

Altered Spontaneous Brain Activity Patterns in Patients After Lasik Surgery Using Amplitude of Low-Frequency Fluctuation: A Resting-State Functional MRI Study

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Objective: Previous studies demonstrated that myopia could result in alterations of brain activity in specific areas. However, whether the visual function could improve by Lasik surgery, with the brain activity alterations also change, is still unknown. Here, we intended to use the amplitude of low-frequency fluctuation (ALFF) technique to investigate the intrinsic brain activity changes in pre-Lasik (PRL) and post-Lasik (POL) patients.

Methods: A total of 15 patients with myopia (nine male and six female) were recruited in our study, who were matched according to age, weight, and height. These patients comprised both the PRL and POL groups, which is self-controlled. The patients all underwent resting-state functional magnetic resonance imaging (MRI), and the spontaneous brain activity changes were recorded by the ALFF technique. The data were recorded and arranged in the receiver operating characteristic (ROC) curve, which presented how intrinsic activities altered in different brain regions. Moreover, by Graphpad prism 8, we can analyze the linear correlation between HADS (Hospital Anxiety and Depression Scale) and ALFF values as well.

Results: Differences in ALFF values existed in brain regions between the same patient before and after the Lasik operation. The regions with increased ALFF values after Lasik surgery were the left parahippocampal gyrus, cerebellar vermis, and left posterior cingulate cortex. The regions with decreased ALFF values after Lasik surgery were the left supramarginal gyrus and right trigonometric inferior frontal gyrus.

Conclusion: We demonstrated significant fluctuations of ALFF values in specific brain areas between the same patients before and after the Lasik surgery. The altered ALFF values reflected the hyperactivity or hypoactivity of the specific brain areas, which may help predict the recovery level of patients' vision after Lasik surgery. Furthermore, based on the experimental results that presented significant activity alterations in specific brain regions, patients could be speculated equipped with a better visual function.

Keywords: myopia, Lasik, resting-state functional MRI, amplitude of low-frequency fluctuation

Introduction

Myopia is a condition in which the eye is loosely regulated, causing parallel light rays to fall in front of the retina after being refracted by the eye's refractive system, eventually forming a blurry image. Myopia has become the most common disease in ophthalmology in recent years,¹ and teenagers are most susceptible to being affected.² The overall prevalence of myopia in adults aged above 40 years is 38.9%

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in Singapore, and that is much lower in Western countries like the US (25.1%).³ And the prevalence is much higher in East Asian children as well; in 12-year-old children, the prevalence of myopia is 62.0% in Singapore and 49.7% in Guangzhou (China), while in the US it is just 20.0%.³ It is a condition that should receive high awareness, as the occurrence of myopia has increased progressively over recent years in our country, and a significant number of teenagers are diagnosed with myopia. Research has shown that the risk factors include genetic, environmental, and dietary factors, as well as lifestyle and other factors.² Although myopia is not a type of organic lesion,⁴ it can cause inconvenience in the daily life of patients. If not managed in a timely manner, reduced vision or even blindness can occur.² Therefore, the prevention, remission, and treatment of myopia are high-priority topics.

Laser surgery has been considered as the main method to correct the refractive system and myopia, the efficacy of which has been widely accepted in clinics.⁴ Laser Assisted in Situ Keratomileusis (LASIK) is the surgery most frequently performed worldwide,⁷ the mechanism of action of which is to change the curvature of the anterior surface of the cornea with a laser, achieving neutralization of the ametropia.⁴ Although the high precision and high resolution of LASIK surgery can guarantee satisfactory therapeutic outcomes and the minor trauma can facilitate a good prognosis after the surgery,⁵ several risk factors exist that may affect the operative results; for example, besides the contingent complications after the surgery, dry eyes are the most common adverse effect.⁶

MRI is a technique that provides advanced and comprehensive brain information, including structural anatomy of the brain as well as information on how it functions, in a harmless and non-invasive manner.⁸ Recently, a more burgeoning and accurate technique has arisen called functional magnetic resonance imaging (fMRI), which compensates for the limitations of MRI by providing information on the activity level of specific parts of the cerebral cortex.⁹ One branch of fMRI is BOLD-fMRI (blood oxygenation level-dependent functional MRI), which can detect spontaneous fluctuations of the BOLD-fMRI signal in a resting state, which is detected by ALFF (amplitude of low-frequency fluctuation).¹⁰ The spontaneous neurological activity detected by ALFF has been accepted to be considered as a valuable parameter in research.¹¹

Previous studies revealed that a close correlation between neuronal activities changed and visual function impaired existed in patients with high myopia.

