motor cortex area, temporal lobe, and some occipital lobe regions, which could reflect the neural plasticity of the cerebral functional network caused by BQD (20). A voxel-based analysis had revealed that BQD chewers had a higher mean fractional amplitude of low-frequency fluctuation (mfALFF) activation of the left cuneus and precuneus than the healthy control (HC) (21). Despite this, no studies have used PerAF to investigate spontaneous changes in brain activity in BQD chewers. We hypothesized that BQD chewers had altered spontaneous brain activity in this investigation, and the PerAF method could detect these alterations. To verify this hypothesis, PerAF was used to detect global spontaneous neural activity in BQD chewers, which may give insight into the neurobiological mechanisms underlying BQD.

METHODS

Ethics Statement

The Research Ethics Review Committee of the Hainan General Hospital, based on the Declaration of Helsinki (2000), formally approved this study. Written informed consent was obtained from each participant before starting the research.

Inclusion and Exclusion Criteria

All participants were Hainanese natives. The following were the BQD chewer's inclusion criteria: (1) the BQD subjects should be between the ages of 18 and 60; (2) the BQD subjects should not use nicotine or have used nicotine only one time or two times a month over the previous 3 years. The Fagerstrom Test for Nicotine Dependence (FTND) was also used to test nicotine addiction status to avoid nicotine's influence; (3) BQD subjects with Betel Quid Dependence Scale (BQDS) > 4, Hamilton Depression Rating Scale-24 item (HAMD-24) $\leq$ 7, and Hamilton Anxiety Rating Scale-14 item (HAMA-14) $\leq$ 7; (4) no contraindication to magnetic resonance imaging

(MRI) examination; no structural lesions and abnormal signals in craniocerebral MRI, and the imaging data was complete; (5) not addicted to any other substances and not taking any antidepressants or other psychotropic or addictive drugs; (6) no systemic illnesses and familial psychiatric history; and (7) right-handed.

The following were the inclusion criteria for the HC participant: (1) age range of 18–60 years; (2) no use of BQ, AN, and cigarette in any form; (3) no MRI contraindications; (4) no structural lesions or abnormal signals in craniocerebral MRI, and the imaging data were complete; (5) no additional substance abuse or addiction, and no antidepressants or other psychotropic or addictive drugs; (6) no systemic illnesses or psychiatric history in the family; and (7) right-handed. Finally, 48 BQD chewers and 35 HC volunteers were recruited from Hainan province's local community.

Questionnaire

We evaluated all subjects with a questionnaire including age, gender, educational status, the daily dosage of BQ, duration of BQD, and the usage of tobacco and alcohol before MRI examination. The BQDS was used to assess BQD. This scale quantitatively defines the degree of dependence of BQD individuals, and it has been tested to have good internal consistency and validity. Thus, BQDS is currently the most widely used BQD evaluation tool (22, 23). On the scanning day, the HAMD-24 and HAMA-14 scales were used to assess depression and anxiety levels.

MRI Data Acquisition

The rs-fMRI data were acquired using a 3-T MRI scanner with a standard 32-channel head coil (TIM Skyra, Siemens Medical Solutions, Erlangen, Germany). During the MRI scanning, all subjects were told to keep their heads motionless, keep their eyes open while thinking of nothing, and use foam paddings to reduce head movement. A gradient-echo echo-planar imaging (GRE-EPI) sequence (repetition time (TR) = 2,000 ms, echo time (TE) = 30 ms, field of view (FOV) = 224 × 224 mm^2, image matrix = 64 × 64, section thickness = 3.5 mm, each brain volume consists of 32 axial slices, each functional run contains 240 brain volumes) was used to obtain the BOLD of whole-brain functional images. A magnetization-prepared rapid gradient-echo (MPRAGE) sequence was used to obtain the high-resolution T1-weighted structural image (TR = 2,530 ms, TE = 2.98 ms, FOV = 256 × 256 mm^2, in-plane matrix = 256 × 256, 192 sagittal slices with a thickness of 1 mm). Then, a routine MRI scanning was performed to rule out any gross cerebral pathology.

Data Preprocessing

The preprocessing of fMRI imaging data was performed using the REST plus V1.2 (http://www.restfmri.net) toolbox, which included: (1) removing the first ten functional volumes; (2) slice timing correction; (3) head motion correction; (4) co-registration, spatial normalization, and resampling to 3 × 3 × 3 mm^3; (5) smoothing the resampled images with an isotropic Gaussian kernel of 6 mm; (6) removal of linear trends; (7) covariate regression with the Friston-24 parameter model (24, 25) to remove the nuisance signals; and (8) band-pass (0.01–0.08 Hz) filtering to remove the influence of high-frequency noise and low-frequency drift. Participants with head motion more than 1.5 mm of maximal translation or 1.5° of maximal rotation were excluded from further analyses. No participants were excluded due to excessive head motion in this research. PerAF was calculated after data preprocessing. PerAF, mPerAF, and zPerAF maps were then generated.

Statistical Analysis

SPSS version 25.0 (IBM Corp, Armonk, NY, USA) was used to compare the demographic and clinical features of BQD chewers and HC volunteers. The normality test was performed before the comparison analysis for continuous variables. An independent two-sample *t*-test was adopted if the variables met the normal distribution criteria. If not, an independent two-sample nonparametric test was used to analyze the data. An independent two-sample *t*-test was adopted for continuous variables (age, HAMA-14, and HAMD-24), a chi-square test was performed for gender, and an independent two-sample nonparametric test was used to analyze years of education. $P < 0.05$ was considered to be statistically significant. The data were shown as mean ± standard deviation (mean ± SD). Two-sample *t*-tests were performed to explore the intergroup differences in PerAF between BQD and HC using the DPABI software. To eliminate potential confounding factors, age, gender, and educational status were regressed out (26). Multiple comparisons were conducted with the Gaussian random field (GRF) correction, voxel-wise $P < 0.01$, and cluster-level $P < 0.001$. Regions of interest (ROIs) were identified as brain regions with statistical PerAF alterations between BQD chewers and HC. We extracted the PerAF values from the ROIs. With a significant level of $P < 0.05$, Spearman's correlation analysis was performed between the PerAF values of ROIs and BQDS, dosage, duration, HAMA-14, and HAMD-24. Age, gender, and educational status were all regressed. A receiver operating characteristic (ROC)

TABLE 1 | Demographics and clinical characteristics of participants.

	BQD ($n = 48$)	HC ($n = 35$)	*P*-value
Gender (males / females)	36 / 12	24 / 11	0.518[a]
Age (year)	37.9 ± 10.9	41.9 ± 11.5	0.110[b]
Education (year)	12.9–15	12.12–15	0.180[c]
BQDS(score)	8.6 ± 3.0	N / A	
BQ dosage (g / d)	70.0 ± 63.2	N / A	
Duration of BQD (year)	13.3 ± 8.5	N / A	
HAMA-14 (score)	1.6 ± 1.8	2.3 ± 1.8	0.075[b]
HAMD-24 (score)	2.0 ± 2.2	2.6 ± 2.5	0.255[b]

Values are expressed as means ± standard deviations except for gender and education, gender is presented as a number, education is expressed as median and inter-quartile range. BQ, betel quid; BQD, betel quid dependence; BQDS, betel quid dependence scale; HAMA-14, hamilton anxiety rating scale-14 item; HAMD-24, hamilton depression rating scale-24 item; HC, healthy control; N / A, not applicable. [a]P-value between the two groups was obtained by chi-square test. [b]P-value between the two groups was obtained by an independent sample t-test. [c]P-value between the two groups was obtained by independent sample non-parametric test.

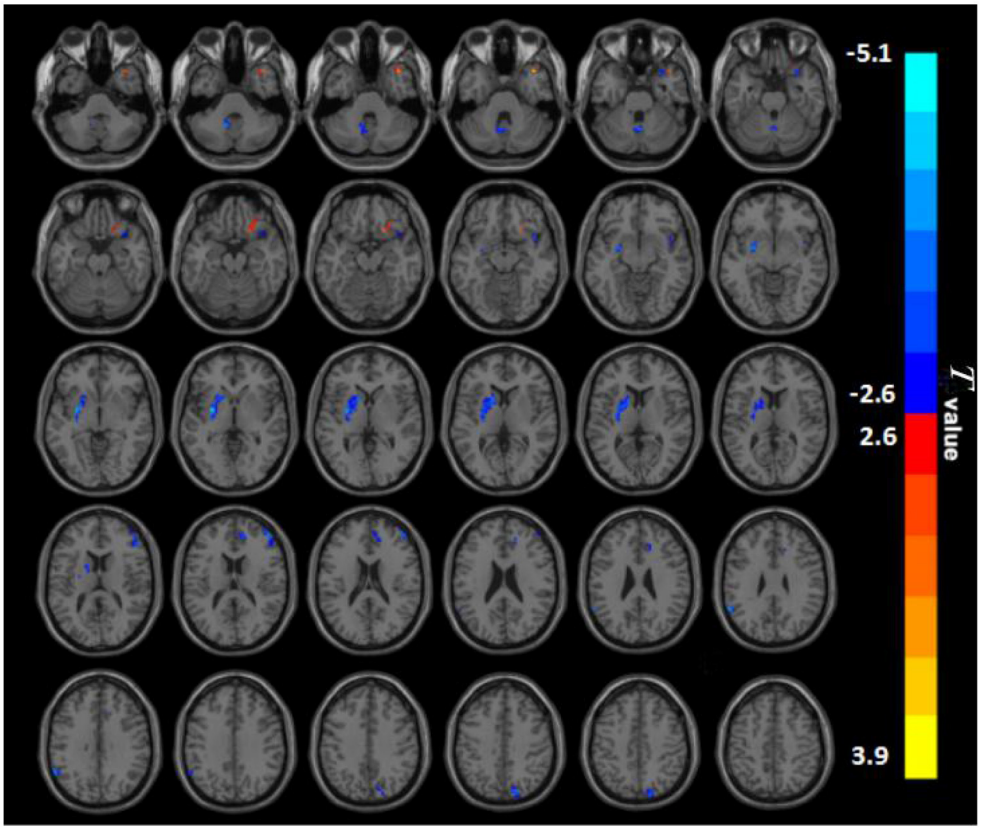

FIGURE 1 | PerAF maps show differences between BQD individuals and HC subjects ($P < 0.05$). The BQD group showed significantly lower PerAF values in the right anterior cingulate cortex, right middle frontal gyrus, right insula, right precuneus, left putamen, left supramarginal gyrus, left cerebellum, but increased PerAF values in the right orbitofrontal, left superiortemporal gyrus relative to both the HC group. BQD, betel quid dependence; HC, healthy control; PerAF, percent amplitude of fluctuation. The color bar represents *T*-values for the two-sample *t*-test. Colors in red and blue indicate significant increase and decrease in the two-sample *t*-test respectively.

curve analysis was conducted to assess the PerAF method's ability to distinguish between BQD chewers and HCs.

RESULTS

Demographics and Clinical Characteristics

Eighty-three participants (48 BQD chewers and 35 control volunteers) were included in the ultimate statistical analysis. The mean age of BQD chewers was 37.9 ± 10.9 years old, and the mean education was 12.1 ± 2.7 years. The mean age of HC volunteers was 41.9 ± 11.5 years old, and the mean education was 12.9 ± 2.6 years. BQD chewers revealed that they had been chewing BQ for an average of 13.3 ± 8.5 years, with a range of 4.8–21.8 years. The mean BQDS score in the BQD group was 8.6 ± 3.0. HAMA-14 and HAMD-24 did not meet clinically meaningful thresholds on average. The demographics and clinical characteristics of BQD and HC subjects were demonstrated in **Table 1**. There was no statistical difference between the BQD chewers and HCs in terms of age, gender, education level, HAMA-14, or HAMD-24 (all $P > 0.05$).

Group Differences in PerAF

Between the two groups, there were significantly distinct patterns of spontaneous neural activity. Compared with HC, BQD chewers exhibited decreased PerAF values in the right anterior cingulate cortex (ACC), right middle frontal gyrus (MFG), right insula, right precuneus, left putamen, left supramarginal gyrus (SMG), and left cerebellum, while that exhibited increased PerAF values in the right orbitofrontal and left superior temporal gyrus (STG) (**Figure 1**; **Table 2**).

Correlation Analysis

There is a negative association between PerAF values of the right MFG, the right ACC, and the duration of BQD ($r = -0.285$, $P = 0.049$; $r = -0.334$, $P = 0.020$; **Figure 2**). No correlation was found between BQDS and spontaneous neural activity alterations. There is also no link found between HAMA-14, HAMD-24, and neural activity alterations, suggesting that anxiety and depressive mood in the BQD group might not be the contributors to the decreased activity of these brain areas.

ROC Curve

The average values of PerAF in the brain regions with spontaneous neural activity changes were extracted for ROC curve analysis. Our data indicated that these BQD-related regional brain activity changes exhibited differences between the BQD chewers and HCs. They might serve as potential sensitive biomarkers for identifying the two groups (**Figure 3**; **Table 3**).

DISCUSSION

This study is the first to identify BQD-induced brain alterations using PerAF. Our results revealed abnormal PerAF in some impulsivity areas (MFG and ACC), cognitive areas (MFG, ACC, precuneus, and the cerebellum), and reward systems such as orbitofrontal, putamen, and insula in the brain in BQD chewers.

TABLE 2 | Brain regions showing differences in PerAF between BQD and HC groups.

Brain region	Voxel	Peak *T* score	MNI coordinates		
			X	Y	Z
BQD < HC					
R.ACC	26	−3.3138	15	45	18
R.MFG	38	−3.9018	45	48	18
R.Insula	32	−3.5913	30	15	−27
R.Precuneus	27	−3.5871	15	−81	42
L.Putamen	207	−5.0539	−30	−3	0
L.SMG	22	−4.451	−60	−51	30
L.Cerebellum	42	−4.2455	−12	−54	−36
BQD > HC					
R.Orbitofrontal	28	3.2213	21	21	−15
L.STG	20	3.9199	39	18	−30

ACC, anterior cingulate cortex; BQD, betel quid dependence; HC, healthy control; L, left; MFG, middle frontal gyrus; PerAF, percent amplitude of fluctuation; R, right; SMG, supramarginal gyrus; STG, superiortemporal gyrus.

T, statistical value of peak voxel showing PerAF values differences between the two groups (negative values: BQD < HCs; positive values: BQD > HCs); MNI, montreal neurological institute coordinate system or template; x, y, z, coordinates of primary peak locations in the MNI space.

Our results suggest that PerAF is an effective tool for investigating spontaneous brain activity alterations in individuals with BQD. Moreover, ROC curve analysis revealed the left putamen, left cerebellum, and left STG had a relatively high area under the curve (AUC) values, indicating that these BQD-related regional brain activity changes might serve as potential sensitive biomarkers for identifying the two groups.

A decreased PerAF value of the right MFG was observed in the BQD group. The MFG, which is encompassed in the dorsolateral prefrontal cortex (dlPFC), is well-known for its role in impulse control and executive functions (27). Dysfunctions in dlPFC have been linked with drug addiction, which leads to compulsive drug taking, yearning, denial of disease, and a loss of enthusiasm to seek medical assistance (28). The cortical thickness of the dlPFC was found to have a significant role in mediating executive function deficits among BQD chewers in a recent study (29). Moreover, dysfunction of functional activity in the dlPFC may relate to diminished cue-induced hankering and responding suppression in BQD (30). The dlPFC is also involved in decision-making (31, 32). A previous fMRI study demonstrated that reduced neural activity in the prefrontal gyrus within methamphetamine-dependent participants was related to maladaptive decision-making (33). Furthermore, dlPFC has to be well known for its contribution to resolving conflicts. For example, in people who had restarted cigarette consumption, dlPFC was associated with enhanced cognitive control and a greater ability to resolve conflicts (34). According to previous studies and observation of this study, we speculated that the decreased PerAF value in the right MFG within the dlPFC may contribute to demonstrating the affects of BQD on cerebrum impulsivity and the cognitive system.

Similar to MFG, ACC is also contained in the impulsive and cognitive systems. According to the prominent hypothesis of control conflict (35), ACC symbolizes the emergence of response conflict, which gives rise to the agglomeration of dlPFC for better cognitive management of subsequent behavior. Moreover, various neuroimaging studies have shown that cognitive management is associated with a special cortical-subcortical network, including ACC and dlPFC (36). Our previous research indicated decreased ALFF and ReHo values in the right rostral ACC in BQD individuals (20). When compared

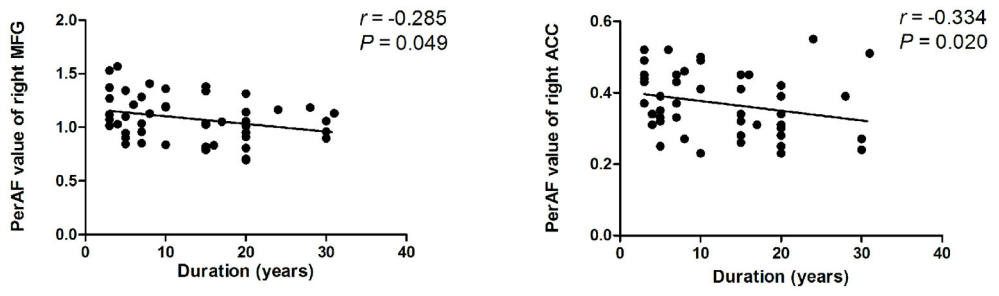

FIGURE 2 | Correlation results between PerAF value and duration of BQD. Spearman correlation analyses reveal that PerAF value of the right MFG, the right ACC showed a negative correlation with duration of BQD in BQD individuals ($P < 0.05$). ACC, anterior cingulate cortex; BQD, betel quid dependence; MFG, middle frontal gyrus; PerAF, percent amplitude of fluctuation.

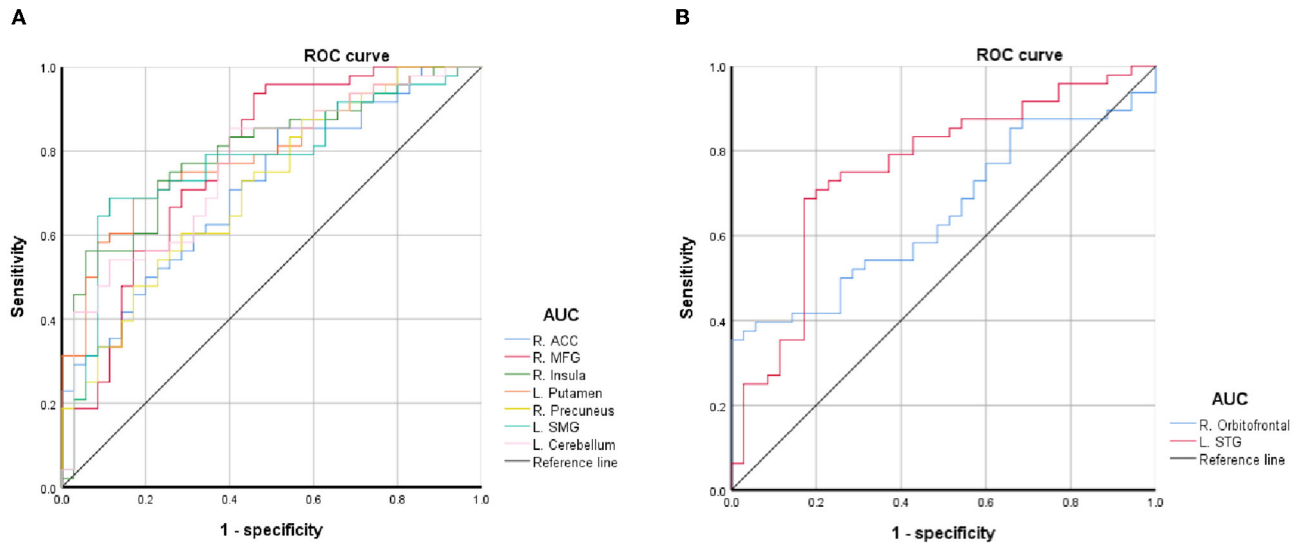

FIGURE 3 | ROC curve of PerAF differences in brain areas. **(A)** Decreased PerAF areas; **(B)** Increased PerAF areas. ACC, anterior cingulate cortex; AUC, area under the curve; L, left; MFG, middle frontal gyrus; PerAF, percent amplitude of fluctuation; R, right; ROC, receiver operating characteristic; SMG, supramarginal gyrus; STG, superiortemporal gyrus.

TABLE 3 | ROC curve for PerAF differences in brain regions between BQD and HC groups.

Brain region	Area under the curve	Sensitivity	Specificity	Cut off points[a]
R.ACC	0.714	85%	49%	0.3701
R.MFG	0.773	94%	54%	1.1879
R.Insula	0.793	56%	43%	0.6892
R.Precuneus	0.713	60%	71%	1.0827
L.Putamen	0.792	69%	83%	0.5490
L.SMG	0.775	69%	89%	0.5740
L.Cerebellum	0.764	85%	60%	0.6910
R.Orbitofrontal	0.652	35%	100%	1.1335
L.STG	0.758	69%	83%	0.8504

ACC, anterior cingulate cortex; BQD, betel quid dependence; HC, healthy control; L, left; MFG, middle frontal gyrus; PerAF, percent amplitude of fluctuation; R, right; ROC, receiver operating characteristic; SMG, supramarginal gyrus.
STG, superiortemporal gyrus. [a]cut off point of mean PerAF signal value.

to HCs, BQD chewers had lower functional connectivity (FC) from ACC to their default mode network (DMN) (37). As far as we know, ACC is also reported to be responsible for the inhibitory control of reward-related behavior (38). The decreased PerAF value in the right ACC and right MFG within the dlPFC in this study may be associated with compulsive BQ chewing behavior, cognitive management, decision-making, and leading to a certain degree of craving.

The duration of BQD was negatively correlated with decreased PerAF of right MFG and right ACC, which demonstrated that the longer a person chewed BQ, the lower their PerAF of MFG and ACC became. Therefore, we deduced that BQD duration might be associated with altered brain function. Given the importance of the frontal cortex and limbic system in the addiction process, it seems sensible to associate functional changes with the duration of BQD. Yet, the correlation result needs further confirmation. The correlation was only found between some brain areas and the duration of BQD, and no correlation was found in BQDS, suggesting that the BQD duration may have a greater impact on brain function or that it may be due to the relatively low BQD score (BQDS, 8.6 $\pm$ 3.0). The small sample size might have accounted for the results.

Besides, lower spontaneous brain activity was observed in the BQD group in the precuneus, which is part of the brain areas of the DMN. The DMN, contained in the cognitive system, is involved in a variety of brain functions which include auditory attention, visuality, memory, language processing, and motor performance (39, 40). As a key component of the DMN, the precuneus is closely associated with determining visual and appetite cues (41, 42), which means that it is involved in visuospatial processing (43). As a result, we contend that the decreased PerAF of the precuneus in BQD chewers may be related to appetite cues and visuospatial processing. Similar to our findings, a recent structural imaging study found a significant decrease in cortical thickness in the precuneus of BQD individuals, which could be related to neurodegeneration caused by chronic BQ chewing (44).

In addition to the alterations in cognitive areas, altered brain activity was found in reward system-associated areas such as orbitofrontal, putamen, and insula in BQD chewers. Increased PerAF was found in the orbitofrontal cortex (OFC) in individuals with BQD, which forms part of the prefrontal cortex (PFC). It is reported that the orbitofrontal network enhances the capacity to control behavior based on potential consequences, whose functional alteration may lead to compulsive drug use and drug relapse in drug addicts (45). Neuroimaging studies have found increased FC of the orbitofrontal in BQD individuals (46) and also in control subjects immediately after BQ chewing (47). This was in line with our notion that BQ chewing and dependence

might affect the brain's reward system. It has also been suggested that the OFC was involved in the processing of rewards and punishments (48), explaining the observed decision-making and goal-driven behavior abnormalities caused by BQD (20). By contrast, a recent fMRI study found that athletes who consumed alcohol or cannabis seemed to have hypoconnectivity in their left OFC relative to nonusers (49), suggesting that different types of psychoactive substances may produce inconsistent neural mechanisms (50).

In our study, the PerAF of the left putamen was decreased. The putamen controls autonomic movements. The putamen injury can interfere with autonomic nervous system functions (51). Furthermore, the putamen within the dorsal striatum influences the acquisition and expression of action-outcome association conditioning (52), which includes the development of habitual compulsive drug dependence. The reduced PerAF of the left putamen in BQD individuals reported here may explain, at least to some degree, the obsessive behavior and drug-seeking in BQD chewers. Similar to other drug dependence users (53, 54), we also found decreased PerAF in BQD individuals in the insula that has been considered a significant structure in generating conscious and interoceptive experiences (55, 56). Similarly, structural imaging studies showed a smaller gray matter volume as insula in addicts (57). Nevertheless, in view of the chicken and egg question, the interpretation of these alterations would still require further investigation.

LIMITATIONS

This study had several limitations. First, we can only observe altered spontaneous brain activity in BQD chewers and are not able to draw direct causal inferences between the BQD and brain activity abnormalities since the study was cross-sectionally designed. Therefore, a longitudinal study design to establish cause-effect relationships should be considered in the future. Second, while the carefully selected samples in this study ensured the comparative specificity of BQ chewing-related problems and limited the use of other addictive substances, alcohol and nicotine consumption are still extremely common in BQD subjects, and their effects could not be fully excluded. Third, the spontaneous brain activity in BQD individuals was evaluated only from single-mode imaging based on the rs-fMRI data. Comprehensive studies on the brain function of BQD using multimodal fusion technology and a more in-depth explanation of genetic mechanisms are lacking. We will study BQD individuals in the future using multimodal fusion technology and genetic imaging to learn more about how gene-environment-brain network-behavior cross-information regulates betel nut addiction at the molecular level.

CONCLUSION

We found altered spontaneous neural activity in the impulsivity (areas in the MFG, ACC), cognitive (MFG, ACC, precuneus, and the cerebellum), and reward (orbitofrontal, putamen, and insula) systems in BQD chewers, and this might underline a neurobiological basis for BQD individuals. Moreover, as a new and reliable method, PerAF might be a potential neuroimaging tool to identify spontaneous brain activity alterations in individuals with BQD.

DATA AVAILABILITY STATEMENT

The datasets in this study are not available currently because the present data is part of an ongoing longitudinal study and most data are still in collection and ought to be protected. Reasonable requests to obtain the data could be emailed to FC, fenger0802@163.com.

ETHICS STATEMENT

This study was formally approved by the Research Ethics Review Committee of the Hainan General Hospital, basing upon the Declaration of Helsinki (2000). The patients/participants provided their written informed consent to participate in this study.

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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ORIGINAL RESEARCH
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Altered EEG Microstates Dynamics During Cue-Induced Methamphetamine Craving in Virtual Reality Environments

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Background: Cue-induced craving is widely considered to be the most important risk factor for relapse during abstinence from methamphetamine (Meth). There is limited research regarding electroencephalography (EEG) microstates of Meth-dependent patients under exposure to drug-related cues. Our objective was to investigate whether EEG microstate temporal characteristics could capture neural correlates of cue-induced Meth craving in virtual reality (VR) environments.

Methods: EEG recordings of 35 Meth-dependent patients and 30 healthy controls (HCs) were collected during eyes-open state and cue-induced state, respectively. Group differences and condition differences in temporal parameters of four microstate classes were compared.

Results: The results demonstrated the greater presence of microstate B in both Meth-dependent patients and HCs during the cue-induced condition, compared to resting state. In addition, for Meth-dependent patients, microstate C occurred significantly less frequently, along with a tendency of increased occurrence for class D during the cue-induced condition, compared to resting state. However, the change direction of class C and class D in HCs was completely opposite to that of Meth-dependent patients. The cue-induced condition also elicited different changes in transition probability between Meth-dependent patients and HCs.

Conclusion: This study explored the features of EEG microstates in Meth-dependent patients during the cue-induced condition, which can improve our understanding of Meth addiction and contribute to the development of effective assessments and intervention tools.

Keywords: methamphetamine dependence, cue-induced craving, resting state, virtual reality, EEG microstates analysis

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INTRODUCTION

Drug craving is a central concept in the research realm of addiction. Although the definition of craving is still debated (1–3), there is no controversy that drug craving is often elicited by a wide range of cues, from internal emotional changes to external drug-related cues (4, 5). Cue-induced craving is believed to be closely related to the maintenance and relapse of addictive behaviors (6, 7). Improving our understanding of the cue-induced craving would increase our knowledge of drug craving and represent potential targets for assessment and intervention.

In recent years, neuroimaging techniques have been increasingly used to identify neural correlates of cue-induced craving (8–11). Functional magnetic resonance imaging (fMRI) studies have revealed several brain networks associated with the cue-induced craving (11, 12). Inherent to the cue-reactivity paradigm, the visual processing network showed enhanced activation levels when exposed to drug-related cues (13, 14). In addition, hyperactivity of reward network and salience network were found when drug-dependent patients were confronted with drug-related cues (12, 15, 16). Somewhat counterintuitively, hyperactivity of executive network and attention network was also reported in drug-dependent patients when exposed to the drug-related cues (11, 12, 17). Since these networks both play a primary role in inhibitory control and self-regulation, the hyper-engagement of the executive and attention network suggests the recruitment of cognitive resources when they were confronting with drug-related cues (11).

Electroencephalography (EEG) is a powerful and popular method for rapid and noninvasive detection of brain signals corresponding to various states from the scalp surface area. EEG microstates, corresponding to specific EEG topographic maps, represent the global and quasi-stable neuronal activity (18, 19). It is impressive that four canonical microstate maps (i.e., labeled classes A, B, C, and D by Lehmann and colleagues) explain consistently around 80% of the total topographic variance in spontaneous EEG (20–22). This has generated much interest about the functional significance of the four EEG microstates (23–28). As expected, it has been well documented that the parameters of microstate changed in different states of consciousness (26, 28, 29), responded to external and internal stimuli (23, 24, 27), and altered in neuropsychiatric disorders [e.g., (21, 22, 30, 31)]. Although far from being conclusive, previous studies have supported the notion that class A and class B are associated with auditory network and visual network (21, 22, 24, 32), respectively, while class C and class D reflect task-negative network and task-positive network, respectively (21, 22, 24, 32).

To date, changes in the EEG microstates have been reported in addictive disorders (33–35). However, it has never been investigated the degree to which specific microstates are influenced by exposure to drug-related cues. In our previous work, Wang et al. (36) developed a methamphetamine (Meth)-related virtual reality (VR) social environment for cue-induced craving assessment. Findings indicated that exposure of Meth-dependent patients to this VR social environment could elicit an increase in heart rate variability (HRV) compared to healthy controls (HCs), and HRV is positively correlated with craving scores. Here, as a continued work, we explored for the first time the potential association between EEG microstates and cue-induced craving. Specifically, we recorded the multichannel EEG signals during VR cue-induced condition (compared to eyes-open resting condition) in Meth-dependent patients (compared to HCs).

For the inherent to the cue-reactivity paradigm, it was predicted that VR cue-induced condition would significantly increase one or more microstate parameters (i.e., duration, occurrence, and coverage) of class B for both Meth-dependent patients and HCs, which is associated with visual network. As mentioned earlier, hyperactivity of attention network was found in drug-dependent patients, reflecting by the recruitment of cognitive resources when exposed to drug-related cues. Thus, it was predicted that the cue-induced condition would significantly increase one or more microstate parameters (i.e., duration, occurrence, and coverage) of class D for Meth-dependent patients, which is associated with the dorsal attention system (21, 32) or task-positive network (22, 24). Finally, since class C is supposed to reflect the task-negative network, we also hypothesized that cue-induced condition would significantly decrease one or more microstate parameters (i.e., duration, occurrence, and coverage) of class C for Meth-dependent patients.

MATERIALS AND METHODS

Participants

A total of 35 male participants with Meth dependence were recruited from the Shiliping Compulsory Rehabilitation Center in Zhejiang Province, China. All Meth-dependent patients had completed more than 1 month of forced detoxification. They were interviewed by an experienced clinical psychiatrist and met the following inclusion criteria: (1) they met the criteria for Meth dependence according to the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV; (2) no evidence of current or previous head injury or central nervous system (CNS) disease; (3) no history of cardiovascular disease; and (4) no other DSM-IV axis I disorder. All patients had no history of antidepressant or/and antipsychotic medication use within 2 weeks.

Notably, 30 age-matched HC male participants were recruited from the local community through advertisement. The HC met the following inclusion criteria: (1) no history of Meth use; (2) no DSM-IV axis I disorder; (3) no evidence of current or previous head injury or CNS disease; and (4) no history of cardiovascular disease.

All participants were over 18 years of age and right-handed, with normal vision and hearing. Written and informed consent was obtained. The study was approved by the local ethics committee of the Seventh Hospital of Hangzhou.

Methods

Assessment Procedure

The participants were first introduced to the equipment (i.e., EEG recording device, VR helmet, and headphones). After participants felt comfortable with all the settings, a 6-min period

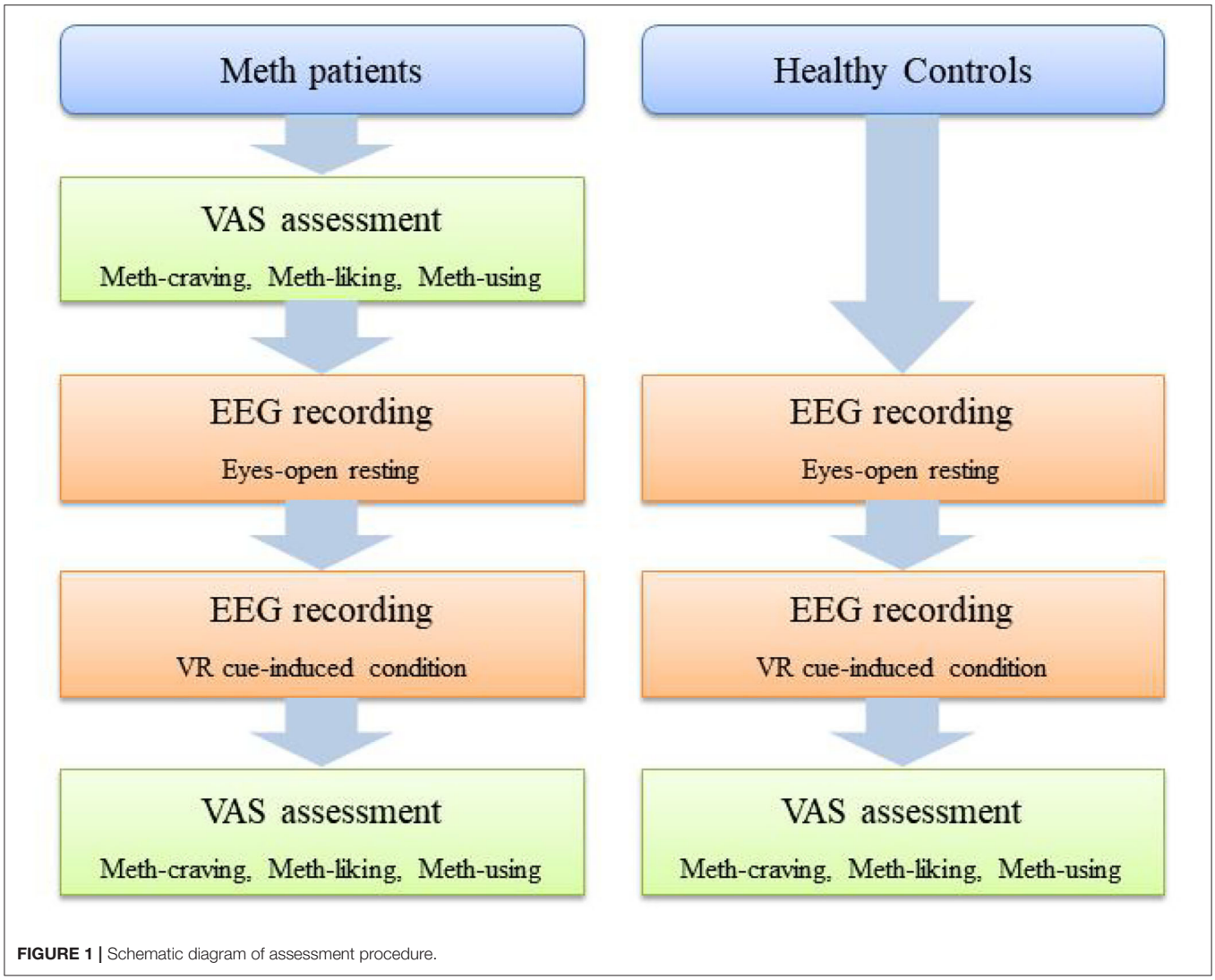

FIGURE 1 | Schematic diagram of assessment procedure.

of resting-state continuous EEG signals (i.e., eyes-open resting-state condition EEG) was recorded. Then, participants were exposed to a VR Meth-cue model (36, 37), with a concurrent recording of EEG signals (i.e., cue-induced EEG). In this VR Meth-cue model, participants were required to watch an 8-min VR video, which simulates a real Meth-related social context, including various Meth-related cues. The details of the Meth-cues VR video and its validity for craving assessment can be found in our previous study (36).

For Meth-dependent patients, before EEG recording and immediately after the VR video, they were asked to answer three questions on a visual analog scale (VAS) (i.e., VAS-craving, VAS-liking, and VAS-using). For HCs, these three VAS questions were only asked after the VR video. The schematic diagram of the assessment procedure is shown in **Figure 1**.

EEG Recording

The experiment was performed in two conditions, namely, resting condition and cue-induced condition. In the resting condition, each subject relaxed in an eyes-opened resting state as measured by EEG for 6 min. In the cue-induced condition, each subject watched an 8-min video, demonstrating Meth use with simultaneous EEG recordings. The scalp EEG data were recorded from a 32-channel EEG system according to the international 10–20 system and referenced to CPz electrode (eego^{TM} mylab, ANT Neuro, The Netherlands). The sampling rate was 2,048 Hz, and all impedances were kept below 10 KΩ.

VAS Assessments

Participants were asked to answer three questions on a VAS, by choosing the most suitable option for each question. The first question was regarding Meth-craving, namely, "How much do you crave Meth/ice right now?" (ranging from 0 to 10, "0" indicated "no carving at all," and "10" indicated "extremely strong craving"). The second question was regarding Meth-liking, namely, "To what extent do you find the Meth/ice pleasant/unpleasant?" (ranging from 0 to 10, "0" indicated "very unpleasant," "5" indicated "neither unpleasant nor pleasant," and

"10" indicated "very pleasant"). The third question was regarding the possibility of Meth-using, namely, "If you have access to Meth/ice right now, how likely would you be to use it?" (ranging from 0 to 10, "0" indicated "certainly not" and "10" indicated "certainly").

EEG Data Preprocessing

Preprocessing was performed using EEGLAB version 13.0b (38) in MATLAB 2016a (The Mathwork, Inc., Natick, US). EEG data were bandpass filtered between 0.1 and 70 Hz with a Butterworth infinite impluse responses (IIR) filter, and eye movement artifacts were removed using independent component analysis (ICA). Data were subsequently segmented into artifact-free 2s epochs before microstate analysis. Bad epochs with excessive muscle activity were rejected. Then, the data were referenced to the average reference, digitally band passed at 2–20 Hz and further downsampled to 250 Hz.

Microstate Analysis

The EEG microstate analysis was performed separately for each group in resting or cue-induced condition. First, global field power (GFP) was computed as the spatial standard deviation of the potential field for each time point of the recording. Microstate configurations remain stable around GFP peaks, which have the highest signal-to-noise ratio and were taken as the original momentary maps (39). Original momentary maps are submitted to atomize–agglomerate hierarchical clustering (AAHC) to calculate individual microstate maps for each subject (40). The number of microstate clusters for this study was preset to four according to previous EEG microstate studies (22).

We then submitted the individual microstate maps into a second AAHC cluster analysis to identify group model maps for Meth-dependent patients with resting condition, Meth-dependent patients with cue-induced condition, HC with resting condition, and HC with cue-induced condition separately. Based on these group models, a "grand-mean" model was calculated, which was then class-labeled into microstates A-D by using minimal global map dissimilarity (GMD) (39). Next, the class-labeled "grand-mean" model maps were used as a template to assign the group model maps to the four class-labeled grand-mean maps. As a final step, the individual microstate maps were sorted into one of the microstates A-D using minimal GMD between the individual microstate maps and the class-labeled group model maps as a criterion. Four parameters were then extracted per microstate, namely, coverage (the percentage of the time covered by each microstate class), duration (the average length of time for each microstate class), occurrence (total number of each microstate class per second), and transition probability (the transition probability between any two EEG microstates). The microstate analysis was performed using EEGLAB (http://www.thomaskoenig.ch/Download/EEGLAB_Microstates/).

