Abnormal Fractional Amplitude of Low-frequency Fluctuation Changes in Patients With Diabetic Optic Neuropathy: a Functional MRI Study

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Abstract

The present study was to assess the spontaneous changes in brain activity in patients with DON using functional magnetic resonance imaging (fMRI). 14 DON patients and 14 healthy controls (HCs) were enrolled. All participants underwent resting-state fMRI (rs-fMRI). The fractional amplitude of low-frequency fluctuation (fALFF) method was applied to evaluate neural activity changes. The Hospital Anxiety and Depression Scale (HADS) was used to assess the anxiety and depression status of participants. The independent sample t test and chi-squared test were applied to analyze demographics of DON patients and HCs. Receiver operating characteristic (ROC) curves were applied to analyze the variation in mean fALFF values between DON patients and HCs. The relationships between the fALFF values of brain regions and clinical behaviors in the DON group were analyzed using Pearson's correlation analysis. In contrast to HCs, the fALFF value of DON patients was significantly higher in the right precentral gyrus (RPCG). However, the fALFF values in right anterior cingulate gyrus (RACG) and left middle cingulate gyrus (LMCG) were markedly decreased in DON patients. The area under the curve (AUC) of ROCs for each brain region showed high accuracy. Pearson's correlation analysis showed that fALFF values of the right anterior cingulate gyrus and left middle cingulate gyrus were negatively correlated with HADS scores, while fALFF values of the left middle cingulate gyrus were negatively correlated with DON disease duration. To sum up, we found abnormal spontaneous brain activities in regions related to cognitive and emotional dysfunction, eye movement disorder, and vision loss in patients with DON. These results may indicate the underlying neuropathological mechanisms of DON, and show that fALFF may be an effective method by which to distinguish patients with DON from healthy individuals.

Introduction

The incidence of diabetes has increased in recent years, making it one of the major diseases affecting public health globally. Diabetes is a metabolic disease with systemic effects. Complications often occur in tissues and organs such as the eyes, kidneys, heart, blood vessels, and nerves[1,2]. Ocular complications include diabetic retinopathy, macular edema, and cataracts. Neuropathy is a complication of diabetes that affects the central and sensory nerve endings, with manifestations such as skin numbness, diabetic nephropathy, and diabetic foot problems. When the optic nerve is involved, it is known as diabetic optic neuropathy (DON). DON is an important clinical blindness factor[3]. DON may be found in the form of optic disc neovascularization, Wolfram syndrome, diabetic papillitis, or anterior ischemic optic neuropathy[4]. Patients with DON have varying degrees of decline in visual function, which will eventually seriously affect their life quality and physical and mental health. Due to anatomical structure, the diagnosis of DON mainly relies on clinical symptoms and routine fundus examination, including visual evoked potential (VEP) and fundus fluorescein angiography (FFA)[5,6]. Figure 1 shows the results of retinal fundus photography and FFA of DON patients. However, the diagnosis of DON is difficult due to the lack of specific clinical symptoms and specific changes in ophthalmic examination results. Compared with other ocular complications, there are few studies focused on DON, and its pathogenesis remains unclear.

Functional magnetic resonance image (fMRI) is based on traditional magnetic resonance imaging. It can make full use of anatomical, imaging, and functional factors, and provide imaging technical support for clinical magnetic resonance diagnosis from a single morphological study combining morphological and functional research. The technology is non-invasive, non-radioactive, and has the advantages of repeatability and high temporal and spatial resolution[7,8]. In the past, fMRI has been applied to various psychoneurotic diseases, providing new perspectives for further understanding of their neuropathological mechanisms[9,10]. The amplitude of low-frequency fluctuation (ALFF) method is a resting state-fMRI (rs-fMRI) approach. Based on blood oxygen level dependent (BOLD) imaging, ALFF indicates the intensity of spontaneous local brain activity in the resting state. Therefore, ALFF may be a useful tool to measure brain activity and to better understand the pathophysiological changes. The fALFF analysis method involves calculation of the ratio of low frequency amplitude to whole brain frequency, and aims to remove the influence of the cerebrospinal fluid noise signal on low frequency amplitude. It is an analysis method for ALFF standardization. In the past, fALFF has been used in post-stroke depression, borderline personality disorder, anorexia nervosa, and Alzheimer's disease[11–14] but has not been applied to DON patients. Therefore, we used the fALFF technique to assess brain activity in DON patients in order to explore the neuropathological mechanism of this disease.

Patients And Methods

Subjects. In total, 14 patients with DON were recruited from the First Affiliated Hospital of Nanchang University (Nanchang, China). The inclusion criteria for DON were: (1) a history of diabetes; (2) evidence of optic neuropathy with papilledema, ischemic optic neuropathy, optic disc neovascularization, or optic atrophy. DON was diagnosed according to clinical manifestations, visual field defect, VEP, and FFA; (3) no systemic diseases such as serious heart, liver, or kidney diseases. The exclusion criteria were: (1) current pregnancy; (2) patients with serious neurological and hematopoietic diseases; (3) patients with ocular or orbital diseases, such as corneal ulcers, glaucoma, or orbital tumor; (4) patients with systemic lesions that might cause optic disc edema; (5) history of psychiatric disease; and (6) contraindications for MRI scans, such as pacemakers or other metal implants.

