Ventral Visual Pathway-Cerebellar Circuit Deficits in Alcohol Dependence: Long- and Short-Range Functional Connectivity Density Study

Lingling Chen1†, Bi-Xia Liu2†, Run Liu3†, Jiyong Zheng4* and Xi-Jian Dai5*

1Department of Pediatric Internal Medicine, Linyi Central Hospital, Linyi, China, 2Department of ICU, Jiangxi Provincial Cancer Hospital, Nanchang, China, 3Department of Radiology, The Affiliated X’ian Central Hospital of Xi’an Jiaotong University, Xi’an, China, 4Department of Medical Imaging, The Affiliated Huaian No.1 People’s Hospital of Nanjing Medical University, Hua’ian, China, 5Department of Medical Imaging, Jinling Hospital, Medical School of Nanjing University, Nanjing, China

Objective: To identify the underlying intrinsic functional connectome changes in patients with alcohol dependence.

Methods: A functional connectivity density (FCD) analysis was used to report on the functional connectivity changes in 24 male patients with alcohol dependence (age, 47.83 ± 6.93 years) and 24 healthy male subjects (age, 47.67 ± 6.99 years). We defined the voxels with a correlated threshold of \( r > 0.25 \) inside their neighborhood (radius sphere \( \leq 6 \) mm) as shortFCD, and radius sphere \( > 6 \) mm as longFCD. We repeated the network analysis using a range of correlation \( r \) thresholds (\( r = 0.30, 0.35, 0.40, 0.45, 0.50, 0.6, \) and \( 0.75 \)) to determine whether between-group differences were substantially affected by the selection of the different R-value thresholds used. A ROC curve was used to test the ability of the FCD in discriminating between the two groups. Pearson’s correlation was used to evaluate the relationships between the FCD differences in brain areas and demographic characteristics.

Results: The covered differences in brain areas in binarized shortFCD were larger than binarized longFCD in both groups. The intra-group FCD differences did not depend on the selection of different thresholds used. Patients with alcohol dependence were associated with the longFCD deficit in the cerebellum posterior lobe, and shortFCD deficit in the ventral system of the visual pathway and increased shortFCD in the left precentral gyrus, right salience network and right cingulate gyrus. A ROC curve demonstrated that these specific brain areas alone discriminated between the two groups with a high degree of sensitivity and specificity. In the alcohol dependence group, the cerebellum posterior lobe, visual association cortex and the salience network displayed significant correlations with demographic characteristics.

Conclusions: The shortFCD analysis was more sensitive than the longFCD analysis in finding differences in the brain areas. The ventral visual pathway-cerebellar circuit deficit appeared to be altered in patients with alcohol dependence.

Keywords: alcohol dependence, functional connectivity density, visual pathway, cerebellar circuit, receiver operating characteristic
INTRODUCTION

Alcohol dependence is the widest substance addiction worldwide, with high morbidity or mortality. Alcohol dependence is a chronic relapsing disorder, characterized by morbid alcohol consumption. Alcohol consumption creates a perception of relief from the negative emotions, but extravagant alcohol consumption may bring numerous adverse health consequences, such as cancer, liver cirrhosis and vehicle accidents (1). However, it may also thereby increase and/or reinforce the likelihood of future drinking behavior (2).

The pernicious effects of alcohol dependence on the brain and behavior are well-recognized. Neuroimaging studies have described diverse pernicious effects from alcohol dependence, including the neurochemical changes and regional functional activity in the brain (3). Resting-state functional MRI (rs-fMRI) can be used to visualize the brain activities associated with oxygenation and blood flow changes, without the need of exposure to radioactive tracers, which is widely used for detection of specific regional brain alterations that can't be identified by traditional MRI examination (4). The advances of fMRI may help us to non-invasively explore the functional organization in the human brain, thus better characterizing the changes of regional neuronal spontaneous brain activity and intrinsic connectivity patterns, to better understand the underlying neural basis of neuropsychiatric disorders.

Functional connectivity studies have revealed abnormal connectivity patterns in individuals with alcohol dependence (5, 6). Seed-based functional connectivity analysis reflects the relationships of time series between a given seed point area and other brain areas (6, 7); however, it is based on a priori hypothesis with the need of a priori definition for the regions of interest. Functional connectivity density (FCD) can be used to identify the distribution of the brain network hubs and unbiasedly search for abnormalities within the whole brain, without the need of a priori hypothesis (7, 8). The FCD can be divided into short-range FCD (shortFCD) and long-range FCD (longFCD) on the basis of the neighboring relationships between brain voxels (9).