The majority of previous studies compared nearsighted patients with healthy groups who were matched with age, sex, and educational status to subjects.^{12,13} Guo et al¹² pointed out that, in high myopia patients compared with 20-paired subjects, decreased ALFF was observed in the bilateral frontal lobe, the right inferior parietal lobule, and the left occipital lobe, while increased ALFF values were in the left caudate nucleus, the occipital lobe, and the thalamus. Besides, Huang et al¹³ discovered the altered regions in high myopia patients that were significantly lower ALFF in the middle temporal gyrus, left inferior frontal gyrus/putamen, right inferior frontal gyrus/putamen/insula, right middle frontal gyrus, and right inferior parietal lobule, and higher ALFF values in the bilateral midcingulate cortex, left postcentral gyrus, and left precuneus/inferior parietal lobule. There were also studies revealing the uncomfortable symptoms and recovery level of visual function after Lasik surgery.^{4,6} However, to our knowledge, our study is the first to combine ALFF to explore the altered-activity regions of the brain after Lasik surgery. The controlled and experimental groups in this experiment were the same clusters of patients and differed according to whether they had undergone Lasik surgery. We hypothesized that altered ALFF values in specific brain regions was detectable in patients after Lasik surgery, which furthermore could give a clue for the recovery level of patient's vision and efficiency of Lasik surgery.

Methods

Subjects

The participants in the study were patients seeking clinical assistance in the Ophthalmology Department of the First Affiliated Hospital of Nanchang University, and we selected 15 of them randomly in this study, including nine males and six females. The inclusion criteria were the following: 1) age between 20–25; 2) patients who decided to undergo Lasik surgery; and 3) patients whose myopia was their only ocular problem. The typical fundus photographs are shown in [Figure 1](#). The exclusion criteria were the following: 1) patients who had completed Lasik surgery or corrective measures previously; 2) patients with other ocular diseases (eg, glaucoma, optic neuritis, strabismus) besides myopia; 3) patients with psychiatric problems (depression, bipolar disorder), cardiovascular diseases, or cerebral infarction diseases; and 4) patients who were addicted to alcohol or abused drugs. These 15 patients comprised the PRL group, and the POL group included the same group of people. This