In addition, 14 healthy people of similar age and education level were enrolled. The main inclusion criteria were: (1) no history of eye disease; (2) no liver, kidney, or heart disease; (2) no history of nervous system diseases; (3) no history of mental illness; (4) no contraindications for MRI scans.

All participants were told about the purpose, procedure, and risks of this study and provided signed informed consent. The current study was conducted in accordance with the Declaration of Helsinki and was approved by the Medical Ethics Committee of the First Affiliated Hospital of Nanchang University.

MRI parameters. A 3-Tesla MRI scanner was used. All participants were asked to breathe smoothly and keep their eyes closed throughout the scan. A 3D metamorphic gradient recalled-echo pulse sequence was used to obtain the functional data and 176 structural images were obtained using the parameters described previously[15]. Functional images (n = 240) were collected using the following scanning

parameters: acquisition matrix, 64x64; field of view, 220x220 mm; thickness, 4.0 mm; gap, 1.2 mm; repetition time, 2,000 msec; flip angle, 90^{o}; echo time, 30 msec and 29 axial.

rs-fMRI data processing. Statistical parameter maps (Statistical Parametric Mapping, SPM5; http://www.fil.ion.ucl.ac. uk/spm) and the REST software (http://www.restfmri.net) were used for fMRI data processing. Initially, the first 10 time-points were removed to balance the signal, then head motion correction was performed. Data with more than 1.5 mm maximum displacement in the x, y, or z-axes or 1.5 ^{o} of angular motion were deleted. Subsequently, functional images were normalized to meet the standards of the Montreal Neurological Institute (MNI) with a voxel size of 3x3x3mm. A linear regression process was applied to remove uncontrollable variables. Finally, the data were filtered in the frequency range of 0.01–0.08 Hz to eliminate the influence of high-frequency physiological noise.

fALFF calculation. For data processing, the REST software was used to calculate the fALFF value of the whole brain, as the ratio of ALFF value in the low-frequency range (0.01–0.08 Hz) to that of the whole frequency range (0–0.25 Hz). This ratio reduced the effect of variance between individuals.

Statistical analysis. SPSS 20.0 software (SPSS, Chicago, IL, USA) was applied for statistical analysis. An independent sample t test and chi-squared test was used to compare data between the two groups. The operating characteristic (ROC) curves were used to assess the accuracy of the fALFF value as a means of distinguishing DON patients from HCs. Pearson's correlation analysis was used to look for any association between regional fALFF values and clinical parameters of DON patients. In all analyses, p value < 0.05 was the threshold for statistical significance.

Results

Demographics and visual measurements. No significant difference was found in age or gender between DON and HC groups (P>0.05). The mean duration of diabetes was 52.14 ± 27.46 days. However, statistically significant differences between the groups were found in best-corrected visual acuity (VA) of each eye, latency (ms), and amplitude (uv) of the monocular VEP from each eye, and the HADS score (P < 0.05). More details are shown in Table 1.

Table 1
Basic informations of participants in the study.

Condition	DON	HCs	t	P-value*
Male/female	6/8	6/8	N/A	> 0.99
Age (years)	54.21 ± 5.16	53.45 ± 5.62	0.226	0.783
Weight (kg)	64.33 ± 7.02	60.83 ± 7.15	0.168	0.915
Handedness	14R	14R	N/A	> 0.99
Duration of DON (days)	52.14 ± 27.46	N/A	N/A	N/A
Best-corrected VA-left eye	0.35 ± 0.25	1.05 ± 0.15	-3.674	0.019
Best-corrected VA-right eye	0.45 ± 0.15	1.05 ± 0.10	-3.932	0.023
Latency (ms)-right of the VEP	123.76 ± 10.16	103.23 ± 5.24	3.617	0.006
Amplitudes(uv)-right of the VEP	6.77 ± 2.34	14.84 ± 1.89	-8.764	0.002
Latency (ms)-left of the VEP	113.42 ± 8.32	101.76 ± 3.66	5.543	0.019
Amplitudes (uv)-left of the VEP	11.35 ± 3.39	15.04 ± 2.68	-3.241	0.007
HADS	8.71 ± 1.02	3.36 ± 0.58	8.397	<0.001

Notes: $p < 0.05$ was set as statistical level.

Abbreviations: DON, diabetic optic neuropathy; HC, healthy control; N/A, not applicable; R, right; VA, visual acuity; VEP, visual evoked potential; HADS, Hospital Anxiety and Depression Scale.

fALFF differences. The fALFF values varied between brain regions. Compared with HCs, patients with DON had higher fALFF value in the right precentral gyrus (RPCG) but lower fALFF values in the right anterior cingulate gyrus (RACG) and left middle cingulate gyrus (LMCG). More details are shown in Table 2 and Fig. 2.

Table 2
Brain areas with significantly different fALFF values between DON patients and HCs.

	Brain areas	T-values	voxel	MNI coordinates		
				x	y	z
DON>HC	RPCG	4.0529	63	18	-27	48
DON<HC	RACG	-3.8260	140	6	45	3
DON<HC	LMCG	-4.0013	68	0	12	33

Notes: The statistical threshold was set at a voxel level P<0.01 and a cluster level of P<0.05.