Alcohol dependence is associated with changes in regional brain activity in several areas, which makes its neurobiological mechanism more complex. Although recent evidence of structural and functional MRI experiments have increased tremendously, identifying several brain regions that are relevant to alcoholism (10, 11), the neurobiological mechanism underlying alcohol dependence remains largely unknown. The shortFCD and longFCD analysis can reveal extra information which cannot be provided by the seed-based functional connectivity analysis and FCD, and have been widely applied into several diseases (12–16). In the current study, we hypothesized that alcohol dependence is associated with distinct connectivity patterns reflected by different shortFCD and longFCD changes, depending on the neighborhood strategy. To test the hypothesis, we utilized the potential biological indicators of shortFCD and longFCD to characterize the intrinsic functional connectivity changes in 24 patients with alcohol dependence relative to 24 status-matched healthy subjects. Next, we used Pearson’s correlation to evaluate the relationships between those brain areas with FCD differences and behavioral characteristics. We also utilized the receiver operating characteristic (ROC) curve to investigate whether the brain areas with FCD differences had the ability to distinguish between patients with alcohol dependence, and the status-matched healthy subjects.

MATERIALS AND METHODS

Subjects

A total of 24 male patients with alcohol dependence (age, 47.83 ± 6.93 years; education, 9.67 ± 3.09; mean ± standard deviation) and 24 male healthy subjects (age, 47.67 ± 6.99 years; education, 8.33 ± 3.21 years) participated in the study.

Patients with alcohol dependence met the diagnostic criteria as defined by the Diagnostic and Statistical Manual of Mental Disorders, version 4 (DSM-IV). All recruited volunteers met the following inclusion criteria as in previous studies (6): (1) first-time visitors, and previously had never taken any benzodiazepine or chlorpromazine medications treatment before; (2) without any history of other substance dependence or abuse (such as marihuana and tobacco addiction) as defined by DSM-IV; (3) without any history of sleep disorders and major psychiatric disorders; (4) right-handed; (5) without any family history of alcohol dependence. Additional exclusion criteria were as follows: (1) a history of head injury with loss of consciousness; (2) the presence of any past or current neurological disease; (3) presence of hepatitis C, hypertension and type 2 diabetes that required medical intervention; (4) aged above 60 years and below 19 years; (5) the presence of any contraindications to an MRI; and (6) any foreign implants, pathological brain MRI findings, and inborn or other acquired diseases. The life history of psychiatric disorders, daily alcohol consumption, severity of alcohol dependence questionnaire (SADQ), mean years of drinking and alcohol use disorders identification test (AUDIT) were recorded by an experienced psychiatrist who worked for more than 10 years. The study was approved by the Medical Research Ethical Committee, in accordance with the Declaration of Helsinki. Written informed consent was obtained from all volunteers.

### Table 1 | Characteristics of alcohol dependent and healthy subjects.

| Alcohol dependent | Healthy subjects | t-value | p-value |
|-------------------|------------------|---------|---------|
| **DEMOGRAPHICS**  |                  |         |         |
| Mean age, years   | 47.83 ± 6.93     | 47.67 ± 6.99 | 0.083 | 0.934 |
| Education, years  | 9.67 ± 3.09      | 8.33 ± 3.21 | 1.466 | 0.149 |
| Years of drink,   | 27.46 ± 10.89    | N/A     | N/A     | N/A   |
|                  |                  |         |         |
| SADQ score        | 20.21 ± 7.09     | N/A     | N/A     | N/A   |
| AUDIT score       | 24.08 ± 5.69     | 2.63 ± 0.97 | 18.2 | <0.001 |
| Daily alcohol     | 237.5 ± 115.39   | N/A     | N/A     | N/A   |

Data are mean ± standard deviation values. SADQ, Severity of alcohol dependence questionnaire; AUDIT, Alcohol use disorders identification test; N/A, Not applicable.
MRI Parameters
We performed the MRI scan on a 3.0-Tesla MR scanner (Siemens). First, 176 high-resolution anatomical images of 3D T1-weighted in an sagittal orientation (repetition time/echo time (TR/TE) = 1,950/2.3 ms, gap/thickness = 0/1 mm, field of view (FOV) = 244 × 252 mm, acquisition matrix = 248 × 256, flip angle = 9°) were collected. Next, we collected 240 functional images (TR/TE = 3,000/25 ms, gap/thickness = 0.5/5.0 mm, flip angle = 90°, acquisition matrix = 32 × 32, FOV = 210 × 210 mm) covering the whole brain.