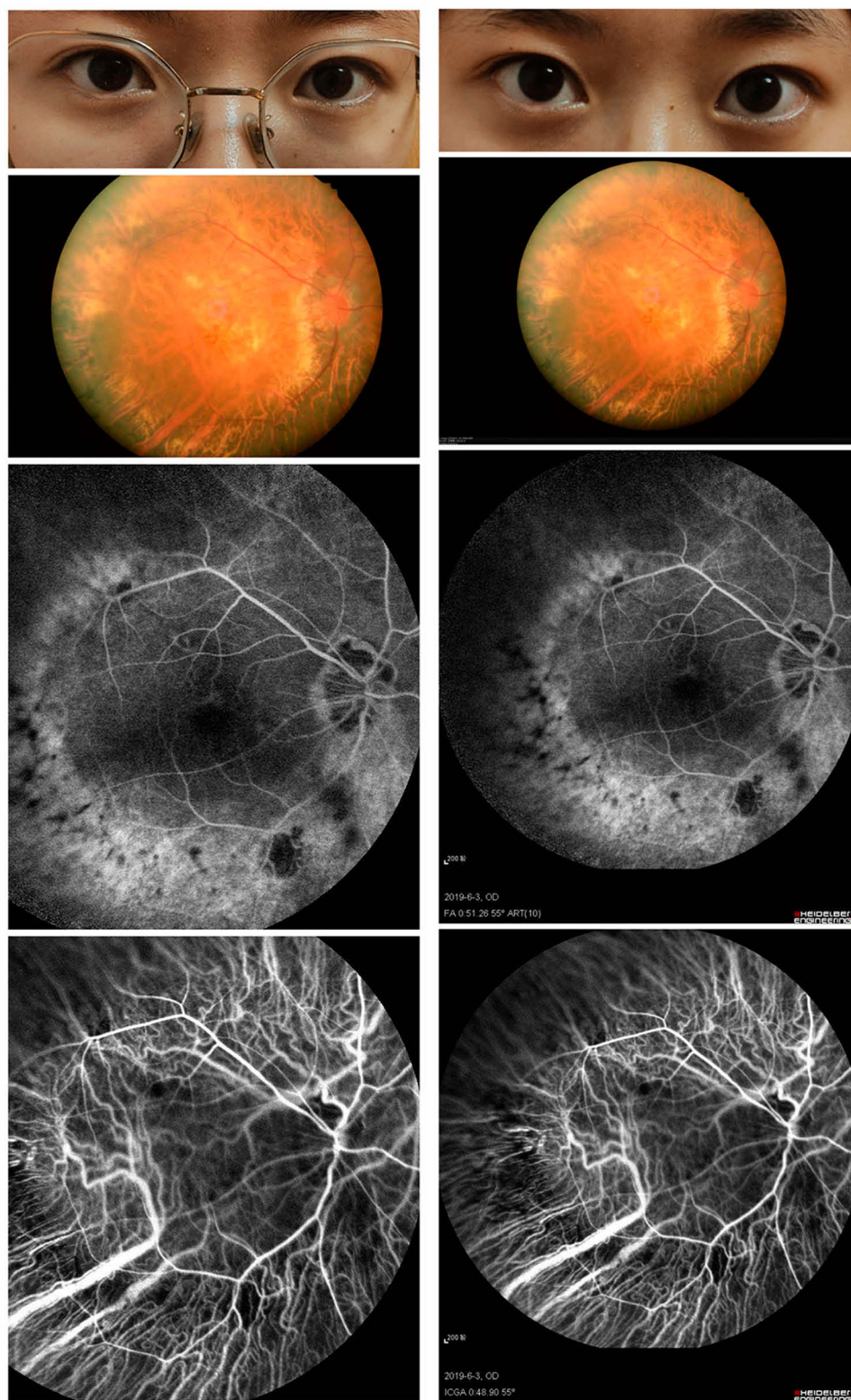


Figure 1 Typical fundus photographs of myopia patients before and after surgery.

study was carried out with permission from the Medical Ethics Committee of the First Affiliated Hospital of Nanchang University. We collected data on participants

who were informed of the study protocol and the potential risks that could occur, volunteered to participate, and signed informed consent forms.

MRI Parameters

Three-Tesla MRI scanners were required for MRI scans. During the process of scanning, the participants were advised to stay still and not open their eyes until the process was complete, which required 15 minutes. A total of 240 images were then collected using the following parameters: 1,900 ms repetition time, 2.26 ms echo time, 1.0 mm thickness, 0.5 mm gap, 256×256 acquisition matrix, 90° flip angle, 250×250 mm field of view, and 29 axial slices.

fMRI Data Processing

We used MRIcro software (<https://www.mccauslandcenter.sc.edu/crnl/mricro>) to identify valuable data to be collected. To maintain magnetization equilibrium, we abandoned the first 10 time points as well. We employed Data Processing Assistant for Advanced Edition of Resting-State fMRI (DPARSF) software to analyze Digital Imaging Communications in Medicine (DICOM) images, performed correction of head movement, normalized the spatial position and slice time, and performed full-width smoothing with a Gaussian kernel of 6×6×6 mm³ at half-maximum based on the rs-fMRI data analysis toolkit (REST) software and Statistical Parametric Mapping software (SPM). Subjects who had at least a 1.5-mm exceeding shift in x, y, or z or 1.5 angular motion were eliminated. To remove the effects induced by head motion, the Friston six head motion parameters were introduced.¹⁴ After head movement had been corrected, a standard echo plane image template was used to ensure the collected data reached the standards of the Montreal Neurological Institute (MNI). To calculate ALFF, the data were further processed by a Gaussian kernel. Additionally, to increase the accuracy of images and reduce the deviation caused by breathing or cardiac noise, the fMRI images were detrended and bandpass-filtered (0.01–0.08 Hz). A previous study had described the process for calculating ALFF in detail, and the ALFF maps of each patient were divided by the mean value of ALFF.¹⁵