Abbreviations: fALFF, fractional amplitude of low-frequency fluctuation; DON, diabetic optic neuropathy; HCs, healthy controls; RPCG, right precentral gyrus; RACG, right anterior cingulate gyrus; LMCG, left middle cingulate gyrus; MNI, Montreal Neurological Institute.

ROC curve. The ROC curve was used to analyze the ALFF values of different brain regions and the area under the curve (AUC) represented the diagnostic accuracy. The AUC of fALFF values in the RPCG, RACG, and LMCG were 0.980, 0.990, and 0.939 respectively (Fig. 3). All of the AUC values are above 0.9, indicating high diagnostic accuracy.

Correlation analysis. Pearson correlation analysis showed that the average fALFF values in the RACG and LMCG were negatively correlated with HADS scores (r=−0.8370, P = 0.0313; r=−0.8582, P = 0.0476, respectively). In addition, disease duration of DON was negatively correlated with fALFF value in the LMCG (r=−0.9403, P = 0.0316). The results are shown in Fig. 4.

Discussion

As one method of rs-fMRI, fALFF has been used to investigate several ophthalmic diseases including primary open-angle glaucoma, primary blepharospasm, retinitis pigmentosa, normal-tension glaucoma, and monocular blindness[16–20] (Table 3). The target of the present research was to assess regional brain function in DON using the fALFF method and to explore the potential pathogenesis of this disease. Our results showed vision deficits in DON patients. In contrast to HCs, we found that the fALFF value of the RPCG in DON patients was increased, while the fALFF values of RACG and LMCG were decreased (Table 4 and Fig. 5).

Table 3
fALFF method applied for ophthalmological diseases.

Author	Year	Disease	Increased fALFF values	Decreased fALFF values
Li T[16]	2014	Primary open-angle glaucoma	LSTG, LIPL, RMCC, RMFG	RMTG, LIG, BC, LPCG, RC/LL, LC/PC
Ni MF[17]	2017	Primary blepharospasm	RCH	-
Huang X[18]	2018	Retinitis pigmentosa	LSFG, LMFG, BSMA	BLG/CAL
Li HL[19]	2020	Normal-tension glaucoma	-	RAG, RPC
Fang JW[20]	2020	Monocular blindness	LPC, BIPL	LAC

Abbreviations: fALFF, fractional amplitude of low-frequency fluctuation; LSTG, left superior temporal gyrus; LIPL, left inferior parietal lobule; RMCC, right middle cingulate cortex; RMFG, right middle frontal

gyrus; RMTG, Right middle temporal gyrus; LIG, left lingual gyrus; BC, bilateral cuneus; LPCG, left postcentral gyrus; RC/LL, right calcarine/limbic lobe; LC/PC, left calcarine/posterior cingulate; RCH, right caudate head; LSFG, left superior frontal gyrus; LMFG, left middle frontal gyrus; BSMA, bilateral supplementary motor area; BLG/CAL, bilateral lingual gyrus/cerebellum anterior lobe; RAG, right angular gyrus; RPC, right precuneus; LPC, left precuneus; BIPL, bilateral inferior parietal lobe; LAC, left anterior cingulate.

Table 4
fALFF changes in brain areas of DON patients and its potential impact.

Brain areas	Study results	Brain function	Anticipants results
RPCG	DON>HC	Primary motor cortex, the control of eye movements	Eye movement
RACG	DON<HC	Part of the limbic system	Depression and anxiety, visual impariment
LMCG	DON<HC	Part of the limbic system	Depression and anxiety, visual impariment

Abbreviations: fALFF, fractional amplitude of low-frequency fluctuation; DON, diabetic optic neuropathy; RPCG, right precentral gyrus; RACG, right anterior cingulate gyrus; LMCG, left middle cingulate gyrus; HCs, healthy controls.

Composed of many pyramidal neurons, the precentral gyrus (PCG) is the cortical motor center. In the brain of anesthetized monkeys, the PCG was found to be involved in the control of defensive actions, including eye movements[21]. A previous study also showed that cortical electrical stimulation of frontal gyrus and PCG could lead to eye movement[22]. Thus, there may be a close link between abnormal activity of precentral gyrus and eye movement disorder. Hong *et al.*[23] demonstrated that the functional activity of the frontal gyrus was reduced in people with restricted eye movement to the designated target In comitant strabismus patients, researchers found a significant reduction of gray matter volume (GMV) in the precentral gyrus and a reduction of the white matter volume (WMV) in the right premotor cortex[24]. Compared with HCs, patients with mild frontotemporal degeneration (FTLD) also showed PCG degeneration and lower eye movement speed[25]. Patients with schizophrenia reportedly have reduced exploratory eye movements and significantly decreased GMV values in the bilateral PCG[26]. In addition, a positive correlation was found between the speed of eye movement and GMV values in the bilateral PCG, indicating that PCG dysfunction may explain eye movement disorder in these patients. Shi *et al.*[27] found that the lower voxel-mirrored homotopic connectivity (VMHC) value in the precentral gyrus of patients with corneal ulcer than in those without suggests that it may in part explain ocular movement dysfunction in this condition. Using the voxel-based degree centrality (DC) method, Cai *et al.*[28] studied changes in the brain functional network of primary angle-closure glaucoma patients and found significantly higher DC values in PCG after than before glaucoma surgery. Voxel-based DC value in this region is also reportedly decreased in patients with comitant exotropia strabismus, and regional homogeneity (ReHo) value in PCG in patients with strabismus and amblyopia is lower than in

controls[29,30]. All of these findings indicate an association between the PCG and eye movements. In the current study, we found higher fALFF value in the PCG, indicating enhanced brain activity in PCG in patients with DON. On this basis, we speculate that increased fALFF value in PCG may reflect a compensatory mechanism for ocular movement disorder in patients with DON.