Data Analysis
First, we deleted the first 10 time points of the functional images because of the possible instability of the initial MRI signal and inadaptation to the scanning environment. The remaining data were conducted as standard for form transformation. The data preprocessing of the remaining data were done with the following steps, including the slice timing, head motion correction and spatial normalization to the Montreal Neurological Institute (MNI) space. The data of volunteers with >1.5 mm maximum translation or/and >1.5° degree of rotation in any directions were dislodged. After which, the remaining images were resampled at a resolution of 3 × 3 × 3 mm^3 during the step of spatial normalization. Linear regression was applied to remove the effects of spurious covariates, including the Friston 24 head motion parameters, white matter signal and cerebrospinal fluid signal. Next, the functional images were entered into linearly detrended and temporal bandpass filter (0.01–0.1 Hz).

Calculation of Long FCD and ShortFCD Calculation Maps
The local and global FCD maps for each individual were calculated in a gray matter (GM) mask. The number of functional connections of a given voxel was considered as a degree of a node in a binary graph. First, we defined the functional connectivity between a given voxel with each of the other voxels in the whole brain with a correlation threshold of r > 0.25 (17). Second, the binarized longFCD and shortFCD were defined based on the neighborhood strategy. We defined the voxels with a correlation threshold of r > 0.25 inside their neighborhood (radius sphere ≤ 6 mm) as shortFCD, and defined the voxels with a correlation threshold of r > 0.25 outside their neighborhood (radius sphere > 6 mm) as longFCD. Next, the shortFCD and longFCD maps of each subject were divided by the mean value so as to convert to Z scores to improve the normality. Finally, the shortFCD and longFCD maps underwent spatial smoothing with a Gaussian kernel of 6 × 6 × 6 mm^3 full-width at half-maximum. The detailed procedure of the shortFCD and longFCD is given in a previous study (8).

In this study, we repeated the network analysis using a range of correlation r thresholds, to determine whether between-group differences were substantially affected by the

![FIGURE 1](image-url)
selection of different r-value thresholds or nodes, used to construct brain networks (7). We used seven r thresholds ($r = 0.30, 0.35, 0.40, 0.45, 0.50, 0.6,$ and $0.75$). Here, we adopted the threshold of $r = 0.45$ to calculate the binarized FCD maps.

**Statistical Analysis**

Data are presented as mean ± standard deviation. The demographic characteristics (age, AUDIT score and years of education) were analyzed with independent sample unpair t-tests. The statistical threshold was set at $p < 0.05$.

Before comparing the between-group differences in the FCD maps, we first used sample t-tests to construct within-group statistical maps of shortFCD and longFCD for patients with alcohol dependence and healthy subjects to identify the network distributions of each group ($p < 0.001$, false discovery rate (FDR) corrected). Then, independent sample unpair t-tests were utilized to evaluate the voxel-wise differences between patients with alcohol dependence and healthy subjects with
TABLE 2 | Binarized FCD differences between alcohol dependent and healthy subjects.

| Condition | Brain regions of peak coordinates | R/L | BA | Voxel volume (mm³) | t-score of peak voxel MNI coordinates | X, Y, Z |
|-----------|----------------------------------|-----|----|-------------------|----------------------------------------|--------|
| longFCD   | Cerebellum posterior lobe         | R   | N/A| 40                | −3.6616                                | 30, −75, −36 |
| shortFCD  | Superior temporal gyrus          | L   | 38 | 83                | −4.8136                                | −39, 21, −33 |
| shortFCD  | Fusiform gyrus, Parahippocampal gyrus | R | 20, 30, 37 | 43                | −3.4763                                | 36, −33, −24 |
| shortFCD  | Lentiform nucleus                | L   | N/A| 43                | −3.6663                                | 15, 12, −6  |
| shortFCD  | Lingual gyrus                    | L   | 18 | 41                | −4.0418                                | −12, −48, −3 |
| shortFCD  | Middle occipital gyrus           | R   | 19, 37 | 88                | −4.0348                                | 54, −69, 6  |
| shortFCD  | Medial temporal gyrus            | R   | 46 | 51                | 4.3969                                 | 18, 48, 9  |
| shortFCD  | Precentral gyrus                 | L   | 6  | 74                | 4.7508                                 | −45, −9, 45 |
| shortFCD  | Cingulate gyrus                  | R   | 31 | 40                | 3.6171                                 | 9, −30, 39  |