Correlation Analysis

Patients completed a Hospital Anxiety and Depression Scale (HADS), the data of which were statistically analyzed. Graphpad prism 8 was used to analyze the linear correlation between HADS and ALFF values of the left parahippocampal gyrus ($P=0.002$) and the left posterior cingulate gyrus ($P=0.144$). After combining the results, a linear correlation graph was generated.

Statistical Analysis

The demographic and clinical variables between PRL and POL groups were compared using SPSS20.0 software (SPSS, IBM Corporation, Armonk, NY, USA) with independent t sample test, the data of which can be considered as significant only when P -values are less than 0.05.

A two-sample t -test was applied by the REST software that aimed to explore the voxel-wise difference between two groups. The statistical threshold of voxel level was set at $P<0.05$ for multiple comparisons by False Discovery Rate (FDR). And AlphaSim corrected at a voxel level of $Q<0.01$ and cluster size >40 .

All the data were recorded by a receiver operating characteristic (ROC) curve that showed how the ALFF values varied after the Lasik surgery. The correlations between the ALFF values of distinct brain areas and the recovery levels in patients after Lasik surgery were evaluated by the Pearson's correlation analysis.

Results

Demographics and Visual Measurements

The age, weight, and height between patients in the PRL and POL groups were well matched. We ensured that the spherical equivalent and astigmatism were not significantly different between the left and right eyes. The details are presented in Table 1.

ALFF Differences

In the POL group, the ALFF values were decreased in the left supramarginal and Frontal_Inf_Tri_R regions (Figure 2 [blue and green] and Table 2). Furthermore, the brain areas that

Table 1 Demographics and Visual Measurements Between PRL and POL Groups

Condition	Lasik-Pre	Lasik-Post	t	P-value
SE-L (diopter)	-10.75±2.75	-0.75±0.25	8.764	0.023
SE-R (diopter)	-11.00±3.25	-0.50±0.25	9.437	0.016
Astigmatism-L (diopter)	-2.50±0.50	-0.25±0.25	5.182	0.044
Astigmatism-R (diopter)	-2.25±1.00	-0.50±0.25	4.032	0.047
Color Vision	15R	15R	N/A	>0.99
Confrontation Visual field	15R	15R	N/A	>0.99

Notes: 2-sample t -test between the same patients before and after the Lasik surgery; Data presented as mean±standard deviation.

Abbreviations: L, left; N/A, not applicable; R, right; SE, spherical equivalent.