The cingulate gyrus consists of four sub regions: anterior cingulate gyrus (ACG), middle cingulate gyrus (MCG), posterior cingulate gyrus, and retrosplenial cingulate gyrus[31]. Having many nerve fiber connections with other parts of the brain, the cingulate gyrus participates in the maintenance of arousal, regulating the body's emotional, memory, cognitive, and executive functions[32]. Using independent component analysis of rs-fMRI, researchers have found significantly lower activation of cingulate gyrus in early Parkinson's disease patients than in healthy controls[33]. Lower functional connectivity in the ACG and MCG were also observed in patients with schizophrenia[34,35]. In a study conducted by Bürger *et al.*[36], whole-brain and a multivariate pattern classification analysis of rs-fRMI were used to study brain activity in patients with emotional disorders. They found significantly decreased ACG activation in patients with major depressive disorder than in those with bipolar disorder or the healthy controls, which potentially indicates impaired bottom-up emotional processing and abnormal automatic emotion regulation. Additionally, patients with chronic tinnitus also had abnormal ALFF value in the cingulate gyrus, which was thought to be related to psychological problems including stress, anxiety, inattention, and insomnia[37]. Consistent with the above results, the present study demonstrated significantly decreased fALFF values in the RACG and LMCG. Additionally, fALFF values in RACG and LMCG were negatively correlated with HADS scores, and fALFF values in the LMCG were negatively correlated with disease duration in DON, indicating that changed brain activities in the RACG and LMCG are associated with emotional dysfunction in DON patients.

However, activity in the cingulate gyrus may also be related to visual function, since ACG receives afferent neurons from the thalamus. Hence, it can be deduced that cingulate gyrus is related to visual function. A previous study found that the ReHo values of the left marginal lobe/ACG and the right marginal lobe/ACG were significantly reduced after eye enucleation[38], suggesting reduced visual signals after the surgery. Similarly, previous research has also found significantly decreased GMV value of RACG in monocular blindness, indicating a disturbance of synchronous nerve activity in these patients[39]. A decrease in fALFF of ACG in monocular blindness further confirmed this conclusion[20]. Zhai *et al.*[40] analyzed the role of perceptual learning in the treatment of amblyopia using fMRI. They reported that activity of the cingulate gyrus and visual cortex were significantly increased in patients after treatment, suggesting deficit of the cingulate cortex as a cause of amblyopia. In the current study, we found lower fALFF value in RACG in DON patients, which suggests that the abnormality of RACG may be part of the neuropathological mechanism of vision loss in DON patients.

ROC curve analysis is often utilized to distinguish diseased from healthy states. In the present analysis, the AUC of fALFF values of the RPCG, RACG, and LMCG were 0.980, 0.990, and 0.939 respectively. All AUC of fALFF values was above 0.9, indicating significant differences in fALFF values between DON and

control groups and suggesting that the changed fALFF values may be potential diagnostic biomarkers for patients with DON.

The present study has some limitations. First of all, the size of samples included in our study is limited. Secondly, the patient may have made subtle movements during the scanning process, which may have affected the stability of MRI results. Finally, our conclusion needs further verification.

Conclusion

In summary, this is the first study to find functional brain activity changes in DON patients using fALFF. The abnormalities in the central anterior gyrus, anterior cingulate gyrus, and middle cingulate gyrus are associated with eye movement, emotional disorder, and vision loss in DON patients. Our findings may help to explain the pathological mechanisms of DON. Furthermore, the fALFF abnormalities may also serve as diagnostic signs for DON patients, which is of great significance.

Declarations

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Author contributions

Designed the study: Y.S., W.L., C.Y., Y.P. Acquired data: W.L., C.Y., H.S. Analysed data: W.L., L.Z. Interpreted results: W.L, Q.L., Q.G., R.L. Drafted manuscript: W.L., C.Y., Y.P. All authors read and approved the final version of the manuscript.

Conflict of Interest Statement

This was not an industry supported study. The authors report no conflicts of interest in this work.

Data availability

Data are available from the authors upon request.

Ethical Statement

All research methods were approved by the committee of the medical ethics of the First Affiliated Hospital of Nanchang University and were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All subjects were explained the purpose, method, potential risks and signed an informed consent form.