The statistical threshold was set at a corrected significance level of an individual two-tailed voxel-wise p < 0.01 using an AlphaSim corrected threshold of cluster p < 0.05. Positive values of t-score indicate the increased longFCD or shortFCD in brain areas, and negative values of t-score indicate the decreased longFCD or shortFCD in brain areas. FCD, Functional connectivity density; shortFCD, Short-range FCD; longFCD, Long-range FCD; R, right; L, left; BA, Brodmann’s area; MNI, Montréal neurological institute; N/A, Not applicable.

RESULTS

Sample Characteristics

The behavioral characteristics of the alcohol dependent and the healthy subjects are presented in the Table 1. Patients with alcohol dependence did not significantly differ from the healthy subjects in mean age (t = 0.083, p = 0.934) and mean education (t = 1.466, p = 0.149). The mean AUDIT score was higher in patients with alcohol dependence than that of the healthy subjects (t = 18.2, p < 0.001). In the alcohol dependence group, the mean duration of drinking history was (mean ± std, 27.46 ± 10.89 years, 7 ~ 45 years), the mean SADQ score was (20.21 ± 7.09), and the mean daily alcohol consumption was (237.5 ± 115.39) ml.

Binarized FCD Differences

One Sample t-Tests

Before comparing the binarized FCD differences between the alcohol dependent and healthy subjects, one sample t-tests were used to construct within-group statistical maps for the alcohol dependence group and the healthy subjects, separately (p < 0.001, FDR corrected). Figure 1 shows the binarized longFCD and shortFCD maps in the alcohol dependence group and the healthy subject group (Figures 1A–D, respectively. We found that the two groups exhibited significantly similar differences in brain areas both in binarized longFCD and shortFCD maps. The covered differences of the brain areas in the binarized shortFCD were larger than the binarized longFCD for both groups. The
covered differences of the brain areas in the binarized shortFCD in the alcohol dependent group were larger than that of the healthy subject group.

Two Sample T-tests
We further analyzed the binarized longFCD and shortFCD differences between the alcohol dependent group and the healthy controls, using several different r-value thresholds (r = 0.30, 0.35, 0.40, 0.45, 0.50, 0.6, and 0.75). We observed highly similar intra-group differences in the binarized longFCD and shortFCD maps when using these different thresholds (Figures 2, 3), indicating that the intra-group differences did not depend on the threshold used; therefore, we only reported the results of the binary network analysis using a threshold of r = 0.45 in which the covered differences in the brain areas were larger.

Compared with the healthy subjects, the alcohol dependent group showed a significant increased binarized longFCD area in the right cerebellum posterior lobe (Table 2, Figure 4A). Compared with the healthy subjects, the alcohol dependent group showed significantly increased binarized shortFCD areas in the left precentral gyrus (BA 6), right medial frontal gyrus (BA 10, 46) in the salience network and the right cingulate gyrus (BA 31), and decreased binarized shortFCD areas in the left temporal pole (BA 38), bilateral visual association cortex (BA 18, 19), left striatum cortex (lentiform nucleus) and the right inferior temporal lobe (fusiform gyrus extending to parahippocampal gyrus) (BA 20, 30, 37) (Table 2, Figure 4B).

ROC Curve
The mean beta value of the binarized shortFCD and longFCD differences in those altered brain areas were extracted (Figure 5). These binarized FCD differences in the brain areas were further used in a ROC curve, to evaluate the ability in distinguishing the alcohol dependent group from the healthy subjects (Figures 6A–C). The area under the curve (AUC) value, of the binarized longFCD difference in the right cerebellum posterior lobe was 0.806. Furthermore, the ROC curve demonstrated that this area alone discriminated the alcohol dependent group from the healthy subjects, with a sensitivity of 70.8% and a specificity of 79.2% (Table 3, Figure 6A). The AUC values, of the binarized shortFCD differences in brain areas were (0.838 ± 0.021; 0.806–0.878). Furthermore, the ROC curve demonstrated that the binarized shortFCD differences in those regional brain areas alone discriminated between the two groups with a high degree of sensitivity (81.26 ± 9.19%; 66.7–91.7%) and specificity (78.64 ± 10.31%; 62.5–95.8%) (Table 3, Figures 6B,C). The AUC value, of the combined areas of visual pathway that discriminated the alcohol dependent group from the healthy subjects, was up to 0.943, accordingly, the sensitivity and specificity were up to 91.7 and 91.7% with a cut-off point of 0.4629 (Table 3, Figure 6D).