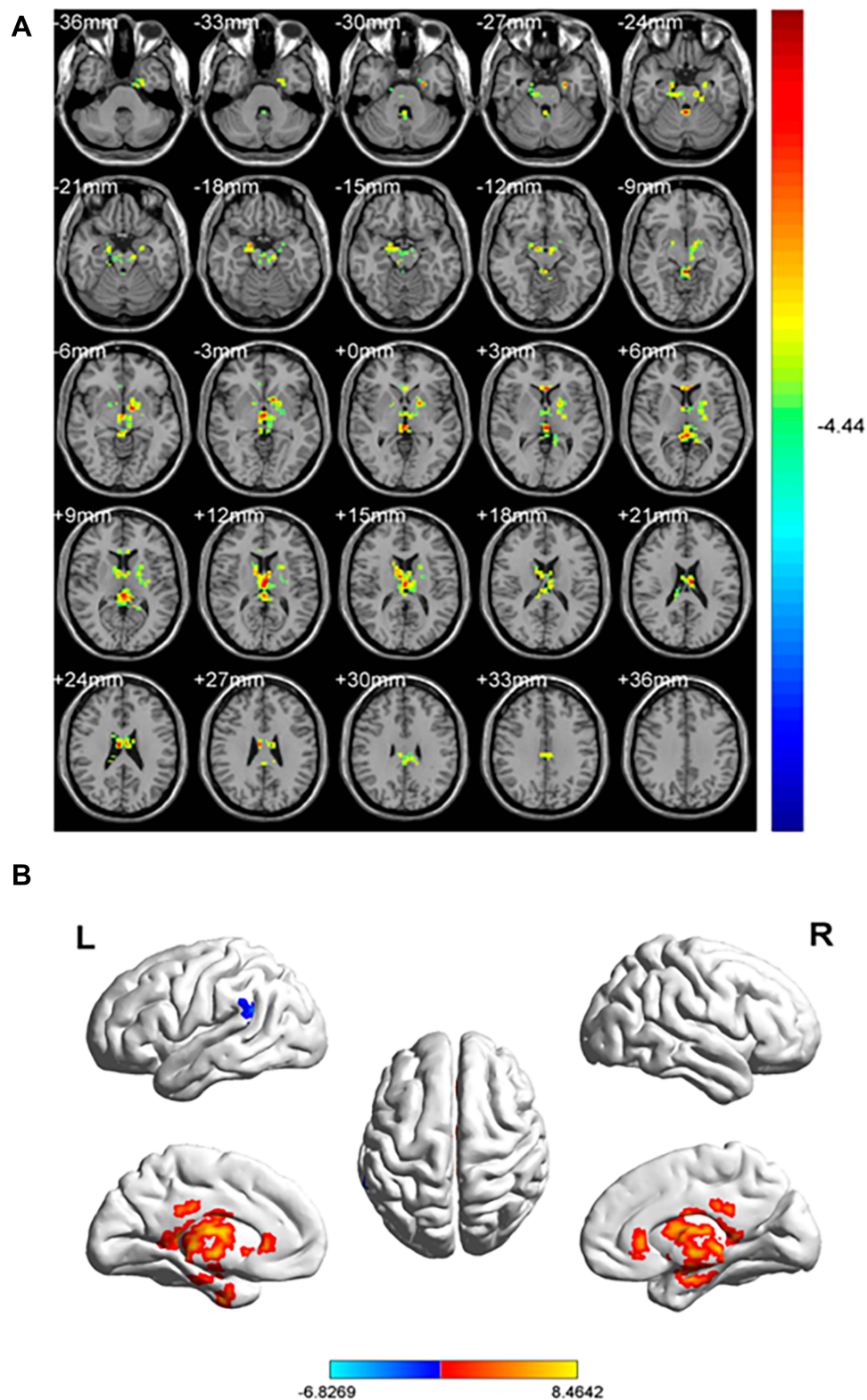


Figure 2 Spontaneous brain activity in the PRL and POL groups. **(A)** The different ALFF regions between the PRL and POL groups. **(B)** Significant difference of brain activity in cerebrum. The red regions indicate higher ALFF values, and the green and blue regions imply lower AFLL values.

Notes: **(A)** The different ALFF regions between the PRL and POL groups. **(B)** significant difference of brain activity in cerebrum. The red regions indicate higher ALFF values, and the green and blue regions imply lower AFLL values.

Abbreviations: ALFF, amplitude of low-frequency fluctuation; L, left; R, right.

Table 2 Brain Areas with Significantly Different ALFF Values Between Groups

Brain Areas	MNI Coordinates			BA	Number of Voxels	T value
	X	Y	Z			
Lasik-post>Lasik-pre ParaHippocampal_L	-24	-6	-27	36	59	6.6240
Vermis_1_2	3	-42	-24	0	27	7.8057
Cingulum_Post_L	-3	-33	30	23	37	5.7116
Lasik-post<Lasik-pre SupraMarginal_L	-63	-51	24	22	17	-5.4842
Frontal_Inf_Tri_R	45	24	21	48	17	-6.8269

Note: The statistical threshold was set at the voxel level with $P<0.05$ for multiple comparisons using False Discovery Rate ($Q<0.01$ and cluster size >15).