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Figures

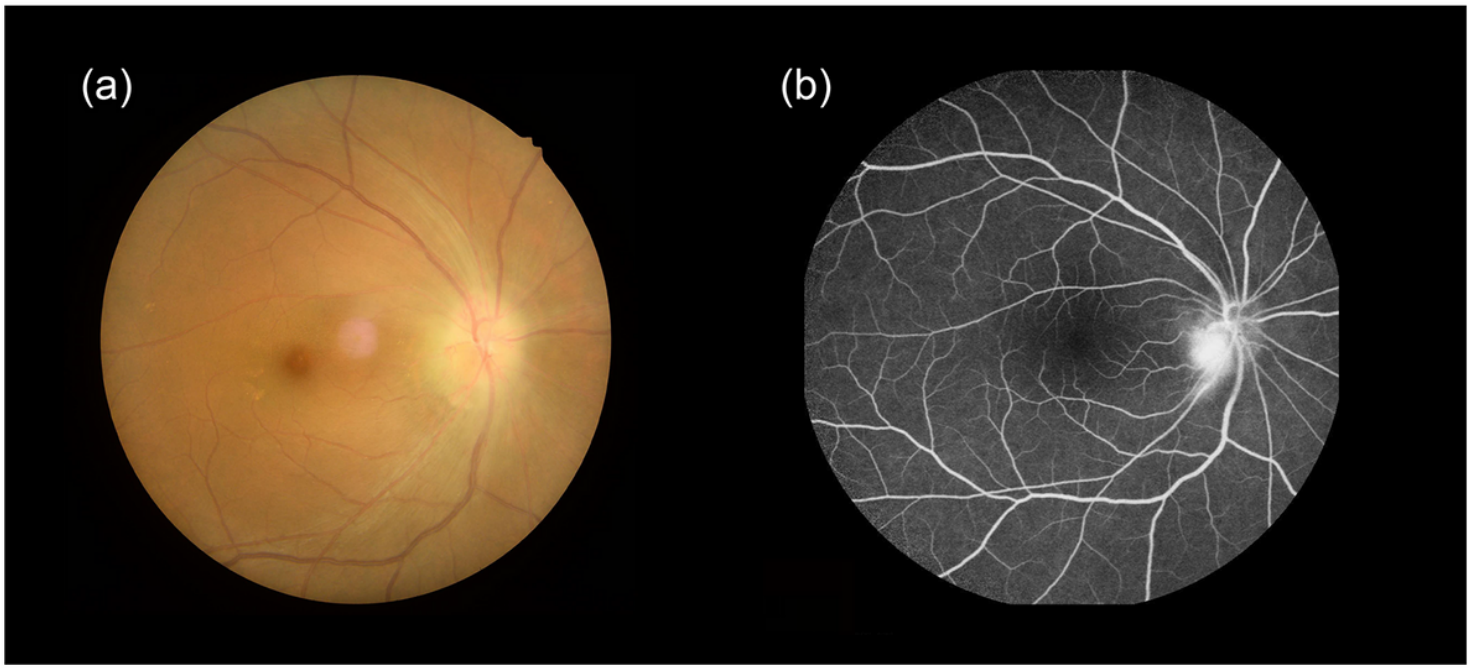

Figure 1

Typical retinal fundus photography (a) and fluorescence fundus angiography (b) images in the DON patients. Abbreviations: DON, diabetic optic neuropathy.