Statistical Analyses

Two-sample *t*-test and paired *t*-test were conducted to compare between groups and between conditions for age and VAS score, respectively. For the EEG microstates (i.e., coverage, occurrence, and duration), separate three-way rmANOVAs were conducted, with conditions (resting vs. cue-induced) and classes (A, B, C, or D) as a within-group factor, and groups (Meth vs. HC) as a between-group factor. For the transition probability between classes, three-way rmANOVAs were conducted, with condition and transition pairs as a within-group factor, and group as a between-group factor. *Post-hoc* multiple comparisons were conducted by family-wise error (FWE) correction.

TABLE 1 | Demographic characteristics and VAS scores.

Variables	Meth ($n = 35$)	HC ($n = 30$)	*t*-value	*P*-value
Age (Years)	33.69 ± 6.46	31.57 ± 9.15	1.06	0.29
Years of Meth-use	9.83 ± 3.83			
Age of first Meth taking	24.14 ± 7.01			
Resting condition VAS				
Meth-craving	2.257 ± 1.559			
Meth-using	4.629 ± 3.606			
Meth-liking	3.886 ± 2.220			
Cue-induced condition VAS				
Meth-craving	4.457 ± 2.254	0.267 ± 0.828	10.225	<0.001
Meth-using	6.371 ± 3.388	0.400 ± 1.192	9.748	<0.001
Meth-liking	4.686 ± 1.859	1.233 ± 1.888	7.401	<0.001

RESULTS

Demographic and VAS

Demographic data of study participants are presented in **Table 1**. There were no significant differences in age between Meth-dependent patients and HC. For Meth-dependent patients, the cue-induced condition had significantly higher VAS scores than the resting state, on Meth-craving ($t = 7.604$, $P < 0.001$), Meth-using ($t = 4.866$, $P < 0.001$), and Meth-liking ($t = 3.357$, $P = 0.002$). After cue-induced condition, Meth-dependent patients had significantly higher VAS scores than HCs, on Meth-craving, Meth-using, and Meth-liking (all *P*-values < 0.001).

EEG Microstate Parameters

Spatial topographic correlations were used to assess between-group and between-condition similarity in microstate class topographies. **Figure 2** depicts the map topographies of microstate of each group in resting or cue-induced condition and spatial topographic correlations. The four independently identified topographies were consistent with previous studies (39, 41) and were similar between conditions and groups, with mean topographic correlations exceeding 0.90.

Outcomes of EEG microstates (i.e., coverage, duration, and occurrence) and their transition probability are shown in **Figure 3**. The diagram of the interaction between group and condition for each class and transition probability are shown in **Figure 4**.

Coverage

The rmANOVAs revealed a significant main effect of class ($F = 6.495$, $P = 0.001$), reflecting by class A had a larger coverage

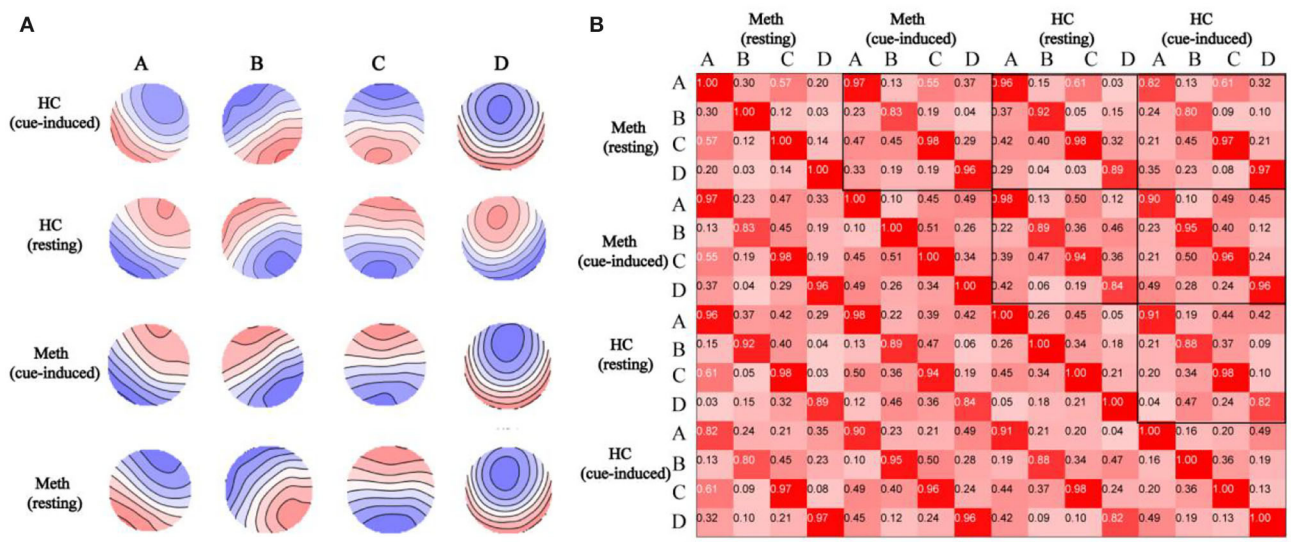

FIGURE 2 | Spatial correlations are depicted between microstate topographies. **(A)** Topographies of microstate in healthy control (HCs) with cue-induced condition, HCs with resting condition, Meth-dependent patients with cue-induced condition, and Meth-dependent patients with resting condition. The topographies are labeled as microstate classes (A–D). Note that polarity (blue vs. red) is ignored in the microstate definition. **(B)** Spatial correlations between microstate topographies (A–D) identified from separate topographies of resting or cue-induced conditions in each group.

than class B ($P = 0.010$) and class C ($P = 0.005$). There was a significant interaction between condition and class ($F = 10.490$, $P < 0.001$), which was mainly explained by a decrease in the coverage of class A ($P < 0.001$), but an increase of the coverage of class B, from resting state to cue-induced condition ($P < 0.001$). No significant main effect of group and condition, and their interaction (all P-values $= 1.000$) were found. In addition, there was a significant interaction between group and class ($F = 6.340$, $P = 0.001$), which was modulated by condition ($F = 6.870$, $P < 0.001$). For the sake of simplicity, further analyses were performed for each class separately.

As shown in **Figure 4**, cue-induced condition elicited different pattern results across classes. For class A and class B, cue-induced condition elicited changes in the same direction for Meth and HC. There was a significant decrease of class A and a significant increase of class B in Meth (both P-values < 0.001), along with a decreasing tendency of class A and an increasing tendency of class B for HC. However, cue-induced condition elicited opposite changes in class C and class D for Meth and HC, respectively. Cue-induced condition elicited a decreasing tendency of class C in Meth, but a significant increase of class C in HC ($P = 0.008$). On the contrary, cue-induced condition elicited an increasing tendency of class D in Meth, but a significant decrease of class D in HC (test for interaction, $P = 0.007$). More details of multiple comparisons are shown in **Figure 3**.

Duration

The rmANOVAs revealed a significant main effect of class ($F = 8.188$, $P < 0.001$), reflecting by class A had a larger duration than class B ($P < 0.003$), class C ($P < 0.001$), and class D ($P < 0.010$). No significant main effect was found for condition ($F = 0.190$, $P = 0.664$) and group ($F = 0.031$, $P = 0.861$). There was a significant interaction between condition and class ($F = 8.442$, $P < 0.001$) (for class A, resting > cue-induced, $P < 0.001$; for class B, class C, and class D, between conditions, all P-values ≥ 0.121). No significant interaction was found between group and condition ($F = 0.031$, $P = 0.861$). There was a significant interaction between group and class ($F = 6.363$, $P = 0.001$), which was modulated by condition ($F = 3.165$, $P = 0.031$). For the sake of simplicity, further analyses were performed for each class separately.

As shown in **Figure 4**, for class A, cue-induced condition elicited a larger decrease of class A in Meth than HC (test for interaction, $P = 0.012$). No significant interaction between condition and group was found for class B (test for interaction, $P = 0.359$) and class C (test for interaction, $P = 0.835$), respectively. A marginally significant interaction between condition and group was found for class D ($F = 3.481$, $P = 0.067$). Cue-induced condition elicited an increasing tendency of class D in Meth, but a decreasing tendency of class D in HC. More details of multiple comparisons are shown in **Figure 3**.

Occurrence

The rmANOVAs revealed a significant main effect of class ($F = 6.101$, $P = 0.001$), reflecting by the occurrence of class C was significantly lower than class A ($P = 0.024$) and class D ($P = 0.014$). No significant main effect of condition ($F = 0.083$, $P = 0.774$) and group ($F = 0.618$, $P = 0.435$) was found. There was a significant interaction between condition and class ($F = 10.969$, $P < 0.001$), which was mainly explained by a decrease in the occurrence of class A ($P < 0.001$), but an increase in the occurrence of class B ($P < 0.001$), from resting state to

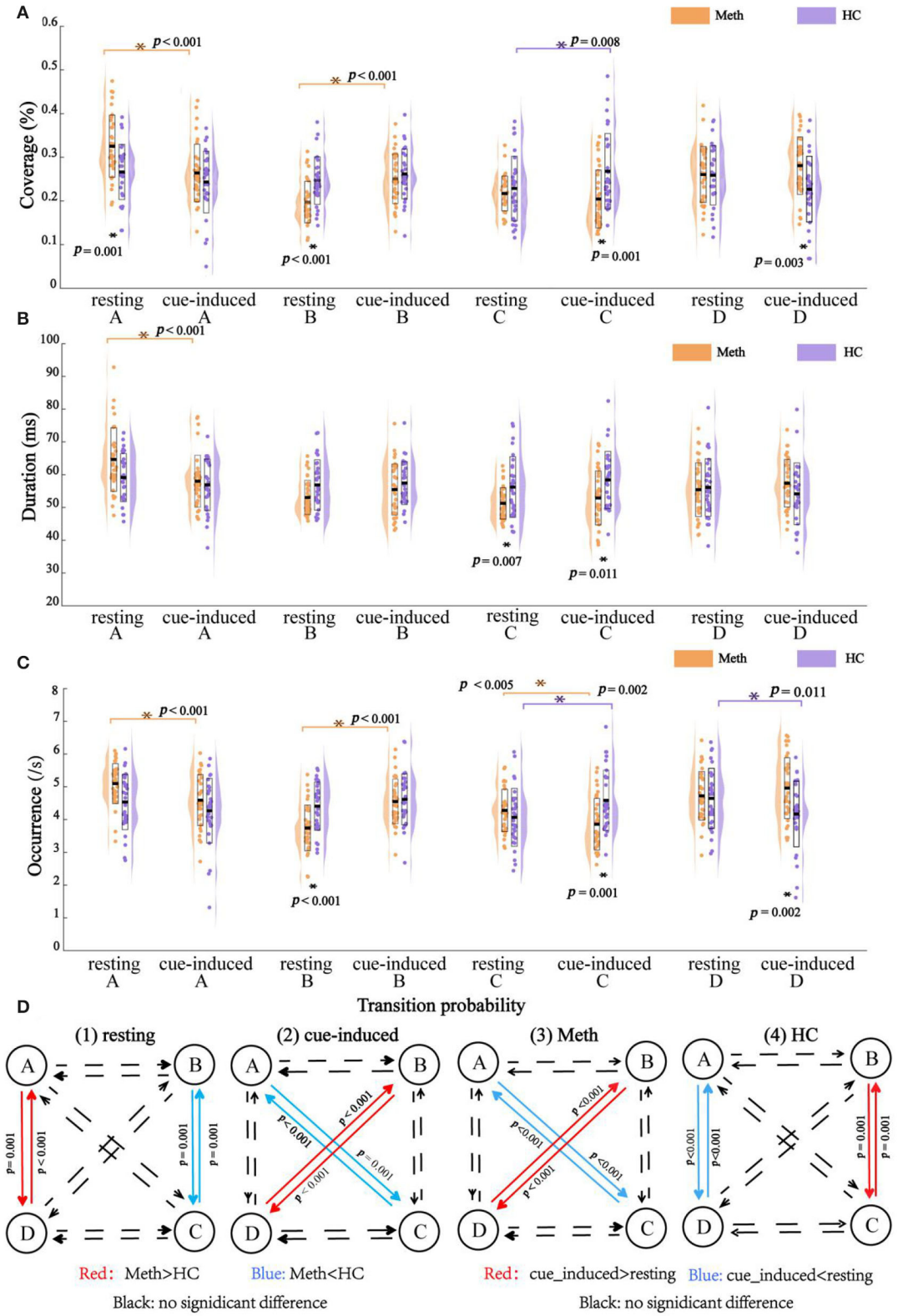

FIGURE 3 | Outcomes of electroencephalography microstates with multiple comparisons. **(A)** Coverage, **(B)** duration, **(C)** occurrence, and **(D)** transition probability. Dots indicate the microstates feature and outline of violin plot represents the kernel probability density estimation. Significant *post-hoc* results between groups or between conditions are marked by asterisks.

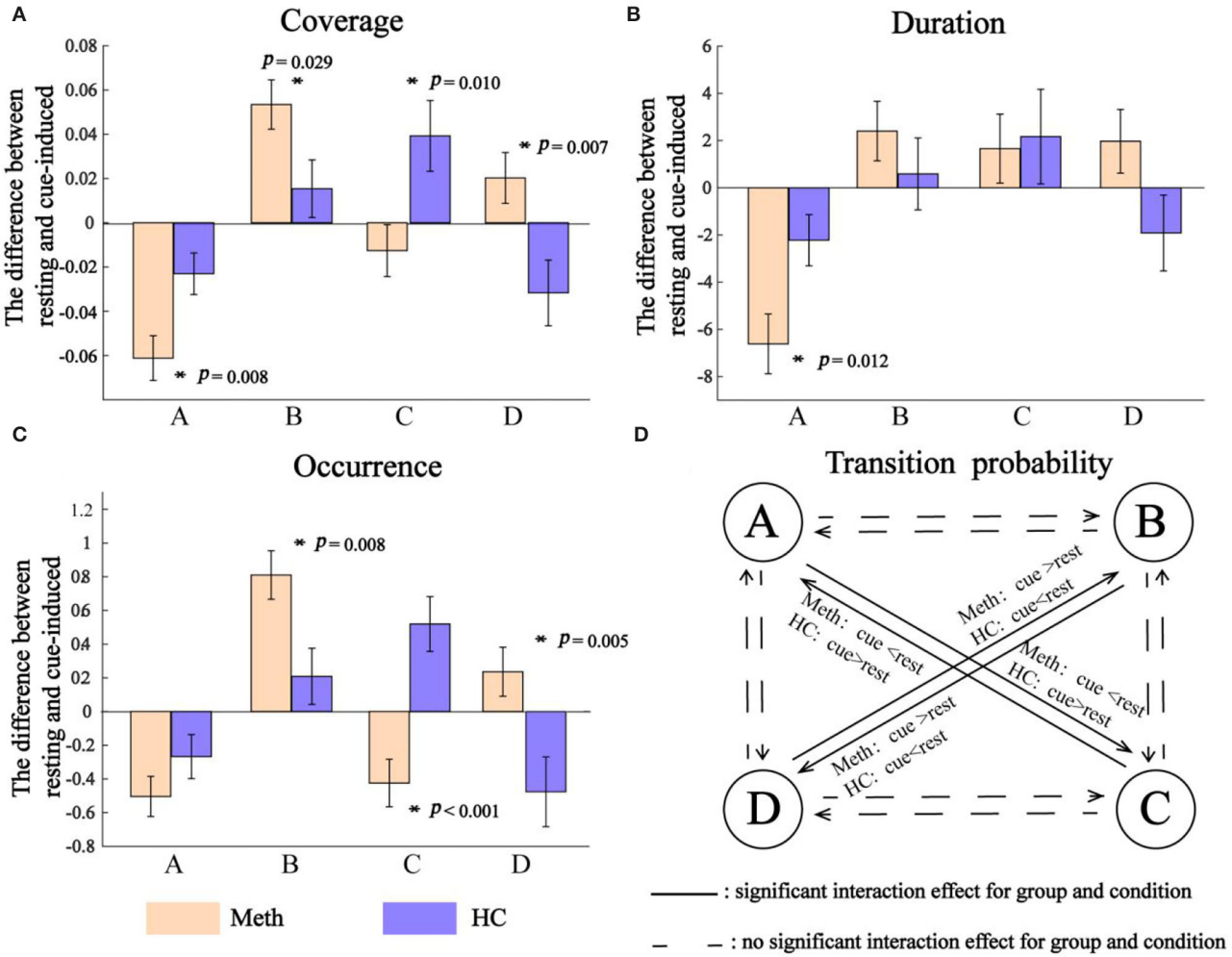

FIGURE 4 | Interaction between group and condition. **(A)** Coverage, **(B)** duration, and **(C)** occurrence. Positive value indicates an increasing microstates feature from resting to cue-induced condition, while negative value represents a decreasing microstates feature. Significant interaction between group and condition is indicated by asterisks. **(D)** Transition probability. The solid line indicates a significant interaction between group and condition for transition probability of A→ C, C→ A, B→ D, and D→ B, respectively.

cue-induced condition. No significant interaction was found between group and condition ($F = 0.142$, $P = 0.707$). There was a significant interaction between group and class ($F = 6.231$, $p = 0.001$), which was modulated by condition ($F = 11.699$, $P < 0.001$). For the sake of simplicity, further analyses were performed for each class separately.

As shown in **Figure 4**, cue-induced condition elicited different pattern results across classes. For class A and class B, cue-induced condition elicited changes in the same direction for Meth and HC. There was a significant decrease of class A and a significant increase of class B in Meth (both $P < 0.001$), along with a decreasing tendency of class A and an increasing tendency of class B for HC. However, cue-induced condition elicited opposite changes in class C and class D for Meth and HC, respectively. The cue-induced condition significantly decreased the occurrence of class C in Meth ($P = 0.005$), but significantly increased the occurrence of class C in HC ($P = 0.002$). On the contrary, cue-induced condition elicited an increasing tendency of class D in Meth, but a significant decrease of class D in HC (test for interaction, $P = 0.011$). More details of multiple comparisons are shown in **Figure 3**.

Transition Probability

The rmANOVAs revealed a significant main effect of transition pairs ($F = 5.510$, $P = 0.002$) (but pairwise comparisons, all P-values ≥ 0.051). No significant main effect of condition ($F = 0.029$, $P = 0.865$) and group ($F = 1.013$, $P = 0.318$) was found. There was a significant interaction between condition and pairs ($F = 10.630$, $P < 0.001$) (for A→ C, C→ A, A→ D, and D→ A, resting > cue-induced, all P-values ≤ 0.011; for B→ C, C→ B, B→ D, and D→ B, resting < cue-induced, all P-values ≤ 0.001; other comparisons between conditions, all P-values $\geq$

0.225). No significant interaction of condition and group ($F = 0.021$, $P = 0.885$) was found. There was a significant interaction between pairs and groups ($F = 6.094$, $P = 0.001$), which was modulated by condition ($F = 10.006$, $P < 0.001$). For the sake of simplicity, further analyses were performed for each transition pair separately.

As shown in **Figure 4**, cue-induced condition elicited changes in transition probability between class B and class D (i.e., B→ D and D→ B) in the opposite direction for Meth and HC (test for interaction, all *P*-values < 0.001). Meth-dependent patients showed a significantly higher transition probability between class B and class D during cue-induced condition compared to resting state (all *P*-values ≤ 0.004), while HC showed a tendency of lower transition probability between class B and class D during cue-induced condition. In addition, cue-induced condition also elicited changes in transition probability between class A and class C (i.e., A→ C and C→ A) in the opposite direction for Meth and HC (test for interaction, all *P*-values < 0.001). Meth-dependent patients showed a lower transition probability between class A and class C during cue-induced condition compared to resting state (all *P*-values < 0.001), while HC showed a tendency of higher transition probability between class A and class C during cue-induced condition. More details of multiple comparisons are shown in **Figure 3**.

DISCUSSION

The goal of this study, for the first time, was to examine the effects of exposure to drug-related cues on EEG microstates under VR environment. Consistent with our hypotheses, we found that both Meth-dependent patients and HCs showed an increase in the coverage and occurrence for class B during cue-induced condition. In addition, for Meth-dependent patients, cue-induced condition elicited a significant decrease of the occurrence for class C, along with an increasing tendency of the occurrence for class D. However, for HCs, the change direction of class C and class D was completely opposite to that of Meth-dependent patients. Finally, cue-induced condition elicited a significant decrease of the A→ C and C→ A transition pairs in Meth-dependent patients, while HC exhibited an increased transition probability. In contrast, cue-induced condition elicited a significant increase of the B→ D and D→ B transition pairs in Meth-dependent patients, while HC exhibited decreased transition probability.

The data support the first hypothesis; that is, one or more parameters associated with microstate class B would increase during cue-induced condition for both groups. For Meth-dependent patients, the coverage and occurrence of class B were significantly higher during cue-induced condition compared to the resting state. For HCs, although no significant differences were found, there was an increasing tendency of the coverage and occurrence of class B, from resting state to cue-induced condition. These are congruent with findings from prior works (24, 32, 42, 43), suggesting the association between microstate class B and visual system. In addition, we found that Meth-dependent patients showed a significant decrease in the coverage, occurrence, and duration for class A from resting state to cue-induced condition. HCs also showed a decreasing tendency of the coverage, occurrence, and duration for class A from resting state to cue-induced condition. Previous studies have proposed that class A is associated with auditory system [e.g., 31, 22]. Thus, we cautiously speculate that the decrease in class A might be interpreted as the relative reduction of auditory input under VR environment. The results of transition probability between classes provide further support for our speculation. Regarding transition probability, we observed a preference for transitions to microstate B (i.e., B→ C, C→ B, B→ D, and D→ B) during cue-induced condition, compared to an eyes-open resting state. In contrast, the transitions to microstate class A (i.e., A→ C, C→ A, A→ D, and D→ A) were significantly lower during cue-induced condition, compared to an eyes-open resting state.

The findings also mostly support the hypotheses with regard to microstate class C and class D. For Meth-dependent patients, cue-induced condition elicited an increasing tendency of the occurrence for class D. In addition, for Meth-dependent patients, we also found a significantly higher transition probability between class B and class D (i.e., D→ B and B→ D) during cue-induced condition, compared to resting state. On the contrary, for Meth-dependent patients, cue-induced condition significantly decreased the occurrence of class C. In addition, the transition probability between class A and class C was significantly lower during cue-induced condition among patients. These findings favor the proposal that class C and class D reflect task-negative network and task-positive network, respectively (22, 24, 43). As reported by previous fMRI studies, Meth-dependent patients showed the hyperactivity of executive and attention networks under exposure to drug-related cues (11, 12, 17, 44, 45). The increase of class D and decrease of class C in Meth-dependent patients could be explained by their recruitment of cognitive resources when exposed to drug-related cues.

Most impressively, our findings revealed that HCs showed completely different result patterns regarding class C and class D. The change direction of class C and class D from resting state to cue-induced condition for HCs was opposite to Meth-dependent patients. Specifically, there were significantly higher coverage and occurrence of class C during cue-induced condition, along with significantly lower occurrence of class D. In addition, there was an increasing tendency of transition probability between class A and class C (i.e., A→ C and C→ A) during cue-induced condition, along with a decreasing tendency of transition probability between class B and class D (i.e., D→ B and B→ D). Taken together, the results with regard to class C and class D favor that "there may be a functionally relevant balance between microstates C and D, and that a preponderance of microstate C may result in a progressive detachment of mental states from environmental input" (22). Previous studies have also evidenced that relaxed, meditative, and hypnotic states were associated with an increase in class C, along with a decrease in class D (22–24, 46, 47). In addition, in our previous study (36), we also found a dissociation of the effects of drug-related cues on HRV between Meth-dependent patients and HCs. Drug-related cues induced a larger HRV for Meth-dependent patients, but a lower

HRV for HCs. The relaxed, meditative, and hypnotic states, as mentioned earlier, are mostly associated with increased activity of parasympathetic nervous, which is manifested as decreasing of HRV. Taken together, the observed findings of the current work and our previous study based on HRV (36) seem toward the association between EEG microstates and HRV. This is still understudied and should be addressed in further studies.

Finally, we also found that there were significant differences between Meth-dependent patients and HCs during resting state. For example, Meth-dependent patients showed a higher coverage of class A compared to HCs, along with a lower coverage and occurrence of class B. Although the discussion of between-group differences in the resting state is beyond the scope of this study, we found that these findings are mostly inconsistent with the previous study by Chen et al. (34). In their study, they reported that Meth-dependent patients showed a lower duration of the microstate classes A and B, compared to HCs. We have speculated that this inconsistency may be related to different conditions between Chen et al.'s study (i.e., eyes-closed resting state) and the present study (i.e., eyes-open resting state). As evidenced by previous study, there were significant differences between eyes-closed state and eyes-open condition on the parameters of microstate (24).

Several limitations of this study should be noted. First, only men were enrolled in the study, and the sample size was small, which limited the generalization of results. Considering that male and female Meth-dependent patients were accommodated separately in China, multicenter and large-scale sample studies will be needed in the future. Second, to maintain context within the broader body of microstate literature, we limited our analyses to 4 model microstate classes for each experimental condition. Despite these drawbacks, our preliminary findings support that immersing patients in a Meth-related virtual social-context environment can successfully affect their EEG microstates. Further studies can build a classifier based on EEG microstate features of the significant differences between HC and Meth-dependent patients to distinguish HC and Meth-dependent patients as a potential, supplementary quantitative diagnostic tool for Meth dependence. In addition, it will be helpful to combine EEG, eye movement trajectory, and ECG, which might further deepen our understanding of the neurophysiological mechanism underlying the Meth craving.

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 Seventh Hospital of Hangzhou. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

QL contributed to data collection, data analysis, writing of original draft, manuscript redaction, and revisions. DL and CH contributed to data collection. ZS contributed to the study design. YW contributed to study design, manuscript redaction, and revisions. All authors contributed to the article and approved the submitted version.

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The Effects of Immediate vs Gradual Reduction in Nicotine Content of Cigarettes on Smoking Behavior: An Ecological Momentary Assessment Study

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Background: In recent years, much research has examined the effects of various interventions and treatments for smoking cessation. The results suggest that interventions targeting changes of nicotine content can help smokers reduce tobacco use or quit smoking. A number of clinical studies show that smokers who received an immediate reduction in nicotine content to very low levels have significantly greater reductions in the number of cigarettes smoked and toxic substance exposure compared to those with gradual reductions. However, from the perspective of smoking craving, whether the immediate and gradual reduction in nicotine content reduce smoking by reducing cravings needs further investigation.

Methods: 74 eligible Participants were randomly allocated to one of the two experimental conditions: (1) immediate reduction to 0.1 mg of nicotine per cigarette (n = 40); (2) gradual reduction from 1.0 (0.8 g ~ 1.2 mg) to 0.1 mg of nicotine per cigarette (n = 34). All participants completed 1-week baseline period during which they smoked their usual cigarette, followed by 16-week of interventions. The primary outcomes included cigarette cravings and number of cigarettes smoked per day (CPD); secondary outcomes included the number of cigarette-free day and emotional states.

Results: Among the 52 participants [51 (98.1%) men; mean (SD) age, 33.44 (6.71) years; mean (SD) CPD, 16.83 (9.94)] who completed the trial, significantly lower cravings for cigarettes were observed in the immediate (n = 25) vs. gradual nicotine reduction group (n = 27) in the morning (t = −2.072, p = 0.039) and after dinner (t = –2.056, p = 0.041). Compared with the baseline daily smoking, the number of cigarettes smoked per day was significantly reduced at the beginning of week 12 in the immediate nicotine reduction group (p = 0.001) and at week 16 in the gradual nicotine reduction group (p < 0.001). The number of participants with any cigarette-free day was not significantly different between the groups (p = 0.198). The number of cigarette-free days was significantly more in the immediate vs. gradual nicotine reduction group (p = 0.027).

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Conclusions: The significantly lower cravings were observed in the immediate vs. gradual nicotine reduction group, and led to faster reduction in the number of CPD, and a significant increase in the number of cigarette-free days.These findings add to the evidence base for reduced nicotine content in cigarettes.

Clinical Trial Registration: ClinicalTrials.gov, identifier: ChiCTR2100048216.

Keywords: immediate nicotine reduction, gradual nicotine reduction, craving, smoking behavior, ecological momentary assessment

INTRODUCTION

Smoking remains one of the leading causes of morbidity and premature death worldwide (1–5). Long-term smoking can affect many systems of the body, resulting in serious and life-threatening diseases, such as ischemic heart disease, chronic obstructive pulmonary disease, tracheal cancer, bronchial cancer, lung cancer, stroke, etc., (5–11). According to data from the Global Burden of Disease (GBD), in 2019, the number of smokers worldwide increased to 1.1 billion, and smoking caused 7.7 million deaths worldwide. In China, the number of smokers reached 341 million (30%), and smoking causes around 2.4 million deaths a year (5). The harmful lifelong consequences of smoking lead to huge public health costs. It is estimated that smoking causes economic losses of over US $500 billion annually worldwide (12).

Nicotine is the main addictive component of cigarettes (2, 13–15). The essence of smoking addiction is nicotine dependence (16) characterized in DSM-IV, by impulsive use, discontinuation difficulty, and withdrawal symptoms after chronic use, and craving which is one of the core symptoms of nicotine addiction (17–22). Craving is common among smokers (23). Long-term use of nicotine can induce changes in the neuroplasticity of the cortex and striatum, thus forming a strong and lasting memory of nicotine addiction, resulting in a continuous craving for cigarettes (24). The existence of craving directly leads to a series of adverse consequences such as smokers' failure to quit smoking or susceptibility to relapse (25, 26). Craving is an important indicator for maintaining addictive behavior and predicting relapse after withdrawal (17, 27–35). Moreover, some studies have found that craving can significantly predict the withdrawal rate after treatment (36, 37), and therefore nicotine craving has become a criterion for estimating the effectiveness of treatment (38). Although the mechanism of craving is not completely clear, it has become an important target for the treatment of smoking addicts (39). The pain point of smoking addiction is mainly manifested in the high relapse rate, and craving is the key factor in precipitating relapse (40, 41). Thus, reducing craving has become the main target in clinical smoking cessation.

Since craving is the main precipitator of relapse, creating new intervention content that targets cravings could greatly enhance the effectiveness of the treatment. In recent years, some researchers have proposed that reducing the content of nicotine in cigarettes is an effective strategy that reduces smoking and improves public health (2, 42–48). A gradual reduction in nicotine content is a potential way to reduce the addiction to cigarettes and promote smokers to quit smoking (42, 44, 49–51). Multiple studies have shown that gradual reduction in nicotine content reduces nicotine intake, without increased exposure to tobacco toxins, and without significant "compensatory" smoking (43, 52–55). The gradual reduction in nicotine content is considered a possible smoking cessation approach (42, 44, 49–51), but it may take a long time to realize the potential health benefits (42, 56). Recent studies have found that reducing nicotine levels faster may be the same even more effective than gradual reduction (45). There is growing evidence showed that immediate reduction in nicotine content reduces the number of cigarettes smoked per day (45, 47, 57, 58), reduces exposure to toxic substances (43, 45, 52, 57–59), reduces nicotine dependence (45, 47, 57, 58), increases smoking cessation attempts (43, 45, 52, 57–59), and "compensatory" smoking is rare compared with the use of traditional nicotine cigarettes (43, 45, 52, 57, 60, 61). A comparison of the two reduction methods showed that the immediate reduction in nicotine content has a significant advantage, as it results in less exposure to toxic substances (60–64), less smoking per day (61), less nicotine dependence (56, 61), and more cigarette-free days (60, 61, 64) over time. The answer to the question of whether the gradual and immediate reduction in nicotine content reduces smoking by reducing craving is still unknown and thus has to be answered. Especially the dynamic changes in cravings shall be assessed, and therefore it is necessary to examine the relationship between daily craving changes and smoking behavior in real-time.

Ecological Momentary Assessment (EMA) is an innovative approach developed for real-time data collection, which greatly improves the field's understanding of the cognition, emotion, and behavior of smokers as they occur in the natural environment (65). The advantages of the EMA approach over retrospective self-reporting include more accurate tracking of smoking frequency and patterns, more detailed capturing of smoking cravings, and high ecological validity of the data (65–67). Since both craving and substance use are situational phenomena related to emotion and environment (68–70), measuring these variables in daily life may lead to more reliable answers. Therefore, in this study, EMA was used to assess daily craving changes and smoking behavior in real-time.

The main goal of this study was to examine the effects of the immediate and gradual reduction in nicotine content in cigarettes on cigarette craving, as well as to observe changes in smoking behavior. The main hypothesis of this research was that significantly lower cravings and lowered number of cigarettes

smoked per day will be observed in the immediate vs. gradual nicotine reduction group for craving.

METHODS AND MATERIALS

This study has been approved by the Medical Ethics Committee of Shougang Hospital of Peking University and has been registered in the Chinese Clinical Trial Registry. All participants provided informed consent after they were qualified to participate.

Study Cigarettes

To avoid the problem that offering free cigarettes increases smoking or increases the use of cigarettes with regular nicotine content, cigarettes consumed in the study were purchased by participants at designated regular tobacco companies. Study cigarettes, both menthol and non-menthol, are all of the same brand. In a study, researchers examined commercial low yield cigarettes and found that little change was seen in plasma cotinine concentration from 0.9 to the 0.4 mg nicotine yield cigarettes, suggesting compensation in smoking behavior. However, significant decreases plasma cotinine concentration and carcinogen exposure biomarker levels were observed when smokers were switched to 0.1 mg nicotine cigarettes, most likely due to the extensive filter ventilation of these "ultra-low yield" cigarettes, too much to be overcome by compensation. In addition, the 0.1 mg nicotine cigarette also produced non-significantly greater withdrawal (54). Other related studies have shown that 0.1 mg nicotine cigarettes can reduce the amount of smoking and exposure to harmful substances (52, 71, 72). Thus, the cigarette with a nicotine content of 0.1 mg was used in the immediate reduction group. Cigarettes with nicotine content of 0.6 mg (43, 53, 73–77), 0.3 mg (53, 57, 73, 75–77) and 0.1 mg (52, 71, 72) were selected in the gradual reduction group.

Participants

Participants were recruited from Daxing District, Beijing, by handing out flyers and advertising on WeChat moments. Inclusion criteria included participants meeting the legal age for buying cigarettes (18 years old); the average daily smoking amount $\geq$5 cigarettes for at least 1 year; no intention to quit smoking in the past 30 days; and stable mental and psychiatric conditions. Exclusion criteria included participants who intend to quit smoking within the next 30 years; regular use of tobacco products other than cigarettes; current use of nicotine replacement or other tobacco products for cessation; symptoms of severe mental or medical illness during the past 3 months; and being pregnant or breastfeeding. A total of 94 people applied for participation, of which 74 eligible participants were included. One blinded researcher (XJC) who had no direct contact with the participants did a computer-generated randomization to assign participants to one of the two groups.

Study Design

This study was a randomized parallel experiment (**Figure 1**). Participants ($N = 74$) were randomly assigned to 1 of 2 experimental conditions: (1) immediate reduction to 0.1 mg of nicotine per gram of tobacco cigarettes ($n = 40$); (2) gradual reduction from 1.0 (0.8 $\sim$ 1.2 mg) to 0.1 mg of nicotine per gram of tobacco cigarettes ($n = 34$).

Procedure

Cigarette smokers were contacted by the researcher and were screened for eligibility over the telephone. Participants were told that the goal of the study was to examine how changes in nicotine content in cigarettes affect smoking behavior over time. They were also told that if they participate in the research, they would need to buy their own cigarettes during the course of the study. Eligible participants completed a baseline period of 1 week and then were randomly assigned to 1 of 2 experimental conditions for 16 weeks. Participants smoked their usual brand of cigarettes during the baseline period and used the cigarettes they were assigned to purchase during the 16-week experimental phase. In the immediate reduction group, the participants were required to smoke 0.1 mg of nicotine per gram of tobacco cigarettes for 16 weeks. In the gradual reduction group, the participants were asked to reduce the nicotine intake once every 4 weeks (0.6 mg of nicotine per gram of tobacco cigarettes were used from week 1 to week 4; 0.3 mg of nicotine per gram of tobacco cigarettes were used from week 4 to week 8; and 0.1 mg of nicotine per gram of tobacco cigarettes were used from week 8 to week 16). During the experiment, participants were told to use the designated brands of cigarettes (Study cigarettes) and try not to use their usual brand of cigarettes (Non-study cigarettes). If both types of cigarettes are used, record them and inform the researchers. Participants were required to buy the designated cigarettes during the experiment to avoid the problem that providing free cigarettes would increase smoking or use of more cigarettes with regular nicotine content. At the end of the experiment, participants were paid according to their compliance with participating in the experiment, everyone was paid for ¥200—¥400.

EMA Assessment

During the baseline and experimental periods, this study used a score table to record the emotional state and craving degree of the participants before and after smoking in the morning and evening. The scoring method was 1–9 points to record emotional state (78), when the degree of craving was assessed, 1 indicated not wanting to smoke at all, and 9 indicated being very eager to smoke (**Table 1**). At the same time, the number of cigarettes smoked per day (study cigarettes and non-study cigarettes) was recorded on the score table.

All participants were asked to put a score table in the cigarette packs (**Table 1**). In the morning when they have their first cigarette, they were asked to timely estimate their emotional state and craving values before and after smoking; in the evening, when smoking their last cigarette, participants were requested to timely estimate their emotional state and craving values before and after smoking and write down the number of cigarettes they smoked that day. The following three ways were used to provide feedback on the daily smoking to the research staff. The first one was to directly send the information on the score table to the staff through WeChat or SMS on the same day; the second one

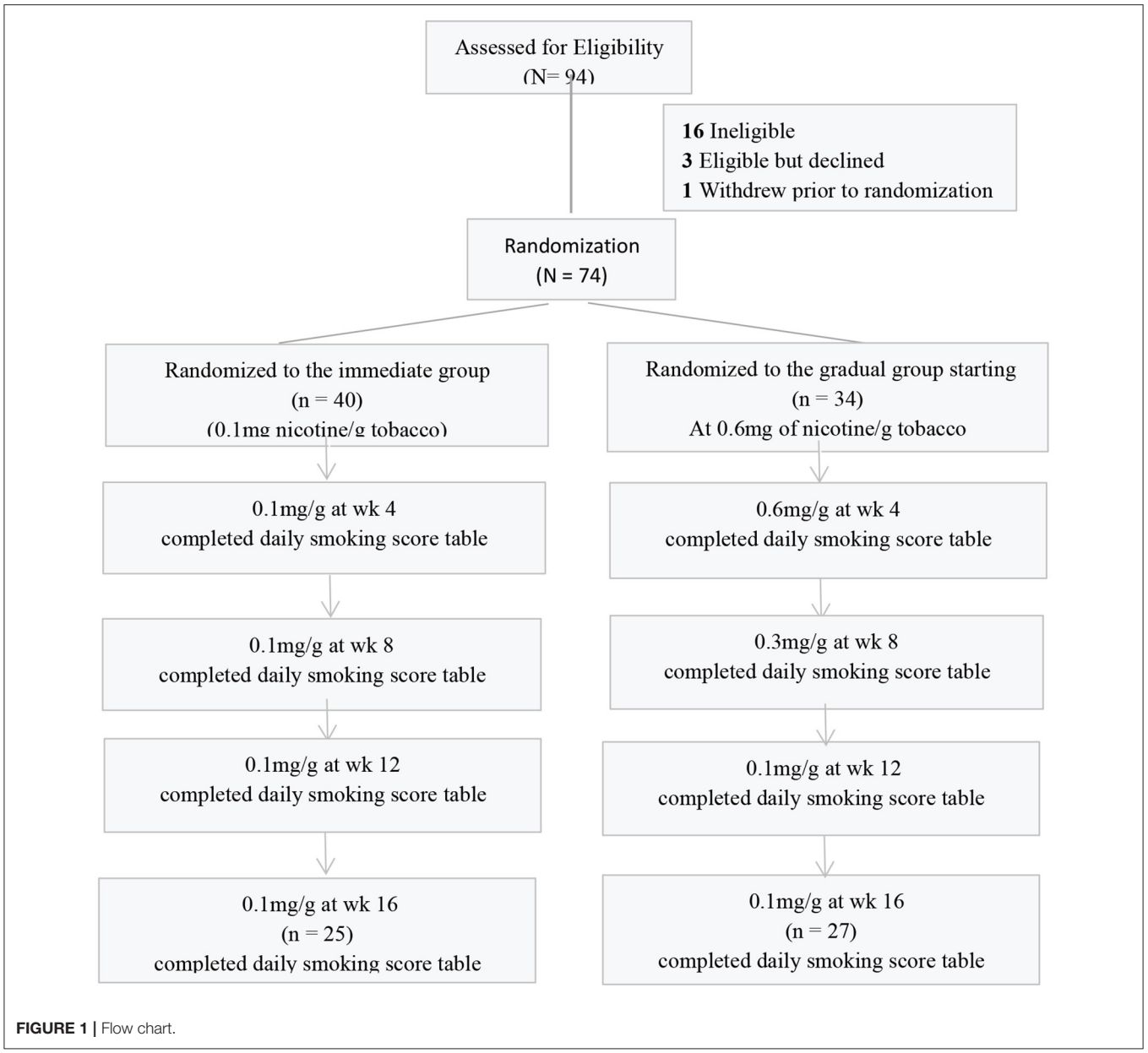

FIGURE 1 | Flow chart.

required the information on the score table to be filled into the questionnaire and submitted to the staff before going to bed every night. The third one required filling in the information on their own score table in an Excel file every day and sending it to the staff regularly.