Pearson’s Correlation Analysis
In the alcohol dependent group, the mean years of drinking displayed a negative correlation with the beta value of the decreased binarized longFCD in the right cerebellum posterior lobe (r = −0.513, p = 0.01; Figure 7A). SADQ showed positive correlations with the beta value of the binarized shortFCD in the left visual association cortex (r = 0.412, p = 0.045; Figure 7B) and AUDIT score (r = 0.63, p = 0.001; Figure 7C), and an approximate positive correlation with the beta value of the increased binarized shortFCD area in the right medial frontal gyrus (r = 0.367, p = 0.077; Figure 7D). Other significant correlations between the beta value of brain areas with FCD differences and clinical features were not found (p > 0.05).

DISCUSSION
The current study is the first to utilize the shortFCD and longFCD analysis to identify alcohol-related intrinsic functional connectivity patterns in 24 patients with an alcohol dependence relative to 24 status-matched healthy subjects, and their correlations with demographic characteristics. Female subjects were excluded to remove the influence of a gender factor (18, 19). A ROC curve was applied to identify the ability of those FCD differences in distinguishing between the two groups. Three results were revealed: (1) the covered differences in the brain areas in the binarized shortFCD were larger than the binarized longFCD for both groups. (2) the intra-group FCD differences did not depend on the selection of different r-value thresholds used. Alcohol dependence in patients was associated with the right cerebellum posterior lobe with significant decreased binarized longFCD, as well as the left precentral gyrus, right salience network and right cingulate gyrus with increased binarized shortFCD, and the left temporal pole, right inferior temporal lobe (BA 20, 37), bilateral visual association cortex and left striatum cortex with decreased binarized shortFCD. (3) Recently, the ROC curve was widely applied in the exploration of the reliability of one neuroimaging approach, as a potential indicator in distinguishing one group from the other group (4, 19, 20). In general, an AUC value between 0.9 and 1 is considered
FIGURE 6 | ROC curve analysis of FCD differences in regional brain areas. (A) Binarized longFCD difference in the right cerebellum posterior. (B) Decreased binarized shortFCD differences. (C) Increased binarized shortFCD differences. (D) Combined areas of visual pathway (right fusiform gyrus, left lingual gyrus, right middle occipital gyrus). ROC, Receiver operating characteristic; FCD, Functional connectivity density; R, right; L, left.

excellent, and a value between 0.8 and 0.9 is considered good. In the present study, the ROC curve revealed good AUC values for those specific brain areas, and further diagnostic analysis demonstrated that those specific brain areas alone discriminated between patients with alcohol dependence and healthy subjects, with a high degree of sensitivity and specificity; (4) In the alcohol dependent group, the cerebellum posterior lobe, visual association cortex and salience network displayed significant correlations with demographic characteristics.

The visual pathway is divided into an object and spatial properties processing pathway (21–23). The object properties processing pathway runs from the striatum cortex down to the occipital lobe and inferior temporal lobe (BA 20, 37), and has been called the ventral system; this system processes properties of objects, such as shape, face, color and size. A decreased FCD value in the functional hubs may reflect less correlated activity and impairment of these functional hubs in facilitating network communications. In alcoholics, the concept of inefficiency includes difficulties in isolating irrelevant information (24), which is necessary for discriminating the targets from the distractors (6). In the present study we found that alcohol dependence was associated with decreased shortFCD areas in the ventral system, which showed a positive correlation with the SADQ score. Furthermore, the combined areas of visual pathway alone discriminated the alcohol dependent group from the healthy subjects, with a sensitivity of 91.7% and a specificity of 91.7%. These findings may reflect disorganization of the visual pathway caused by extravagant alcohol consumption, leading to inefficiency in information transmission from one place to another in the ventral system.

Morphology-anatomical studies have found decreased gray matter volumes in the cerebellum (25, 26) in patients with an alcohol dependence. These changes have shown to be predictive of the relapse risk, suggesting a significant role of
decreased gray matter volumes in the cerebellum in clinical outcomes in alcohol dependence (27). Similarly, resting-state functional connectivity studies also showed consistent findings of decreased functional connectivity in the cerebellum (28–30).