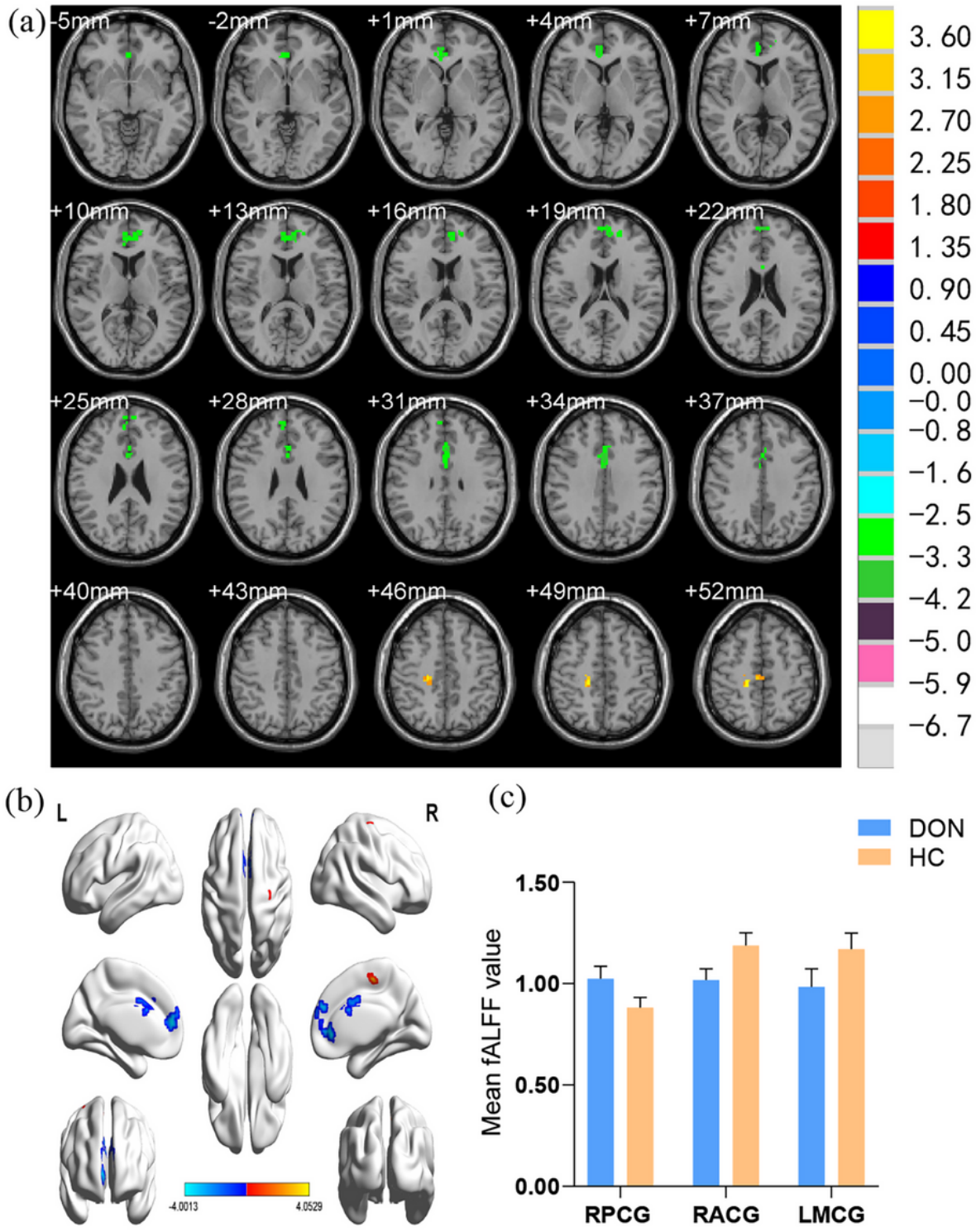

Figure 2

Spontaneous brain activity in the DON group and HCs measured using the fALFF method. (a) A significant difference between DON and control group was observed in the RPCG, RACG, and LMCG regions (z.2.3, P=0.01, cluster=40 voxels, Alphasim corrected). (b) The stereoscopic form of the cerebrum. (c) The mean fALFF values between the two groups. Abbreviations: DON, diabetic optic neuropathy; HCs,

healthy controls; fALFF, fractional amplitude of low-frequency fluctuation; RPCG, right precentral gyrus; RACG, right anterior cingulate gyrus; LMCG, left middle cingulate gyrus.