Questionnaire Assessment

The following measures were taken at the baseline and after the intervention: Fagerström Test for Nicotine Dependence (FTND) (57), WHO Quality of Life-BREF (WHOQOL-BREF) (79); Self Rating Anxiety Scale (SAS) (80); Self-rating Depression Scale (SDS) (81). Profile of Mood States (POMS) (82). Demographic data and smoking history were collected at baseline.

FTND: A total of 6 items, and the score of the scale ranges from 0 to 10, with higher values indicating greater dependence. The degree of nicotine dependence can be divided into five levels: very low dependence (0–2), low dependence (3–4), medium dependence (5), high dependence (6–7), and very high dependence (8–10).

WHOQOL-BREF: An international scale developed by the World Health Organization to measure an individual's health-related quality of life. 5-point scale was used to measure the quality of life from four aspects: physical health, psychological, social relationships and environment. The higher the score in each area, the better the quality of life, and the most likely area scores are 35 (physical health), 30 (psychological), 15 (social relationships) and 40 (environment).

TABLE 1 | Daily smoking score table.

The number of non-study cigarettes	The number of study cigarettes	Time	Emotion		Craving degree	
			Before smoking	After smoking	Before smoking	After smoking
		Morning				
		Evening				

SAS: It is used to evaluate the anxiety symptoms of adults. Compiled by W.K.Zung in 1971, there were 20 items and four grades.

SDS: It is used to evaluate the depressive symptoms of adults. Compiled by WilliamW.K.Zung in 1965, with 20 items and four grades. The severity of depression was measured from 4 aspects: psycho-emotional symptoms (2 items), somatic disorders (8 items), depressive psychological disorders (8 items) and psychomotor disorders (2 items).

POMS: The Chinese Profile of Mood States (POMS) revised by Zhu Beili (83) contains 40 items with a grade of 5. It contains seven dimensions: tension, anger, fatigue, depression, energy, panic and self-related emotions. The reliability is between 0.60 and 0.82.

Statistical Analysis

SPSS 21.0 data analysis software was used for statistical analysis. The primary end points of this study need to be evaluated continuously every day, and the intervention time is as long as 16 weeks. Most of the dropouts dropped out of the experiment in the first few days of the intervention period, and the real-time data of the primary end points were greatly missing, and there was no late follow-up data. Thus, the analysis method used in this study is per-protocol (PP) analysis, that is, participants with good compliance and completion of the study were analyzed. Chi-square test and independent-sample *T*-test were used to analyze the differences demographic characteristics of the two groups of participants. A Chi-square test was used to compare the completion rates at week 16. Changes in cigarette craving and emotion were analyzed using a generalized linear mixed model, and the number of cigarettes was analyzed using repeated-measures analysis of variance. A Chi-square test and negative binomial regression analysis were used to analyze the number of participants with any cigarette-free day, the number of cigarette-free days among all participants. Subjective reports were analyzed by an independent sample *T*-test.

RESULTS

Demographic Characteristics and Smoking History

A total of 74 participants (40 in the immediate reduction group and 34 in the gradual reduction group) were eligible, and 52 participants completed the experiment (25 in the immediate reduction group and 27 in the gradual reduction group). The completion rate of the immediate reduction group was 62.5%, and the completion rate of the gradual reduction group was 79.4%. The dropout rate of the immediate group is 37.5% ($n = 15$), and that of the gradual group is 20.6% ($n = 7$). In the later stage of follow-up, the dropout of participants in the immediate group was mainly due to the poor adaptability of some participants to very low nicotine cigarettes, and adverse events such as dizziness and nausea occurred in the first few days of using very low nicotine cigarettes, resulting in negative emotions of participants, which resulted in participants quit the intervention. The gradual group being resistant to changing cigarettes during the nicotine content change from 0.6 mg to 0.3 mg nicotine cigarettes, resulting in more dropout.

Table 2 shows the demographics and smoking history of the two groups. There is no significant difference between the two groups in demographics and smoking history, indicating that the participants in the two groups were similar (**Table 2**).

Smoking Cravings and Emotional Change in the Immediate Reduction vs. Gradual Reduction Group

The changes of cigarette craving after getting up in the morning and after dinner (before smoking-after smoking) were analyzed by generalized linear mixed model. The results showed that the smoking cravings after getting up in the morning were significantly lower in the immediate vs. gradual nicotine reduction group (12 week, $t = -2.091$, $p = 0.038$; 16 week, $t = -2.072$, $p = 0.039$) after 12 weeks of intervention; and the smoking cravings after dinner were significantly lower in the immediate vs. gradual nicotine reduction group (16 week, $t = -2.056$, $p = 0.041$) after 16 weeks of intervention. Smoking cravings were significantly lower in the immediate reduction group at 4 week ($t = 5.789$, $p < 0.001$), 8 week ($t = 6.386$, $p < 0.001$), 12 week ($t = 5.227$, $p < 0.001$), and 16 week ($t = 4.861$, $p < 0.001$) of intervention vs. baseline smoking cravings after getting up in the morning. Smoking cravings were not significantly different in the gradual reduction group at 4 week ($t = 1.593$, $p = 0.112$) of intervention vs. baseline smoking cravings after getting up in the morning, but were significantly reduced at 8 week ($t = 3.440$, $p = 0.001$), 12 week ($t = 2.442$, $p = 0.015$), and 16 week ($t = 2.464$, $p = 0.014$) of intervention. Smoking cravings were significantly lower in the immediate reduction group at 8 week ($t = 2.385$, $p = 0.018$) of intervention vs. baseline smoking cravings after dinner, but no significant difference were observed at other time periods (4 week, $t = 1.837$, $p = 0.068$; 12 week, $t = 1.559$, $p = 0.120$; 16 week, $t = 1.865$, $p = 0.063$). Smoking cravings were significantly lower in the gradual reduction group at 8 week ($t = 2.183$, $p = 0.030$) of intervention vs. baseline smoking cravings after dinner, but no significant difference were observed at other time periods (4 week, $t = 1.545$, $p = 0.124$; 12 week, $t = 1.435$, $p = 0.153$; 16 week, $t = 1.358$, $p = 0.0176$) (**Figures 2A,B**).

The emotional changes of smoking after getting up in the morning (before-after smoking) were analyzed. The results showed that there was no significant difference between the immediate reduction group and the gradual reduction group ($t = -1.285$, $p = 0.200$). The emotional changes of smoking after

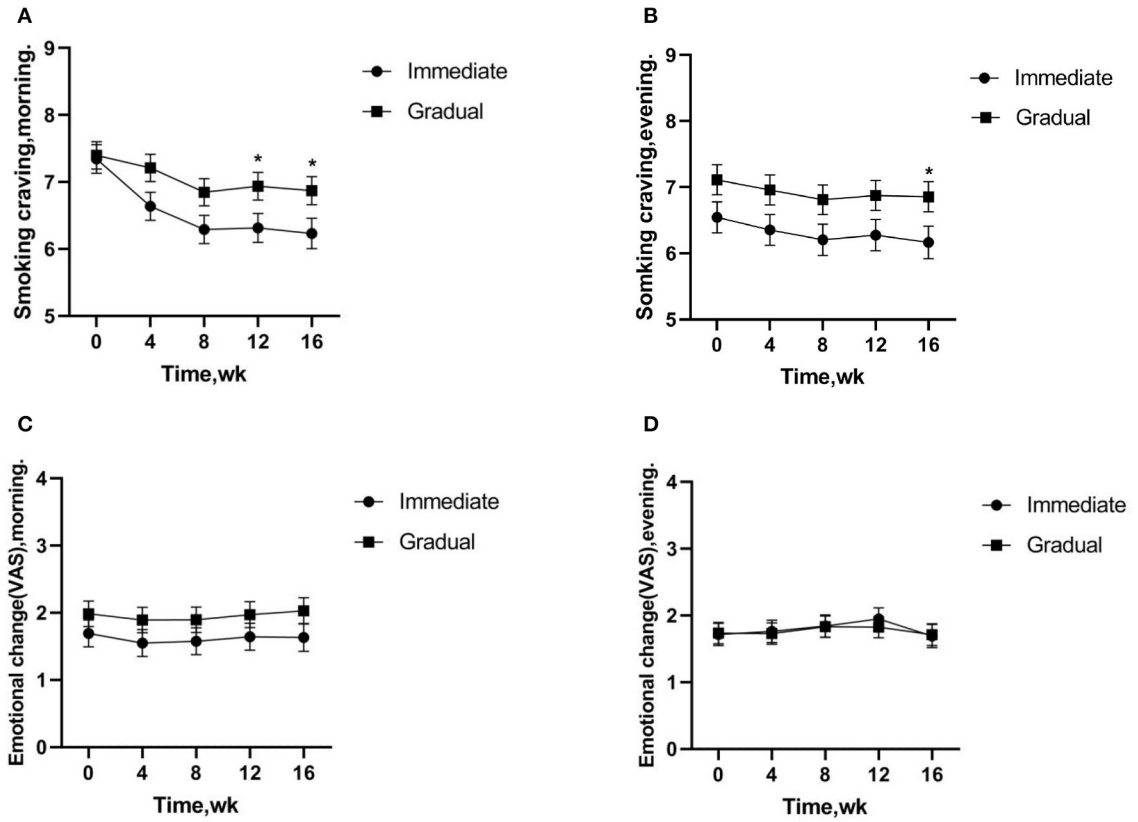

FIGURE 2 | (A,B) Smoking cravings indicate changes in cravings before-after smoking after getting up in the morning and after dinner, respectively. **(A,B)** Significantly lower cravings were observed in the immediate vs. gradual nicotine reduction group. **(C,D)** The emotional changes of smoking indicate changes in cravings before-after smoking after getting up in the morning and after dinner, **(C,D)** there was no significant difference between the immediate reduction group and the gradual reduction group. *$p < 0.05$.

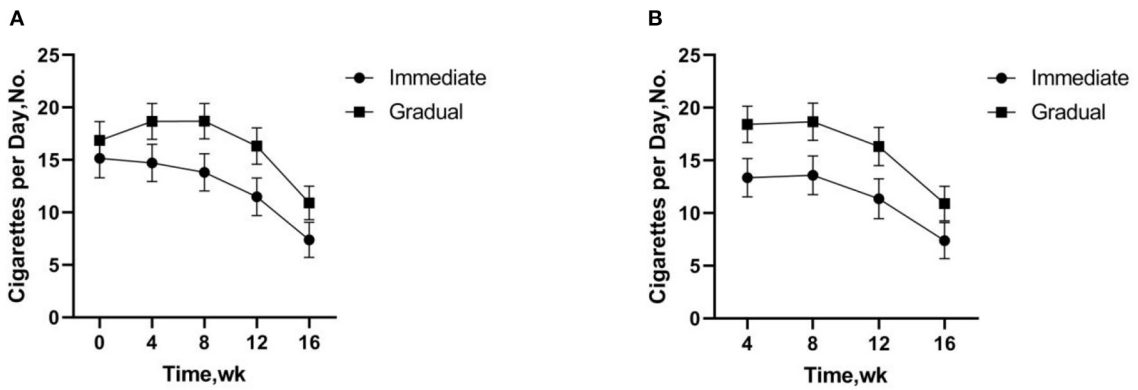

FIGURE 3 | (A) Total cigarettes per day includes study cigarettes and non-study cigarettes. **(B)** Study Cigarettes are low nicotine content cigarettes used in the experiment. **(A,B)** There was no significant difference in the number of cigarettes per day between the immediate and gradual reduction group, but the number of cigarettes per day fewer more quickly in the immediate reduction group compared to baseline.

TABLE 2 | Demographics and smoking history.

	Immediate reduction group $n = 25$ (%)	Gradual reduction group $n = 27$ (%)	χ^2	p
Male	25 (100)	26 (96.3)	0.944	0.331
Married	21 (84)	21 (78)	0.324	0.569
Education				
≤High school	7 (28)	10 (37)	0.482	0.488
>High school	18 (72)	17 (63)		
	M (*SD*)	**M (*SD*)**	***F***	***p***
Age	34.48 (5.77)	32.48 (7.46)	0.560	0.288
Cigarettes per day	15.88 (10.89)	17.7 (9.09)	1.693	0.514
Years of regular smoking	14.72 (7.22)	13.74 (7.58)	0.000	0.634
FTND[a]	3 (2.68)	3.81 (2.68)	0.230	0.278
WHOQOL-BREF[b]				
Physical health	56.71 (10.07)	53.17 (11.31)	1.833	0.240
Psychological	55.5 (11.52)	57.72 (12.27)	0.029	0.506
Social relationships	58.67 (14.92)	58.33 (14.80)	0.062	0.936
Environment	52.75 (9.65)	55.90 (13.04)	2.260	0.330
Brief POMS[c]	111.36 (16.51)	107.37 (15.99)	0.326	0.380
SAS[d]	38.35 (7.68)	42.04 (8.23)	0.041	0.102
SDS[e]	52 (9.56)	53.47 (8.10)	0.186	0.551

[a] *The FTND scale ranges from 0 to 10, with higher scores indicating greater nicotine dependence.*
[b] *The WHOQOL-BREF produces scores for four domains related to quality of life: physical health, psychological, social relationships and environment, with higher scores indicating better the quality of life.*
[c] *The Brief POMS, with higher total of emotional disturb indicating a more negative emotional state, that is, a more disturb, upset or dysfunctional mood.*
[d] *The SAS includes 20 items, with higher scores indicating greater anxiety level.*
[e] *The SDS includes 20 items, with higher scores indicating greater depression level.*

dinner (before-after smoking) were analyzed. The results showed a similar effect pattern ($t = 0.121$, $p = 0.904$) (**Figures 2C,D**).

Total Number Cigarettes per Day (CPD) Change in the Immediate Reduction vs. Gradual Reduction Group

Two-factor repeated measurement ANOVA was used to compare the reduction effect of different intervention groups after 16 weeks of intervention. Significantly fewer numbers of total CPD were smoked in the immediate reduction group at weeks 12 ($p = 0.001$) and 16 ($p < 0.001$) vs. baseline smoking. Significantly increased numbers of total CPD were smoked in the gradual reduction group at weeks 4 ($p = 0.006$) and 8 ($p = 0.025$) vs. baseline smoking, but significantly fewer at weeks 16 ($p < 0.001$). The same effect pattern was observed in the study of cigarettes for several weeks.The number of cigarettes significantly fewer in both the immediate ($p < 0.001$) and gradual reduction group ($p < 0.001$) at the weeks 16 (**Figure 3**).

The Number of Cigarette-Free Days in the Immediate vs. Gradual Reduction Group

A Chi-square test was used to analyze the number of participants with any cigarette-free day. The number of participants with any cigarette-free day was not significantly different between the immediate vs. gradual reduction group ($p = 0.198$). Negative binomial regression analysis was used to analyze the number of cigarette-free days among all participants. The number of cigarette-free days among all participants was significantly higher in the immediate vs. gradual reduction group ($p = 0.027$) (**Table 3**).

Dependence, Quality of Life and Emotional Symptoms in the Immediate vs. Gradual Reduction Group

At week 16, significantly lower FTND scores were observed in the immediate vs. gradual reduction group (4.629, $p = 0.000$). There were no significant differences between the immediate vs. the gradual nicotine reduction group in four areas of WHOQOL-BREF scores: physical health ($t = 1.324$, $p = 0.191$), psychological ($t = -0.723$, $p = 0.473$), social relationships ($t = 0.093$, $p = 0.926$), and environment ($t = -0.966$, $p = 0.339$) at week 16. Total scores of emotional disturbances as assessed by the POMS were not significantly different for the immediate vs. gradual reduction group at week 16 ($t = 0.817$, $p = 0.418$). Anxiety symptoms scores as assessed by the SAS ($t = -1.622$, $p = 0.111$) and depression symptoms scores as assessed by the SDS ($t = -0.687$, $p = 0.495$) were not significantly different for the immediate vs. gradual reduction group at week 16 (**Table 4**).

TABLE 3 | Cigarette-free days at week 16.

Measures	Immediate	Gradual	Immediate vs. Gradual	
	No. (%) or Mean (SD)	No. (%) or Mean (SD)	Estimated OR/IRR (95% CI)	*p*
Any cigarette free day during weeks 0-16, No. (%)[b]	17 (68%)	18 (66.7%)	1.06 (0.33, 3.39)	0.918
Count of cigarette free days during weeks 0-16, mean (SD)[c]	24.40 (30.22)	13.00 (20.18)	0.533 (0.30, 0.93)	0.027[a]

[a]*p < 0.05 for the significant difference.*
[b]*Odds ratio (OR) was estimated based on unadjusted analysis; no abstinence being assumed for days with missing Interactive Voice Response (IVR) data.*
[c]*Incidence rate ratio (IRR) was estimated based on unadjusted negative binomial regression; no abstinence being assumed for days with missing IVR data.*

TABLE 4 | The questionnaire measures at week 16.

Measure	Immediate vs. gradual reduction group	
	Mean difference (95%CI)	*p*
FTND	0.166 (0.44, 1.10)	0.000
WHOQOL-BREF		
Physical health	3.95 (−2.04, 9.93)	0.191
Psychological	−2.37 (−8.96, 4.22)	0.473
Social relationships	0.38 (−7.89, 8.65)	0.926
Environment	−3.13 (−9.65, 3.38)	0.339
Brief POMS	3.65 (−5.32, 12.61)	0.418
SAS	−3.44 (−7.71, 0.82)	0.111
SDS	−1.66 (−6.53, 3.20)	0.495

DISCUSSION

The present study aimed to examine (1) smoking behavior, cravings and emotional change among smokers in their daily life using Ecological Momentary Assessment (EMA) (84–86); (2) compare the effectiveness of immediate vs. gradual reduction intervention; and (3) explore whether two different nicotine reduction methods affect smoking behavior by reducing craving from a mechanism point of view.

In this study, the immediate nicotine reduction compared with gradual nicotine reduction was associated with a faster decrease in cigarette cravings, lowered cigarette cravings, faster reduction in the CPD, and more cigarette-free days over time. However, the immediate nicotine reduction caused a higher dropout rate. The use of low nicotine cigarettes had no effect on the quality of life and emotional state of the participants.

When these 2 methods were compared in this study, the results demonstrated that with immediate nicotine reduction, the smoking craving reduction could be realized sooner than gradual nicotine reduction. Therefore, the immediate reduction method is possible to facilitate cessation of cigarettes as quickly as possible. Immediate reduction method is more effective than gradual reduction method, because nicotine immediate reduction method is more conducive to promote smokers to quit smoking, faster to achieve potential public health effects. In a large clinical trial involving 1,250 smokers from 10 academic institutions, immediate reduction in nicotine may achieve positive public health effects more quickly (61).

The results of the comparison of both approaches have shown that cigarette cravings were reduced faster and significantly in the immediate reduction group. This is consistent with other research (61, 87). However, previous studies used the smoking craving scale to assess cravings periodically during the intervention, rather than continuously assessing the dynamic changes of cravings during the intervention (61). Some studies suggested that craving is an instantaneous state that changes constantly, so it may be inaccurate to assess craving over a long period of time (88). In addition, some researchers suggest that craving is a measurable continuous state (89). Therefore, in this research, we utilized EMA, to continuously assess the dynamic changes of participants' cravings over the course of intervention. The results demonstrated that cravings were significantly lower and decreased faster in the immediate nicotine reduction group.

There was no significant difference in the number of CPD between the immediate and the gradual reduction group in the study. The results of this study are not consistent with those of previous studies (61, 64). Previous studies have shown that significantly fewer numbers of CPD in the immediate than the gradual reduction group (61, 64). One possible explanation is that the duration of this study was only 16 weeks, so there were no significant differences in smoking reduction between both interventions. Another possible explanation is that the sample size of this study was too small. Compared with the baseline, both the immediate and the gradual reduction groups were able to significantly reduce the number of CPD after 16 weeks of intervention, with a faster reduction in the number of CPD in the immediate reduction group. The results have shown that the number of CPD in the immediate reduction group was reduced during the intervention and significantly reduced at week 12, while the number of CPD in the gradual reduction group increased at weeks 4 and 8 and significantly reduced at week 16. There was a temporary increase in smoking in the gradual reduction group, possibly due to compensatory smoking in moderate nicotine cigarettes (45, 52, 53, 61).

In the comparison between the immediate vs. gradual reduction group, the results demonstrated significantly more cigarette-free days among all participants in the immediate reduction group. The results are consistent with those of Hatsukami et al. (61). Both intervention methods had no effect on the quality of life and emotional state of the participants, indicating that switching to low nicotine cigarettes may be more acceptable by participants who participated in the entire intervention.

There was a higher drop-out in the immediate group. However, the withdrawal rate was no different between the immediate vs. the gradual reduction group. Other studies have shown that immediate nicotine reduction is less satisfying (61, 87), leading to more severe withdrawal symptoms (56, 61) and a higher subjects' attrition rate (61, 87) than gradual nicotine reduction. The reason why there was no difference in compliance between the immediate and the gradual reduction group may be due to the gradual nicotine reduction group being resistant to changing cigarettes during the nicotine content change from 0.6 mg to 0.3 mg nicotine cigarettes, resulting in more subjects dropout. In this study, participants were free to choose blended / flue-cured 0.6 mg nicotine cigarettes. However, when switched to 0.3 mg nicotine cigarettes, only the blend cigarettes were available. 75.0% of the participants in this study were flue-cured cigarette users, and discomfort caused by different types of cigarettes made it easier for participants to drop out during this process.

Limitations

This study has several limitations. Firstly, the duration of this study was only 16 weeks, and the long-term effects of the two nicotine reduction methods were uncertain (61). Secondly, there was no follow-up at the end of the study. Third, the relatively small number of participants could limit the universality of the findings (43, 52). Furthermore, the average level of education of the participants was higher, and the universality of the findings is limited. Likewise, the selectivity of cigarette types was limited, which may affect the measurement of various outcomes. Moreover, the monitoring of adverse events (any negative changes in physical or mental health) and the measurement of withdrawal reaction were not carried out during the study.Seventh, in this study, most of the participants were male and there was only one female who completed the study, the study didn't compare the effectiveness of the two methods in male and in female, future studies could further compare the effectiveness of the two methods in male and in female. In addition, in this study, there is a lack of objective indicators to measure the intervention effect, and it may not be very comprehensive and objective to evaluate the intervention effect only from the self-report. in the future study, the intervention effect can be measured from multiple perspectives, and the mode of combining physiology with self-report can be adopted. Biomarkers such as cotinine can be used for physiological indicators. As well, in terms of efficacy analysis, per-protocol (PP) analysis may overestimate the efficacy. The interpretation of the results of this study is more applicable to participants who are more compliant with low nicotine cigarettes.

CONCLUSIONS

Among smokers, the immediate nicotine reduction group led to a faster and significant decrease in cigarette cravings, a faster reduction in the number of CPD, and a significant increase in the number of cigarette-free days among all participants in the gradual reduction group.

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

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

AUTHOR CONTRIBUTIONS

QL and YL contributed in conceptualization and methodology. YL supervised the study design and implementation. QL designed the experiment, analyzed data, wrote the paper, and revised the article. ZL designed experiment and collected data. XC provided methodological and substantive support throughout the manuscript process. MG, XC, and XL revised the article. All authors contributed to the article and approved the submitted version.

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Correlates of Aggression in Men With Methamphetamine Use Disorder: Childhood Trauma and Methamphetamine-Use Characteristics

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Background: Aggression is common among individuals with methamphetamine use disorder (MAUD) and constitutes a serious public health issue. The current study aimed to examine associations of methamphetamine-use characteristics and childhood trauma with aggression in men with MAUD.

Methods: This cross-sectional study was conducted from December 2017 to August 2018. MAUD patients recruited from a compulsory drug rehabilitation center (n = 360) and healthy comparison subjects (n = 604) completed a survey that measured aggression and childhood trauma, using the Chinese version of Buss-Perry Aggressive Questionnaire (AQ-CV), and the short form of Childhood Trauma Questionnaire (CTQ-SF), respectively. MAUD patients also provided information on methamphetamine-use characteristics such as the age of MAUD onset, MAUD severity, and co-occurring alcohol use disorder (AUD) and tobacco use disorder (TUD) using standard or self-designed questionnaires. Chi-square tests and *t*-tests were used to compare childhood trauma and aggression between the MAUD and comparison groups. Multiple linear regressions were conducted to determine correlates of overall aggression and its five sub-scales among the MAUD group.

Results: The MAUD group had higher childhood trauma and aggression scores than the comparison group. Within the MAUD group, age of MAUD onset, having severe MAUD, co-occurring AUD, co-occurring TUD, and childhood trauma were associated with overall aggression, with slightly different correlates found for its five sub-scales.

Conclusions: Our study shows a high level of childhood trauma and aggression in the MAUD group. Both methamphetamine-use characteristics (age of MAUD onset, severe

MAUD, co-occurring AUD/TUD) and childhood trauma were associated with aggression in MAUD. Our findings provide useful information on potential risk factors for aggression and inform future longitudinal research to establish causal relationships between these factors and aggression to guide further prevention and treatment programs.

Keywords: substance-related disorders, addictive behaviors, aggression, methamphetamine use disorder, childhood trauma, men, China

INTRODUCTION

Methamphetamine use disorder (MAUD) has become a serious public health problem globally. According to the 2021 World Drug Report, roughly 27 million people (0.5% of the global adult population aged 15–64 years) were estimated to have used amphetamines in 2019, with methamphetamine (MA) being the most used amphetamine-type stimulant (ATS) in East and South-East Asia (1). The China Drug Trend Report 2019 shows that 2.148 million people have illicit substance use disorders (SUDs) and 55.2% (1.186 million) of them have MAUD, indicating that MA is the most abused illicit substance in China (2). According to the China Drug Trend Report 2020, although the COVID-19 pandemic may have created conditions for or prevented drug abuse, MA remains the main abused illicit substance in China (3).

Aggression has been well documented as a common phenomenon among people with SUDs, especially MAUD (4, 5). Aggression is defined as behavior that is elicited by provocation, driven by anger, and intended to harm another person (6). Aggression represents a state phenomenon or a disposition to behave aggressively across various situations and over repeated occasions (7). Aggression may take many forms, ranging from relatively minor acts (e.g., pushing) to severe acts (e.g., stabbing or killing). Aggressive cognitions (e.g., hostile attitudes, beliefs, or thoughts) and emotions (e.g., feelings of anger) often serve as important precursors to aggressive behaviors. The relationship between MAUD and aggression is complex and likely explained by a wide range of psycho-socio-biological factors, among which MA-use characteristics and childhood trauma have been studied and reported (8–10).

Several MA-use characteristics have been identified as affecting aggression among patients with MAUD, including age at first use of MA, duration and frequency of MA use, route of MA administration, psychotic symptoms, and combined use of other substances such as alcohol. Early age at first use of MA increased the likelihood of engaging in MA-related violence after adjusting for other factors and socio-demographics (11, 12). In addition, chronic MA use (13), more frequent MA use (14), and MA injection (15) were all linked to difficulties controlling anger and the exhibition of more aggressive behaviors. Furthermore, MA use may increase positive psychotic symptoms such as hallucinations and delusions, which may create a sense of danger or threat and thus lead to defensive or pre-emptive aggression (14, 16, 17). Therefore, positive psychotic symptoms may further strengthen the risk of aggression in people with MAUD (5). Finally, the combined use of other substances, particularly alcohol, is common in people with MAUD, especially in nightlife environments where aggressive behaviors often occur and have thus been associated with aggression (18, 19). In summary, MA-use characteristics, including early age at first MA use, chronic and frequent MA use, MA injection, positive psychotic symptoms, and combined use of alcohol, all may contribute to aggression among people with MAUD.

Compared to MA-use characteristics, childhood trauma has been less studied as a potential risk factor for aggression among patients with MAUD, although data indicate positive associations between childhood trauma and aggression in samples from prisons (20), colleges (21), and the general population (22). Childhood trauma involves any adverse experiences that may harm or threaten the health and welfare of a child under the age of 16 years and may include physical, mental, or sexual abuse, or neglect (23). Experiencing childhood trauma has been identified as a risk factor for both aggressive behaviors and substance use problems such as MAUD (24, 25). A recent review (26) demonstrated that experiences of childhood trauma were associated with SUDs (including MAUD), which in turn increased the likelihood of aggression and violence. These findings provide further evidence regarding the links between and mechanisms underlying childhood trauma and aggression among individuals with MAUD.

Despite some evidence documenting associations of MA-use characteristics and childhood trauma with aggression among individuals with MAUD, there have been several methodological concerns that may limit a comprehensive understanding of such associations. First, most studies focused only on individuals with MAUD and did not compare childhood trauma and aggression with a control comparison group. Second, most studies tended to treat aggression as a unidimensional construct, rather than as a spectrum of behaviors that range from verbal to physical and psychological aggression (27). Since most studies focused on only one aspect of aggression, it remains unclear whether certain dimensions may differ from others with respect to patterns of associations. Third, while the association between MA-use characteristics and aggression among patients with MAUD has been studied, relatively less is known about associations between childhood trauma and aggression among patients with MAUD. To our knowledge, no study has ever included both MA-use characteristics and childhood trauma as potential risk factors to examine their effects comprehensively and simultaneously on aggression among patients with MAUD. Fourth, most previous studies on aggression among patients with MAUD were conducted in Western countries, and less is known about situations in Asian countries like China, where MAUD has been a major issue with increasing prevalence.

In light of the research gaps identified above, we conducted the current study to compare childhood trauma and aggression in MAUD patients and healthy control subjects, to examine associations of MA-use characteristics and childhood trauma with aggression in MAUD patients. This study addresses the above-mentioned limitations of previous studies by adding a healthy control group, including a broader measurement of aggression to assess its various dimensions, examining both childhood trauma and a range of MA-use characteristics, and involving a sample of male patients with MAUD recruited from a compulsory drug rehabilitation center in China.

METHOD

Participants and Procedures

Participants in the MAUD group were conveniently sampled from a compulsory drug rehabilitation center in Changsha, China, between December 2017 and August 2018. The inclusion criteria for the MAUD group were: 1) male (since aggression is much more common among male MAUD patients, and all MAUD patients in the study site were male), 2) age ranging from 18 to 60 years, 3) at least 14 days of detoxification at the time of study participation to avoid the negative effects of acute MA withdrawal symptoms (usually peaks within 24 hours after MA abstinence and resolves over the next two weeks) on data collection quality, 4) the core illicit substance abused was MA, and 5) DSM-5 diagnosis of MAUD. The exclusion criteria were: 1) other lifetime/current illicit SUDs such as heroin and ketamine (except for alcohol and tobacco), 2) unable to understand research content, 3) diagnosis of so-called organic brain diseases or severe mental disorders (e.g., schizophrenia, major depression, bipolar disorder).

We recruited a gender-, age-, and education-matched healthy control group that was drawn nationally from a mobile phone survey conducted between January 2022 and February 2022. The control group comprised of healthy participants aged between 18 and 60 years with no history of mental illness or SUD (except possibly for tobacco). Individuals with severe physical or mental illness that could impair cognitive performance or inability to read Chinese and give informed consent were excluded from both groups. We screened a total of 376 MAUD patients and 905 healthy control subjects and finally recruited 360 eligible MAUD patients and 604 eligible healthy control subjects who completed the study, providing valid questionnaires based on completion time and quality of the survey answers.

The study was approved by the Ethics Committee of The Second Xiangya Hospital, Central South University. A semi-structured interview and self-reported questionnaire survey were administrated to each participant by two psychiatrists who validated the raters' consistency. All participants were provided information about the purpose and procedure of the study and were informed that they were free to quit at any stage of the study and any information we obtained was confidential, for research only. Informed consent to participate was obtained from each participant before they completed the study.

Materials and Measures

Demographics and MA-Use Characteristics

Semi-structured interviews and questionnaires were used to collect demographics for both groups and MA-use characteristics for the MAUD group only. Demographic data included gender, age, education, marital status, and employment (full-time job, part-time job, or unemployment). MA-use characteristics included the age of first MA use, age of MAUD onset, duration of MA use (years from the age at first MA use to age at the time of enrollment), the longest period of MA abstinence (specifically referring to the longest voluntary MA detoxification/abstinence period rather than during compulsory treatment), MA-induced paranoia (a positive answer of the item: "Have you ever had a paranoid experience when you were using MA?"), the severity of MAUD (having six or more DSM-5 criteria for stimulant use disorder was considered severe MAUD, while two to five criteria was considered mild to moderate), co-occurring alcohol use disorder (AUD) [a score of eight or more on the Alcohol Use Disorders Identification Test (AUDIT) measured for the past year before entering the Compulsory Drug Rehabilitation Center (28, 29)] and co-occurring tobacco use disorder (TUD) [a score of six or more on the Fagerström Test for Nicotine Dependence (FTND) measured for the past year before entering the Compulsory Drug Rehabilitation Center (30, 31)].

Childhood Trauma

Childhood trauma was evaluated in both groups using the validated Chinese version of the Childhood Trauma Questionnaire (32) that was based on the short form of the Childhood Trauma Questionnaire (CTQ-SF) to measure traumatic experiences before the age of 16 (33, 34). It is a self-administered instrument that includes 28 items rated on a 5-point Likert-type scale ranging from 1 "never" to 5 "always." The CTQ-SF assesses five aspects of traumatic experiences, including emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect. For the purpose of the current study, the total score was calculated, with higher scores reflecting more severe childhood trauma. In the current MAUD sample, the Cronbach's α of the questionnaire was 0.87.

Aggression

The severity of aggression was measured in both groups using the Chinese version of the Buss-Perry Aggressive Questionnaire (AQ-CV) (35) adapted from Aggression Questionnaire (AQ) developed by Buss and Perry (36) and also validated in individuals with MAUD (19, 37) to measure current level of aggression. The AQ-CV is a self-reported questionnaire of 30 items that assesses five domains of aggression: physical aggression (seven items), verbal aggression (five items), anger (six items), hostility (seven items), and self-aggression (five items), with each rated on a 5-point Likert scale of 1 "extremely uncharacteristic of me" to 5 "extremely characteristic of me." The score of each sub-scale was the sum of the scores of the items contained, and then mathematically transformed to a centesimal score, with higher scores reflecting higher aggression. The total score of AQ-CV is the centesimal score of sum of all item scores and reflects participants' levels of overall aggression. In the MAUD sample

TABLE 1 | Sample characteristics and group comparisons.

Variables	MAUD group	Control group	c^2/t	*p*
	n = 360	*n* = 604		
Demographics				
Male	360 (100)	604 (100)		
Age	33.65 (6.38)	33.27 (8.95)	−0.77	0.439
Junior high school or above	283 (78.6)	493 (81.6)	1.30	0.254
Married	159 (44.2)	446 (73.8)	84.98	<0.001
Full-time job	227 (63.1)	518 (85.8)	66.24	<0.001
MA-use characteristics				
Age of first MA use	26.49 (7.06)	–	–	–
Age of MAUD onset	30.00 (6.56)	–	–	–
Duration of MA use (years)	7.16 (3.57)	–	–	–
Longest period of MA abstinence (months)	12.52 (14.25)	–	–	–
MA-induced paranoia	145 (40.3)	–	–	–
Severe of MAUD	278 (77.2)	–	–	–
Co-occurring AUD	166 (46.1)	–	–	–
Co-occurring TUD	126 (35.0)	–	–	–
CTQ-SF total score	38.70 (11.83)	35.50 (9.57)	−4.34	<0.001
Emotional Abuse, EA	7.12 (2.59)	6.76 (2.45)	−2.14	0.032
Physical Abuse, PA	6.63 (2.93)	5.81 (1.89)	−4.75	<0.001
Sexual Abuse, SA	6.16 (2.30)	5.43 (1.31)	−5.51	<0.001
Emotional Neglect, EN	10.38 (4.97)	9.56 (4.53)	−2.54	0.011
Physical Neglect, PN	8.41 (3.41)	7.93 (2.87)	−2.21	0.027
AQ-CV total score	34.41 (14.98)	24.00 (14.66)	−10.58	<0.001
Physical Aggression, PAG	40.94 (20.20)	22.41 (16.32)	−14.77	<0.001
Verbal Aggression, VAG	38.46 (15.79)	28.28 (16.94)	−9.427	<0.001
Self-aggression, SAG	29.09 (17.99)	17.04 (16.70)	−10.53	<0.001
Anger, A	36.00 (20.19)	28.06 (20.39)	−5.87	<0.001
Hostility, H	27.43 (16.78)	24.01 (17.53)	−2.98	0.003

MA, methamphetamine; MAUD, methamphetamine use disorder; AUD, alcohol use disorder; TUD, tobacco use disorder; CTQ-SF, the short form of the Childhood Trauma Questionnaire; AQ-CV, the Chinese version of the Buss-Perry Aggressive Questionnaire.

in this study, the Cronbach's α for the AQ-CV was 0.92, and the Cronbach's α values for the five sub-scales ranged from 0.58 for verbal aggression to 0.80 for physical aggression and anger.

Statistical Analysis

First, descriptive statistics were used to describe demographics, childhood trauma, and aggression for both groups, as well as MA-use characteristics for the MAUD group only. Each continuous variable was described by mean (M) and standard deviation (SD) for normal distributions. The categorical variables were described by numbers and proportions. Second, independent sample *t*-tests for continuous variables and Chi-square tests for categorical variables were used to compare the demographics and the severity of childhood trauma and aggression between the MAUD group and the control group. Third, simple linear regression analyses were used to initially identify relationships between aggression and demographics, MA-use characteristics, and childhood trauma in the MAUD group. Next, multivariate linear regression analyses were applied to identify independent relationships with aggression in the MAUD group. In light of the differences in the overall and five dimensions of aggression, we conducted six regression models with overall aggression, physical aggression, verbal aggression, self-aggression, anger, and hostility as dependent variables, and all significant variables in the previous univariate analyses as independent variables. All analyses in this study were executed in IBM SPSS Statistics 23.0 software, and the two-tailed significance level was set as $p < 0.05$. Of note, given the clinical relevance of MAUD onset, we designated age of MAUD onset instead of age and age of first MA use as the independent variable in the multivariate linear regression models to avoid multicollinearity.

RESULTS

Sample Characteristics and Group Comparisons

Table 1 presents sample characteristics and group comparisons between the MAUD group and the control group.

The MAUD group was all male (100%), with a mean age of 33.65 ± 6.38 years (range: 19–53), predominantly with junior high school or above education (78.6%), not married (55.8%), and with full-time jobs (63.1%). Regarding MA-use

characteristics, the MAUD group had a mean of 26.49 years for first MA use age, 30.00 years for MAUD onset age, 7.16 years for MA use duration, and 12.52 months for the longest period of MA abstinence. Most had severe MAUD (77.2%) and large minorities had MA-induced paranoia (40.3%), co-occurring AUD (46.1%), and co-occurring TUD (35.0%). The MAUD group had a total mean score of 38.70 for childhood trauma, with its five sub-scales ranging from 6.16 for sexual abuse to 10.38 for emotional neglect. The MAUD group had a mean score of 34.41 for aggression, with its five sub-scales ranging from 27.43 for hostility to 40.94 for physical aggression.