The cerebellum posterior lobe is associated with the regulation of coordinating movement, and was particularly vulnerable to alcoholism-related damage (18, 19, 31). The cerebellar circuit has been associated with motor control function and motor behavior, which are disrupted by alcohol intoxication (6, 32). Poor regulation of coordinating movement, balance and emotional changes are core characteristics of alcohol dependence (31, 33). Our results revealed that alcohol dependence was associated with a decreased longFCD in the cerebellum posterior lobe, which has shown altered functional connectivity in several studies in patients with alcohol dependence (6, 32–35), and may therefore support our results. Furthermore, the cerebellum posterior lobe was significantly correlated with the number of years of alcohol consumption. Therefore, we speculated that the decreased functional connectivity in the cerebellum posterior lobe may be interpreted as a functional impairment caused by long-term alcoholism, which may be one of the main reasons for impaired driving behavior in alcoholics.

In the present study we found significantly increased shortFCD in several brain areas. There are two prevalent speculations (6). One explanation of this finding could be interpreted as brain compensation, in which the alcoholic brain utilizes additional resources to help achieve the same level of performance as before. Long-term noxious effects of alcohol consumption on the human brain structure, both in gray and white matter, have widely been studied (36, 37). Another explanation of this finding could be interpreted as an enhanced

### TABLE 3 | ROC curve for FCD differences in brain areas between alcohol dependent and healthy subjects.

| Brain area                        | AUC  | Sensitivity, % | Specificity, % | Cut off Point*         |
|----------------------------------|------|----------------|----------------|------------------------|
| R_Cerebellum Posterior Lobe      | 0.806| 70.8           | 79.2           | 0.353                  |
| L_Superior Temporal Gyrus        | 0.833| 87.5           | 70.8           | −0.798                 |
| R_Fusiform Gyrus                 | 0.833| 79.2           | 75             | −0.6405                |
| L_Lentiform Nucleus              | 0.844| 83.3           | 75             | −0.0515                |
| L_Lingual Gyrus                  | 0.832| 91.7           | 62.5           | 0.2195                 |
| R_Middle Occipital Gyrus         | 0.826| 70.8           | 87.5           | 0.84                   |
| R_Medial Frontal Gyrus           | 0.878| 66.7           | 95.8           | −0.277                 |
| L_Precentral Gyrus               | 0.851| 91.7           | 83.3           | −0.173                 |
| R_Cingulate Gyrus                | 0.806| 79.2           | 79.2           | 0.5965                 |
| R_Fusiform Gyrus + L_Lingual Gyrus + R_Middle Occipital Gyrus | 0.943| 91.7 | 91.7 | 0.4629 |

*Cut-off point of mean FCD signal value. ROC, Receiver operating characteristic; FCD, Functional connectivity density; AUC, Area under the curve; R, Right; L, Left.

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**FIGURE 7 | Pearson’s correlation among characteristics of alcohol dependent and beta value of FCD differences in brain areas.** In the alcohol dependent group, mean years of drinking negatively correlated with right cerebellum posterior lobe (A). SADQ correlated with left lingual gyrus (B), AUDIT score (C), and right medial frontal gyrus (D). FCD, Functional connectivity density.
neural effort to offset the noxious effects of alcohol consumption on the human brain structure.

CONCLUSIONS

In summary, the longFCD and shortFCD analysis might serve as a biological indicator to observe the underlying intrinsic functional connectome changes in patients with alcohol dependence, with a high degree of discriminating ability. Specifically, the shortFCD analysis is more sensitive than the longFCD analysis in finding the differences in brain areas. In the present study, we found that the ventral visual pathway-cerebellar circuit deficit appears to be altered in patients with alcohol dependence, showing that alcohol dependence was associated with the longFCD deficit in the cerebellum posterior lobe, and the shortFCD deficit in the ventral system of the visual pathway. Our data highlights the role of functional connectivity and provides a new insight to better understand the neurobiological mechanisms that underlie alcohol dependence. These findings may help clinicians develop targeted intervention and prevention strategies. However, the relatively small sample size limited the significance. Future studies with a larger sample size are necessary to corroborate our findings.

AUTHOR CONTRIBUTIONS

LC and B-XL wrote the main manuscript text. LC, JZ, and X-JD conceived and designed the whole experiment. B-XL, RL, and X-JD collected the data. JZ and X-JD analyzed the data. X-JD revised the manuscript.

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