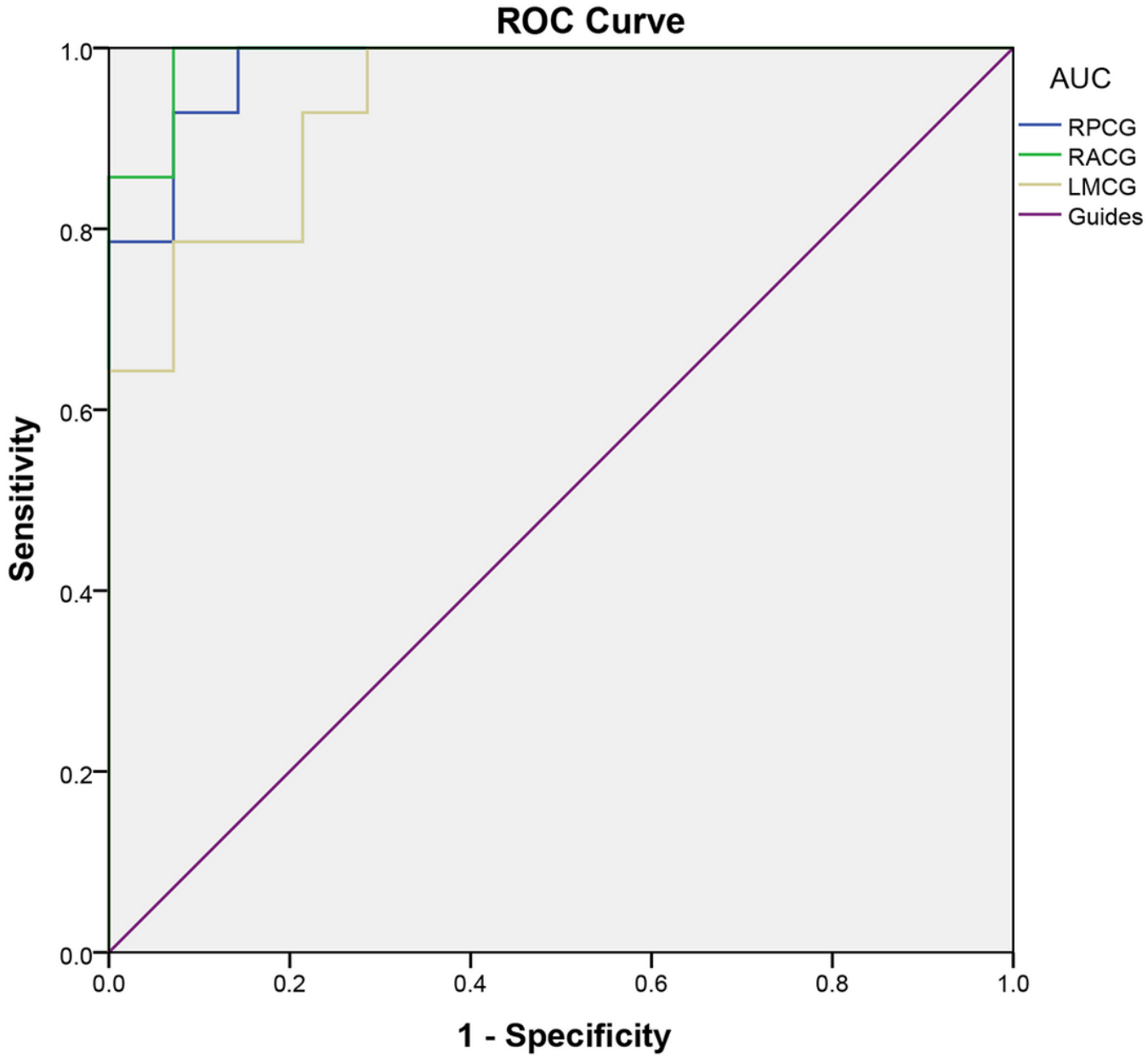

Figure 3

ROC curves of the fALFF values for brain regions with significant difference between DON and HC groups. The AUC of fALFF values of the RPCG, RACG, and LMCG were 0.980, 0.990, and 0.939 respectively. Abbreviations: ROC, receiver operating characteristic; fALFF, fractional amplitude of low-frequency fluctuation; DON, diabetic optic neuropathy; HC, healthy control; AUC, area under the curve; RPCG, right precentral gyrus; RACG, right anterior cingulate gyrus; LMCG, left middle cingulate gyrus.