The matched control group had comparable ages and education levels with the MAUD group, but significant differences existed in other demographic characteristics, childhood trauma, and aggression. Compared to the control group, the MAUD group was less likely to be married (44.2% vs. 73.8%, $p < 0.001$), and have full-time jobs (63.1% vs. 85.8%). The MAUD group also scored higher on childhood trauma (38.70 $\pm$ 11.83 vs. 35.50 $\pm$ 9.57, $p < 0.001$) and aggression (34.41 $\pm$ 14.98 vs. 24.00 $\pm$ 14.66, $p < 0.001$), including on each sub-scale.

Univariate Analyses

Table 2 shows the univariate analyses of the associations of demographics, MA-use characteristics, and childhood trauma with aggression in the MAUD group. The total aggression score was negatively associated with age ($\beta = -0.14$, $p < 0.01$), junior high school or above education ($\beta = -0.13$, $p < 0.05$), being married ($\beta = -0.13$, $p < 0.05$), age of first MA use ($\beta = -0.13$, $p < 0.05$), and age of MAUD onset ($\beta = -0.18$, $p < 0.01$). The total aggression score was positively associated with having MA-induced paranoia ($\beta = 0.18$, $p < 0.01$), severe MAUD ($\beta = 0.21$, $p < 0.001$), co-occurring AUD ($\beta = 0.14$, $p < 0.05$), co-occurring TUD ($\beta = 0.23$, $p < 0.001$), and the childhood trauma total score ($\beta = 0.20$, $p < 0.001$), as well as each of its five sub-scales ($\beta =$ 0.14–0.17, $p < 0.01$). Slight differences existed in the associations of the five sub-scales of aggression with demographics, MA-use characteristics, and childhood trauma, with details shown in **Table 2**.

Multivariate Analyses

Table 3 shows the results of six multivariate linear regressions to determine independent factors linked to aggression and its five domains. Age of MAUD onset was negatively correlated with overall aggression, while having severe MAUD, co-occurring AUD, co-occurring TUD and childhood trauma were positively associated with overall aggression. Slightly different correlates were found for its five domains. Physical aggression was inversely associated with junior high school or above education and age of MAUD onset, and positively associated with severe MAUD, co-occurring AUD/TUD and childhood trauma. Verbal aggression was positively associated with severe MAUD and co-occurring TUD. Self-aggression was inversely associated with being

TABLE 2 | Univariate analyses of aggression.

Variables	Overall aggression	PAG	VAG	SAG	A	H
Demographics[a]						
Age	−0.14**	−0.19***	−0.05	−0.11*	−0.10	−0.09
Junior high school or above	−0.13*	−0.12*	−0.07	−0.11*	−0.11*	−0.11*
Married	−0.13*	−0.10	−0.04	−0.16**	−0.06	−0.15**
Full-time job	−0.02	−0.03	−0.01	−0.03	−0.05	0.02
MA-use characteristics[a]						
Age of first MA use	−0.13*	−0.21***	−0.06	−0.09	−0.10	−0.04
Age of MAUD onset	−0.18**	−0.23***	−0.09	−0.14**	−0.14**	−0.10
Duration of MA use (years)	0.01	0.08	0.02	−0.02	0.01	−0.07
Longest period of MA abstinence (months)	0.02	0.01	−0.05	0.06	−0.01	0.04
MA-induced paranoia	0.18**	0.10	0.05	0.22***	0.15**	0.21***
Severe MAUD	0.21***	0.21***	0.12*	0.13*	0.20***	0.17**
Co-occurring AUD	0.14*	0.17**	−0.02	0.19***	0.05	0.14*
Co-occurring TUD	0.23***	0.24***	0.15**	0.21***	0.21***	0.12*
CTQ-SF total score[a]	0.20***	0.15**	0.06	0.20***	0.18**	0.22***
Emotional Abuse, EA	0.17**	0.12*	0.06	0.19***	0.12*	0.19***
Physical Abuse, PA	0.15**	0.16**	0.04	0.13*	0.12*	0.14*
Sexual Abuse, SA	0.16**	0.12*	0.06	0.18**	0.11*	0.17**
Emotional Neglect, EN	0.14**	0.10	0.05	0.11*	0.16**	0.15**
Physical Neglect, PN	0.14**	0.08	0.03	0.15**	0.12*	0.16**

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

[a]*Standardized coefficient* β.

MA, methamphetamine; MAUD, methamphetamine use disorder; AUD, alcohol use disorder; TUD, tobacco use disorder; CTQ-SF, the short form of the Childhood Trauma Questionnaire; PAG, physical aggression; VAG, verbal aggression; SAG, self-aggression; A, anger; H, hostility.

TABLE 3 | Multivariate linear regression analyses of aggression.

Aggression domains	Correlates	*β*	*T*	*p*
Overall aggression				
	Age of MAUD onset	−0.11	−2.04	0.043
	Severe MAUD	0.15	2.80	0.005
	Co-occurring AUD	0.11	2.16	0.031
	Co-occurring TUD	0.18	3.55	<0.001
	Childhood trauma	0.15	2.91	0.004
Physical aggression				
	Junior high school or above	−0.12	−2.34	0.020
	Age of MAUD onset	−0.17	−3.39	0.001
	Severe MAUD	0.15	2.89	0.004
	Co-occurring AUD	0.13	2.59	0.010
	Co-occurring TUD	0.20	4.00	<0.001
	Childhood trauma	0.10	2.02	0.044
Verbal aggression				
	Severe MAUD	0.11	1.99	0.048
	Co-occurring TUD	0.13	2.50	0.013
Self-aggression				
	Married	−0.11	−2.29	0.023
	MA-induced paranoia	0.16	3.23	0.001
	Co-occurring AUD	0.16	3.28	0.001
	Co-occurring TUD	0.15	3.07	0.002
	Childhood trauma	0.14	2.71	0.007
Anger				
	Severe MAUD	0.14	2.64	0.009
	Co-occurring TUD	0.17	3.40	0.001
	Childhood trauma	0.14	2.73	0.007
Hostility				
	Married	−0.12	−2.39	0.017
	MA-induced paranoia	0.14	2.78	0.006
	Severe MAUD	0.13	2.53	0.012
	Co-occurring AUD	0.13	2.63	0.009
	Childhood trauma	0.17	3.31	0.001

MA, methamphetamine; MAUD, methamphetamine use disorder; AUD, alcohol use disorder; TUD, tobacco use disorder.

married and positively associated with MA-induced paranoia, co-occurring AUD/TUD, and childhood trauma. Anger was positively associated with severe MAUD, co-occurring TUD and childhood trauma. Hostility was inversely associated with being married and positively associated with MA-induced paranoia, severe MAUD, co-occurring AUD and childhood trauma.

DISCUSSION

Childhood Trauma and Aggression in MAUD

Our findings showed higher levels of childhood trauma and aggression in the MAUD group than the matched healthy control group. The higher level of childhood trauma in the MAUD group was consistent with previous studies showing high childhood trauma among people who use MA (38) and exposure to childhood trauma being related to subsequent initiation of MA use (39, 40). Childhood trauma has been established as an important factor linked to substance abuse including MA abuse (10, 41). Exposure to childhood trauma may increase vulnerability to emotional distress and mental disorders, and people with childhood trauma may resort to substance abuse as a coping mechanism to deal with psychological impacts of childhood trauma (42, 43). The higher level of aggression in the MAUD group was consistent with previous studies showing positive associations between substance abuse, aggression, and violence (44–46). The strong link between MAUD and aggression may be explained by multiple factors, including altered brain function, decreased ability to inhibit impulsive behaviors, or impaired social cognition, among others (8).

Associations Between Childhood Trauma and Aggression

Results from the study generally support positive associations between childhood trauma and aggression, reflected in all aggression sub-scales except for verbal aggression. This finding was consistent with previous studies showing childhood trauma as a factor linked to adult aggression in other populations such as prisoners (20), college students (21), and the general population (22). This result also aligns with proposed mechanisms linking childhood trauma to aggression through substance abuse (26). Experiencing childhood trauma may lead to significant changes in brain structure and neuroendocrine function, such as decreased gray matter volumes of the caudate nucleus, hippocampus, and amygdala, and lower cortisol levels during arousal (47, 48), promoting emotional disorders and aggression. Our findings suggest that childhood trauma may constitute an independent, significant, and important risk factor for aggression among patients with MAUD, even after controlling for MA-use characteristics and demographics. Thus, special attention may be needed to address coping with childhood trauma when designing intervention programs targeted at limiting aggression among patients with MAUD.

Relationships Between Demographics and MA-Use Characteristics and Aggression

Our study found significant associations between a range of MA-use characteristics, certain demographics and aggression among patients with MAUD. To be specific, the age of MAUD onset, having severe MAUD, and co-occurring AUD/TUD were associated with overall aggression, while other factors including having MA-induced paranoia, junior high school or above education and being married were associated with specific dimensions.

The negative association between the age of MAUD onset and aggression was consistent with prior studies showing that younger age of MA use and MAUD onset were associated with aggression, especially physical aggression (11, 12). Studies have shown that the early age of MA use and related MAUD onset increased the likelihood of engaging in MA-related violence, after adjusting for socio-demographics and other potential confounders (11). Additionally, age at first perpetration of violence against another person was positively associated with age at first use of MA, and with age at which the person regularly used MA (12). Therefore, when treating and managing patients with

younger onset ages of MAUD, special attention may need to be given to assessing and preventing physical aggression.

Although previous studies have shown that chronic MA use (13) and more frequent MA use (14) were associated with aggression, our study failed to identify such associations. Instead, multivariate regression analyses showed a higher level of aggression among patients with severe MAUD than those with mild to moderate MAUD. These findings may reflect greater MA-use severity associated with chronic and more frequent use rather than duration and frequency of MA use *per se*. One study found a MA dose-dependent increase in aggressive behavior, with the risk probability of aggression increasing from 9 to 52% in relation to various degrees of MA use ranging from abstinence to heavy use (49). Our findings add further evidence to the potential dose-response relationship between MA-use severity and aggression in MAUD.

The finding that co-occurring AUD/TUD was associated with aggression among patients with MAUD was consistent with previous studies showing links between alcohol and tobacco use and aggression (19, 50, 51). For instance, a study showed that simultaneous alcohol and MA use was associated with elevated odds of MA-related aggression and hostility, with an adjusted odds ratio of 2.74 (19). Moreover, MA, alcohol, and tobacco all may affect cognitive functioning and increase the likelihood of perceiving an environmental stimulus as threatening, potentially leading to impulsive and aggressive responses to the perceived threats (19). Our findings suggest that co-occurring AUD/TUD were common and associated with aggression among people with MAUD, and we believe that this warrants further attention in clinical settings.

Our study also found other MA-use characteristics and demographics associated with specific dimensions of aggression. Compared with patients without MA-induced paranoia, patients who have experienced MA-induced paranoia showed a higher degree of self-aggression and hostility. This finding was in keeping with previous studies showing positive associations between positive psychotic symptoms (e.g., hallucinations and paranoia) and hostility (52) and self-aggressive behaviors including suicide (53). These findings suggest that paranoia is an important factor linking MAUD with aggression that warrants particular attention. Regarding demographics, our study found junior high school or above education and being married to be possible protective factors mitigating against physical aggression, self-aggression and hostility, in line with prior studies (54–56). The link between education and aggression may be bidirectional; patients with higher educational attainment may have better health literacy and resources to control aggressive tendencies, while those with aggressive behaviors may be at increased risk of poor school performance, thus leading to lower academic achievement (57). The findings speculatively suggest that strengthening education among MAUD patients may help with the prevention and reduction of aggression. Regarding marital status and aggression, an explanation may be that individuals showing higher levels of hostility and self-aggression were more likely to experience dissolution or termination of their relationships as a result of their aggression. The potentially bidirectional relationship between marital status and aggression should be further tested in future longitudinal study designs.

Limitation and Strengths

Several limitations warrant consideration. First, we used a convenience sample from a compulsory drug rehabilitation center in Changsha City of Hunan Province in China, which may not represent other MAUD populations from other organizations or from the community and in other areas of China. Future multi-center studies that include MAUD populations from various organizations and communities in various parts of China are needed to get a more representative sample. Second, we only included male MAUD patients in our study since aggression is much more common among male MAUD patients, and all MAUD patients in the Compulsory Drug Rehabilitation Center in our study were male. Future studies should include both male and female MAUD patients to examine gender differences of aggression among MAUD patients. Third, the cross-sectional nature of the study precluded making causal inferences. Future longitudinal studies are needed to examine temporal relationships. Fourth, the extent to which specific aspects of aggression link to specific sample characteristics warrants study in larger samples as strengths of between-domain relationships were not tested statistically. Fifth, data collection of certain questionnaires such as the Childhood Trauma Questionnaire is based on recall of traumatic childhood experiences that require full cognitive function. MAUD patients may have cognitive impairments that affect their accurate memories of past experiences and thus affect the accuracy of data collection quality. Future research may consider assessing and controlling for the cognitive function of the MAUD patients to reduce such potential recall bias. Finally, we failed to find any interaction (moderation and/or mediation) between childhood trauma and all eight selected MA-use characteristics on aggression in our study (results not shown here). On the one hand, this may indicate that both childhood trauma and MA-use characteristics work independently on aggression. On the other hand, this may imply that childhood trauma affect aggression through other MA-use characteristics not included in the current study. Future studies are needed to further explore potential underlying mechanism of childhood trauma on aggression.

Despite these limitations, our study is the first to our knowledge to compare childhood trauma and aggression between MAUD and control groups and to explore various dimensions of aggression among male patients with MAUD in China. The inclusion of a control group helps to better understand associations between MAUD, childhood trauma, and aggression. Exploration of various dimensions of aggression facilitates a better understanding of specific relationships. Furthermore, this study examined the associations between a wide range of potential risk factors and aggression among patients with MAUD. These factors were selected based on previous studies with a solid theoretical background and included mainly MA-use-related characteristics (age, duration and frequency of MA use, psychotic symptoms, and concurrent alcohol and tobacco use) and childhood trauma (emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect). Our study observed positive associations of both MA-use characteristics and childhood trauma with aggression among patients with MAUD. As China is the most populous country worldwide and has prevalent MAUD, the findings have implications for a large group

of people and expand the literature beyond WEIRD (White, educated, industrialized, rich, and democratic) countries (58). The extent to which the findings extend to other jurisdictions warrants direct examination.

CONCLUSIONS

This study showed higher levels of childhood trauma and aggression in MAUD patients than in healthy control subjects from the general population. Both MA-use-related characteristics (mainly age of MAUD onset, severe MAUD, and co-occurring AUD/TUD), and childhood trauma were associated with aggression among patients with MAUD. The results provide novel and useful information on potential risk factors for aggression and its various dimensions and inform future longitudinal research to establish causal relationships between these factors and aggression to guide further prevention and treatment programs on aggression.

DATA AVAILABILITY STATEMENT

The data supporting the conclusions of this article will be made available by the corresponding authors, with any reasonable reasons. Requests to access the data should be directed to WH, weihao57@csu.edu.cn.

ETHICS STATEMENT

This study was approved by the Ethics Committee of the Second Xiangya Hospital, Central South University. All participants understood the purpose and procedure of the study and provided their informed consent.

AUTHOR CONTRIBUTIONS

WH and TL designed the study. XZ supervised the whole process. HS and QD provided support for data collection of MAUD group. ML and YW performed the semi-structure interviews and data collection for MAUD group with the participation of CY and TF. LP collected healthy control group data. WL and XF assisted in the data analysis and interpretation. ML managed the literature search and drafted the manuscript. MP directed the manuscript and revised it. All authors contributed to the article and approved the submitted version.

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The Status and Prescription Patterns of Opioid Utilization in a Large Comprehensive Teaching Hospital in China According to the Anatomical Therapeutic Chemical Classification/Defined Daily Dose Methodology

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Fang T, Zhang X, Hao W and Deng Q (2022) The Status and Prescription Patterns of Opioid Utilization in a Large Comprehensive Teaching Hospital in China According to the Anatomical Therapeutic Chemical Classification/Defined Daily Dose Methodology. Front. Psychiatry 13:913640. doi: 10.3389/fpsyt.2022.913640

Background: Few studies have analyzed opioid consumption and the average daily dose and duration for different patients in hospital settings in China. The aim of this study was to measure the status of and trends in prescribed opioids and the prescribing patterns at the Second Xiangya Hospital.

Methods: The data were obtained from the prescribed medicine database of the inpatient department. We included patients who were >18 years old and received any level of opioid analgesic between 2012 and 2017. The international Anatomical Therapeutic Chemical Classification/Defined Daily Dose (ATC/DDD) methodology was used to standardize the consumption rates. All opioid units were converted into morphine equivalents (MEs) to analyze the specific opioid usage.

Results: The consumption of prescribed opioids increased from 3.16 to 3.74 DDD/100 bed-days (+18.3%) from 2012 to 2017. Both cancer and noncancer patients had similar administration routes and median daily dosages in MEs, but cancer patients had longer treatment durations (median: 5 days vs. 1 day, respectively). The median average daily dose and treatment duration for all patients were 15 MEs/day and 2 days, respectively, for oral administration, 100 MEs/day and 1 day for parenteral administration, and 47.14 MEs/day and 5 days for both oral and parenteral administration.

Conclusion: Although there was a tendency toward an increase in opioid consumption, the overall level of consumption in the Second Xiangya Hospital remained relatively low. Thus, it is urgently necessary to increase the availability of opioids and alter prescription habits for them in order adopt the current concept of pain management developed by the World Health Organization (WHO).

Keywords: opioid consumption, prescription pattern, pain management, cancer patient, opioid accessibility, addiction

INTRODUCTION

The solemn commitment made at the 1961 Single Convention on Narcotic Drugs was amended by the 1972 Protocol and is stated as follows: "To make adequate provisions to ensure and not unduly restrict the availability of drugs that are considered indispensable for medical and scientific purposes." However, this commitment to opioids has yet to be fulfilled. The most recent data show that in low- and middle-income countries, the incidence of many diseases that require analgesics, especially cancer, is widespread and increasing (1). Opioids are essential for the control of moderate to severe pain, as they can bind to and directly excite opioid receptors in the central nervous system after entering the human body, thereby enhancing or replacing endogenous opioid peptides to regulate pain (2). In the three-step analgesic ladder recommended by the World Health Organization (WHO) for pain relief, patients with moderate to severe pain benefit from the use of opioids (3). However, approximately 5.5 billion people still have limited or no access to narcotic medicines, such as opioid analgesics, meaning that 75% of the world's population does not have access to proper pain relief treatment (4). In middle- and low-income countries, the prevalence of chronic pain in adults is 33 and 56% in elderly individuals (1). Pain may result in substantial financial burden and can negatively impact quality of life (5).

Opioid use is a two-sided coin that can lead to the development of iatrogenic addiction while treating pain. Unfortunately, global opioid use is unevenly distributed, and approximately 92% of the morphine used worldwide is administered in developed countries, such as the United States, which comprise only 17% of the world's population (4). According to International Narcotics Control Board's (INCB's) report in 2019, developed countries such as the United States have an opioid supply that is large enough to meet more than 1,000% of their demand. Opioid abuse and addiction caused by medical reasons, such as treating chronic pain, have had serious consequences that cannot be underestimated in many countries and regions, especially in the United States. Over the past 15 years, the rate of opioid analgesic use in the United States has soared. From 1999 to 2011, oxycodone consumption increased by almost 500% in the United States. In addition, the mortality rate due to opioid overdose nearly quadrupled. According to the U.S. Centers for Disease Control and Prevention, the unprecedented increase in opioid consumption has led to "the worst drug overdose crisis in U.S. history." In a speech at the White House on 26 October 2017, U.S. President Donald Trump highlighted the epidemic of opioid abuse in the United States, claiming that opioids kill hundreds of people every day and declaring a national public health emergency. Contrary to the epidemic of abuse in the United States, opioid use for medical purposes in China is extremely inadequate. This unequal distribution of supplies is unequitable to those living in developing countries, as it deprives them of access to medical care, including palliative care. Accordingly, public attention should be given to the imbalance in the availability of opioid analgesics (6).

The medicinal use of opioids in China is quite conservative. China accounts for approximately 20% of the world's population. In 2016, the consumption of medical morphine in China equaled only 1.8 tons, accounting for 4.98% of global consumption (36.2 tons) (4). According to the 2016 INCB, China's defined daily doses for statistical purposes (S-DDDs) rank 89th in the world and 22nd in Asia.

According to the WHO's recommendations, levels less than 200 S-DDDs (per million people per day) are considered inadequate, and levels less than 100 S-DDDs are considered very inadequate. Recent research showed that the total consumption of prescribed opioids in China in 2016 was 78.64 S-DDDs (7). As an essential medication for the control of moderate to severe pain, opioid use is considered an indicator for pain management by the WHO. Thus, pain relief in China is still at a lower level than in other countries.

In China, the number of new cancer patients with severe pathological pain has substantially increased (8). In addition to increasing cancer-related needs, the adequacy of opioid analgesic consumption for severe pain has been lower than the adequacy of consumption measure (ACM) value calculated based on INCB statistics, which ranked it at a "very poor" level from 2006 to 2016. Moreover, a survey conducted in 30 hospitals in Beijing reported that only 9.48% of 589 cancer patients achieved pain relief. To date, there are few convincing clinical data focusing on current opioid-based pain control strategies for patients, and the therapeutic strategy of opioids prescribed for cancer-related pain has not been appropriately addressed in China. Based on these statistics, opioid consumption and the prescription patterns of opioid usage for patients should be analyzed.

Some Chinese studies have focused on the consumption of opioids in hospitals without considering the hospital occupancy index and number of beds. This makes comparisons among different hospitals very difficult. At this critical juncture, China needs not only national data on opioid consumption in terms of S-DDD but also data from hospitals to show the status and trends in opioid prescriptions and related prescribing habits. Collection and analysis of these data are critical for understanding the reasons for low consumption of opioids in China. Hence, the aim of this study was to show the opioid consumption status, trends and prescribing patterns in a teaching hospital as a representative example in China.

MATERIALS AND METHODS

Data Sources

This was a retrospective, descriptive and analytical cross-sectional study performed at the Second Xiangya Hospital, Central South University, in Hunan, China. The data were obtained from the prescribed medicine database of the inpatient department. We extracted the required data from medical records in the hospital database. The collected data included age, sex, hospital ward, diagnosis [the cause of hospitalization, using the 10th revision of the International Classification of Diseases (ICD-10) to determine diagnoses], surgical condition and information about opioid administration, such as the generic name of the opioid analgesic, dosage, route of administration, and treatment duration.

Patients with or without a cancer diagnosis who were aged >18 years and received any opioid analgesic in the 6-year period from 2012 to 2017 were included. Opioid analgesics included parenteral and oral opioids. The parenteral opioids included fentanyl, remifentanil, morphine, tramadol, pethidine, and transdermal fentanyl, and the oral opioids included tramadol (sustained release), oxycodone (sustained release), morphine (sustained release), codeine phosphate, and a combination of paracetamol and tramadol hydrochloride tablets. The study followed the tenets of the Declaration of Helsinki for research involving human subjects, and all participants signed an informed consent form.

Statistical Analysis

To determine the opioid consumption of the hospital population, the current study used the international Anatomical Therapeutic Chemical Classification/Defined Daily Dose (ATC/DDD) method. The DDD is the assumed average maintenance dose per day for a drug administered for its main indication in adults and is a unit of measurement defined by the WHO Collaborating Centre (WHOCC) for Drug Statistics Methodology (9). DDDs provide a fixed unit of measurement independent of price, currency type, package size, and strength, enabling researchers to assess trends in drug consumption and perform comparisons between population groups. In our study, the DDDs represent the values per 100 inpatients per day and were calculated with the following formula (10):

$$f(x) = a_0 + \frac{\begin{array}{c}Number\ of\ units\ delivered \\ in\ a\ fixed\ period\ (mg) \times 100\ beds\end{array}}{\begin{array}{c}DDD\ (mg) \times Number\ of\ days\ in\ the\ period \\ \times\ Number\ of\ beds \times Hospital\ occupancy\ index\end{array}}$$

$$= DDD\ /100\ bed - days$$

To further estimate doctors' prescribing patterns for different patients, this study converted all oral and parenteral opioid units into morphine equivalents (MEs) for analysis and comparison. The prescribing patterns, including the average daily dose (MEs/day) and the treatment duration, are described with violin plots. Multiple prescriptions for one patient during the 6-year period were considered a complete data record. All statistical analyses were performed using SPSS v. 21.0 for Mac (Chicago, IL, United States), and violin plots were generated with the R (v.3.2.5) program. Values were determined to be significant at $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

RESULTS

A total of 147,814 patients, including 67,343 cancer patients (average of 2.57 prescriptions per patient) and 80,471 noncancer patients (average of 2.50 prescriptions per patient), received opioid treatment during the evaluated time span, based on 374,164 medical records that were extracted.

The Tendency of Opioid Consumption

As demonstrated in **Figure 1A**, there was an increasing trend with fluctuations in the consumption of opioids in this hospital. From 2012 to 2014, opioid consumption decreased from 3.16 DDD/100 bed-days to 2.64 DDD/100 bed-days. Then, consumption increased remarkably starting in 2014 and reached 3.74 DDD/100 bed-days in 2017. **Figure 1B** shows the consumption of oral and parenteral opioids based on DDD/100 bed-day units during the observed 6-year period. From 2012 to 2017, the consumption of parenteral opioids increased by 0.35 DDD/100 bed-days (+14%). For oral opioids, the rate of consumption increased from 0.80 to 1.03 DDD/100 bed-days (+29%).

The top three most consumed opioids were remifentanil, fentanyl, and tramadol, of which remifentanil and fentanyl were administered via the parenteral route and tramadol was administered orally. **Figure 2** shows that the consumption of fentanyl and its analogs accounted for a significant proportion (approximately 65%) of the hospital's total opioid consumption, and remifentanil accounted for 65–86% of the consumption of fentanyl and its analogs from 2012 to 2017.

The Prescription Patterns of Opioids for Both Cancer and Noncancer Patients

We classified patients based on their diagnosis as either cancer or noncancer patients, and we used violin plots to evaluate the distributions of daily dosages, treatment durations, etc. **Figure 3** shows that both cancer and noncancer patients had similar routes of administration and approximately the same median average daily dose. The following data represent the median dosage and median duration. Among the cancer patients, 5.86% received oral administration (15 MEs/day, 5 days), 87.07% received parenteral administration (100 MEs/day, 1 day), and 7.07% received both oral and parenteral administration (43.33 MEs/day, 5 days). Among the noncancer patients, 6.76% received oral administration (15 MEs/day, 1 day), 79.82% received parenteral administration (100 MEs/day, 1 day), and 13.42% received both oral and parenteral administration (51.25 MEs/day, 5 days).

For all patients with cancer or noncancer diagnoses, the average daily dose for oral administration (median of 15 MEs/day) was significantly lower than that for parenteral administration (median of 100 MEs/day). The average daily dose for patients who used both oral and parenteral opioids (median of 47 MEs/day) was between these two values.

For treatment duration (**Figure 3B**), the number of days in which a patient received opioids over the 6-year study period and the distribution frequency of different routes of administration were similar. During the 6-year observation period, the treatment duration of noncancer patients (median of 1 day) was significantly lower than that of cancer patients (median of 5 days). Moreover, there was an apparent difference between the treatment duration in the patients receiving only parenteral opioids (median duration of 1 day) and those receiving both oral and parenteral opioids (median duration of 5 days).

The Mann–Whitney test was used to evaluate the differences between the cancer and noncancer groups, including the route of administration, average daily dose and treatment duration. The

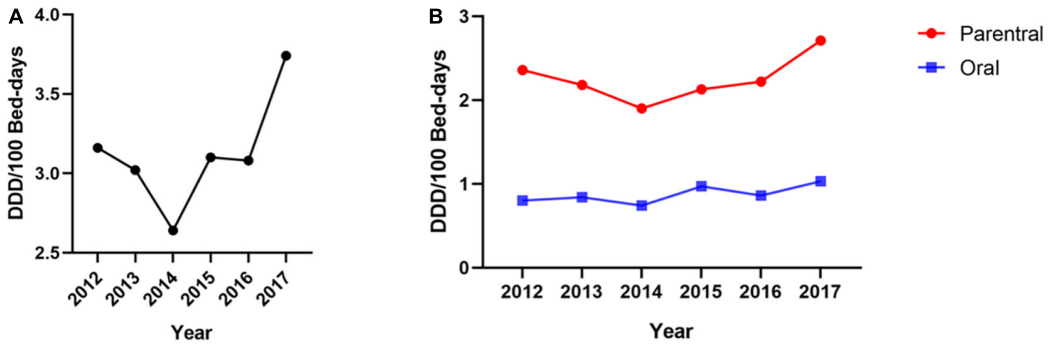

FIGURE 1 | Opioid consumption from 2012–2017 [unit: defined daily dose (DDD)/100 bed-days]. **(A)** The trend of opioid consumption during the 6-year study period. **(B)** The trends of oral and parenteral opioid consumption during the 6-year study period.

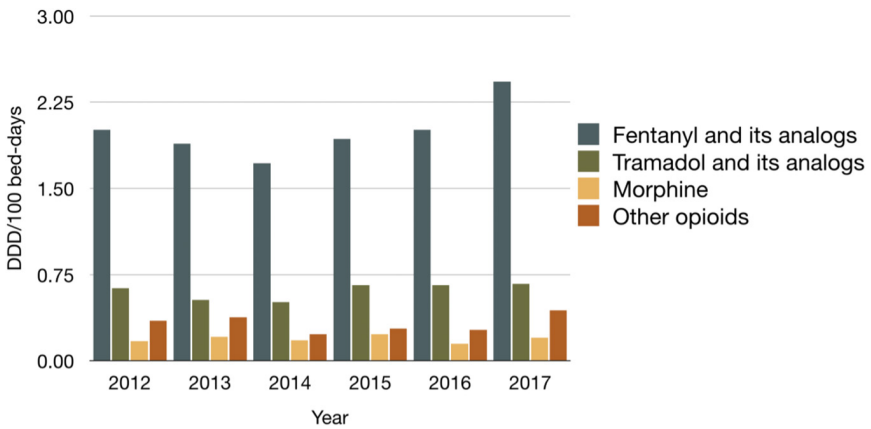

FIGURE 2 | The consumption of all opioids during the 6-year study period.

results showed that all differences between the two groups were significant ($P < 0.001$). However, large sample sizes can easily make small differences in data statistically significant. For this analysis, the differences had limited clinical significance.

DISCUSSION

There is concern about opioid prescriptions and their potential for global harm, such as addiction (11); however, opioid analgesics are still one of the most effective painkillers. In developing countries, the prevalence of chronic pain in adults is 33%, and 56% in the elderly (1). The effective treatment of people with chronic or cancer-related pain is still limited by an inadequate understanding of the importance of pain therapy or inadequate access to treatment with narcotics (4).

In this large-sample retrospective study, a slight overall increase in opioid consumption was observed in this hospital from 2012 to 2017. However, the consumption of opioids in this hospital did not exceed 4.0 DDD/100 bed-days during the whole 6-year observation period. Based on the doctors' prescribing patterns, the average daily dose for both cancer and noncancer patients was low for the different administration routes, and the treatment durations were short, especially for cancer patients, at only 5 days.

Given that there is no international standard for measuring the level of hospital opioid consumption, comparisons were made between our collected data and existing foreign published studies on drug utilization and opioid consumption evaluations in hospital settings based on the ATC/DDD system. We confirmed the hypothesis of our study, that is, opioid consumption at this hospital was relatively low compared with the consumption rates reported in other studies, which included two hospitals and four databases from Europe, East Asia, and North America (12–17). One study using the same measurement unit (DDD/100 bed-days), carried out in a hospital in Madrid, Spain, revealed that opioid consumption had a remarkable increasing tendency, from 22.3 DDD/100 bed-days in 2011 to 26.5 DDD/100 bed-days in 2015 (16). Another study in Iran evaluated parenteral opioid analgesic utilization in a referral teaching hospital in 2013 (17) and the number of patients who received parenteral opioid analgesics (41.185 DDD/100 bed-days) was nearly 21.9 times higher than that in this hospital (1.88 DDD/100 bed-days) in 2013. As a large, comprehensive, university-affiliated hospital, the authors believe that the consumption of opioids in this hospital must be greater than that in other local hospitals. The low level

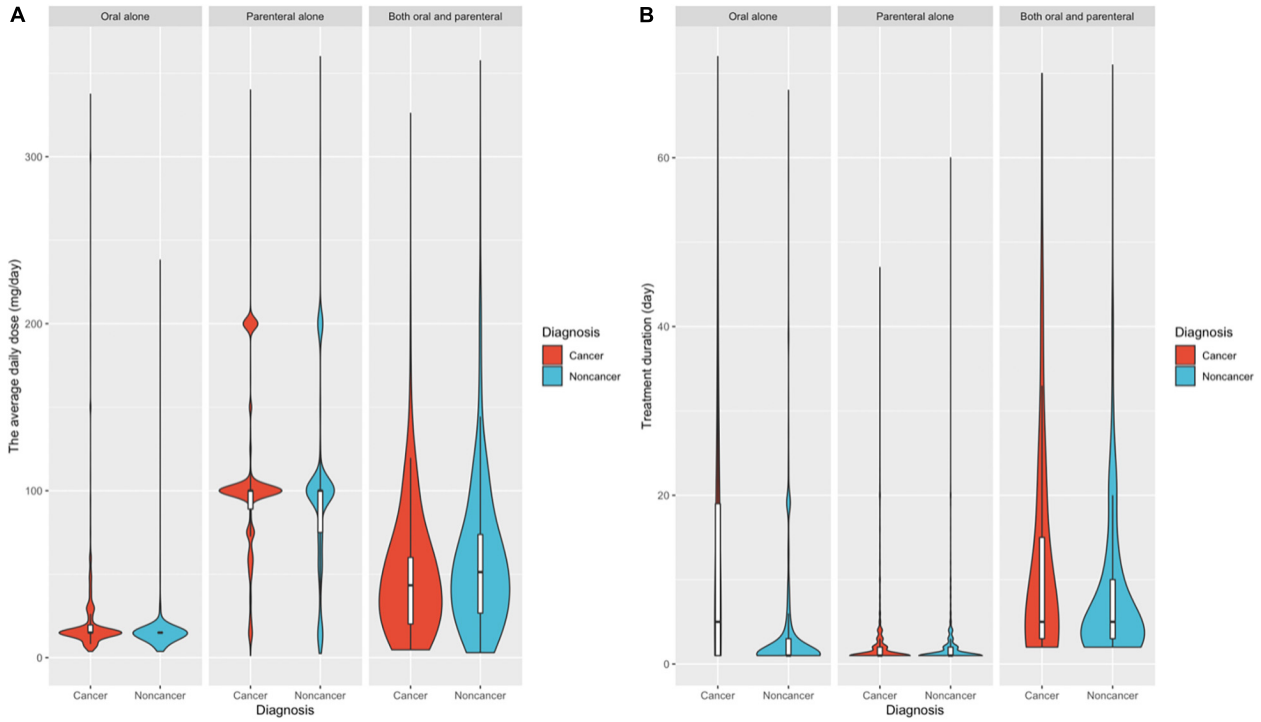

FIGURE 3 | Distribution frequency of the average daily dose **(A)** and treatment duration **(B)** in patients with cancer and noncancer diagnoses.

of opioid consumption was also consistent with national data (4), which show that the consumed number of opioids (<100 S-DDDs) is relatively low compared with the rest of the world.

The low level of opioid consumption in China may be attributable to multiple factors, such as policies, regulations, culture, and awareness (18). As the WHO report stated, the Chinese government's fear of opioid abuse could be one reason for the limited availability of medical opioids (4). Recent research has revealed that there is no evidence showing a precise relationship between high medical opioid consumption and the abuse of prescription opioids (19). Additionally, the fear of opioid abuse is due to an insufficient understanding of opioids and a lack of practical and reasonable clinical guidelines. According to research conducted in China that administered the Knowledge and Attitudes Survey Regarding Pain (KASRP) to doctors and nurses, the doctors and nurses did not receive passing scores (the authors of this questionnaire suggested that a score of 80% was considered a passing score) (20). Finally, China has a unique history. By the beginning of the 20th century, 85–95% of the total global opium was consumed and misused by Chinese people (18). As a result, both patients and their family members fear nonmedical use, addiction to opioids, and opioid-induced side effects, which may have led to insufficient medical use (21).

In a study assessing the basic knowledge of cancer pain management in a Chinese hospital, oncologists correctly answered only 59.7% of the knowledge questions (22), while in various other studies, oncologists scored between 31 and 68% (23–25), indicating an insufficient understanding of opioid use for cancer patients in this hospital. In this study, cancer patients received similar daily doses and had similar routes of administration, which may not be consistent with the clinical treatment guidelines. Both cancer and noncancer patients had similar prescription patterns. Last but not least, the treatment duration for all patients was relatively short, especially for cancer patients. The reason may be related to the fear of side effects and drug addiction. These fears are in accordance with the findings by Jeon et al. (26), who reported that 90.6% of doctors worried about difficulties in controlling the side effects of strong opioids.

Finally, only the sustained-release formulations of oral opioids were administered in this hospital. The WHO guidelines recommend the use of immediate-release opioid formulations in the early stage of treatment to enable rapid titration to the optimal dose for individualized treatment. The lack of an immediate-release formulation means that titration to the optimal dose cannot be accurately achieved.

Recommendations for Rationalizing Opioid Use in China

The two extremes of the opioid crisis, overuse and the lack of analgesia caused by underuse, demonstrate the difficulty of opioids as a formal medical method of analgesia in clinical application. Whether it is opioid abuse in the United States or China's lack of analgesia, the essence of the problem is that a balance between medical use and the prevention of abuse has not been found. Based on the analysis of existing research results, the authors believe that the following recommendations

should be employed to improve the accessibility and rational administration of opioids:

1. Legislation, monitoring, and advocacy: comprehensive and balanced regulations to address the rational use of opioids and addiction-related problems should be adopted. The medical use of opioids, especially for palliative care for cancer patients, should not be restricted because of opioid addiction concerns. Moreover, based on lessons learned from the opioid crisis in North America (27), controlling the activities of pharmaceutical enterprises and strengthening drug monitoring after marketing are very important.
2. Updated guidance: the current guidance for opioid prescriptions and pain treatment should be updated based on international experiences, scientific evidence, and clinical practice.
3. Professional training: professional training should include comprehensive knowledge of relevant topics, such as pain management, pharmacological effects, and the addiction potential of opioids, as well as the early identification, assessment, and treatment of opioid use-related abuse and addiction.
4. Establishment of a government coordination program: it is important to have a coordinated program for pain management, opioid prescription management and drug abuse prevention to balance the treatment needs of pain patients and opioid addiction and diversion.
5. Public education: public education should include overcoming the fear of opioid use by providing scientific information on the rational use of opioids to change knowledge and attitudes toward opioids and the risk of opioid addiction.

Limitations

This study has limitations. The findings are based only on medical records used to represent overall opioid use in this hospital. The authors are not aware of individual pain relief responses without further investigation of the patients. Additionally, this study only described part of the current situation in one hospital, and further research is needed to provide a complete picture and guide clinical practice.

CONCLUSION

Our study demonstrated that despite a positive trend in 2012–2017, opioid consumption in this hospital was still at a relatively low level. Both cancer and noncancer patients had similar administration routes and dosages, but the cancer patients had a longer treatment duration (median: 5 days vs. 1 day, respectively). The median average daily dose and treatment duration for all patients were 15 MEs/day and 2 days for those who received oral administration, 100 MEs/day and 1 day for those who received parenteral administration, and 47.14 MEs/day and 5 days for those who received both oral and parenteral administration, respectively. The authors also found that the cancer patients did not receive personalized pain management. We should recognize that opioids are indispensable for medical and scientific purposes and that the availability of opioids for such purposes should not be excessively restricted. China's regulations, the availability of opioids and the management of access to pain relief must be further strengthened. Following this, guidelines for personalized pain treatment should be established for patients with different diagnoses.