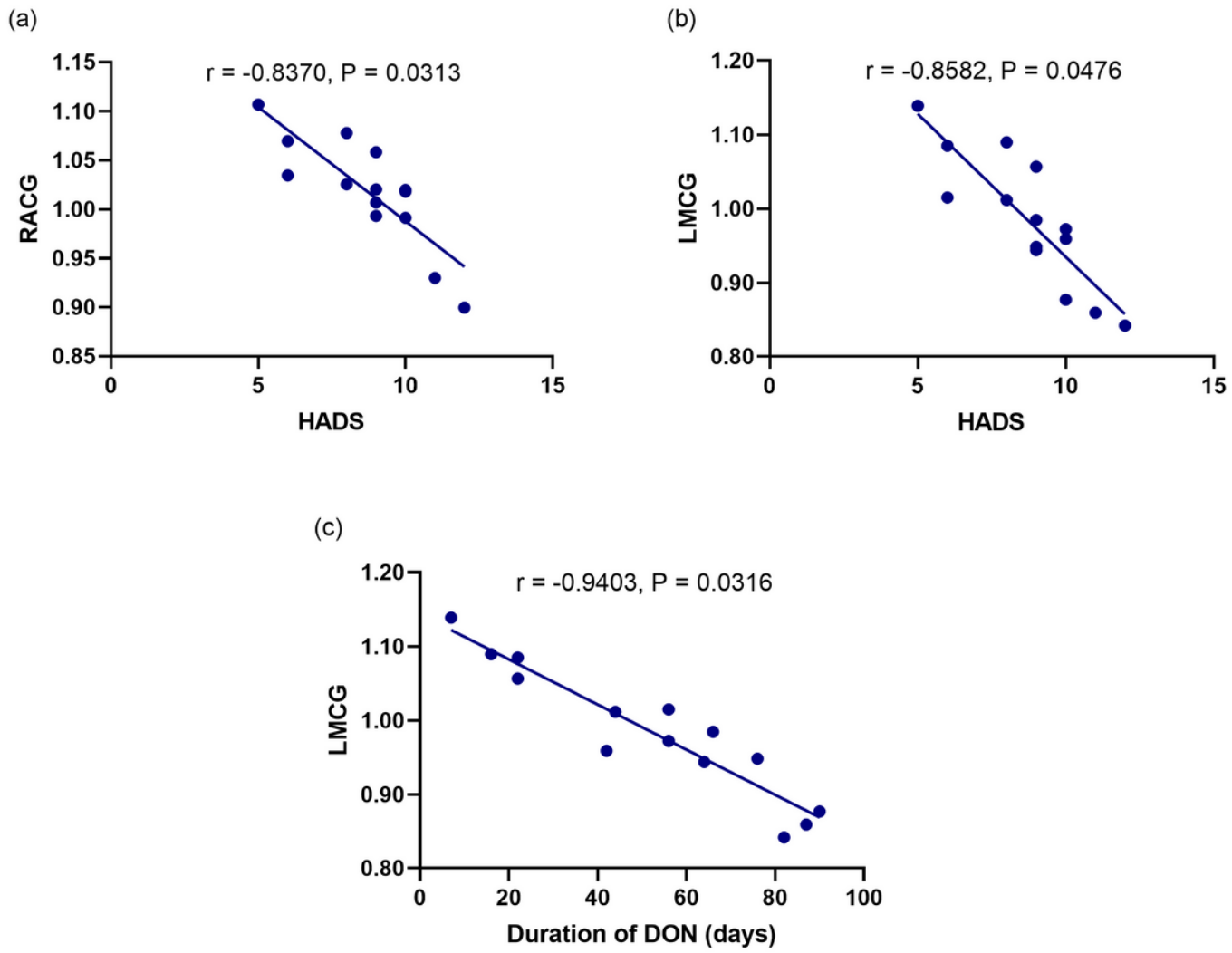

Figure 4

Pearson's correlation analysis of relationships between the fALFF values of brain regions and clinical behaviors in DON group. (a) The mean fALFF value of the RACG showed negative correlations with the HADS score (r=−0.8370, P=0.0313); (b) there was negative correlation between mean fALFF value of the LACG and HADS score (r=−0.8582, P=0.0476); (c) The mean fALFF value of the LMCG was negatively correlated with the duration of DON (r=−0.9403, P=0.0316). Abbreviations: fALFF, fractional amplitude of low-frequency fluctuation; DON, diabetic optic neuropathy; RPCG, right precentral gyrus; HADS; Hospital Anxiety and Depression Scale; RACG, right anterior cingulate gyrus; LMCG, left middle cingulate gyrus.

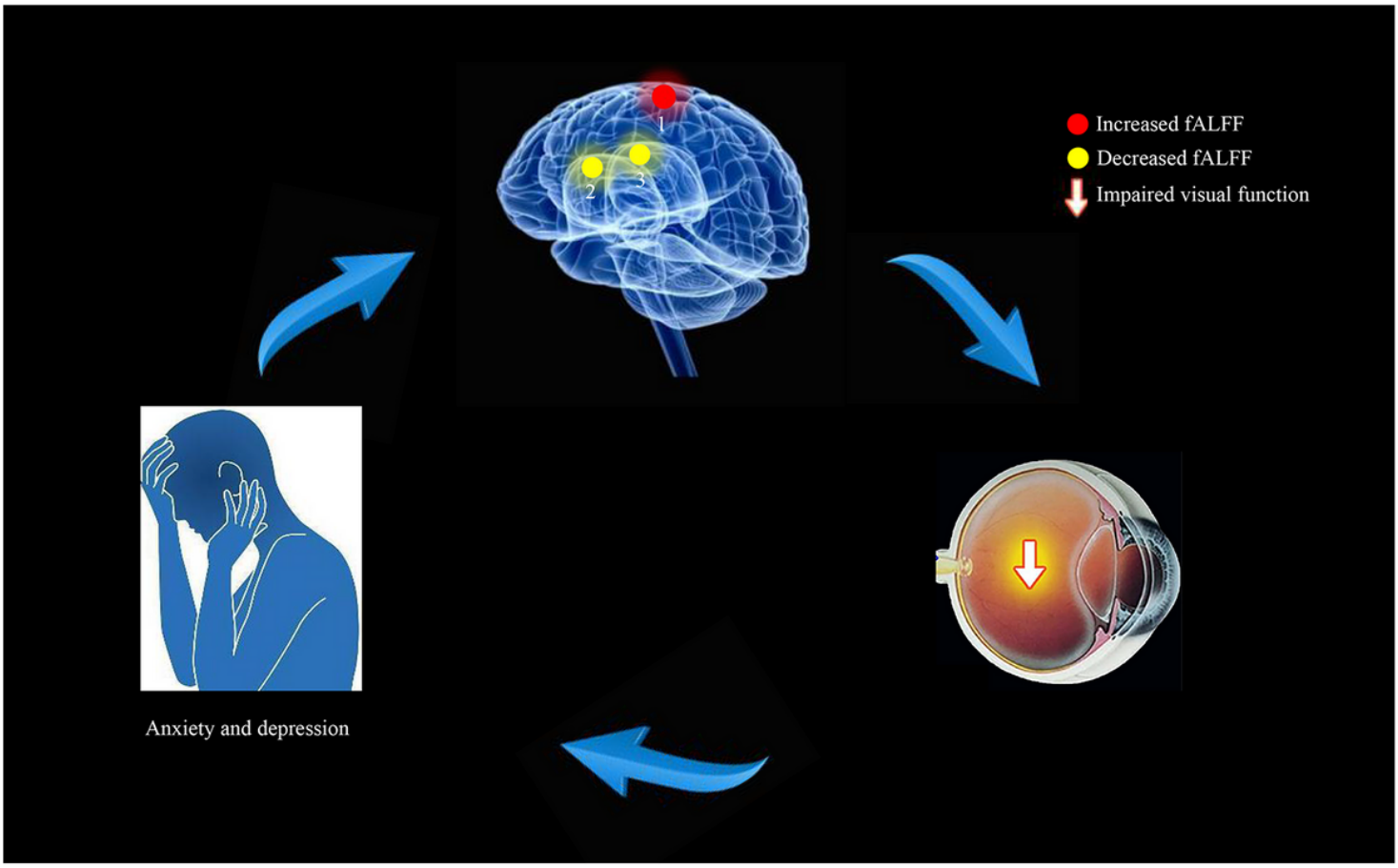

Figure 5

Correlations between mean fALFF values and behavioral performance. The fALFF value in brain regions in DON patients was associated with impaired visual function and emotional disorder. Abbreviations: fALFF, fractional amplitude of low-frequency fluctuation; DON, diabetes optic neuropathy.