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

The studies involving human participants were reviewed and approved by Ethics Committee of the Second Xiangya Hospital, Central South University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

TF and WH designed the study and wrote the manuscript. TF, XZ, and QD performed the experiments and analyzed the data. All authors contributed to the article and approved the submitted version.

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Prevalence and Correlates of Risky Drinking Among the Oldest-Old in China: A National Community-Based Survey

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Aims: To investigate the prevalence and correlates of risky drinking in Chinese elderly people aged 80 and over.

Methods: Data were obtained from the Chinese Longitudinal Healthy Longevity Survey (CLHLS) conducted in 2018. A total of 10,141 respondents aged 80 years or older were included in this analysis. Risky drinking was defined as drinking > 2 drinks per day. The participants were divided into no risky drinking, past risky drinking, and current risky drinking groups. The prevalence of risky drinking, daily dosage, and type of alcohol beverages were assessed. The correlates of risky drinking were analyzed using logistic regression.

Results: The prevalence of past and current risky drinking was 6.2 and 4.4%, respectively. A total of 12.2% of males and 2.1% of females reported past risky drinking, and 8.9% of males and 1.4% of females reported current risky drinking. The median of the daily dosage of the past risky drinking group was 4.5 and 4 drinks in males and females, respectively, and were 4 and 3.3, respectively, of the current risky drinking group. Strong liquor was the most popular alcohol beverage in all groups. Men who were older or had white-collar work were less likely to be past risky drinkers, while those with smoking in past or current or heart disease were more likely to be past risky drinkers. Women who smoked in the past were more likely to be past risky drinkers. Men with older age or living in the urban areas or with heart disease were less likely to be current risky drinkers. Women with higher education or with heart disease were less likely to be current risky drinkers. Women with current smoking were more likely to have current risky drinking.

Conclusions: Our findings indicated that risky drinking among the oldest-old was not rare in China. The correlates of past and current risky drinking were different. Men and women had various correlates of risky drinking as well. Those with higher socioeconomic status seemed less likely to be risky drinking. More attention should be given to risky drinking among the oldest old, and sex-specific intervention may be needed.

Keywords: alcohol use problem, the oldest old, risky drinking, prevalence, national community-based survey

INTRODUCTION

China is facing great challenges in aging, as it has the largest and fastest aging population worldwide (1). In 2019, there were 164.5 million individuals aged 65 years old and over and 26 million aged 80 years old and over (the oldest-old) in China. It is estimated that there will be 115 million oldest-old individuals by 2050 (2). The rapid increase in the oldest-old population is of particular concern, since the oldest-old are the most susceptible to disease and disability (3), and they often need daily-living assistance as well as medical care, resulting in a heavy burden on the health care system, society, and families (4).

At the same time, rapid economic development and urbanization in China have also resulted in the increased alcohol use over the past 40 years (5). It is well-known that risky drinking affects the alcohol users' health, especially in those of old age. Previous studies reported that risky drinking was associated with increased morbidity, medical burden, and all-cause mortality (6). On the other hand, family members may hold the belief that risky drinking later in life does not exist or does not need treatment and will therefore overlook risky drinking among older adults (7). Health care workers may refrain from asking about risky drinking but focus more on their physical complaints (7). In fact, risky drinking seems to be not rare among the elderly. Germany reported 6.5% risky drinkers among older adults aged 75 years and over, including 12.1% male and 3.6% female (8). The Epidemiologic Catchment Area Study (ECA) in the United States estimates that alcohol abuse in the group aged 65 and over ranged from 1.9 to 4.6% for men and from 0.1 to 0.7% for women (9). The overall prevalence of heavy drinking among middle-aged and older adults was 7.23% in China (10). However, the evidence of the prevalence of risky drinking among the oldest-old is limited, and no evidence has been seen in the Chinese oldest-old population using a representative sample.

It is globally agreed upon that men are more likely than women to be risky drinkers due to cultural values, norms, and drinking patterns (11, 12). Chinese culture similarly has a long history of alcohol consumption among men. Therefore, it is necessary to explore the correlates of risky drinking in males and females. Except for sex and smoking, the correlates of risky drinking in previous studies were inconsistent. Some studies found that younger age, living with spouses, higher socioeconomic status, worse physical status, and greater anxiety were associated with risky drinking (5, 8, 13, 14), while other studies showed that lower education were associated alcohol use or depression was not associated with drinking (8, 15). Understanding the risky drinking of the older population can help medical practitioners to recognize and offer focused assessment.

The Chinese Longitudinal Healthy Longevity Survey (CLHLS) is a national community-based cohort study with the largest sample of oldest-old individuals in China. In this study, we investigated the prevalence of risky drinking and to explored its correlates based on the CLHLS 2018 survey.

METHODS

Study Design and Participants

In this study, we obtained data from the CLHLS 2018 survey. The CLHLS is a national, ongoing cohort study of community-dwelling Chinese older adults from 1998. Follow-up occurred every 2–4 years, and the most recent (eighth) survey was completed in 2018. It is conducted in 866 highly diverse counties and cities selected from 23 of China's 31 provinces and covers ∼85% of China's older population (4, 16). The CLHLS invited all centenarians in the sampled sites to voluntarily participate in the study and adopted a targeted random-sample design to interview approximately equal numbers of male and female non-agenarians, octogenarians, and young-old (aged 65–79 years) living near the centenarians to ensure representativeness (4). This design serves our aim of investigating the prevalence and correlates of risky drinking among the oldest-old in China. The surveys were administered through face-to-face interviews in participants' homes by trained interviewers with a structured questionnaire. There are details about CLHLS elsewhere (4, 16). The CLHLS study was approved by the Research Ethics Committee of Peking University (IRB00001052-13074), and all participants or their proxy respondents provided written informed consent.

Given that the present study focused on the current prevalence and correlates of risky drinking among the oldest-old, the data used were from the CLHLS 2018 survey. Participants who were aged 80 and over and completed the questions relating to alcohol drinking (including drinking status, drinking type, and dosage) were included. Participants who were younger than 80 and had no information about drinking were excluded.

Measures

Definition of Risky Drinking

First, participants were grouped into never drinking, past drinking and current drinking groups according to their drinking status based on the question "Do you drink in the past?" and "Do you drink at present?". Second, the past and current drinkers' daily dosage of pure alcohol consumption was calculated by multiplying the alcohol content (according to the alcohol content in different types of alcohol typically seen in China: strong liquor 53%, weak liquor 38%, beer 4%, grape wine 12%, rice wine 15%) and the amount drunk per day (the unit is "liang," equivalent to 50 g). Third, the daily dosage was transferred into standard drinks, that is, per 10 g of pure alcohol equivalent to one standard drink (5). Risky drinking was defined as drinking alcohol above 2 drinks per day in old adults (17). Finally, participants with risky drinking were divided into past risky drinking and current risky drinking groups according to their drinking status, and others were grouped into the no risky drinking group.

Potential Correlates of Risky Drinking

Potential correlates of risky drinking in the oldest-old were selected according to previous studies (5, 13, 14, 18). The sociodemographic characteristics were sex, age, residence (urban, rural), ethnic group (han, others), marriage [in marriage, not in marriage (never married/widowed/divorced)], living

arrangement (with families, alone), education (no schooling, schooling), occupation (no work, agriculture, white collar), and financial status (poor, middle, rich). Behaviors and health status were performing exercises (never, past, current), smoking (never, past, current), body mass index (BMI), falls (yes, no), hypertension (yes, no), diabetes (yes, no), dyslipidemia (yes, no), heart disease (yes, no), cerebrovascular disease (yes, no), gastrointestinal ulcer (yes, no), hepatitis (yes, no), Parkinson's disease (yes, no), epilepsy (yes, no), depressive symptoms (yes, no), anxious symptoms (yes, no) and sleep quality (good, not good). BMI was divided into four groups according to the Asian criteria: underweight-BMI < 18.5 kg/m^2, normal weight-BMI > 18.5 to 23 kg/m^2, overweight-BMI between 23–24.9 kg/m^2, and obesity-BMI > 25 kg/m^2 (19). Depressive symptoms were divided into two groups with a cutoff of 10 on the 10-item Center for Epidemiologic Studies Depression Scale (CES-D-10) (20). An anxious state was divided into two groups using the cutoff 10 of the Generalized Anxiety Disorder-7 (GAD-7) (21).

Data Analysis

The descriptive results of sociodemographic variables, behaviors and health status, prevalence of risky drinking, daily dosage and type of alcohol were presented as frequencies (percentages) for categorical variables and means and standard deviations (SDs) or medians (p25, p75) for continuous variables according to the distribution of the variable. For group comparisons, one-way analysis of variance or non-parametric test was used for continuous variables, while the chi-square test was used for categorical variables. The factors with a *P*-value <0.1 in the univariate analysis and those identified in previous studies were included in the multinomial logistic regression analysis (**Supplementary Table 1**). Odds ratios (ORs) and 95% confidence intervals (CIs) were used in data interpretation for regression analyses. *P*-value <0.05 was considered statistically significant. All analyses were performed with SPSS 20.0 (IBM SPSS Inc., Chicago, IL, USA).

RESULTS

Description of Sociodemographic and Health Status

Of the 10,141 old adults, 4,077 (40.2%) males and 6,064 (59.8%) females, and the mean (SD) age was 92.32 (7.75). Overall, 55% lived in urban areas, 77.4% were not in marriage (never married/widowed/divorced), 76.8% were living with families, 63% had no schooling experience, and 53.3% had worked in agriculture. For the behavior and health status, 37% of the sample reported currently doing exercises, 12% were current smokers, 17% were obese, 25.3% had fall experience, 40.2% had hypertension, 7.7% had diabetes, 17.5% had heart disease, 11.3% had cerebrovascular disease, 49.4% had depressive symptoms, 11.6% had anxious symptoms, and 2.9% did not have good sleep. Compared to females, males were more likely to live in urban areas, be in marriage, live with others, be educated, be white-collar workers and have better financial status, do exercises currently, smoke, have a higher BMI, suffer cerebrovascular disease and Parkinson's disease but less likely to experience falls, suffer hypertension, depressive symptoms, anxious symptoms, and sleep trouble (see **Table 1**).

Prevalence, Dosage, and Type of Risky Drinking

There were 77.2% of people who never drank, 11.2% had past drinking, and 11.6% were current drinkers. A total of 89.4% of participants reported no risky drinking, 6.2% reported past risky drinking, and 4.4% reported current risky drinking. Of the male respondents, 12.2% reported past risky drinking, and 8.9% reported current risky drinking. The corresponding figures for females were 2.1 and 1.4%, respectively. Males showed a higher prevalence of past and current risky drinking than females ($P < 0.001$) (**Figure 1**).

Among past risky drinkers, the daily dosage was 4.5 (4, 8) drinks for the total population, 4.5 (4, 8) drinks for males, and 4 (3, 8) drinks for females. Among current risky drinkers, the daily dosage was 4 (3, 6) drinks for total population, 4 (3.6, 6) drinks for the males, and 3.3 (3.4, 4.7) drinks for females. Among past risky drinkers, 481(77.0%) drank no <4 drinks per day, 390 (78.2%) for males and 91 (72.2%) for females. Among current risky drinkers, 310 (69.2%) drank no <4 drinks per day, 268 (74.0%) for males and 42 (48.8%) for females. The past risky drinking group drank more than the current group (**Table 2**).

Among those who drank in the past or at present, the most preferred alcohol beverage was strong liquor in all groups, which was 46.9, 46.4, and 46.6% in the no risky, past risky, and current risky drinking groups, respectively, as well as in males and females (**Table 3**).

The Correlates of Risky Drinking

Compared with no risky drinkers, males (OR = 3.30, 95% CI: 2.33–4.66) and people who smoked (OR = 6.40, 95% CI: 4.68–8.76 for the past and OR = 3.26, 95%CI: 2.28–4.66 for the current) or had cerebrovascular disease (OR = 1.40, 95% CI: 1.01, 1.93) were more likely to have past risky drinking. In males, people with older age (OR = 0.97, 95% CI:0.95–0.99) and white-collar occupations (OR = 0.63, 95% CI:0.42–0.95) were less likely to have past risky drinking, but with heart disease (OR = 1.38, 95% CI:1.01–1.90) and smoking (OR = 6.33, 95% CI: 4.57–9.618 for the past and OR = 3.56, 95% CI: 2.34–5.40 for the current) were more likely to have past risky drinking. In females, those with past smoking were more likely to have past risky drinking (OR = 6.17, 95% CI: 3.50–10.86) (**Table 4**).

Compared with no risky drinkers, people who were older (OR = 0.96, 95% CI: 0.94–0.99), living in urban areas (OR = 0.68, 95% CI: 0.50–0.93), having schooling experience (OR = 0.71, 95%CI: 0.537–0.946) and having heart disease (OR = 0.49, 95% CI: 0.31–0.78) were less likely to have current risky drinking. Men (OR = 5.822, 95% CI: 3.705–9.148) and people who smoked (OR = 2.63, 95% CI: 1.74–3.97 for the past and OR = 4.44, 95% CI: 3.44–7.13 for the current) were more likely to have current risky drinking. In males, those of older age (OR = 0.95, 95% CI: 0.92–0.97), living in urban areas (OR = 0.67, 95% CI: 0.50–0.90), and heart disease (OR = 0.58, 95% CI: 0.35–0.94) were

TABLE 1 | Characteristics of 10,141 participants and compared between male and female.

Variables	Total sample (*N* = 10,141)	Gender		*p*
		Male (*N* = 4,077)	Female (*N* = 6,064)	
Sociodemographic characteristics				
Age, year (Mean ± SD)	92.32 ± 7.75	90.61 ± 7.13	93.46 ± 7.94	**<0.001**
Residence, urban (%)	5,622 (55.0)	2,364 (58.0)	3,258 (53.7)	**<0.001**
Ethnic group, Han (%)	8,249 (81.3)	3,296 (80.8)	4,953 (81.7)	0.29
In marriage, no (%)	1,160 (77.4)	2,423 (60.0)	5,337 (88.9)	**<0.001**
Living with family member, yes (%)	7,784 (76.8)	3,187 (78.2)	4,597 (75.8)	**0.006**
No Schooling, yes (%)	5,461 (63.0)	1,249 (36.5)	4,212 (80.4)	**<0.001**
Occupation, *n* (%)				
No work	3,827 (37.7)	1,493 (36.6)	2,334 (38.5)	**<0.001**
Agriculture	5,410 (53.3)	1,920 (47.1)	3,490 (57.6)	
White-collar	904 (8.9)	664 (16.3)	240 (4.0)	
Financial status, *n* (%)				
Poor	1,140 (11.4)	426 (10.6)	714 (12.0)	
Middle	6,909 (69.1)	2,649 (65.8)	4,260 (71.3)	**<0.001**
Rich	1,949 (19.5)	952 (23.6)	997 (16.7)	
Behavior and health status				
Exercises, *n* (%)				
Never	5,516 (55.7)	2,090 (52.5)	3,426 (57.9)	
Past	716 (7.2)	168 (4.2)	548 (9.3)	**<0.001**
Current	3,665 (37)	1,723 (43.3)	1,942 (32.8)	
Smoking, *n* (%)				
Never	7,316 (73.1)	1,929 (47.8)	5,387 (90.3)	
Past	1,484 (14.8)	1,171 (29)	313 (5.2)	**<0.001**
Current	1,204 (12.0)	936 (23.2)	268 (4.5)	
Body Mass Index, *n* (%)				
Low	2,864 (28.5)	886 (21.9)	1,978 (32.9)	
Normal	4,160 (41.4)	1,767 (43.7)	2,393 (39.8)	
Overweight	1,318 (13.1)	650 (16.1)	668 (11.1)	
Obesity	1,713 (17.0)	740 (18.3)	973 (16.2)	**<0.001**
Fall, yes (%)	2,512 (25.3)	910 (22.8)	1,602 (27.0)	**<0.001**
Hypertension, yes (%)	3,739 (40.2)	1,459 (38.8)	2,280 (41.2)	**0.025**
Diabetes, yes (%)	687 (7.7)	292 (8.0)	395 (7.4)	0.291
Dyslipidemia, yes (%)	341 (3.9)	133 (3.7)	208 (4.0)	0.522
Heart disease, yes (%)	1,578 (17.5)	604 (16.5)	974 (18.1)	0.052
Cerebrovascular disease, yes (%)	1,015 (11.3)	486 (13.3)	529 (9.9)	**<0.001**
Gastrointestinal ulcer, yes (%)	398 (4.5)	147 (4.1)	251 (4.8)	0.119
Hepatitis, yes (%)	24 (0.3)	11 (0.3)	13 (0.2)	0.609
Parkinson, yes (%)	90 (1.0)	47 (1.3)	43 (0.8)	**0.025**
Epilepsy, yes (%)	26 (0.3)	11 (0.3)	15 (0.3)	0.857
Depressive symptom, yes (%)	4,458 (49.4)	1,787 (47.6)	2,671 (50.7)	**0.003**
Anxious symptom, yes (%)	1,035 (11.6)	326 (8.8)	709 (13.7)	**<0.001**
Sleep quality, good (%)	7,429 (97.1)	3,022 (97.7)	4,407 (96.8)	**0.023**

SD, standard deviation. Data were presented as the mean ± SD, and n (%). P-values were obtained using the two-tailed two-sample t-tests for age, and using two-tailed Chi-square test for other variables.

less likely to have current risky drinking. Males who smoked (OR = 2.53, 95% CI: 1.64–3.90 for past and OR = 4.34, 95% CI: 2.92–6.44 for current) were more likely to have current risky drinking. In females, those with higher education (OR = 0.30, 95% CI: 0.09–0.93) and heart disease (OR = 0.23, 95% CI: 0.05–0.97) were less likely to have current risky drinking. Females with current smoking were more likely to have current risky drinking (OR = 8.41, 95% CI: 4.42–16.00) (**Table 4**).

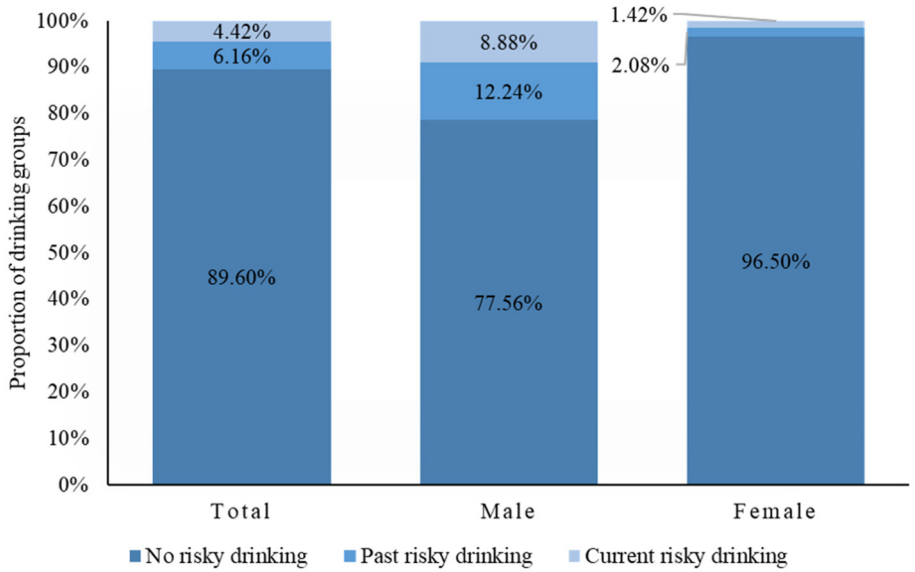

FIGURE 1 | Proportion of different risky drinking groups among whole sample and different genders.

TABLE 2 | Daily dosage of alcohol drinking stratified by risky drinking group and sex.

	Median drinks per day	Daily dosage group *n* (%)		
	(p25, p75)	drinks≤2	2< drinks<4	≥4 drinks
Total sample				
Total	0	9,068 (89.5)	282 (2.8)	799 (7.8)
No risk drinking	1.5 (0.6,2)	9,068 (100)	–	–
Past risky drinking	4.5 (4, 8)	–	144 (23.0)	481 (77.0)
Current risky drinking	4 (3, 6)	–	138 (30.8)	310 (69.2)
Male				
Total	0 (0,2)	3,216 (78.9)	203 (5.0)	658 (16.1)
No risk drinking	0	3,216 (100)	–	–
Past risky drinking	4.5 (4, 8)	–	109 (21.8)	390 (78.2)
Current risky drinking	4 (3.6,6)	–	94 (26.0)	268 (74.0)
Female				
Total	0	5,852 (86.5)	79 (1.3)	133 (2.2)
No risk drinking	0	5,852 (100)	–	–
Past risky drinking	4 (3, 8)	–	35 (27.8)	91 (72.2)
Current risky drinking	3.3 (3,4.7)	–	44 (51.2)	42 (48.8)

Data were presented as the median (p25, p75) and n (%).

DISCUSSION

In our study, there were 6.2% past risky drinkers and 4.4% current risky drinkers. A total of 12.2% of males and 2.1% of females were past risky drinkers and 8.9% of males and 1.4% of females were current risky drinkers. Compared with females, males were more likely to have both past and current risky drinking. Among past and current risky drinkers, males had higher daily alcohol dosage than females. Except for female current risky drinkers, most risky drinkers drank no <4 drinks per day. Males of older age and white-collar occupation were less likely to have past risky drinking, but were more likely to have past risky drinking if they smoked or had heart disease. Females were more likely to have past risky drinking if they smoked in the past. Males of older age, living in urban areas, and having heart disease were less likely to have current risky drinking, but were more likely to have current risky drinking if they smoked. Females with educational experience were less likely to have current risky drinking, but if they smoke in the current, they were more likely to be current risky drinkers.

TABLE 3 | Type of alcohol beverages stratified by risky drinking group and sex among people who drank (n = 3,270).

	Total sample				Gender							
					Male				Female*			
	Total	No risky drinking	Past risky drinking	Current risky drinking	Total	No risky drinking	Past risky drinking	Current risky drinking	Total	No risky drinking	Past risky drinking	Current risky drinking
Strong Liquor	1,536 (46.9)	1,343 (46.9)	111 (46.4)	82 (46.6)	751 (48.8)	584 (49.3)	96 (48.0)	71 (46.4)	785 (45.2)	759 (45.3)	15 (38.5)	11 (47.8)
Weak Liquor	768 (23.4)	667 (23.3)	52 (21.8)	49 (27.8)	329 (21.4)	245 (20.7)	41 (20.5)	43 (28.1)	439 (25.3)	422 (25.2)	11 (28.2)	6 (26.1)
Grape wine	66 (2.0)	60 (2.1)	2 (0.8)	4 (2.3)	26 (1.7)	21 (1.8)	1 (0.5)	4 (2.6)	40 (2.3)	39 (2.3)	1 (2.6)	0 (0)
Rice wine	556 (17.0)	488 (17.1)	39 (16.3)	29 (16.5)	259 (16.8)	198 (16.7)	36 (18)	25 (16.3)	297 (17.1)	290 (17.3)	3 (7.7)	4 (17.4)
Beer	180 (5.5)	151 (5.3)	22 (9.2)	7 (4)	107 (7.0)	80 (6.8)	20 (10.0)	7 (4.6)	73 (4.2)	71 (4.2)	2 (5.1)	0 (0)
Others	153 (4.7)	152 (5.3)	13 (5.4)	5 (2.9)	57 (3.7)	57 (4.9)	6 (3.0)	3 (2.0)	96 (5.5)	95 (5.7)	7 (17.9)	2 (8.7)

Data were presented as the percentage.

In our study, 11.6 % of elders were current drinkers and the prevalence of current risky drinking was 4.4% for all participants, 8.9% for males, and 1.4% for females. In a Chinese adult study, 68.2% were current drinkers, and 15% were risky drinkers (22). The prevalence of current risky drinking found in our study was also lower than that in several Western countries (21% for total, 20–12.1% for men and 9–3.6% for women) (8, 23, 24). The lower prevalence in our study compared with other data may be partly due to our older sample (average age 92.32 years). It is known that risky drinking is a behavior related to early mortality (8), namely, risky drinkers are more likely to die at a younger age than those with no risky drinking. Hence, survival bias may partially explain the lower prevalence of risky drinking in our study. In addition, the criteria for risky drinking and drinking are inconsistent in diverse studies and cultures (5, 17, 25, 26). In view of the different criteria for risky drinking and the lack of consensus about the criteria for risky drinking in the Chinese oldest-old (27), we chose 2 drinks as the cutoff based on previous literature (17). Most studies assessed drinking without distinguishing current from past risky drinking (8, 9, 23, 28, 29), while we differentiated past risky drinking from current risky drinking to clarify the association of correlates with past risky drinking. It was found that 70% non-drinkers were ex-drinkers, which may have a contaminating effect of drinking on health (25, 26). Thus, it is essential to classify risky drinking into past and current risky drinking. Consistent with previous reports (27, 30, 31), males had a higher prevalence of drinking or risky drinking. They drank faster with larger amounts than women, which mainly resulted from the cultural value and norms (12). Overall, our results indicated that past and current risky drinking in the oldest-old Chinese was not rare, especially among men. Considering risky drinking related to many health problems, it is necessary for families and professionals to pay attention to the oldest-old's drinking status.

In our study, the median number of drinks consumed by the oldest-old was 4.5 and 4 in the past and current risky drinking groups, respectively. It was found that among risky drinkers, most elders drank at higher dosages (28), which was consistent with our findings. We found that strong liquor was the most widespread alcohol beverage, and strong liquor and weak liquor, as the top two alcohol beverages, accounted for more than 60% of all alcohol beverages, while beer accounted for no more than 10%. In one study from the United Kingdom, it was shown that among a large sample of older people (75 years and over), relatively few elders drank more than 4 drinks per day, and half of them drank wine, 30% beer, and only 12% liquor (32). The difference in drinking patterns between different countries may be related to different cultures. The WHO reported that liquor accounted for 57% of alcohol beverages and beer accounted for 34% among young drinkers (aged 15 above) (33). The difference in preference for alcohol types in younger and older populations in China may result from young people bringing a more Western style of drinking into traditional drinking patterns. Consequently, the higher daily dosage and type of strong liquor should push health care and policy-makers pay more attention to the oldest old population and their drinking patterns.

TABLE 4 | Association between related factors and risky drinking in whole sample and different sex (n = 10,141).

Variables	Past risky drinking			Current risky drinking		
	Total OR (95% CI)	Male OR (95% CI)	Female OR (95% CI)	Total OR (95% CI)	Male OR (95% CI)	Female OR (95% CI)
Sociodemographic characteristics						
Age	0.99 (0.97, 1.01)	**0.97 (0.95, 0.99)***	1.02 (0.99, 1.06)	**0.96 (0.94, 0.99)***	**0.95 (0.92, 0.97)***	1.01 (0.96, 1.07)
Male	**3.30 (2.33, 4.66)****	–	–	**5.82 (3.70, 9.14)****	–	–
Residence (Urban)	0.89 (0.68, 1.16)	0.84 (0.62, 1.14)	1.09 (0.64, 1.84)	**0.68 (0.50, 0.93)***	**0.67 (0.50, 0.90)***	0.88 (0.41, 1.88)
Marriage (Yes)	1.07 (0.79, 1.45)	1.05 (0.76, 1.47)	0.88 (0.32, 2.39)	1.38 (0.97, 1.97)	1.35 (0.93, 1.97)	1.53 (0.45, 5.18)
Schooling≥1	0.78 (0.61, 1.00)	0.81 (0.59, 1.11)	0.70 (0.31, 1.54)	**0.71 (0.53, 0.94)***	0.81 (0.57, 1.15)	**0.30 (0.09, 0.93)***
Occupation						
Agriculture	1.09 (0.79, 1.51)	0.96 (0.66, 1.40)	1.57 (0.80, 3.06)	0.83 (0.57, 1.20)	0.83 (0.55, 1.27)	0.82 (0.35, 1.94)
White collar	0.76 (0.47, 1.23)	**0.63 (0.42, 0.95)***	3.87 (0.96, 15.62)	0.76 (0.42, 1.37)	0.67 (0.37, 1.24)	2.81 (0.28, 27.84)
Financial status						
Middle	1.08 (0.70, 1.68)	1.29 (0.75, 2.20)	0.69 (0.32, 1.47)	1.29 (0.76, 2.18)	1.4 (0.77, 2.52)	1.08 (0.35, 3.31)
Rich	1.45 (0.88, 2.40)	1.63 (0.89, 2.99)	1.14 (0.47, 2.80)	1.14 (0.61, 2.14)	1.29 (0.6, 2.59)	0.64 (0.13, 3.12)
Behavior and health status						
Smoking						
Past	**6.40 (4.68, 8.76)****	**6.33 (4.57, 9.61)****	**6.17 (3.50, 10.86)****	**2.63 (1.74, 3.97)****	**2.53 (1.64, 3.90)****	1.22 (0.15, 9.55)
Current	**3.26 (2.28, 4.66)****	**3.56 (2.34, 5.40)****	**2.08 (0.88, 4.92)****	**4.44 (3.44, 7.13)****	**4.34 (2.92, 6.44)****	**8.41 (4.42, 16.00)****
Body mass index						
Underweight	1.14 (0.83, 1.57)	1.00 (0.68, 1.48)	1.35 (0.77, 2.37)	1.11 (0.76, 1.62)	0.91 (0.59, 1.42)	2.27 (0.97, 5.31)
Overweight	1.06 (0.74, 1.53)	1.09 (0.72, 1.64)	0.94 (0.40, 2.22)	0.96 (0.61, 1.51)	1.01 (0.63, 1.61)	0.42 (0.05, 3.44)
Obesity	0.96 (0.67, 1.38)	1.09 (0.72, 1.64)	0.64 (0.27, 1.51)	1.36 (0.90, 2.06)	1.37 (0.88, 2.15)	1.62 (0.55, 4.75)
Hypertension (Yes)	0.98 (0.73, 1.31)	0.93 (0.66, 1.30)	1.12 (0.63, 1.98)	0.95 (0.67, 1.35)	0.93 (0.63, 1.36)	0.99 (0.41, 2.34)
Diabetes (Yes)	0.82 (0.54, 1.24)	0.87 (0.55, 1.37)	0.54 (0.16, 1.83)	0.57 (0.31, 1.07)	0.59 (0.31, 1.15)	0.45 (0.06, 3.46)
Heart disease (Yes)	1.28 (0.90, 1.82)	**1.38 (1.01, 1.90)***	0.86 (0.40, 1.84)	**0.49 (0.31, 0.78)***	**0.58 (0.35, 0.94)***	**0.23 (0.05, 0.97)***
Cerebrovascular disease (Yes)	**1.40 (1.01, 1.93)***	1.38 (0.96, 1.98)	1.48 (0.71, 3.09)	0.97 (0.67, 1.50)	0.98 (0.29, 3.28)	1.80 (0.49, 6.60)
Depressive state (Yes)	0.89 (0.68, 1.15)	0.90 (0.66, 1.21)	0.85 (0.50, 1.43)	0.96 (0.71, 1.30)	0.99 (0.71, 1.39)	0.80 (0.37, 1.73)
Anxious state (Yes)	1.19 (0.80, 1.77)	1.22 (0.75, 1.98)	1.16 (0.56, 2.39)	0.82 (0.47, 1.43)	0.64 (0.32, 1.29)	1.53 (0.55, 4.26)

*OR, odds ratio; CI, confidence interval. *$p < 0.05$; **$p < 0.001$.*

In this study, older men were less likely to be risky drinkers both in the past and in the current, which is in line with similar findings from previous community-based studies (14, 28). One reason was that people may stop drinking after the negative outcome of drinking, which may increase with age (25, 26). In addition, the total body water and fat of elders decreased, metabolic ability of alcohol in the liver worsened, and blood alcohol concentration easily increased after drinking, resulting in decreased tolerance to alcohol, so elders may lower their consumption with age (34). Additionally, there may be more alcohol use limitations to drug combination as people get older (34). No association was shown between age and risky drinking among women. The sex difference may result from the lower dosage and drinking method of females (12).

In our study, it is shown that males living in urban areas and having been white-collar workers and females with schooling experience were less likely to have risky drinking. This was in accordance with another study of CLHLS, which showed that males living in rural areas were more likely to be drinkers (12). Data from the WHO showed that a generally higher level of economic wealth was greatly associated with increased levels of alcohol consumption and lower abstention rates (33). Another study also observed that older people were more likely to drink more if they had better socioeconomic status (35). The discordance may be partially due to culture and society. In rural areas in China, homemade rice wine is popular because of its affordable price and traditional customs (36, 37). In addition, many people in rural areas prefer herbal wine to treat diseases or symptoms, as the health care resources are less abundant (12, 36). The association between occupation and risky drinking in females was opposite to males, which may be due to the relatively small sample in females with working backgrounds, thus, it was not possible to test the schooling/no schooling differences.

Smoking, whether in the past or at present, showed a strong association with risky drinking, which has been well-documented in other studies (8, 32, 33). It is well-known that smoking and drinking frequently coexist (8, 38). We found that males with heart disease were more likely to have past risky drinking, which is in accordance with other studies (39, 40). This finding may indirectly support the evidence that risky drinking in the past increases the risk of heart disease. On

the other hand, both males and females with heart disease were less likely to have current risky drinking. This may be due to the "sick quitters" who must stop drinking when they have a physical disease (39, 40). Addressing the correlates for risky drinking in the oldest-old population is of utmost importance and the public should not ignore this problem. Focus should be paid to these people with such correlates in practice.

The strengths of this study are as follows: (1) To the best of our knowledge, this is the first study to examine the prevalence and correlates of risky drinking among the oldest-old in China, with the largest representative sample of community-dwelling people aged 80 and over. (2) We divided risky drinking into past and current risky drinking, which provided the opportunity to describe and explore their correlates, respectively, and our results supported the necessity of this classification. (3) We have analyzed abundant possible correlates of past and current risky drinking.

There were several limitations in this study. The findings of this study could not be generalized to all populations except for community-dwelling oldest-old individuals. Moreover, the information was collected by self-report, which may result in recall bias, especially for past drinking dosage. However, previous evidence supported the validity of this self-report method (41). Additionally, as this is a cross-sectional study, the causal relationship of correlates and risky drinking cannot be drawn based on the present findings. We had no information about the medical treatment of the oldest-old which may be another correlate to risky drinking.

CONCLUSIONS

Risky drinking of elders aged 80 years and over was not rare, especially in males. The correlates of past and current risky drinking were different. Men and women had various correlates of risky drinking as well. Those with higher socioeconomic status were less likely to be risky drinking. This study has filled the gap of risky drinking among the oldest old in China. More attention should be given to the issue of risky drinking among the oldest old, and sex-specific intervention may be needed.

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 at: https://www.icpsr.umich.edu/icpsrweb/DSDR/studies/36179.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Research Ethics Committee of Peking University (IRB00001052-13074). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YQ and XL designed concept, analyzed data, interpreted data, and prepared manuscript. XY designed concept, interpreted outcome, and reviewed manuscript. TW, YZ, HW, and BL interpreted outcome and revised the manuscript. All authors have read and approved the manuscript and ensure that this is the case.

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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.2022.919888/full#supplementary-material

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ORIGINAL RESEARCH
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The Personality Traits and P300 of Offspring of Parents With Alcohol Dependence Differ Depending on Current Risky Drinking: A Preliminary Case-Control Study

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Aims: The aim of this study was to investigate the personality traits, and P300 component in the offspring of parents with alcohol dependence (OPAD) currently engaged in risky drinking and those not engaged in risky drinking, and to further explore the correlates of problematic alcohol use.

Methods: A case-control study was conducted according to the cutoff of the Alcohol Use Disorder Identification Test (AUDIT). The frequency of the TaqIA polymorphism of the dopamine receptor D2 gene associated with alcohol dependence was compared between the two OPAD groups. Tridimensional Personality Questionnaire (TPQ), The Alcohol, Smoking, and Substance Involvement Screening Test (ASSIST), and the MINI-International Neuropsychiatric Interview (M.I.N.I.) were measured or interviewed in OPAD not engaged in risky drinking (resilient; $n = 35$) and those currently engaged in risky drinking (vulnerable; $n = 20$). P300 was measured to test the possible electrophysiological differences. The correlates of alcohol use were analyzed.

Results: Vulnerable OPAD showed higher novelty seeking subscale scores (NS4; 4.45 $\pm$ 2.012 vs. 3.31 $\pm$ 1.728, $P < 0.05$) and harm avoidance subscale scores (HA4; 5.3 $\pm$ 2.319 vs. 3.66 $\pm$ 2.461, $P < 0.05$) than resilient OPAD, while the total scores of each dimension showed no significant difference. OPAD engaged in risky drinking showed more tobacco use than OPAD resistant to risky drinking. OPAD with risky drinking showed a shorter P300 latency than resilient OPAD on Fz electrodes. AUDIT scores of OPAD were correlated with P300 latency.

Conclusions: P300 differed between OPAD with and without risky drinking and alcohol use was associated with P300 latency, indicating that P300 may be used in the early detection of vulnerable OPAD and early intervention in the future.

Keywords: alcohol dependence, offspring of parents with alcohol dependence, risky drinking, tridimensional personality, P300

INTRODUCTION

Alcohol dependence is a chronic mental disease that has both genetic and environmental determinants (1). Large numbers of offspring have parents with alcohol dependence, resulting from the high prevalence of alcohol dependence (2). Evidence shows that OPAD have a 2.5-to 4.4-fold increase in the possibility of developing risky drinking (3) and are more likely to have other negative social and mental outcomes (3–5). Causal associations between offspring drinking and parent alcohol use have even been reported (6, 7). In fact, similar to all other factors, parent alcohol use elicits a heterogeneous influence: some offspring remain resilient, but others indulge in drinking. However, studies on the specific characteristics differentiating vulnerable OPAD from resilient OPAD the differences are limited.

Genetic studies have shown that people with TaqIA of the dopamine receptor D2 gene (DRD2A1) is significantly associated with alcohol dependence (8). Many independent meta-analyses of alcohol dependence and controls have shown this association (9, 10). However, another study did not find an association between DRD2A1 and alcohol dependence (11). There was also evidence that the DRD2A1 was associated with alcohol use among young children of parents with alcohol dependence (12), while the difference in DRD2A1 between OPAD with and without risky drinking remains unclear.

Tridimensional personality, especially novelty seeking, differs between people with alcohol dependence and without alcohol dependence (13). Some studies have indicated novelty seeking personality traits as precursors of alcohol dependence (14, 15). Studies reported higher novelty seeking scores in the children of parents with alcohol dependence, but most used healthy controls for comparison (16, 17). However, there is still a lack of studies focusing on differentiating the personality trait between OPAD with and without risky drinking.

P300 event-related potential (ERP) has been shown to reflect an objective physiological basis of cognitive functions (18), such as attention-dependent information processing and stimulus categorization, which are impaired in a wide range of neurological and psychiatric disorders. The latency of the P300 is associated with stimulus evaluation time, reflecting processing speed (19), while the amplitude of the P300 is related to the intensity of processing (20). Previous evidence has indicated P300 as an endophenotype for alcohol dependence (21, 22). Some researchers found an abnormal amplitude of P300 during the No-Go condition in OPAD compared to control groups (23, 24). Evidence from functional magnetic resonance imaging (fMRI) studies has suggested that OPAD students with current alcohol problem showed greater activity of the middle frontal gyrus and reduced activation of the posterior cingulate in response to visual working memory and emotional processing tasks (25). However, the differences in P300 in OPAD with and without risky drinking are still obscure and require further investigation.

To better understand the biological factors that may be associated with the outcomes for OPAD, this study aimed to investigate the genetic, psychological and P300 characteristics in the OPAD currently engaged in risky drinking and those not engaged in risky drinking, and further explore the correlates of problematic alcohol use.

METHODS

Participants

Fifty-five young adults were enrolled through advertisements on Wechat at Peking University Sixth Hospital. Participants completed the first screening to ensure whether they met the criteria for an offspring of a parent with alcohol dependence (OPAD) by asking "Did your father drink continuously for more than 1 year in your childhood and were there negative impacts on his physical and/or mental health, or an impact on his work or that of others?" according to the International Classification of Diseases, tenth edition (ICD-10) alcohol dependence criteria. Affirmations of all these three screening questions that indicated the participants were more than likely to be the offspring of parents with alcohol dependence. We only recruited offspring of fathers with ICD-10 alcohol dependence, in order to avoid the confounding effect of maternal alcohol abuse during pregnancy. All participants were right-handed and aged 18–45 years. They were excluded if they had a history of severe physical or neurological disease, conscious-loss or learning disability, or maternal alcohol use, and excluded if either parent had a history of schizophrenia, bipolar disorder or dementia.

All experimental procedures received approval from the Institutional Review Board of Peking University Sixth Hospital (No. 202046). All the participants provided written informed consent in writing at the beginning of the initial screening and participants were compensated for 100 RMB as their transportation allowance.

Procedures

All participants were instructed to refrain from using any psychoactive substances, including alcohol, tobacco, caffeine, and sedative medications 24 h before testing. Each of them was interviewed and measured during three sessions, including gene sampling, psychological assessment, and EEG acquisition, which were all conducted by two well trained psychiatrists.

Genotyping

The 55 participants were instructed to clean their mouths using pure water and then provide oral swabs; DNA was extracted using standard techniques. DNA was used in the polymerase chain reaction as a template for the determination of DRD2 TaqI A alleles. DRD2 TaqI A genotyping was performed as described in another study (26). Psychiatrists who conducted the psychological assessment and ERP measurement were blinded to the identity of the samples. The A1A1 genotype was indicated by the uncleaned 310 bp fragment; the A1A2 genotype was indicated by three fragments: 310, 130, and 180 bp; and the A2A2 genotype was indicated by two fragments: 130 and 180 bp. There were eight A1A1, 25 A1A2 and 22 A2A2 genotypes. A dichotomous group variable ($A1^+$ or $A1^-$) was designated according to the genotype carried by participants. The $A1^+$ group included eight A1A1 genotypes and 25 A1/A2 genotypes.

Assessment of Risky Drinking and Mental Health

The Alcohol Use Disorder Identification Test (AUDIT) was used to divide all the participants into the risky drinking and no risky drinking groups. Participants who met the criteria for the no risky drinking group had a score below 7 ($n = 35$) and those in the risky drinking group had score of 7 or greater ($n = 20$) according to the cutoff of 7 (27), which has been tested in China and found to be the best score to identify "risky drinking" in the Chinese population. The item-level content validity index was 0.83 and Cronbach's alpha was 0.782 (28).

Tridimensional Personality Questionnaire was a self-report instrument used to measure the personality tendency of OPAD, which included three dimensions (Novelty Seeking, NS; Harm Avoidance, HA; Reward Dependent, RD) and 12 subscales (NS1: exploratory excitability vs. rigidity; NS2: impulsiveness vs. reflection; NS3: extravagance vs. reserve; NS4: disorderliness vs. regimentation; HA1: anticipatory worry and pessimism vs. optimism; HA2: fear of uncertainty vs. confidence; HA3: shyness vs. gregariousness; HA4: fatigability and asthenia vs. vigor; RD1: sentimentality vs. insensitivity; RD2: persistence vs. irresoluteness; RD3: attachment vs. detachment; RD4 dependence vs. independence). TPQ is a normed and validated 100 item true-false questionnaire (29), and it has accepted validity and reliability in China (30). The Alcohol, Smoking, and Substance Involvement Screening Test (ASSIST) in this study was mainly used to assess other psychoactive substance use other than alcohol, such as tobacco, cannabis, cocaine, amphetamines, inhalants, sedatives, hallucinogens, opiates and other miscellaneous drugs. Concurrent validity was demonstrated by significant correlations between ASSIST scores ($r = 0.59$–0.88). The construct validity was 0.48–0.76 (29). The MINI-International Neuropsychiatric Interview (M.I.N.I.) were conducted in screening for mental disorders to rule out possible confounders. The criterion validity was ranged from 0.764 to 0.880, the concurrent validity within interviewers was 0.94 ($P < 0.01$), and the retest validity was 0.97 ($P < 0.01$) (30).

Auditory Oddball Paradigm

The paradigm consisted of frequently presented standard tones (90%), and infrequent target tones requiring a button press (10%). Standards (50 ms) and targets (100 ms) were 1,000 Hz, 75 dB pure tones. The task comprised a fixed pseudorandom sequence of 750 stimuli, with an interstimulus interval of 475–525 ms, divided into three blocks. Participants were instructed to press a response key to target tones only, using their right hand.

EEG Acquisition and P300 Extraction

Participants sat in front of a computer monitor and wore the headset for EEG recording during the oddball task (31). The EEG recording was digitized at 5,000 Hz from a 64-channel EEG system (Brain Products GmbH, Munich, Germany). The impedance of all electrodes was kept below 20 kΩ.

Signals were analyzed offline with the MATLAB R2014a (The Mathworks, Natick, MA, USA)-based EEGLAB toolbox (http://sccn.ucsd.edu/eeglab/). All recorded artifact-free EEG data were resampled to 500 Hz, referenced to an average of channels, and bandpass filtered in the range of 1–45 Hz to avoid the interference of 50 Hz signals. The time series were segmented into epochs with time- locked to the target auditory stimulus onsets (−200 to 500 ms) and baseline corrected (−200 to 0 ms). Epochs with excessive artifacts were removed. The data were then decomposed to perform an independent component analysis (ICA) *via* the runica algorithm. ICA components associated with vertical eye movements, heartbeats and other obvious artifacts were visually identified and removed according to their spatial, spectral, and temporal properties.

Participant ERP averages were calculated for target stimuli. P300 was identified as the most positive peak in a 235–400 ms window following stimulus onset at the Fz/Cz electrodes, where the P300 showed the largest amplitudes.

Data Analysis

Demographic, personality and psychological health assessment, the amplitude and latency of P300 and the frequency of genotypes were analyzed with SPSS 26.0. All statistical tests were set at a significant level of $P < 0.05$. Categorical variables and continuous variables were compared between no risky drinking group and the risky drinking group by the chi-squared test, Wilcoxon signed-rank test and t test. We performed linear regression analysis to examine the relationships between AUDIT scores and other variables which showed different significantly between groups in the univariate analysis. These variables were used as independent variables in the linear regression analysis and the AUDIT scores were used as the dependent variable.

RESULTS

Demographic Status, Clinical Feature, and Genotypes Among OPAD Based on Current Risky Drinking

The OPAD with risky drinking were similar in demographic status, including age, sex, ethnic group, areas where they were born, educational level, occupation, and marriage. With regard to clinical information, the risky drinking group showed higher scores on the AUDIT and ASSIST (tobacco) than the no risky drinking group. There was no significant difference in the genotype ratio between the two groups. No other psychoactive substances were used as measured by ASSIST (**Table 1**).

Tridimensional Personality for Offspring of Parents With Alcohol Dependence Based on Current Risky Drinking

There were no significant differences in the total scores of novelty seeking, harm avoiding and reward dependence between the two groups. The risky drinking group of OPAD showed higher scores on NS4 and HA4 (uncorrected; **Table 2**).

P300 for Offspring of Parents With Alcohol Dependence Based on Current Risky Drinking

Compared to OPAD without risky drinking, the latency of P300 at Fz in OPAD with risky drinking was shorter ($P = 0.0153$).

TABLE 1 | Demographic status, clinical features and genotypes among offspring of parents with alcohol dependence based on current risky drinking.

	No risky drinking ($N = 35$)	Risky drinking ($N = 20$)	$t/Z/\chi^2$	*P*-Value
Demographic data				
Age	28.8 ± 5.47	30.8 ± 5.45	−1.36	0.174
Sex (male/female)	6/29	8/12	3.504	0.061
Ethnic group (Han/other)	33/2	18/2	0.347	0.616
Born in city/country	27/8	18/2	1.414	0.297
Educational level	17.4 ± 2.64	17.8 ± 2.29	−0.019	0.985
Occupation (unstable/stable)	2/33	2/18	0.347	0.616
Marriage (not in marriage/in marriage)	25/10	13/6	0.053	0.817
Clinical information				
AUDIT score	0 (0, 2)	8 (7, 12)	−6.26	**<0.001**
ASSIT-tobacco	0 (0, 0)	3 (0, 14.75)	−3.174	**0.002**
M.I.N.I.				
Alcohol use disorder	0/35	4/16	7.549	**0.014**
Depression	15/20	13/7	2.497	0.097
Dysthymia	3/32	3/17	0.540	0.377
Mania	1/34	2/18	1.259	0.297
Panic disorder	1/34	1/19	0.167	0.599
Agoraphobia	2/33	1/19	0.013	0.703
Social phobia	2/33	2/18	0.347	0.616
Psychosis	2/33	2/18	0.347	0.616
Anorexia nervosa	1/34	3/17	2.783	0.131
Bulimia nervosa	1/34	4/16	4.526	0.053
Generalized anxiety disorder	2/33	5/15	4.262	0.086
Antisocial personality	0/35	1/19	1.782	0.364
Genotype				
A1+/A1−	22/13	11/9	0.327	0.567

Data were presented as the mean ± SD, median (p25, p75) and ratio. P-values were obtained using the two-tailed two-sample t-tests for age and educational level, using two-tailed non-parametric test for AUDIT score and ASSIST (tobacco) score, and using two-tailed Chi-square test for other variables.
AUDIT, Alcohol Use Disorder Identification Test; ASSIT, Alcohol, Smoking, and Substance Involvement Screening Test; M.I.N.I., MINI-International Neuropsychiatric Interview.
Statistical significant values are shown in bold font.

TABLE 2 | Personality of offspring of parents with alcohol dependence based on current risky drinking.

	No risky drinking ($N = 35$)	Risky drinking ($N = 20$)	*t*-Value	*P*-Value
Novelty seeking score	13.46 ± 4.468	15.05 ± 5.605	−1.027	0.304
Extravagance vs. reserve (NS1)	3.94 ± 1.662	4.2 ± 1.936	−0.792	0.428
Impulsiveness vs. reflection (NS2)	2.97 ± 1.843	2.85 ± 1.789	−0.621	0.534
Extravagance vs. reserve (NS3)	3.23 ± 1.784	3.75 ± 2.337	−0.742	0.458
Disorderliness vs. regimentation (NS4)	3.31 ± 1.728	4.45 ± 2.012	−1.986	**0.047**
Harm avoidance score	17.51 ± 6.464	19.65 ± 6.046	−0.991	0.322
Anticipatory worry/pessimism vs. optimism (HA1)	5.31 ± 2.398	5.75 ± 2.314	−0.732	0.464
Fear of uncertainty vs. confidence (HA2)	4.66 ± 1.454	4.65 ± 1.872	−0.428	0.668
Shyness vs. gregariousness (HA3)	3.89 ± 2.083	3.95 ± 2.139	−0.027	0.979
Fatigability and asthenia vs. vigor (HA4)	3.66 ± 2.461	5.3 ± 2.319	−2.292	**0.022**
Reward dependence score	17.38 ± 3.162	17.15 ± 3.2	−0.235	0.892
Sentimentality vs. insensitivity (RD1)	3.91 ± 1.055	3.8 ± 1.196	−0.226	0.821
Persistence vs. irresoluteness (RD2)	5.18 ± 1.866	4.9 ± 1.483	−0.732	0.464
Attachment vs. detachment (RD3)	6.41 ± 2.476	6.6 ± 2.415	−0.307	0.759
Dependence vs. independence (RD4)	1.88 ± 1.2	1.85 ± 1.268	−0.111	0.912

Data were presented as the mean ± SD. P-values were obtained using the two-tailed two-sample t-tests for all the variables.
Statistical significant values are shown in bold font.

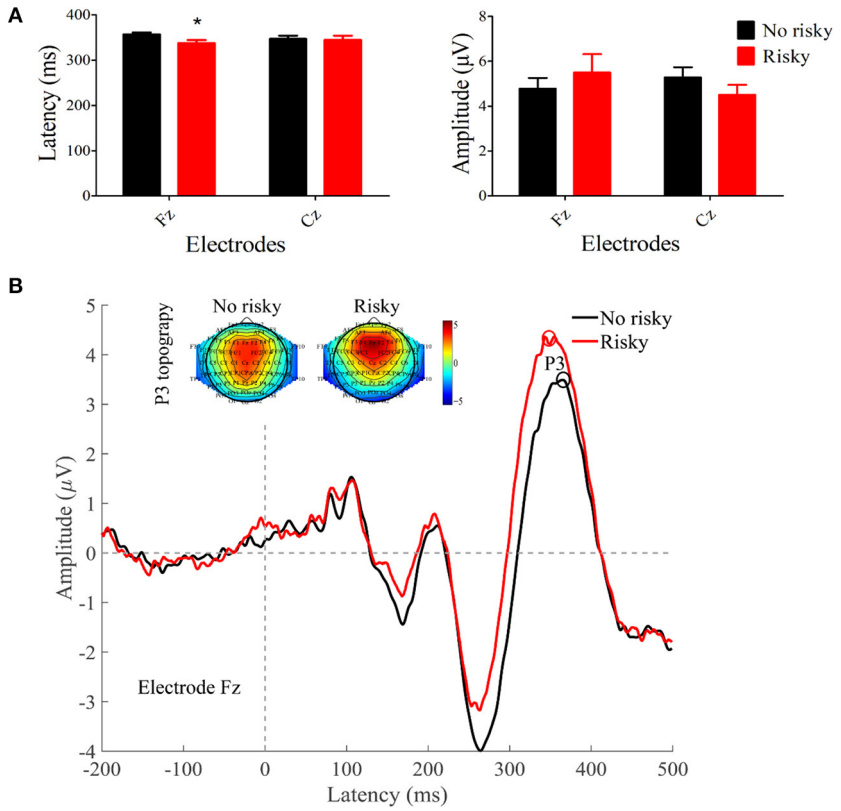

FIGURE 1 | Comparison of P300 latency and amplitude between OPAD with and without risky drinking respectively. **(A)** Comparison of P300 latency and amplitude between two groups on electrodes of Fz and Cz; **(B)** Grand-averaged ERP waveforms in response to target stimuli in the first halves and the second halves as a function of group. Topographic maps of the P300 (235–400 ms) are shown. *$P < 0.05$.

There were no significant differences in the amplitude of P300 (**Figure 1**).

Associations Between Risky Drinking and Psychophysical Characteristics

The linear regression analysis showed that the AUDIT score was negatively associated ($r = -0.31$, $P = 0.0224$) with the latency of P300 at Fz electrode (**Figure 2**). In addition, AUDIT also showed a correlation with disorderliness vs. regimentation (NS4; $r = 0.32$, $P = 0.016$; **Figure 3**) and fatigability and asthenia vs. vigor (HA4; $r = 0.27$, $P = 0.049$; **Figure 4**).

DISCUSSION

In the current study, differences in personality and P300 were observed in OPAD currently engaged in risky drinking, compared with those without risky drinking. These differences included higher NS4 and HA4 scores, higher ASSIST (tobacco) scores and shorter latency of P300. Furthermore, the AUDIT scores were negatively correlated with the latency of P300 and positively correlated with NS4 and HA4 scores.

In this study, the total scores of novelty seeking, harm avoidance and reward dependence personality did not differ between OPAD with and without risky drinking, while OPAD with risky drinking showed more significant disorderliness (NS4) and fatigability and asthenia (HA4) than OPAD without risky drinking. The division of participants into groups based on their current risky drinking is justified by the observation that OPAD who do not have risky drinking at a young adult age are likely to represent resilient individuals, and OPAD with risky drinking are considered to be vulnerable individuals (25). Previous studies have showed that higher novelty seeking scores in the children of parents with alcohol dependence than in healthy controls (16, 17). Another study also found that novelty-seeking personality traits were more significant in families with high density of addiction than in those with a low density of addiction, and in unaffected people with positive family history of addiction, compared to people without positive family history (32–34). In a study investigating the three-dimensional personality between people with and without alcohol dependence, the former had a more apparent novelty-seeking personality than the control group (13). One study reported that compared with offspring of parents without alcohol dependence, OPAD had lower reward dependence scores and no significant difference in harm avoidance scores (17). Other studies showed that there were mixed results in comparing the differences of personality between unaffected people with and without family history of alcohol dependence (34, 35). The discordance may result from the

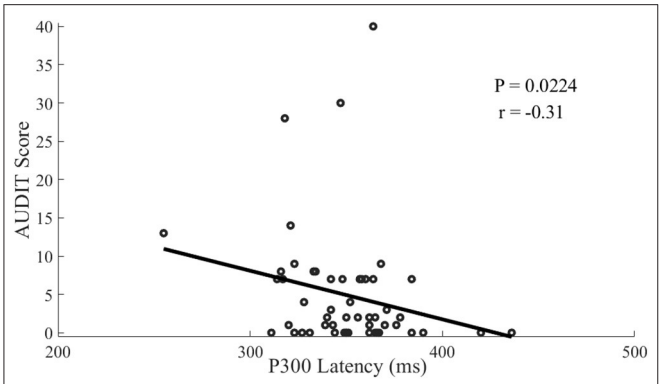

FIGURE 2 | Scatterplot of the correlation between AUDIT scores and P300-lantancy among offspring of parents with alcohol dependence.

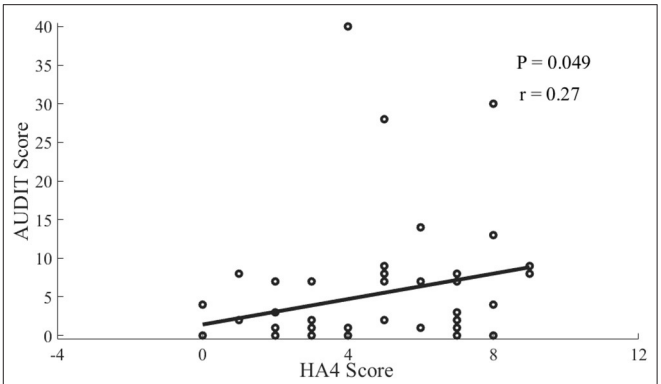

FIGURE 4 | Scatterplot of the correlation between AUDIT scores and HA4 Score among offspring of parents with alcohol dependence.

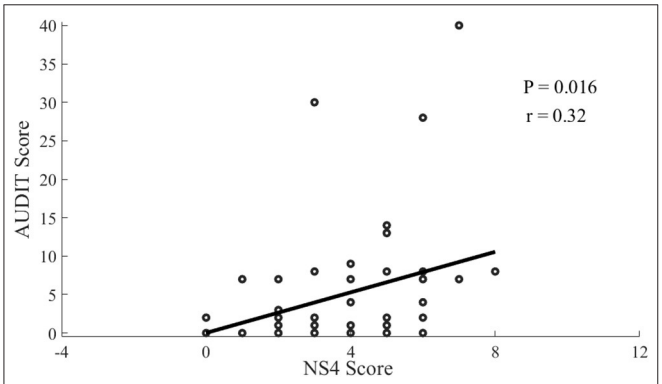

FIGURE 3 | Scatterplot of the correlation between AUDIT scores and NS4 Score among offspring of parents with alcohol dependence.

different sample, study design, and assessment tools, in which the difference between groups among OPAD would not be as significant as the difference between probands and health controls, but it is meaningful to explore the possible precursor before the syndrome bursts out. However, the subtle higher NS4 and HA4 in OPAD with risky drinking can hint at the profile of these vulnerable people who were more disorderly and fatigable, which may help the clinician to understand and make more appropriate intervention strategies.

The risky drinking participants in the current study showed not only risky drinking but also significantly more tobacco use than the no risky drinking group. Prior studies also showed that both alcohol and other substances were used in OPAD, which may result from the need to reduce people's negative feelings (36, 37). Therefore, the current results indicated the possibility that OPAD with risky drinking may also use other substances to manage their possible emotional problems, which should be explored in the future.

This study showed the alteration in latency, not the amplitude of P300 in OPAD with risky drinking than in OPAD without risky drinking. Some previous studies found lower (24) or higher (38) amplitudes of P300 in OPAD than in healthy controls. Some reports did not find a significant difference in the latency of P300 between OPAD and healthy controls (21, 22). Our findings differ from those of other studies. This may be because all the subjects included in this study were OPAD and had no previously diagnosed psychological illnesses, which decreased the discrepancy while the comparison between OPAD and healthy controls would be more significant. As shorter P300 latency was associated with better information processing (39, 40), our different result may indicate that OPAD with risky drinking may process stimulus tasks faster than OPAD without risky drinking, which may be a compensatory mechanism of OPAD to risky drinking. The long-term outcome of the cognition under the impact of risky drinking should be further explored.

In our study there were significant negative correlations between AUDIT scores and P300 latency, while other studies didn't find such correlation (21, 22), possibly because other studies compared the OPAD with the healthy controls. The correlations in this study may result from that alcohol use at an early stage possibly increases the sensitivity and excitability of cerebral cortex (disinhibition), supported by the evidence of greater activity of middle frontal gyrus in young OPAD with risky drinking in response to visual working memory and emotional processing tasks (25). There were positive correlations between AUDIT scores and NS4 and HA4 in this study, which was not in accordance with other studies (16, 17), mainly due to different sampling as well. It indicated that the disorderliness and fatigability may increase the possibility of alcohol use among the OPAD, and vice versa, which can be explained by the mechanism of self-medication (36).

There are scarce studies focusing on vulnerability for offspring of parents with alcohol dependence and exploring possible psychophysiological mechanisms, especially controlling the genetic and other confounders. The results may provide a special and specific research direction for future studies in determining psychological and psychophysiological endophenotypes that can help with early warning and intervention. The current study further detected the difference between two relatively homogeneous groups of OPAD, and this should be more difficult in that both groups are already at risk and they are likely

to be similar across many features. There are several possible limitations as follows. First, the probands were diagnosed according to the self-report of the OPAD, which may result in recalling bias, although we thoroughly checked the symptoms and criteria items to lessen the bias. Second, we do not include comparison groups with risky and no risky drinking whose parents have no history of alcohol use disorders, which may distinguish the impact from current risky drinking or the risk conferred from family. Selection bias may exist in that the participants were mostly had a high education level (as shown in **Table 1**) and stable occupation, which represented for higher socioeconomic status and may be protective factors. Further studies should recruit more representative participants from the general public. Another limitation of this study is the small sample size and unbalanced number of two groups, resulting in a more cautious explanation of the findings, because a small dataset is sensitive to deviation from the general population.

CONCLUSIONS

In the current study, tridimensional personality and P300 latency may respectively distinguish offspring of parents with alcohol dependence based on their risky drinking and P300 latency was significantly correlated with AUDIT scores. Therefore, these findings may be used in the future early detection and intervention.

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

The studies involving human participants were reviewed and approved by Institutional Review Board of Peking University Sixth Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YQ and JW conducted the statistical analysis and drafted the original manuscript. XY conceived the study and revised the manuscript. YZ, TW, and BL collected data and reviewed the manuscript. All authors had final responsibility for the submission and all of them read and approved the final version of the manuscript.

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Whole Transcriptome Sequencing of Peripheral Blood Shows That Immunity/GnRH/PI3K-Akt Pathways Are Associated With Opioid Use Disorder

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Background: Opioid use disorder (OUD), which is most commonly exhibited as addiction, is a persistent chronic disease that places a burden on families and society. Various peripheral traits have been linked to OUD in the past, but research on this topic is insufficient.

Methods: Seven male patients with OUD and 7 male healthy controls with matched demographic and clinical data were enrolled in this study. Peripheral blood RNA was used to construct an rRNA-removed library and a small RNA library. The peripheral transcriptomic differences between the two groups were investigated using RNA-seq. Differentially expressed messenger RNAs (mRNAs), long non-coding RNAs (lncRNAs), circular RNAs (circRNAs) and microRNAs (miRNAs) were identified by bioinformatics methods, and functional enrichment analysis with differentially expressed RNAs was performed to investigate the potential biological mechanisms of OUD.

Results: A total of 229 mRNAs (115 upregulated, 114 downregulated), 416 lncRNAs (191 upregulated, 225 downregulated), 17 circRNAs (16 upregulated, 1 downregulated) and 74 miRNAs (42 upregulated, 32 downregulated) were differentially expressed between the OUD group and the healthy control group. Functional enrichment analysis with differentially expressed mRNAs showed that immunity, GnRH secretion, and PI3K-Akt signaling pathways were associated with OUD. Immunity-, JAK-STAT-, and insulin-related pathways were enriched in functional enrichment analysis of target genes predicted by differentially expressed miRNAs.

Conclusion: We identified hundreds of differentially expressed genes that were enriched in immunity, GnRH secretion and PI3K-Akt signaling pathways. Some genes with significant changes might be used as potential biomarkers for progression and treatment of OUD.

Keywords: OUD, RNA-seq, lncRNA, immunity, GnRH secretion, PI3K-Akt signaling pathway

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INTRODUCTION

Opioid use disorder (OUD) is a chronic relapsing disorder characterized by loss of control of opioid drugs use, compulsive use, and continued use despite harm (1). These drugs include prescription painkillers such as morphine and illegal drugs such as heroin. According to an estimate by the Global Burden of Disease study in 2016, there were 26.8 million people with OUD worldwide (2). Opioid use outside of its appropriate clinical applications can directly cause physical harm and potential health sequelae, such as virus infection due to shared syringes. In addition, opioid abuse is associated with wider societal costs, a high divorce rate, reduced employment and a high crime rate (3). The physical and social harms caused by the abuse of opioids have become an increasingly serious public health issue.

Multiple brain regions, such as the nucleus accumbens (NAc), central nucleus of the amygdala (CeA) and prefrontal cortex (PFC), as well as the peripheral system, have been reported to be associated with addiction (3). Previous studies have revealed that metabolism, endocrine systems, immune systems and mitochondria play important roles in the process of long-lived behavioral abnormalities associated with addiction (4–8). Opiate addiction leads to autophagy-mediated dysfunction of mitochondria, such as a decrease in mitochondrial DNA (mtDNA) copy number in the hippocampus and peripheral blood and an increase in reactive oxygen species (ROS), which contributes to cell damage and apoptosis (6). In addition, recent studies have suggested that many cytokines and regulatory T cells in peripheral blood were dysregulated in patients with heroin addiction compared to healthy controls (8–11). Women with addiction had lower oxytocin levels in their peripheral blood, which could be used as a biomarker for predicting the intensity of social anxiety in female patients with heroin withdrawal (12). Overall, although there are many studies on the peripheral blood characteristics of addiction, fewer studies have focused on the systematic peripheral changes in OUD.

With the advances of high-throughput sequencing, we can now systematically investigate the transcriptome profile, including mRNAs, long non-coding RNAs (lncRNAs), circular RNAs (circRNAs) and miRNAs, in peripheral blood. In the present study, we enrolled 7 patients with OUD and 7 healthy controls and analyzed the transcriptome expression of peripheral blood by RNA sequencing. We identified hundreds of differentially expressed transcripts (mRNAs, lncRNAs, circRNAs, and miRNAs), and enrichment analysis with these differentially expressed transcripts suggested that several pathways might participate in the mechanisms of OUD.

MATERIALS AND METHODS

Participants and Ethics Statement

A total of 14 male participants, 7 patients with OUD and 7 healthy control subjects aged 40–50 years, who met the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5), diagnostic criteria for Opioid Use Disorder 1 (**Supplementary Table S1**). A problematic pattern of opioid use leading to clinically significant impairment or distress, as manifested by at least two of the following, occurring within a 12-month period: Opioids are often taken in larger amounts or over a longer period than was intended. All of them had nicotine dependence, were recruited from Wuhan Mental Health Center, Wuhan, China. We excluded subjects with the following criteria: (1) the subjects had polydrug use; (2) the subjects had a serious physical illness; (3) subjects with primary mental illness in the OUD group and those with mental illness in the control group. All participants provided written informed consent before enrollment. The protocols and recruitment procedures described in the present study were approved by the Research Ethics Committee of Wuhan Mental Health Center (Ky2022.02.04). The history of heroin use and current medication use was obtained via self-report and electronic medical records. Five milliliters of blood was drawn from each of the participants with an empty stomach at the same time of day in the morning.

Library Construction and Transcriptome Sequencing

Total RNA was extracted from the blood using the PAXgene® Blood RNA Kit (Qiagen, Hombrechtikon, Switzerland) according to the handbook's instructions. The purity, concentration and integrity of total RNA were assessed using a NanoPhotometer® spectrophotometer (Implen, CA, USA), Qubit® RNA Assay Kit with a Qubit® 2.0 Fluorometer (Life Technologies, CA, USA) and RNA Nano 6,000 Assay Kit of the Agilent Bioanalyzer 2,100 system (Agilent Technologies, CA, USA), respectively. In addition, RNA degradation and contamination were monitored on 1% agarose gels. To get the overview of whole transcriptome including the long transcripts (mRNAs, lncRNAs and circRNAs) and small RNAs (miRANs), the rRNA-removed library and small RNA library were constructed separately. For the rRNA-removed library, a total amount of 3 μg RNA per sample was used as input material for the RNA sample preparations. Ribosomal RNA was removed by an Epicenter Ribo-zeroTM rRNA Removal Kit (Epicenter, USA). Sequencing libraries were generated using the NEBNext® UltraTM RNA Library Prep Kit for Illumina® (NEB, USA) following the manufacturer's recommendations, and index codes were added to attribute sequences to each sample. The library preparations were sequenced on an Illumina HiSeq platform to generate 150 bp paired-end reads. For the small RNA library, a total of 2 μg of total RNA was isolated from each sample using the NEBNext® Multiplex Small RNA Library Prep Set For Illumina® (NEB, USA) according to the manufacturer's instructions. Small RNA library preparations were sequenced on a NovaSeq 6,000 platform, and 50 bp single-end reads were generated.

RNA-Seq Data Processing

For rRNA-removed library sequencing data, the raw sequencing reads were first processed to remove sequencing adapters and low-quality reads using Trimmomatic (version 0.39) with default parameters (13). For mRNA and lncRNA quantification, the clean reads were mapped to the human reference genome GRCh38 using HISAT2 (version 2.2.1) (14) and then sorted by samtools (version 0.11.0) (15) with the sort function by genome position. FeatureCounts (version 2.0.1) (16) was used

to count reads mapped to specific meta-features (mRNA or lncRNA). We downloaded the genome reference data and gene annotation file (Release 35) from the GENCODE website (https://www.gencodegenes.org/) (17). Since the gene annotation file from GENCODE contained only some lncRNAs, we downloaded the lncRNA annotation file from the NONCODE database (http://www.noncode.org/) (18, 19) and combined all the lncRNAs from the two databases. In total, 173,112 transcripts from 96,409 lncRNA genes were analyzed in this study. We quantified circRNA expression using the CIRIquant (version 1.1.1) process (https://ciri-cookbook.readthedocs.io/en/latest/CIRIquant_0_home.html) (20). Briefly, the clean reads were first mapped to the human reference genome using bwa (version 0.7.17) (21). The backsplice junction reads were identified and quantified by CIRI2 (version 2.0.6) (22, 23) and CIRIquant (20). For small RNA analysis, the miRNAs were quantified by miRDeep2 (24) using human miRNAs from miRbase (25) as a reference.

Differential Expression and Functional Enrichment

We merged all the mRNA, lncRNA and circRNA count files into one file because they were sequenced in one library. The DESeq2 (26) R package was used to conduct the differential expression analysis (mRNAs, lncRNAs, circRNAs and miRNAs). DEseq2 first fitted the reads count with negative binomial distribution model, and then conducted differential expression analysis by Ward test, which used the estimated standard error of a $\log_2$fold change to test if it is equal to zero. We used the multifactor design formula "design = ~age + height + weight + yearofedu + group" in DESeq2 to rule out the effect of confounding factors, including age, height, weight and years of education. Differentially expressed RNAs were defined with the following criteria: upregulated or downregulated 1.5 times and p-value < 0.01. Gene set enrichment analysis (GSEA) was used to conduct Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses with the ClusterProfiler (27) R package. Functional enrichment of genes targeted by differentially expressed miRNAs was conducted on the miEAA 2.0 website (28, 29). miEAA is a web-based application that provides miRNA function enrichment by their targeted genes.

Protein-Protein Interaction and Network Analysis

All differentially expressed mRNAs were used to construct a PPI network by STRING V11.5 (30, 31). To improve the network quality, we set the minimum required interaction score as high confidence (0.7). To construct the coexpression network, we selected the differentially expressed genes from the PI3K-Akt signaling pathway and GnRH secretion pathway and calculated the Pearson correlation coefficient of these genes with other RNAs (mRNA, lncRNA, circRNAs and miRNAs). Only the gene pairs with Pearson correlation coefficients >0.85 or <-0.85 were considered to be coexpressed. We also selected differentially expressed miRNAs and mRNAs to construct the network of miRNA-targeted mRNAs by Watson Crick pairing of nucleotides. miRWalk 2.0 was used to predict the differentially expressed miRNA-targeted mRNAs (32). To improve the prediction accuracy, we reserved genes that were also predicted by TargetScan (33). Because there were too many predicted target genes, we only showed the differentially expressed genes targeted by differentially expressed miRNAs. All the networks are displayed with Cytoscape (34).

RESULTS

Hundreds of Genes Were Differentially Expressed Between Patients With OUD and Healthy Controls

We obtained whole transcriptome data from 7 patients with OUD and 7 healthy control subjects. The raw sequencing data of every sample from rRNA-removed sequencing and small RNA sequencing were more than 13 G (bases) and 10 M (reads), respectively. The OUD group and healthy control group were distinct, according to the results of principal component analysis (**Figures 1A**, **2A**). A total of 229 mRNAs (115 upregulated, 114 downregulated), 416 lncRNAs (191 upregulated, 225 downregulated), 17 circRNAs (16 upregulated, 1 downregulated) and 74 miRNAs (42 upregulated, 32 downregulated) were differentially expressed between the OUD group and the healthy control group (**Figures 1B,C**, **2B,C**; **Supplementary Tables S2, S3**).

Differentially Expressed Genes Were Enriched in the Immune System and PI3K-Akt Signaling Pathway

Next, we conducted GO and KEGG gene function enrichment analyses with the differentially expressed genes. Many immune-related biological processes were enriched as a result of GO enrichment, including the response to virus (enrichment score = 0.47, p value = 5.24×10^{-8}) and the production of molecular mediators of the immune response (enrichment score = 0.46, p-value = 1.58×10^{-6}) (**Figure 1D**). GnRH (gonadotropin-releasing hormone) secretion (enrichment score = −0.54, p value = 2.37×10^{-3}), taste transduction (enrichment score = −0.61, p-value = 9.68×10^{-4}), and the PI3K-Akt signaling pathway (enrichment score = −0.40, p-value = 1.06×10^{-3}) were significantly enriched according to KEGG enrichment results (**Figure 1F**). These results suggested that the immune process was positively associated with OUD, whereas GnRH secretion and the PI3K-Akt signaling pathways were negatively associated with OUD.

PPI Network and Coexpression Network

Differentially expressed genes constituted a large PPI network and several small PPI networks (**Figure 1E**). The proteins in the largest network, such as IFIT5, ISG15, IFIH1, and OAS1, were mostly associated with immunity. A smaller network, which included MCM10, CDCA2, and PBK, was linked to DNA replication and DNA stability. In particular, we found that three proteins, GRIK4, GRIN2B, and GRIK5, which encode subunits of glutamate receptors, could interact with each other. The genes

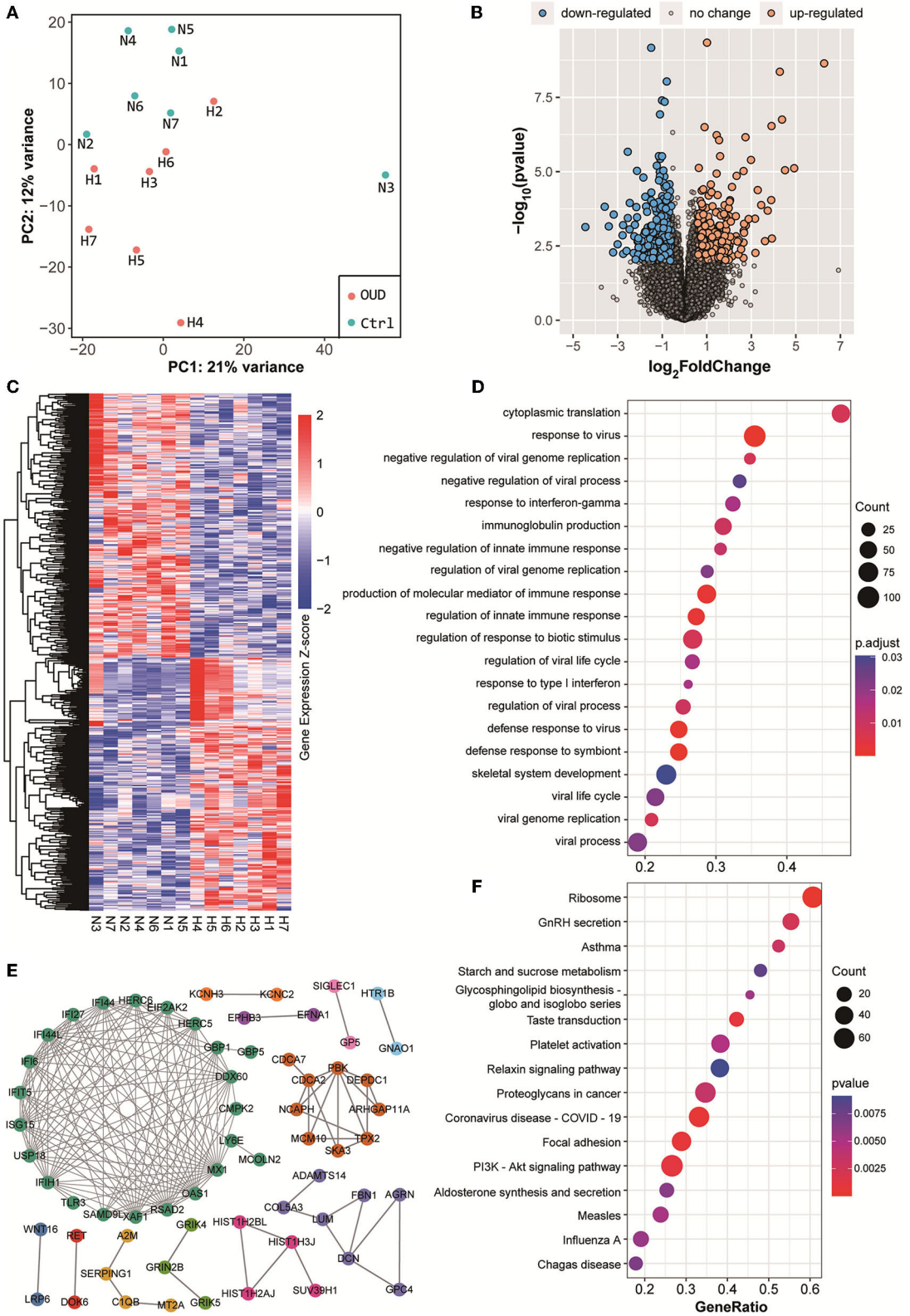

FIGURE 1 | Results of rRNA removed RNA-seq. **(A)** Principal component analysis results of all samples. The red dot in the figure represented the OUD group and blue dot represented the control group. **(B)** Volcano plot of all expressed genes. We set the threshold of differential expression as $|\log_2 FoldChange| > 1.5$ & $p\text{-value} < 0.01$. The blue dot, orange dot and gray dot represented the down-regulated genes (OUD/Control), up-regulated genes and non-significant changed genes,

(Continued)

FIGURE 1 | respectively. **(C)** Expression heatmap of all differentially expressed RNAs, including mRNA, lncRNAs and circRNAs. **(D)** GO biological process enrichment analysis results with differentially expressed mRNAs. **(E)** Protein-protein interaction results of differentially expressed genes. Gene names rendered in different colors indicated that they were in different protein interaction networks. **(F)** KEGG pathway enrichment analysis results with differentially expressed mRNAs.

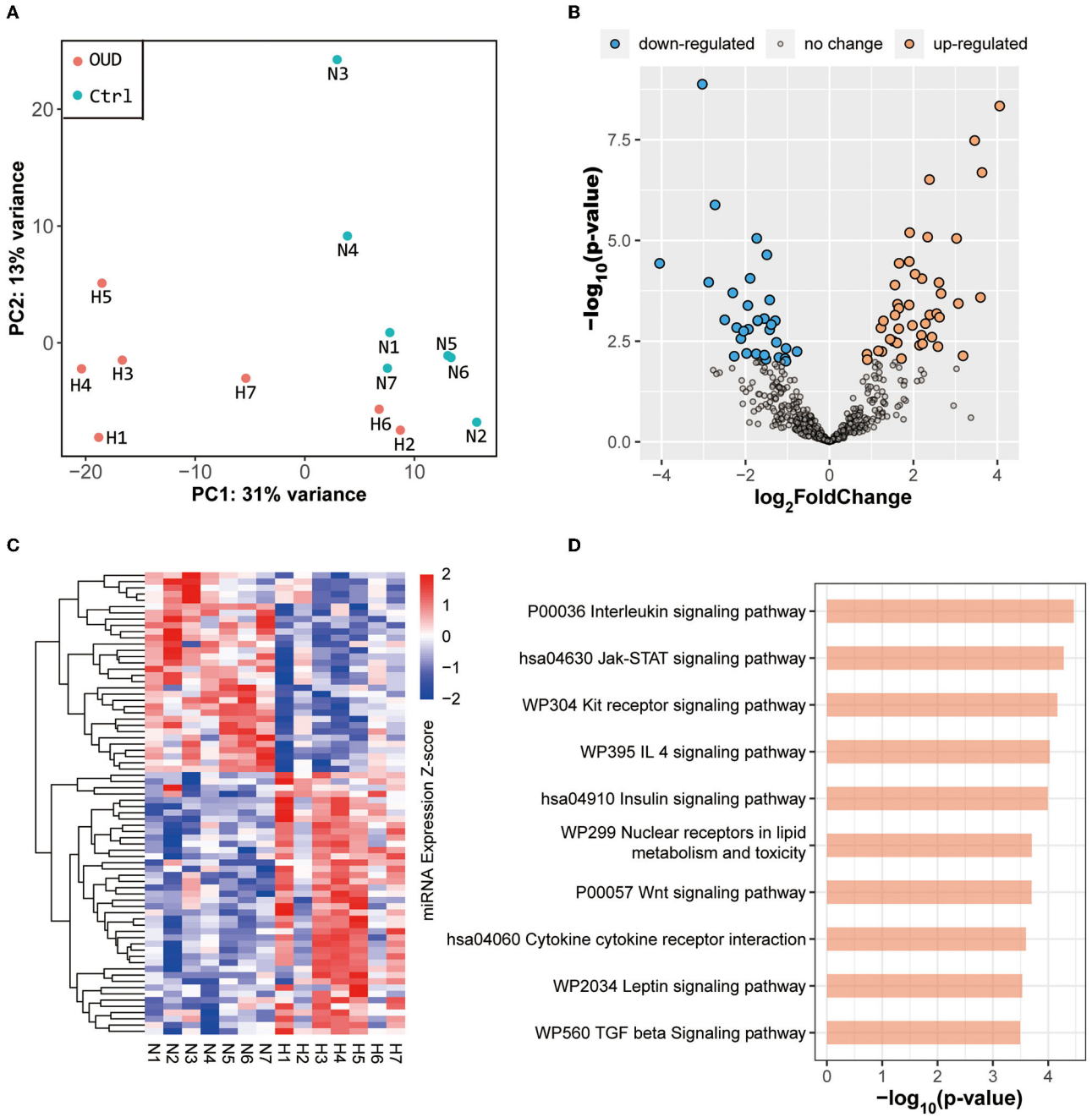

FIGURE 2 | Results of small RNA sequencing. **(A)** Principal component analysis results of all samples. The red dot in the figure represented the OUD group and blue dot represented the control group. **(B)** Volcano plot of all expressed miRNAs. We set the threshold of differential expression as $|\log_2 FoldChange| > 1.5$ & $p\text{-value} < 0.01$. The blue dot, orange dot and gray dot represented the down-regulated miRNAs (OUD/Control), up-regulated miRNAs and non-significant changed miRNAs, respectively. **(C)** Expression heatmap of all differentially expressed miRNAs. **(D)** KEGG enrichment result of targeted genes predicted by differentially expressed miRNAs.

differentially expressed in the PI3K-Akt signaling (**Figure 3A**) and GnRH secretion pathways (**Figure 3B**) were found to be coexpressed with many additional differentially expressed mRNAs and ncRNAs in the coexpressed network. A number of differentially expressed mRNAs were targeted by differentially expressed miRNAs (**Figure 3C**). Some of these differentially

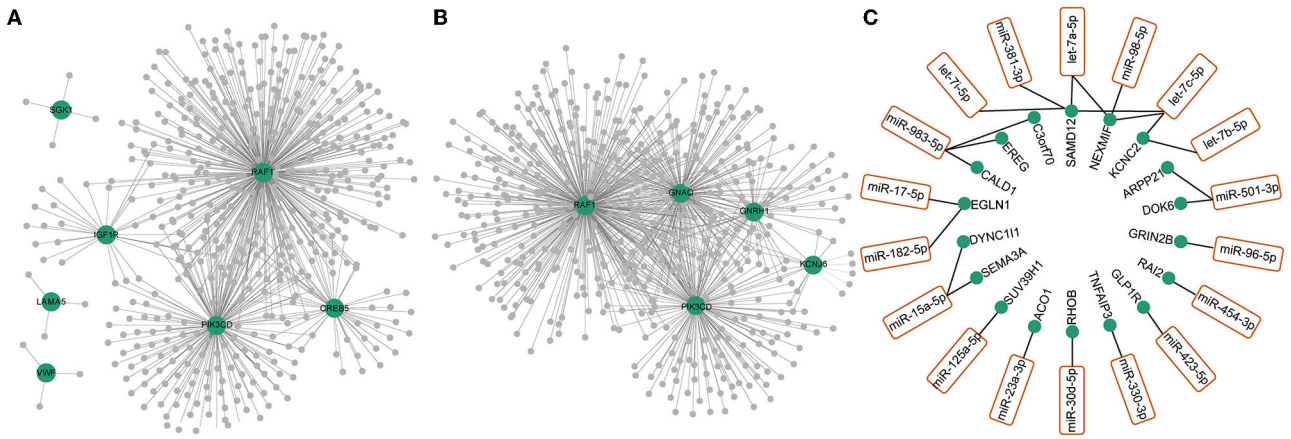

FIGURE 3 | Co-expression network of differentially expressed RNAs (mRNAs, lncRNAs, circRNAs and miRNAs) with differentially expressed RNAs in GnRH secretion **(A)** and PI3K-Akt signaling pathway **(B)**. The green dots represented the differentially expressed mRNAs in GnRH secretion **(A)** and PI3K-Akt signaling pathway **(B)**. The gray dots represented the differentially expressed ncRNAs (lncRNAs, circRNAs, miRNAs) coexpressed with differentially expressed mRNAs in these two pathways. **(C)** Differentially expressed miRNAs (orange box) and their targeted mRNAs (green dots).

expressed mRNAs were involved in immunity or PI3K-Akt signaling pathways, such as *EREG*, *TNFAIP3* and *RHOB*. In addition, we noted that some of these genes were associated with brain function or related disorders, such as neuronal development (*C3orf70*, *DOK6*, *GRIN2B*), cognitive or intellectual disorder (*RAI2*, *ARPP21*), schizophrenia and Alzheimer's disease (*SEMA3A*), and epilepsy (*NEXMIF*, *KCNC2*, *SAMD12*, *GRIN2B*). These results further suggested that the immune system, PI3K-Akt signaling and GnRH secretion pathways might be related to opioid addiction. Moreover, some miRNAs played key roles in the process of addiction and led to addiction-related symptoms by working together with targeted mRNAs.

Functional Enrichment of Genes Targeted by Differentially Expressed miRNAs

The predicted genes targeted by differentially expressed miRNAs were mainly enriched in several pathways, including the interleukin (p-value $= 3.45 \times 10^{-5}$), Jak-STAT (p-value $= 5.27 \times 10^{-5}$), insulin (p-value $= 1.02 \times 10^{-4}$) and Wnt (p-value $= 2.00 \times 10^{-4}$) signaling pathways (**Figure 2D**). The results of functional enrichment of differentially expressed miRNAs and differentially expressed mRNAs were partially consistent, especially in immune-related pathways. Moreover, although the Jak-STAT pathway is not directly related to immunity, the expression of cytokines, including interleukin and interferon, is regulated by Jak-STAT (35, 36). This evidence, in line with the mRNA enrichment results, further confirmed that the immune system was indeed involved in the pathogenesis of OUD.

DISCUSSION

In this study, we analyzed the whole transcriptome characteristics of peripheral blood samples from patients with OUD and healthy control subjects. We found that many genes in the immune system were differentially expressed. Immunity has long been reported to be associated with opioid addiction, as injection use of heroin contributes significantly to virus transmission (37, 38). This finding might be due to the sharing of injection equipment among drug users (39, 40). However, higher viral loads have also been detected in virus-infected heroin users than in infected non-heroin users (41), and opioid abuse has been proposed to undermine IFN-mediated antiviral innate immunity and enhance virus replication *in vitro* (42–44). Recent studies also suggested that heroin dependence could suppress adaptive immune responses by reducing the proliferation of regulatory T cells and the secretion of proinflammatory cytokines (9, 10). Moreover, many cytokines, including interleukin and interferon, fluctuate significantly in patients with OUD compared with healthy controls (8). All this evidence suggests that immune changes caused by opiate abuse might come from two sources: sharing injection equipment and reduction of immunity. When treating patients with OUD, it is also important to consider the patient's immunity.

We also found that opioid addiction could suppress GnRH secretion. Opioids can bind to opioid receptors in the hypothalamus, pituitary and testis to modulate gonadal function (5). Previous researchers have also shown that opioids could decrease plasma testosterone levels by suppressing hypothalamic GnRH and luteinizing hormone release (45). Moreover, the endocrine system, including growth hormone, prolactin, luteinizing hormone, testosterone, estradiol and oxytocin, has been reported to be affected by opioids through the hypothalamic-pituitary-adrenal (HPA) axis (45). In our study, we also found that the synthesis and secretion of aldosterone (enrichment score $= -0.48$, p-value $= 6.44 \times 10^{-3}$), which is an adrenocortical hormone, was related to opioid addiction. Therefore, our studies further indicated that opioids could affect the endocrine system.

The PI3K-Akt signaling pathway is associated with many human diseases and can regulate a variety of important cellular

pathways, such as the mammalian target of rapamycin (mTOR), immune regulation, insulin and mitogen-activated protein kinase signaling pathways (46–50). Previous studies showed that selectively blocking the spinal dopamine D2 receptor (D2DR) could attenuate morphine tolerance in mice by inhibiting PI3K-Akt-MAPK signaling, and activation of PI3K-Akt signaling could promote the development of morphine tolerance (51, 52). In addition, electroacupuncture could delay the occurrence of morphine tolerance in rats by downregulating the protein expression of phosphorylated PI3K and phosphorylated Akt, which are two key molecules in the PI3K-Akt pathway (53). A recent study showed that the PI3K-Akt signaling pathway was activated after biphalin, a dimeric opioid peptide, and this effect could be reversed by opioid receptor inhibitors (54). Our findings that many genes in the PI3K-Akt pathway were differentially expressed, in line with previous studies, further suggested that PI3K-Akt participated in the pathogenesis of OUD and could be used as a potential therapeutic target.

In the present study, many ncRNAs were differentially expressed, suggesting that ncRNAs might be involved in the molecular pathogenesis of OUD. ncRNA has important regulatory functions and has been implicated in a variety of human diseases (55, 56). Not all differentially expressed ncRNAs were involved in the pathogenesis of OUD, and most of them might only be byproducts of OUD. However, regardless of whether these ncRNAs are directly related to OUD, ncRNAs with obvious expression changes, such as hsa-let-7i−3p ($\log_2$FC $= -4.05$, p-value $= 3.72 \times 10^{-5}$) and hsa-miR-151a-3p ($\log_2$FC $= -4.06$, p-value $= 4.62 \times 10^{-9}$), might serve as biomarkers for progression or treatment of OUD.

Our study has several limitations. First, the sample size in our study was small, as we only collected 14 samples and could not conduct weighted gene coexpression network analysis (WGCNA) (57). Although we identified hundreds of OUD-related genes, the results need to be verified in a larger cohort. Second, age, history of drinking and smoking were matched between the two groups, but clinical statistics, including duration, type and dose of drug use, were inconsistent within the OUD group. These confounders, although difficult to measure, could affect the results and should not be neglected. Third, we identified many ncRNAs that were differentially expressed, but we did not determine their possible biological functions. This study was only an exploratory study, and functional experiments are needed to verify the results in the future.

In summary, we identified hundreds of differentially expressed genes associated with OUD that were enriched in immunity, GnRH secretion and PI3K-Akt signaling pathways. The results of this study will help to further explain the pathogenesis of OUD and provide potential biomarkers for the treatment of OUD, but the findings need to be verified in studies with more samples.

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: Gene Expression Omnibus database under the accession number GSE198123.

ETHICS STATEMENT

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

AUTHOR CONTRIBUTIONS

XL and GW designed the study. QD, S-SP, XY, CL, and YH recruited the participants and collected the blood samples. QD and S-SP analyzed and explained the data. GW drafted the manuscript. All authors contributed to manuscript revision, read, 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.2022.893303/full#supplementary-material

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The modulation of mRNA levels of MAOA by electroacupuncture and psychotherapy in patients with pathological internet use

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Objective: The aim of this study was to observe the efficacy of electroacupuncture (EA) and psychotherapy (PT) effect on the mental status, sleep quality and impulsive trait in patients with pathological internet use, and to observe the changes of Monoamine oxidase type A (MAOA) messenger Ribonucleic acid (mRNA) levels in each group.

Methods: A total of 60 PIU patients were included for the present study. These patients were randomly divided into two groups: EA group and PT group. Baihui, Sishencong, Hegu, Neiguan, Shenmen, Taichong, Sanyinjiao and Xuanzhong were selected for acupuncture in the EA group, while group psychotherapy combined with individual psychotherapy was used for intervention in patients in the PT group. Young's Internet addiction Test (IAT), Yale-Brown Obsessive-Compulsive Scale (Y-BOCS), Self-Rating Anxiety Scale (SAS), Self-Rating Depression Scale (SDS), Barratt Impulse Scale (BIS-11) and Pittsburgh Sleep Quality Index (PSQI) were used to observe the severity of Internet addiction, mental status, sleep quality and impulsive trait of all patients at baseline and 40th days of treatment; and MAOA mRNA data were collected at baseline and 40th days of treatment.

Results: Electroacupuncture and psychological intervention effectively reduced IAT, SAS, SDS, Y-BOCS, BIS and PSQI scores of PIU patients. After 40 days treatment, the MAOA expression of the PT group was increased, and there was no significant change in EA group. The correlation analysis indicated that IAT scores were positively correlated with SAS, SDS, Y-BOCS, BIS and PSQI at baseline. In addition, after treatment the EA group showed that the change in IAT scores was positively correlated with the change in Y-BOCS and BIS scores, and the PT group showed that the change in IAT scores was positively correlated with the change in SDS, BIS and PSQI scores.

Conclusion: The present study showed that electroacupuncture and psychological intervention can improve severity of Internet addiction, mental status, sleep quality and impulsive trait of PIU patients. Simultaneously, neurobiological changes may be the underlying mechanisms of psychotherapy for internet additcion.

KEYWORDS

electroacupuncture, psychotherapy, pathological internet use, MAOA, mRNA level

Introduction

In the past decades, with the rapid development of computer and Internet technology, the Internet has changed the way we communicate, exchange information, and participate in real-time events thousands of miles away. It has become an indispensable part of people's life, work and entertainment. Although the Internet has brought more convenience to our lives, the improper use of the Internet may cause serious damage to society, work and personal psychology (1–3). This brings an emerging problem — pathological internet use (PIU) or internet addiction disorder (IAD), which is a behavioral addiction (4). This can be defined as "creating mental, social, school and/or work difficulties in one's life by using the Internet" (5). Some research have revealed that the incidence of PIU is increasing, and tends to involve younger subjects (6–8), leading to a series of family and social problems. Therefore, PIU has received increasing attention, and is gradually being recognized in the field of public health.

At present, the etiopathogenisis and pathogenesis for PIU have not been elucidated, and its treatment remains in the exploratory stage. The treatments for PIU at home and abroad are mainly the following: psychotherapy, drug therapy or the combination of both. These interventions have been proven to reduce the time for using the internet, and improve the psychological state of Internet addicts (9, 10). Although these treatments have curative effects, they also have some side effects, especially drug therapy (11, 12). If Internet addicts administer related therapeutic drugs for a long period of time, this may lead to serious adverse reactions. Therefore, some researchers have used traditional Chinese medicine to intervene in PIU, and our previous studies also showed that electroacupuncture can improve the clinical symptoms of Internet addicts (13, 14). More and more studies used fMRI to research the mechanism of acupuncture because for fMRI can visualize the effects of acupuncture (15, 16). Previous studies have confirmed that acupuncture has a regulatory effect on the structure and function of brain regions in substance addicts (17, 18). Similarly, our previous findings suggest that acupuncture on PIU individuals can regulate functional connectivity of reward and habit systems (19), and this result is related to the current research results of Internet addiction (20). In a review study, Weinstein and Lejoyyeux (21) reported that Internet addiction is associated with the brain regions associated with reward and cognitive control network. Liu et al. (22) also suggested that the functional change of brain in PIU patients may be relative to reward pathways. Therefore, the modulation effect of acupuncture on brain regions in PIU individuals, which might be the underlying mechanisms of acupuncture on PIU. A large number of studies revealed that psychotherapy can reduce the online time, negative mood, compulsive behavior and so on, but these studies only by using relevant scales to explore the effect of psychotherapy on PIU individuals (23–25).

Dopaminergic and serotoninergic systems are closely related to reward pathway. One study showed that dopamine (DA) played an important role in PIU (26). Hou et al. (27) reported that the individuals with PIU had a decreased level of expression of dopamine transporter in the striatum compared to controls. Luo et al. (28) study indicated that serotonin (5-HT) level was related with PIU. Furthermore, MAOA plays a crucial role in the metabolism of 5-HT, DA and norepinephrine (NE) (29). Low MAOA activity may raise the levels of 5-HT and DA that produce abnormal neurotransmitter system development and behavior (30). Recent research proved that high-activity of the MAOA gene can make the rapid catalyzation of 5-HT and NE, thereby leading to depression (31). Juanes et al found that after long-term drinking, the rhesus macaque MAOA expression in blood decreased and the level of dopamine in cerebrospinal fluid increased (32). Therefore, we speculated that there may be a mechanistic link between MAOA gene and PIU, and our previous research confirmed this hypothesis (33).

Although acupuncture and psychotherapy can improve the symptoms of PIU patients, it is not clear whether acupuncture or psychotherapy can affect the levels of MAOA with PIU patients. In order to provide a new perspective for the study of therapeutic mechanism of PIU, we had made current study. This study aimed to assess (a) the association between MAOA levels of mRNA and therapy; (b) the difference between EA and PT; (c) whether there was an association between changes in MAOA expression in PIU patients and clinical indicators.

Materials and methods

Participants

The aim of the study was to evaluate the impact of different treatment methods on PIU patients, so we choose difference between two dependent means (matched pairs) as statistical test, an effect size of d = 0.6 with power = 0.85 (α = 0.05; two-tailed) to calculate the sample size by Gpower. G-Power calculated that 27 sample sizes were needed for each group, finally each group recruited 30 participants, considering the dropout rate. Participants were recruited in this study came from University of Electronic Science and Technology, Chengdu University of Traditional Chinese Medicine and Sichuan Vocational and Technical College of Communication. All participants in our study were native Chinese speakers. Patients were diagnosed as PIU based on the Beard's Diagnostic Questionnaire (5). The inclusion criteria were as follows: (1) conformed to diagnostic criteria; (2) aged between 18 and 30 years; (3) right-handed; (4) fMRI examination without contraindications; (5) have signed informed consent. The exclusion criteria include the following: (1) having undergone any form of therapeutic intervention; (2) having any other organic or mental illnesses; (3) having a history of drug addiction or alcohol; (4) acupuncture treatment allergy; (5) pregnant or breast-feeding women; (6) left-handed. This study underwent ethical scrutiny and was approved by the Ethics Review Board of the Affiliated Hospital of Chengdu University of Traditional Chinese Medicine (Permission number: 2016KL-005) and it has been registered on Chinese Clinical Trial Registry: the registration number is ChiCTR-INR-16008102.

In this study, eligible subjects were numbered by a researcher who was not involved in the experimental process and assigned to two groups using a randomized digital table produced by SAS 8.0 software. In order to better implement the blind method, we did not inform participants of the specific content of the treatment at the time of recruitment. Only when participants were confirmed to join a group, they can be told which treatment they would receive. In addition, participants in each group were unaware of the other groups. When performing analysis, statisticians were not informed about the group allocation.

Questionnaire

The following scales were used to examine the subjects' the severity of pathological internet use, anxiety and depression symptoms, compulsiveness, impulsiveness and sleep condition.

Young's Internet Addiction Test (IAT) (34) is a self-report questionnaire which consists of 20 items to measures the degree of pathological internet use. Participants use this 5-point scale ranging from 1 (never) to 5 (always) to report the frequency with which they engaged in listed Internet behaviors during the past year. The possible score varies from 20 to 100. Several studies have proved that Young's internet addiction test have adequate validity and reliability (35, 36).

Self-Rating Anxiety Scale (SAS) (37) is widely used to measure participants's anxiety state. It consists of 20 items, which are scored on a 4-point Likert scale. The total score was calculated by the raw score multiplied by 1.25. Because the Chinese version of the SAS demonstrates adequate reliability and validity in past studies, so the Chinese version of the questionnaire is used in the present study (38, 39).

Self-Rating Depression Scale (SDS) is used to ascertain the individuals's situations about depression which compiled by William Zung (40). The SDS questionnaire has 20 self-report questions, which are rated on a 4-point scale. The total score was calculated by the raw score multiplied by 1.25. The Chinese version of the SDS has been widely used, and the reliability and validity has been verified in previous studies (41, 42).

Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) is a self-report measure that consists of 10 core items to test the severity of obsessive-compulsive symptoms (43). Each item with five response categories, rating from 0 (no symptoms) to 4 (severe symptoms). The Chinese version of Y-BOCS has been found to have adequate reliability in Chinese samples study, with Cronbach's alpha a = 0.83 (44).

Barratt Impulsiveness Scale (BIS-11) is a self-report questionnaire consisting of 30 items, which are rated on a 4-point scale (1 = rarely/never; 4 = almost always/always), evaluate the impulsivity of individuals (45). This scale includes three impulsiveness subscales: motor, non-planning, and attentional impulsiveness. The Chinese version of the BIS-11 was verified previously (46).

Pittsburgh Sleep Quality Index (PSQI) is the most commonly used instrument to assess the subjective sleep quality of individuals in clinical (47). This scale consists of 19 items measuring 7 components of sleep, including subjective sleep quality, sleep onset latency, total sleep duration, sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction (48). Each component is scored from 0 to 3 points, and each component are summed to get a total score (range 0–21). The Chinese version of the PSQI was verified previously (49).

The patient need a quiet environment to completed clinical assessments. At the same time, the participants needed to remain awake and attentive and follow the professional's instructions.

Intervention program

Electroacupuncture Treatment: Hwato brand disposable acupuncture needles (size 0.30 × 40 mm, 0.30 × 25 mm) and G6805 type multichannel electroacupuncture apparatuses were used. According to our previous studies on acupuncture

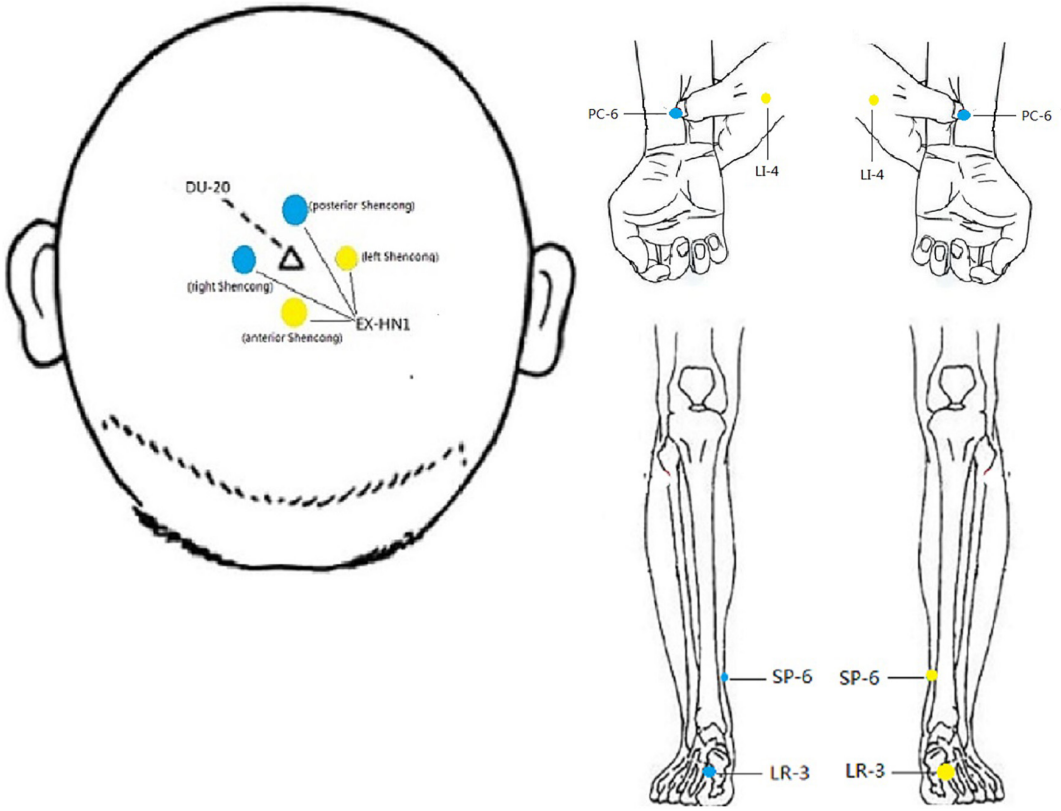

FIGURE 1
Location of acupoints receiving stimulation. Yellow represents the left, Blue represents the right. The left and right acupoints used alternately, that means stimulating the left acupoints for this time and the right acupoints for next time.

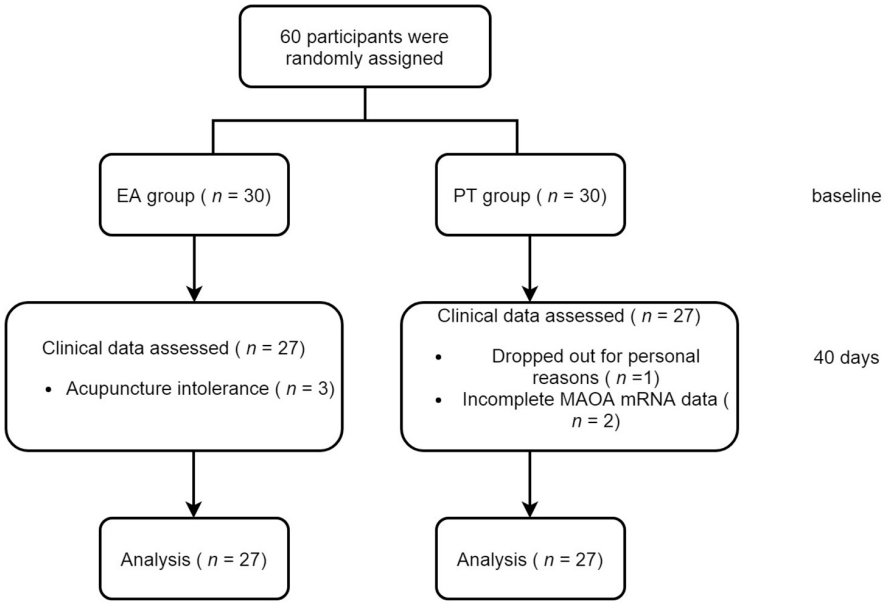

FIGURE 2
Flow chart.

for PIU (13, 14), participants received acupuncture at Baihui (DU-20, located in the center of the top), Sishencong (EX-HN1, a group of four points at the vertex, 1.0 *cun* anterior, posterior and lateral to Baihui, respectively), and bilateral Hegu (LI-4, located between the first and second metacarpal bone, approximately in the midpoint of the second metacarpal radial side), Neiguan (PC-6, located 2 *cun* above the transverse crease of the wrist, between palmaris longus and flexor carpi radialis tendons), Shenmen (HT-7, located ulnar side of the transverse crease of the wrist, in radial depression of the flexor carpi ulnaris tendon), Taichong (LR-3, located in the depression anterior to the meeting point of the first and second metatarsals), Sanyinjiao (SP-6, located 3 *cun* directly above the tip of the medial malleolus, on the posterior border of the medial aspect of the tibia) and Xuanzhong (GB-39, located 3 *cun* above the lateral malleolus, at the anterior edge of the fibula). Acupuncture was performed by an experienced acupuncturist according to the guidelines of acupuncture. Manipulations: After routine disinfection, perpendicularly insert 0.3 mm × 40 mm acupuncture needles, 0.5–1.0 *cun* into LI-4 and PC-6, 1–1.5 *cun* into SP-6 and GB-39, and 0.3–0.5 *cun* into LR-3 and HT-7, and horizontally insert 0.3 mm × 25 mm acupuncture needles, 0.5–0.8 *cun* into DU-20 and EX-HN1. Following needle insertion, uniform reinforcing-reducing manipulations of twirling, lifting, and thrusting were conducted on all needles to reach de qi (De qi refers to the process of the patient produces acupuncture acid, hemp, bilge sensation, and the doctor's heavy and tight sensation coming from beneath the needle, is considered by acupuncture achieved effect important condition). Three pairs of electrodes from the electric stimulator were connected separately to the needle handles at two points of EX-HN1(the anterior and left Shencong, or the posterior and right Shencong), LI-4 and PC-6, LR-3 and SP-6 (The left and right acupoints used alternately, **Figure 1**). Stimulus parameters: The frequency of the rarefaction wave was 2 Hz, and the condensation wave was 100 Hz, with a waviness width of 0.3 ms, and the intensity output was gradually adjusted from 0 mA to the extent of the subject's maximum tolerance. The stimulation lasted for 30 min. Participants received 1 treatment session every other day, 10 sessions as 1 treatment course, 20 sessions in total.

Psychological Treatment: Teamwork and individual counseling were performed by a nationally accredited psychologist. The main steps are as follows: (a) At the first meeting, IAD patients were allowed to be acquainted with each other by participating in a game designed by the psychologist. This would allow these patients to form team consciousness, which is conducive for the subsequent group therapy. The psychologist should understand the past situation of participants, especially their major life events, in order to determine the source of their negative emotions and bad character; (b) During the group psychotherapy, group play therapy was adopted to enhance mutual trust. At the same time, the psychologist should objectively evaluated the internet related events of internet addiction individuals (such as online games, online interpersonal communication, etc.), and compared their past and present situation, in order to let them know that they had deviation cognition on the Internet. During the whole process, the psychologist should allow patients to learn how to actively and correctly deal with problems, and establish a correct behavior pattern; (c) The psychologist formulate a reasonable schedule for patients, according to their own situation, and supplemented this with transference and self-control therapy, thereby lenabling them to determine their positive and beneficial interests. These patients received treatment every 4 days, and each treatment lasted for 2 h. 5 times was considered as one treatment course, and all patients received 2 treatment courses.

Detection of the relative mRNA expression levels of MAOA gene

The samples of peripheral vein blood were collected from all patients before and after treatment and stored in EDTA anticoagulant tube. Mononuclear cells were isolated using a gradient centrifuge (Thermo, Waltham, MA, United States).

Total RNA Extraction: (1) Whole blood mononuclear cells, about 10ˆ6 were added with 1 ml Trizol, and were repeatedly blown to resuspend; (2) The sample was kept at room temperature for 5 min and fully cracked, and the experiment continued in the following steps; (3) Add 0.2 ml chloroform, mixed for 15 s, and placed at room temperature for 2 min; (4) Centrifuged at 13,000 rpm at 4°C for 15 min, and the upper aqueous phase was absorbed into the EP tube pretreated by DEPC; (5) Add 0.5 ml isopropyl alcohol, reversed and mixed for several times, and precipitate on ice or for 10 min; (6) The supernatant was centrifuged at 13,000 rpm at 4°C for 15 min, and then discarded; (7) Add 1 ml 75% ethanol and mixed it by volute rotation; (8) Centrifuge at 12,000 rpm at 4°C for 5 min, then discard the supernatant. Then use a centrifuge for instantaneous centrifugation, carefully suck up the liquid; (9) Open the lid and dry the RNA for a while, add 20 μl DEPC water to dissolve it, and freeze at -80°C; (10) 1% agarose gel electrophoresis was performed on the total RNA extracted in the end. The RNA integrity was detected using the Agilent 2200 Bioanalyzer (Agilent, CA, United States).

Reverse Transcription: 1 μg of total RNA was reverse-transcribed into 20 μl first-strand cDNA by using the Fermentas cDNA synthesis kit (RevertAidTM, Fermentas, United States) according to the manufacturer's instruction.

Real-Time PCR: Nucleotide primers for real-time PCR amplification were designed using primer blast software on the National Center for Biotechnology Information website. Primers used for real-time PCR are as follows: β-actin: forward, 5'-GAAGATCAAGATCATTGCTCCT-3' and reverse, 5'-TTGCTGATCCACA-3' (amplicon size, 111-bp). MAOA:

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TABLE 1 General characteristics and clinical characteristics of the participants.

	EA group pretreatment (n = 27)	EA group posttreatment (n = 27)	PT group pretreatment (n = 27)	PT group posttreatment (n = 27)
Gender, male (n,%)	20 (74.07)	/	22(81.48)	/
Age (years)	22.44 ± 2.50	/	21.2 ± 1.78	/
Internet age (years)	7.93 ± 3.09	/	7.88 ± 2.58	/
IAT	65.26 ± 14.63	45.85 ± 12.61*	62.30 ± 11.03	44.26 ± 11.73▲
SAS	48.11 ± 11.15	40.63 ± 8.70*	48.48 ± 10.55	43.11 ± 9.75▲
SDS	55.19 ± 10.90	45.67 ± 7.96*	54.22 ± 9.63	49.33 ± 10.44▲
Y-BOCS	11.33 ± 9.11	6.00 ± 6.59*	12.15 ± 6.38	6.30 ± 5.86▲
BIS-11	75.00 ± 9.35	69.85 ± 9.99*	75.96 ± 9.36	68.19 ± 9.26▲
PSQI	7.92 ± 2.74	5.52 ± 3.07*	8.07 ± 2.84	4.74 ± 2.19▲

IAT, Young's Internet Addiction Test; SAS, Self Rating Anxiety Scale; SDS, Self Rating Depression Scale; Y-BOCS, Yale-Brown Obsessive-Compulsive Scale; BIS-11, Barratt Impulse Scale; PSQI, Pittsburgh Sleep Quality Index. *Comparison in the EA group between pre- and posttreatment, $P < 0.05$; ▲Comparison in the PT group between pre- and posttreatment, $P < 0.05$.

forward, 5'-CTGCCATCATGGGCTT-3' and reverse, 5'-TTGCTGATCCACA-3' (amplicon size, 154-bp). For real-time PCR, the reaction volume was 25 μl/tube (2 × TaqMan Real-time PCR Mix 12.5 μl + PCR primer pair 1.2 μl + fluorescent probe 0.6 μl + ddH2O 7.7 μl and cDNA template 3 μl). The reaction was performed on an FTC-3000QPCR system (Funglyn Biotech, Toronto, Canada). Reaction conditions were as follows: pre-denaturing at 95 °C for 10 min, denaturation at 95 °C for 10 s, annealing at 53 °C for 30 s, and 45 cycles of extension for 30 s at 60 °C. After the reaction, the amplification curves of target gene and internal reference gene were obtained, and the Ct value was calculated. We use the way of Delta-delta Ct to detect the relative mRNA expression in cells.

Statistical analyses

All data were analyzed by SPSS Statistics 21.0 software and GraphPad Prism 7 Software. If data conforms to the normal distribution, then Independent sample I-test was used for inter group comparison, and Paired sample t-test was used for intra group comparison; and if the data follows a non-normal distribution, we used Non-parametric test to compare in this case. While Chi-square test was used for categorical variable (e.g., gender). When doing correlation analysis, the data must be tested for K-S normality. Pearson correlation coefficient was used to analyze the normal distribution, and Spearman correlation coefficient was used to analyze the non-normal distribution. $P < 0.05$ was considered to be statistically significant.

Results

A total of 60 PIU subjects which were randomly divided into the EA group and the PT group were recruited in our study initially. There were three subjects in the EA group who dropped out due to acupuncture intolerance; and three subjects was excluded in PT group, one for personal reasons and the other two for incomplete MAOA mRNA data. Hence, a total of 54 patients were completed, and the total completion rate was 90%. Flow of participants in the study is shown in Figure 2.

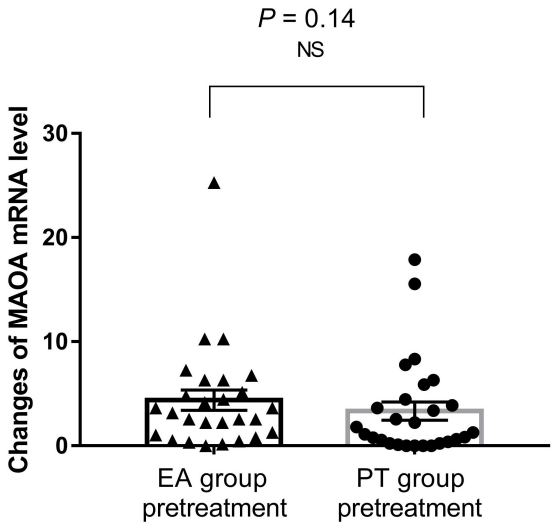

FIGURE 3
Results of mRNA levels of MAOA in the two groups before treatment. Bars represent means, error bars represent SEM, triangles and circles represent individual data points. NS, indicates not statistically.

Demographic characteristics and clinical measures

As shown in Table 1, there were no significant differences in demographics and clinical characteristics data between the

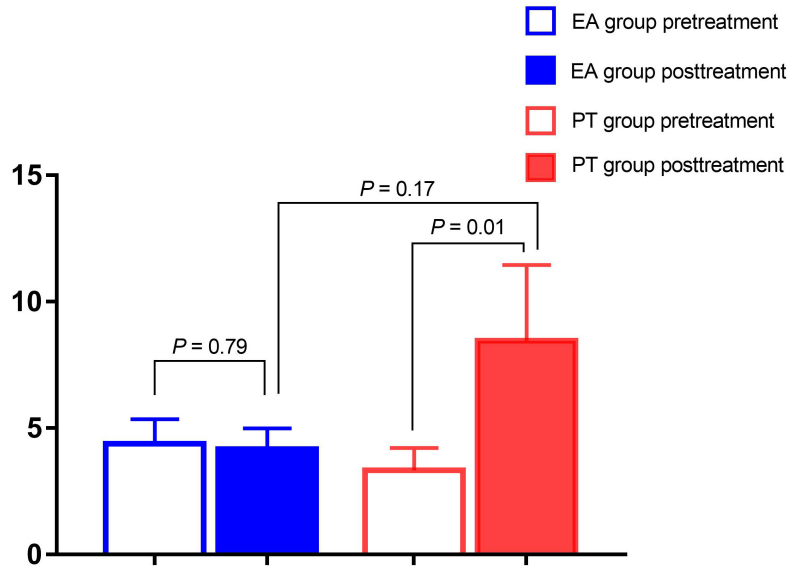

FIGURE 4
Results of mRNA levels of MAOA pre-and post-treatment.

EA group and the PT group at baseline ($P > 0.05$), that means the two groups were comparable for the severity of illness. After 40 days of treatment, the scores of IAT, SDS, SAS, Y-BOCS, BIS-11, and PSQI were decreased in both groups ($P < 0.05$). After 40 days of treatment, there was no significant difference in the scores of IAT, SAS, SDS, Y-BOCS, BIS and PSQI between the two groups ($P > 0.05$).

MAOA mRNA level results

Figure 3 shows that there was no significant difference in MAOA expression between patients in the EA group and the PT group at baseline ($P > 0.05$).

Figure 4 shows that the MAOA expression of the PT group was increased with that before treatment ($P < 0.05$). In contrast, the EA group has no significant change ($P > 0.05$). On the 40th day of treatment, there was no significant difference in the MAOA mRNA levels between the two groups.

Association between MAOA mRNA levels and clinical scale scores

There was no remarkable association between the relative expression levels of MAOA and IAT, SAS, SDS, Y-BOCS, BIS-11, or PSQI scores in patients with PIU ($P > 0.05$). IAT scores were positively correlated with SAS scores ($r = 0.36$, $P = 0.007$), SDS scores ($r = 0.292$, $P = 0.032$), Y-BOCS scores ($r = 0.475$, $P = 0.000$), BIS scores ($r = 0.383$, $P = 0.004$) and PSQI scores ($r = 0.354$, $P = 0.009$), respectively. (See Table 2).

Table 3 lists the correlations between the changes of expression of MAOA and changes in clinical scores in each group after treatment. The change of MAOA in the EA group was positively correlated with PSQI scores ($r = 0.428$, $P = 0.026$). Table 4 shows the correlations between the changes of IAT scores and changes in other clinical scores in each group after treatment. The chage of IAT scores in the EA group was positively correlated with Y-BOCS ($r = 0.391$, $P = 0.044$), and BIS scores ($r = 0.501$, $P = 0.008$); while the chage of IAT scores in the PT group was positively correlated with SDS scores ($r = 0.432$, $P = 0.024$), BIS scores ($r = 0.507$, $P = 0.007$), and PSQI scores ($r = 0.444$, $P = 0.02$).

Discussion

Recently, some studies indicated that PIU has strong association with internalizing and externalize disorders (50, 51). On the basis of a biopsychosocial model, Cerniglia thought that IAD as a result of a mutual influence of individual, psychological profile, and social environment (52). Studies have shown that depression, anxiety and impulsive are common comorbid diseases of PIU (53, 54). Simultaneously, previous studies have reported that excessive time spent online can reduce the required night sleep with internet addiction patients, furthermore leading to sleep disorders (55, 56). Our previous research also found that PIU individuals had higher SDS, SAS and BIS-11 scores, and more poor sleep quality compared with individuals without PIU (33). In present study, the correlation analysis results indicated that IAT scores of PIU individuals

TABLE 2 Correlation between MAOA mRNA levels and clinical measures at baseline.

	MAOA	IAT	SAS	SDS	Y-BOCS	BIS	PSQI
MAOA	1	−0.024	0.003	−0.017	−0.226	0.06	0.139
IAT	−0.024	1	0.36**	**0.292***	**0.475****	**0.383****	**0.354****
SAS	0.003	**0.36****	1	**0.729****	**0.34***	**0.367****	**0.573****
SDS	−0.017	**0.292***	**0.729****	1	**0.309***	**0.448****	**0.525****
Y-BOCS	−0.226	**0.475****	**0.34***	**0.309***	1	0.254	0.243
BIS-11	0.06	**0.383****	**0.367****	**0.448****	0.254	1	**0.397****
PSQI	0.139	**0.354****	**0.573****	**0.525****	0.243	**0.397****	1

IAT, Young's Internet Addiction Test; SAS, Self Rating Anxiety Scale; SDS, Self Rating Depression Scale; Y-BOCS, Yale-Brown Obsessive-Compulsive Scale; BIS-11, Barratt Impulse Scale; PSQI, Pittsburgh Sleep Quality Index.
*$P < 0.05$; **$P < 0.01$. Bold values indicate statistical significance.

TABLE 3 Correlation between MAOA change and clinical index change.

	Change in IAT	Change in SAS	Change in SDS	Change in Y-BOCS	Change in BIS	Change in PSQI
EA						
Change in MAOA	0.031	−0.174	−0.235	−0.286	0.233	**0.428***
PT						
Change in MAOA	−0.008	0.299	0.31	0.054	0.322	0.143

IAT, Young's Internet Addiction Test; SAS, Self Rating Anxiety Scale; SDS, Self Rating Depression Scale; Y-BOCS, Yale-Brown Obsessive-Compulsive Scale; BIS-11, Barratt Impulse Scale; PSQI, Pittsburgh Sleep Quality Index.
*$P < 0.05$. Bold values indicate statistical significance.

TABLE 4 Correlation between IAT score change and clinical index change.

	Change in MAOA	Change in SAS	Change in SDS	Change in Y-BOCS	Change in BIS	Change in PSQI
EA						
Change in IAT	0.031	0.293	0.112	**0.391***	**0.501****	0.145
PT						
Change in IAT	−0.008	0.344	**0.432***	0.26	**0.507****	**0.444***

IAT, Young's Internet Addiction Test; SAS, Self Rating Anxiety Scale; SDS, Self Rating Depression Scale; Y-BOCS, Yale-Brown Obsessive-Compulsive Scale; BIS-11, Barratt Impulse Scale; PSQI, Pittsburgh Sleep Quality Index.
*$P < 0.05$; **$P < 0.01$. Bold values indicate statistical significance.

were positively correlated with scores of SAS, SDS, Y-BOCS and PSQI which were consistent with the results of previous studies. (57, 58). In a systematic review, Liu et al. (25) found that psychotherapy can make positive change to psychoticism, compulsive Internet use and interpersonal issues for patients with internet addiction. Moreover, some researchers reported that electro-acupuncture can improve mental symptoms in patients with Internet addiction disorder (59). In our study, both the two treatment measures could reduce the degree of Internet addiction, the symptoms of depression and anxiety, and improve sleep quality, impulsive and compulsive states in patients with PIU. Although after treatment there was no significant difference in the scores of IAT, SAS, SDS, Y-BOCS, BIS and PSQI between the two groups, the correlation analysis of the EA group showed that the change in IAT scores was positively correlated with the change in Y-BOCS and BIS scores, and the correlation analysis of the PT group showed that the change in IAT scores was positively correlated with the change in SDS, BIS and PSQI scores. Our results indicated that improvement in anxious symptoms, impulsiveness and sleep quality in PIU patients was associated with improvement in Internet addiction severity, and prompting the mechanism of the two interventions may be different.

MAOA gene is located on the short arm of the X chromosome, and some scholars found the genetic variation in MAOA effects on emotion, behavior and substance dependence. One study indicated that lower MAOA activity is associated with impulsive aggressive behavior (60). Du et al. suggested the MAO-A gene polymorphisms may be involved in the pathogenesis of major depression (61). Fite et al. study revealed that MAOA variant has association with tobacco and cannabis use (62). MAOA catalyzes the degradation of monoamine neurotransmitters, including 5-HT, DA and NE (29). In previous studies showed IAD was related to the dopamine (DA) system and serotonin (5-HT) systems, as like other substance addiction (52, 63). Therefore, MAOA is an important candidate gene for investigating PIU. Our study did not observe the MAOA levels was correlated with IAT scores, SAS scores, SDS scores, Y-BOCS scores, BIS scores and PSQI scores. Interesting after 40 days of treatment, we only found the MAOA expression of the PT group was increased, and there was no significant change in EA group. The reason

may be related to the difference of treatment mechanism between the two groups. According to our previous study (19), it is speculated that electroacupuncture maybe improve symptoms by regulating reward regions of PIU patients. Furthermore, after treatment, the upregulation of MAOA gene expression in the PT group may be affected by monoamine neurotransmitters. MAOA gene activity is associated with the metabolism of monoamine neurotransmitters (32). Luo et al. (28) were summarized that platelet 5-HT level was negative related with the degree of IAD. Current research showed that psychotherapy can increase the ability of serotonin and its receptors in patients with obsessive-compulsive disorder and depression. Lissemore et al. (64) demonstrating psychotherapy can increase serotonin synthesis capacity in patients with obsessive-compulsive disorder. Karlsson et al. (65) research found that increased 5-HT1A receptor density in multiple cortical regions after psychotherapy treatment in patients with major depressive disorder. So PIU patients may cause the surge of serotonin after receiving psychotherapy, then up regulate the expression of MAOA through feedback mechanism.

So far as we know, this is the first study to examine the effect of acupuncture and psychotherapy on the mRNA levels of MAOA in PIU patients. Simultaneously, this is also the first study investigating the correlation with MAOA mRNA levels and Clinical Scale Scores in PIU patients. Although we cannot determine the difference in efficacy between EA and PT, according to our results we found that the reduction degree of internet addiction is related to the improvement of negative emotions, impulsiveness and sleep quality. In addition, EA and PT maybe has different mechanism on PIU patients. Combined with our previous research results (19), the underlying mechanisms of electroacupuncture on PIU maybe by regulating functional connectivity of reward and habit systems. While, psychotherapy may work by regulating the MAOA gene and its related neurotransmitter. To confirm our hypothesis, future studies should consider assessing a larger cohort of participants, increasing the course of treatment and follow-up time and adding the detection of neurotransmitters related to MAOA. Meanwhile, explore the correlation between these factors, so as to provide a basis for better explaining the mechanism of electroacupuncture and psychotherapy.

Limitations

This study have limitations. Firstly, participants were recruited from college or university and the sample size of our study was relatively small that will limit the generalization of the current results. Secondly, PIU can be divided into many categories: Internet gaming disorder, Internet pornography addiction, Internet shopping addiction and so on, but we did not classify the participants. Thirdly, estimation of the follow-up effect of interventions were lacked in our research.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee, Affiliated Hospital of Chengdu University of Traditional Chinese Medicine, China (NO. 2016KL-005). The patients/participants provided their written informed consent to participate in this study.

Author contributions

TZ and YD conceptualized the study, designed the plan, and managed the project. TZ supervised the study and revised the manuscript. CZ, LZ, and CW conducted experiments. YD and CZ analyzed the data. YD wrote the first draft of the manuscript. All authors read and approved 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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Gray matter volume alterations in subjects with overweight and obesity: Evidence from a voxel-based meta-analysis

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Background: Obesity is a multi-systemic disease with complex etiology. And consistent evidence indicated obesity or overweight subjects render brain structure changes. Increasing evidence indicates these subjects have shown widespread structural brain gray matter volume (GMV) changes. However, results from other neuroimaging studies have been inconsistent. Consequently, the question remains whether body mass index (BMI), a gold standard to define obesity/overweight, is associated with brain structural changes.

Methods: This study will apply an updated meta-analysis of voxel-based GMV studies to compare GMV changes in overweight and obese subjects. Online databases were used to build on relevant studies published before May 2022. The updated Seed-based d Mapping with Permutation of Subject Images (SDM-PSI) explores GMV changes in individuals with overweight and obesity and further examines the correlation between GMV and obesity-related variables, specifically body mass index (BMI).

Results: This research included fourteen studies and provided a whole-brain analysis of GMV distribution in overweight and obese individuals. It revealed lower GMV in brain regions, including the left putamen and right precentral gyrus, in individuals with overweight and obesity compared to lean controls. Further, meta-regression analyses revealed GMV in the left middle occipital gyrus was negatively correlated with the BMI of the whole sample.

Conclusion: GMV decreased was reported in reward circuit processing areas and sensorimotor processing areas of individuals with overweight and obesity diagnoses, suggesting an underlying structural basis for reward processing and sensorimotor processing dysregulation in overweight and obese subjects.

Our results also suggest that GMV in occipital gyrus, a key region for food visual and gustatory encoding, is negatively associated with BMI. These results provide further evidence for the dysregulated reward circuit in individuals with overweight and obesity.

KEYWORDS

overweight and obesity, BMI, gray matter volume, voxel-based morphometry, meta-analysis

Introduction

The prevalence of obesity diagnoses has been rising rapidly, as an estimated of 650 million people worldwide are currently identified as medically overweight. Kowning that obesity is highly correlated with other issues, including an increased risk of type 2 diabetes, respiratory problems, cardiovascular disease, mood-related disorders, and negative effects on one's quality of life, it is considered one of the leading factors of death (1). Despite the understanding of the harmful effects of obesity is increasing, the etiopathology of weight gain and obesity has remained unclear. Research suggests that the impairment of psychological circuits, such as the reward system which negatively reinforces food craving, can motivate people to engage in food-seeking, overeating, and other forms of substance abuse (2–7). Such responses are particularly pronounced in subjects who are overweight and obese (8).

Compensatory overeating might be one of the leading causes to obesity (maybe the most frequent). To understand the brain structural changes of compensatory overeating and BMI increase, neuroimaging has been used to compare subjects with obesity and healthy controls (HCs) in structural magnetic resonance imaging (MRI) analyses (9, 10). In patients with obesity, reduced regional gray matter volume (GMV) is frequently reported in precentral gyrus, medial prefrontal cortex, inferior frontal gyrus, and cerebellum, while the left middle frontal gyrus, cuneus, and inferior occipital gyrus showed an increase in GMV (9, 10). Other studies imply that the striatum plays a specific role in obesity as presenting high-calorie foods to individuals with an obesity diagnosis leads to greater activation in reward processing areas including caudate, putamen, amygdala, and orbitofrontal cortices, cognitive control related-areas including the prefrontal cortex and anterior cingulate cortex, and sensorimotor processing areas, particularly precentral gyrus (11–14). Research by Rothemund et al. (15) reported that, in overweight and obese subjects, decreased activation in striatal areas during food consumption compared with striatal enhanced activation to high-caloric food-related cues might be the reason for overeating and consequent weight gain (16). Therefore, striatal hypo-activity in response to food intake is considered to be one of the primary mechanisms of compensatory overeating and BMI increase (17, 18). The striatal region is a dopamine-rich region in the brain and thus a key hub of the reward circuit. Patients with obesity have a substandard availability of dopamine D2 receptors in the striatum, which could lead to an increased inability to control overeating impulses (19). Moreover, while body mass index (BMI) is deemed the gold standard for measuring obesity, BMI negatively correlates with GMV in the striatum(e.g., caudate, putamen), emphasizing BMI may be an important risk factor for GMV decreased (20). However, the previous systematic reviews and large-scale evidence are insufficient for exploring GMV changes related to obesity.

Consequently, different from the Anisotropic Effect-Size Seed-Based d Mapping (AES-SDM) (9, 10) and Activation likelihood estimation (ALE) (21), we have taken an innovative approach, a newly released version of Seed-based d Mapping with Permutation of Subject Images (SDM-PSI), to provide a better understanding of the GMV changes in subjects with overweight and obesity. According to the developer's statement, it uses a standard permutation test to evaluate whether effects are null or not and generates more reliable results than previous SDM-meta versions. This method not only avoids the drawbacks of alternative procedures used in current coordinate-based meta-analyses methods (22, 23), but also obtains the following advantages: (a) accounting for both increases and decreases of the outcomes so that contradictory findings cancel each other (24); (b) using random-effects modeling to estimates effect size and thus guaranteeing reliability (25); (c) using subject-based permutation tests equal to those of FSL "randomized" tools (26); (d) using a threshold-free cluster enhancement (TFCE) statistics (27). Considering that BMI is not normally distributed in overweight/obesity and lean individuals, we will primarily focus on the group comparison of the GMV alterations between individuals with overweight and obesity and control subjects who are considered lean. The newest meta-analysis was published in 2018 and did not take the new meta-method. In these meta-analysis, the author has reported the GMV loss in left, middle, and right inferior frontal gyrus (including the insula), precentral gyrus, temporal cortex, and the cerebellum, and increased GMV in the left middle frontal gyrus, left cuneus, left inferior occipital gyrus, in overweight and obesity individuals compared with HCs (9). However, it did not analyze the correlations between GMV alterations and obesity-related

variables such as BMI any further. For this study, we found only two relevant meta-analyses and reviews (74 percent of the articles were the same) that explored the negative correlations between GMV in the medial prefrontal cortex, left temporal pole, bilateral cerebellum and right orbitofrontal cortex and BMI in patients with obesity (10, 21). In addition, they included the studies as follows: waist circumference (28) and eating behavior (29) rather than BMI associated with GMV alternations, BMI-related regional GMV reductions in first-episode mania patients (30), major depression (31) and adolescent (32), and etc. The mixed sample size may confound the group comparison results. Thus, in the current studies, we excluded all studies above. Finally, the studies we included provided mean BMI, from the range of BMI in the original study, we see that overweight individuals were also included in the obese subjects, thus, we named all participants as overweight/obesity individuals.

In this meta-analysis, we will use the voxel-based meta-analysis via the novel algorithm (e.g., PSI) to identify morphometric changes in overweight and obese subjects compared with lean subjects. Secondly, we will analyze the correlation between GMV difference and BMI among the sample pool. With regard to changes in brain structure for individuals with overweight and obesity, we include voxel-based statistical analysis to compare local differences in GMV after spatial normalization is taken into account (33). From the literature reviewed above, based on models of addiction that habitual overeating leading to weight gain and obesity marks the progressive change of the striatum (34–36), we hypothesize that, consistent with previous research, GMV of the overweight and obese group will significantly decrease, especially in reward processing areas, such as putamen, and sensorimotor processing areas such as precentral gyrus, Secondly, we hypothesize that there is a correlation between BMI, as an obesity-related indictor, and GMV loss.

Materials and methods

Data source

Systematic and comprehensive searches of the PubMed[1], Embase[2], CENTER (Cochrane Library)[3] and Google Scholar[4] databases were conducted based on studies published till May 2022. The included keywords are as follows: obesity; overweight; voxel-based morphometry; VBM; volumetry; morphometry; structural MRI or gray matter. In addition, manual searches were performed among the reference sections of the review articles and retrieved studies.

The inclusion criteria are as follows: (1) the study included subjects with obesity vs. lean controls; (2) using VBM to analyze whole-brain GMV differences of subjects with overweight and obesity; (3) the results reported statistical parametric maps and peak coordinates of the GMV alterations which were normalized into the Montreal Neurological Institute (MNI) or Talairach space (TL); (4) peer-reviewed studies; (5) all subjects provided informed consent; (6) participants aged $\geq$ 18. In addition, the authors of published studies were contacted by email when necessary information was not provided in the studies.

The exclusion criteria are as follows: (1) studies deal with seed voxel–based analysis, region-of interest (ROI), white matter changes or cortical thickness evaluations only rather than MRI whole-brain VBM; (2) review articles, theoretical papers, meta-analysis or animal experimental studies; (3) without lean controls; (4) participants aged<18; (5) when t- or z-maps were unavailable, consistent statistical thresholds throughout the brain were not used or peak coordinates were not reported (37) (**Figure 1**).

To evaluate the quality of perceived studies criteria was applied as follows: (1) lean controls compared with obesity/overweight subjects; (2) method of diagnosis; (3) demographic data; (4) samples size(When studies with sample size $<$ 10, we scored as 0; sample size $>$ 30, we scored as 2; the middle section is marked 1); (5) the use of GM volume covariates; (6) whole brain analysis; (7) MRI machines and smooth kernels (8) standard spatial coordinates (e.g., MNI coordinates or TL). Each criterion was independently estimated by two independent reviewers who scored as 0, 1 or 2 if the criteria were unfulfilled, partially met or fully met, respectively, and any study scoring $>$ 8.0 was included in the meta-analysis. Although not specifically designed as an evaluation tool, this checklist provided an objective and strict indication of each study that included in our analysis (38).

Voxel-wise meta-analysis

Regional differences in GMV between patients with overweight and obesity and lean individuals were analyzed with SDM-PSI[5] (22). Details of the SDM-PSI method have been described previously (22). The procedure is briefly summarized as follows. First, the method of SDM-PSI combined the coordinates of cluster peaks with the effect sizes of significant differences between subjects with overweight and obesity and lean individuals to create whole-brain effect-size and signed maps, which were used to perform voxel-wise random effects meta-analyses (23, 38). Then, the SDM-PSI thresholds' parameters were used in our analysis (full

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2 www.embase.com
3 https://www.cochrane.org/
4 http://scholar.google.com/
5 http://www.sdmproject.com/

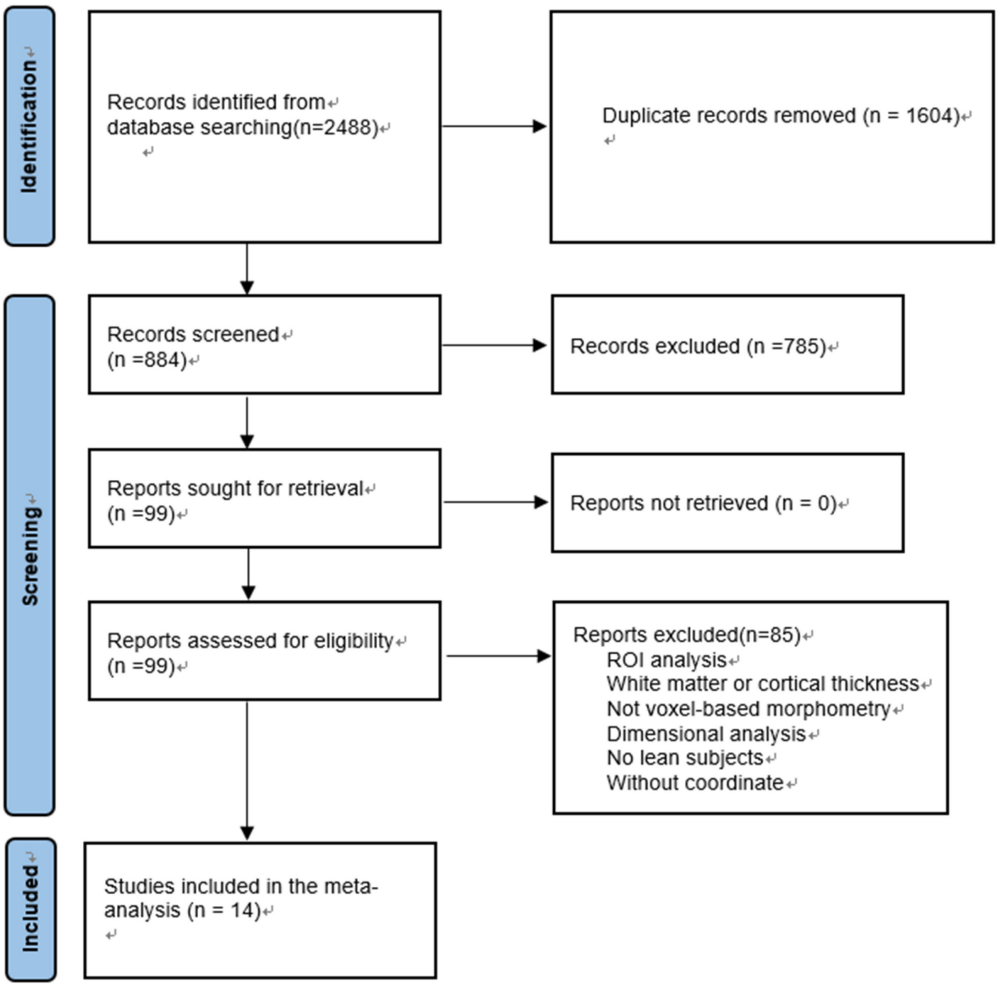

FIGURE 1
Flowchart of the selection of VBM studies in subjects with overweight and obesity for meta-analysis.

width at half maximum = 20 mm, voxel thresholds: TFCE CORRECTED > p, peak height thresholds: peak TFCE CORRECTED > 0.0500, extent threshold of clusters size > 1 voxels) (22). Next, mean map was obtained in regional GMV between subjects with overweight and obesity and lean individuals by voxel-based calculation of the mean of the study maps, weighted by the square root of the sample sizes of each study, so that studies with large sample sizes contributed more to the final map (39); Furthermore, reliability was confirmed by jackknife sensitivity analysis to assess the reproducibility of the results. Heterogeneity analyses were used to determine significant, unexplained differences of studies. Egger tests were performed to identify the asymmetry of funnel plots to examine conflicting studies and potential publication bias (40). The aim of the meta-regression analysis was to explore BMI was associated with the pooled effect size of the GMV difference between overweight/obese and the lean subjects.

Results

The sample characteristics

The present research included 14 structural MRI studies on overweight and obesity based on the search strategy. In total, there were 361 subjects with overweight and obesity (males = 107; females = 254; mean age range: 15.0 – 70.0 years; BMI rang: 26.20 – 43.10) and 419 controls (male = 188; female = 231; mean age range: 16.1–70.0 years; BMI rang: 20.96–24.0). **Table 1** illustrated the demographic information.

Regional gray matter volume differences

The pooled SDM-PSI meta-analysis map revealed significant lower GMV in subjects with overweight and obesity

TABLE 1 Description of the demographic and clinical characteristics in overweight and obesity subjects and lean subjects in the meta-analysis.

Study	Overweight and obesity subjects				Lean subjects				Magnetic field	Software	Smooth kernel
	Sample/ Female	Age (mean/SD)	BMI	Hand (left/right)	Sample/ Female	Age (mean/SD)	BMI	Hand (left/right)			
Brooks et al. (77)	59/34	70	33.0(0.3)	NA	97/52	70	22.5(0.2)	NA	1.5	SPM	8
Haltia et al. (78)	30/18	37(12)	33.0 (4.3)	NA	16/8	37 (21)	22.2 (1.6)	NA	1.5	SPM	12
Honea et al. (79)	72/49	38.9 (8.2)	35.6 (3.6)	NA	22/18	36.8 (10.9)	21.6 (1.6)	NA	3.0	SPM	10
Jauch-Chara et al. (80)	15/0	24.7(0.66)	36.3(1.04)	NA	15/0	24.6(0.69)	23.2(0.38)	NA	3.0	SPM	8
Karlsson et al. (81)	23/18	47.30 (8.90)	43.1(3.74)	NA	22/15	46.45 (9.45)	24.0(2.28)	NA	1.5	SPM	10
Mathar et al. (82)	19/8	27.0	33.6	(0/19)	23/12	25.1	21.8	(0/23)	3.0	SPM	8
Nouwen et al. (83)	13/12	15.0(1.9)	NA	NA	20/14	16.1(1.9)	NA	NA	3.0	SPM	6
Pannacciulli et al. (43)	24/13	32(8)	39.4(4.7)	NA	36/11	33(9)	22.7(2.2)	NA	1.5	SPM	8
Schienle et al. (84)	21/21	22.90 (2.59)	28.30 (3.40)	NA	21/21	22.57 (2.69)	22.34 (1.93)	NA	3.0	SPM	8
Shott et al. (85)	18/18	28.67 (8.30)	34.78(4.44)	NA	24/24	27.42 (6.28)	21.64(1.26)	NA	3.0	SPM	8
Smucny et al. (86)	28/14	30.29(3.81)	26.19(2.90)	NA	25/12	31.32(3.45)	20.96(1.99)	NA	3.0	SPM	8
Tuulari et al. (87)	47/42	44.9 (9.0)	42.2(4.0)	NA	29/23	45.9 (11.8)	23.2(2.8)	NA	1.5	SPM	8
Wang et al. (88)	31/7	39.58 (1.93)	34.38 (0.69)	(5/26)	49/21	29.55 (1.41)	21.87 (0.29)	(7/42)	3.0	SPM	8
Zhang et al. (89)	20/0	20∼28	33.56(3.53)	NA	20/0	20∼28	21.48(1.43)	NA	3.0	SPM	8

BMI: body mass index; SPM: statistical parametric morphometry.

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in the brain areas of the left putamen and right precentral gyrus (**Figure 2A**) with p-value less than 0.05 corrected by threshold-free cluster enhancement (TFCE) compared to lean group. **Table 2** shows the peak coordinates and the cluster breakdown. No brain areas with increased GMV in individuals with overweight and obesity were observed in the present study.

Meta-regression analysis

The results of the meta-regression analysis revealed that GMV in the left middle occipital gyrus (MNI coordinate: −22, −98, 10; 180 peak voxels; SDM z = - 3.745; $p = 0.00099$) yielded a significant negative correlation with BMI of the current samples (**Figure 2B**), with adjustment *p*-value less than 0.05 corrected by using of TFCE statistics.

Sensitivity, heterogeneity and publication bias

As shown in **Table 2**, the whole-brain jackknife sensitivity analysis of the meta-analysis revealed that GMV reduction in the left putamen was highly replicable, as this result was preserved when each study was removed. GMV decreased in the right precentral gyrus remained significant in 13 out of 14 combinations. Heterogeneity analysis results were reflected by the funnel plot and Egger tests, and the funnel plots did not reveal any publication bias (p:0.763 > 0.05) (see **Supplementary Materials**, **Supplementary Figure 1**).

Discussion

This meta-analysis study revealed GM reduction in overweight and obese individuals, using a novel meta method of SDM-PSI based on 14 VBM studies. The results indicated lower GMV in the left putamen and right precentral gyrus in overweight and obese individuals in comparison with lean subjects, which is partly consistent with previously published meta-analysis studies (9). Moreover, for the purpose of this study, previous results were replicated, indicating that GMV in the left occipital gyrus was negatively associated with BMI in overweight and obese subjects (41, 42).

Gray matter volume decreased in the putamen and precentral gyrus

This study revealed the reduced GMV in overweight and obese individuals in the putamen and precentral gyrus, in comparison with lean subjects, which aligns with previously published reviews (2, 9). The putamen is an important part of the striatum, and its structural change has been consistently observed in other empirical studies (43, 44). Accordingly, the putamen is understood to be able to code behavioral contingencies to obtain a specific reward with abundant dopamine D2 receptors (D2R), and overeating reduces dopamine D2R density, D2R sensitivity as well as reward sensitivity to food. Specifically, the morphometric decline in putamen is accompanied by the lower D2R availability among overweight and obese individuals (45). As a result, the reduced D2R availability in the striatal reward circuitry may result in increasing food craving compensatory and the resultant weight gain (46). In addition, previous studies also demonstrate that higher BMI was associated with down-regulated D2R in obese individuals (47), and correlated with the putamen hypo-activity in response to receipt palatable food in obese/overweight subjects (18). Consequently, the change in dopamine neuro-circuitry of the striatum may increase their susceptibility to opportunistic overeating while making food intake less rewarding (47).

In addition to the dopamine reward circuit, the putamen also performs a key role in the highly salient information processing, and is also involved in the origination, generation, and sequencing of motor behaviors (48). Consequently, the ability of information processing and behavior control would be limited by the development of putamen GMV changes (49), namely in the way that it develops an imbalance between autonomic processing and reward processing to overeat when food stimuli are present (49). Specifically, GMV loss in the putamen region would reduce the ability of self-control. The relationship between GMV loss in putamen, the reward circuitry, and behavior control may result from the reduced functional activation observed in the brain region.

Moreover, the function of the putamen is suggested to be lateralized (50, 51). That is, in healthy individuals, D1R, D2R, and D3R binding and dopamine synthesis capacity are higher on the right putamen than the left putamen (52), whereas the binding between the D1R and the dopamine transporter is higher in the left putamen than the right putamen (53). One of the potential hypotheses for such selective lateralization is suggested to be related to handedness, as putamen plays a key role in motor execution (54). Accordingly, the phenomenon might be related to handedness/hemispheric dominance. There is empirical evidence supporting this hypothesis. Research by Jang et al. (55) found that GMV in right putamen are larger in the left-handed subjects than in the right-handed subjects. As a result, the influence on putamen and behavior control would more salient to the left putamen in right-handed subjects. However, this deduction remains theoretical because the current analysis found little information on the patients' handedness. The specific GMV reduction in the left putamen may diverse when patients have different dominate hands.

Additionally, this study reported consistent GMV loss in the right precentral gyrus (PCG). PCG controls motor activity and

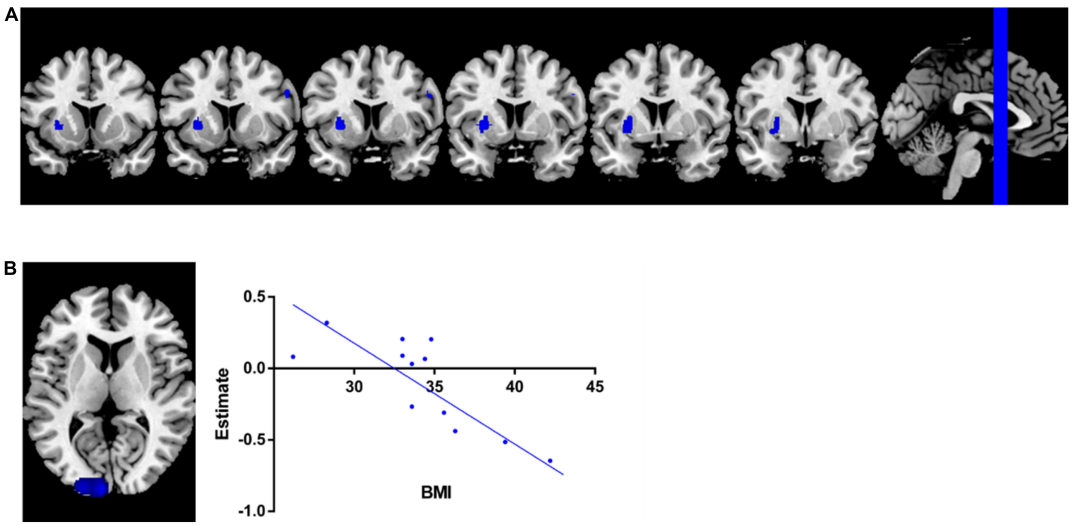

FIGURE 2
Meta-analysis results. **(A)** Regions showed lower GMV in overweight and obesity subjects than leans group. **(B)** Meta-regression analysis indicated that GMV in left middle occipital gyrus was significantly negatively associated with BMI in overweight and obesity individuals.

TABLE 2 Lower gray matter volume in subjects with overweight and obesity compared with controls in the meta-analysis.

Anatomical regions	MNI coordinates x, y, z	SDM-z value	*P** value	Number of voxels	Jackknife sensitivity
obesity < controls					
Left lenticular nucleus, putamen	−28,6,2	−5.004	0.0019	182	14/14
Right precentral gyrus, BA 44	52,8,30	−4.848	0.023	13	13/14

MNI = Montreal Neurological Institute; SDM = signed differential mapping; BA = Brodmann area; * The p-value was adjusted via threshold-free cluster enhancement(TFCE) ($p < 0.05$).

involves the execution of the elaborative motor activity (56). The recent studies have suggested that the relationship between motor control and obesity may be influenced by participants' habits such as eating style (57) and preferences (58). Obese subjects took less time to plan the movement and more time to perform the movement in the face of more preferred food, and with worse motor control compared with lean subjects (59). The areas in PCG forming the sensorimotor networks (SMN) were understood to integrate information with the executive functions, perception, and somesthesis (60). Thus, the GMV reduced in PCG in overweight/obese subjects will have an effect on their ability to intergrade the perceived information, particularly in relation to perceptions of the size, weight, and shape of food, and further influenced their self-control to consume the food (61, 62). This may explain why obese patients are more likely to display overeating behavior and develop obesity. Thus, sensorimotor control might be considered a key pathology in overweight and obesity individuals. A pronounced correlation between sensorimotor and reward-related areas has been widely identified in obesity-related studies and further demonstrated that neural activity in sensorimotor regions is more dependent on reward-related regions. The reward-related regions, such as the putamen and caudate, interconnected with the primary motor cortex (precentral gyrus), which perform a fundamental role in sensorimotor control (63). In addition, the previous studies have estimated that it is possible that variations in total intracranial volume (64) and PCG (65) supporting sensorimotor control precede the development of overeating and obesity. Moreover, neuroanatomical differences may be a consequence of obesity. A longitudinal study found that sustained high BMI relates to greater progressive declines in GMV over 5 years, and obesity may contribute a vicious cycle of overeating behaviors (66). Specifically, the current meta-analysis has shown that loss GMV in PCG in obesity individuals, a region related to sensorimotor control. Reduced GMV in this area could hypothetically relate to limited control over food intake, leading to increased overeating. In turn, the accumulation of body fat caused by overeating might cause further structural damage in PCG.

This study identified deficits of GMV in the putamen and PCG, which is consistent with the previous meta-analysis studies (9), suggesting that areas in the putamen and PCG are the potential neuroanatomical biomarkers in overweight/obese subjects. However, GMV loss in the frontal gyrus, temporal cortex, and subcortical area was unconfirmed. One possible reason is that the observed group effect

disappeared with the increased sample size, suggesting that GMV loss of these brain regions cannot be stable markers of overweight/obese subjects.

Negative association between BMI and occipital gyrus

In the meta-regression analysis section of this study, it was ascertained that GMV in the left middle occipital gyrus was negatively associated with the BMI of the whole sample, indicated that BMI was negatively related to the pooled effect size of the GMV difference between overweight/obese subjects compared to the lean subjects in the left middle occipital gyrus, suggesting that higher BMI was related to greater GMV reduction in the left middle occipital gyrus, which is similar to findings in previous studies (41, 67). The occipital gyrus is one of the key brain areas involved in the neural processing of visual food stimuli in overweight and obese individuals (68). Similarly, empirical studies suggested that significant thinning of the cortex in the occipital gyrus is associated with increasing BMI (41, 42). Kullmann et al. (69) reported that increased body weight in overweight and obese individuals has the potential to change neural processing in high-level visual areas, such as the occipital cortex. Furthermore, the occipital gyrus also performs a key role in object recognition, which can rapidly discern the energetic value of food in relation to salience tracking of high-energy and low-energy food (70). As excess energy intake may contribute to a hyper-responsivity of reward and gustation regions, as a prompt for food intake, this suggests that the obese/overweight subjects are more likely to overeat (71). However, the result of the meta-regression is different from the results of the between-group comparison that GMV decreased in the putamen and precentral gyrus in the obese/overweight subjects. The possible reasons are that many other variables might influence the results, such as gender (44, 72), age (73), body fat percent (74), participants' habits (75), possible psychiatric comorbidities (31). In addition, the low number of studies in literature fulfilling the eligibility criteria was also an important factor. Further studies are needed to elucidate brain morphological structure in overweight/obesity subjects.

Limitations

It is important to highlight several limitations of this study. Firstly, the data is based on collated analysis which has been extracted from published studies, as opposed to the original data, which may result in less accurate results (76).More importantly, the results of the meta-regression analysis study and the results of the subgroup analysis for the obesity group and lean group (e.g., 11 studies reported brain structure differences between the obesity group (BMI $>$ 30) and lean group, and 2 studies reported overweight group (25 $<$ BMI $<$ 30) and lean group, while one study did not clearly report the BMI range) should be considered carefully, as this study incorporated only a small number of studies that fulfilling the eligibility criteria and data availability was limited, future studies need to expand the sample size for further validation the GMV changes in subjects with overweight/obesity. Similarly, it was not possible to conduct a subgroup analysis of obesity grade, age, gender, and co-morbidity. Finally, longitudinal studies are necessary to examine whether the current brain structural changes in the putamen and precentral gyrus are causes or consequences of being overweight and obese.

Conclusion

In summary, the present research reported the most robust structural reduced of the GMV in the putamen and precentral gyrus in overweight and obese individuals. Moreover, GMV in the left occipital gyrus was negatively associated with the BMI of our samples. Our results are replicated with previously published brain structural findings in overweight and obese subjects and suggest that these patients are accompanied with brain abnormalities.

Data availability statement

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

Author contributions

LL and HY were responsible for the study concept and design. HY, WW, YM, and M-LL collected the data, analyzed data, and interpreted the results. LL wrote the manuscript. MZ, SL, TL, and QW provided critical revision of the manuscript. All authors read and 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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2022.955741/full#supplementary-material

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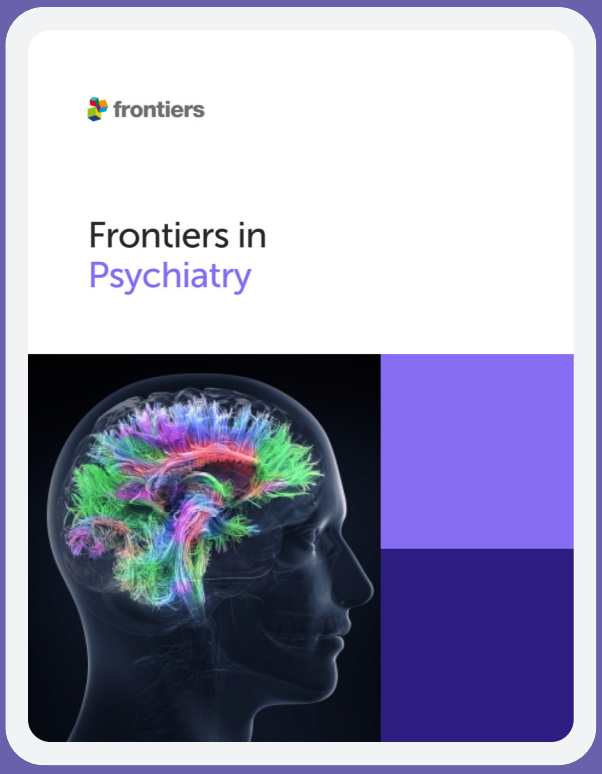

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