Abbreviations: ALFF, amplitude of low-frequency fluctuation; BA, Brodmann area; L, left; MNI, Montreal Neurological Institute; R, right; Cingulum_Post_L, left posterior cingulate cortex; Frontal_Inf_Tri_R, right trigonometric inferior frontal gyrus.

showed increased ALFF values were ParaHippocampal_L, Vermis_1_2, and Cingulum_Post_L (Figure 2 [red] and Table 2). The mean values of ALFF are presented in Figure 3.

ROC Curve

It can be recognized from the diagnostic markers that differences in ALFF existed between the POL and PRL groups, as assessed by the ROC curve. The areas under the curve are presented in Figure 4 and are as follows: SupraMarginal_L (0.980, $P<0.001$), Frontal_Inf_Tri_R (0.980, $P<0.001$), Cingulum_Post_L (0.987, $P<0.001$), ParaHippocampal_L (0.987, $P<0.001$), and Vermis_1_2 (0.987, $P<0.001$).

Correlation Analysis

The results of the linear analysis showed that the values of HADS in the left parahippocampal gyrus ($r=0.53$, $P=0.002$) and left posterior cingulate cortex ($r=0.16$, $P=0.144$) were both negatively correlated with ALFF signal values in these two brain areas (Figure 5).

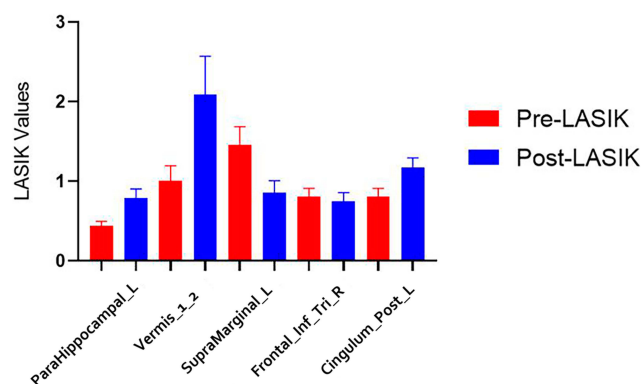


Figure 3 The mean ALFF values between PRL and POL groups.

Abbreviations: ALFF, amplitude of low-frequency fluctuation; L, left; R, right; Cingulum_Post_L, left posterior cingulate cortex; Frontal_Inf_Tri_R, right trigonometric inferior frontal gyrus.

Discussion

To our knowledge, this is the first study aimed at determining whether differences in ALFF values in brain regions exist between the same patient before and after the Lasik operation, as well as where the regions are located and how the values vary.

Analysis of Low ALFF Values in the POL Group

The areas that showed lower ALFF values after Lasik were the left supramarginal gyrus (SMG) and right trigonometric inferior frontal gyrus (Figure 6).

The SMG comprises part of the parietal lobe, which is known as Brodmann area 40,¹⁶ and it is equipped with the functions of melody processing, short-term memory,^{17,18} and visual word recognition.¹⁹ ALFF reflected activities of neurons in particular regions of the brain: increased ALFF values indicated a more active state and vice versa.¹⁷ A previous study claimed that it detected an overactive turbulence in SMG for patients with monocular blindness.²⁰ Instead, we recorded lower ALFF values in POL patients, which may reflect a rather normal state of SMG after Lasik surgery as well as an improved visual function, indicating the surgery should be efficient. Furthermore, Zeng et al¹⁷ discovered that neuronal activities of SMG were decreased in post-stroke depression (PSD) patients, suggesting abnormality of SMG contributed to outcomes of depression. This study may link the potential mechanism of depression with hypoactivity of SMG, and as we detected a decreased ALFF value in POL patients, it may suggest the emotional state of patients was not cheered up after the surgery.

The inferior frontal gyrus (IFG), located in the frontal lobe, has the function of language understanding,²¹ and the triangular part of it is the grammar center.²² Besides, the right

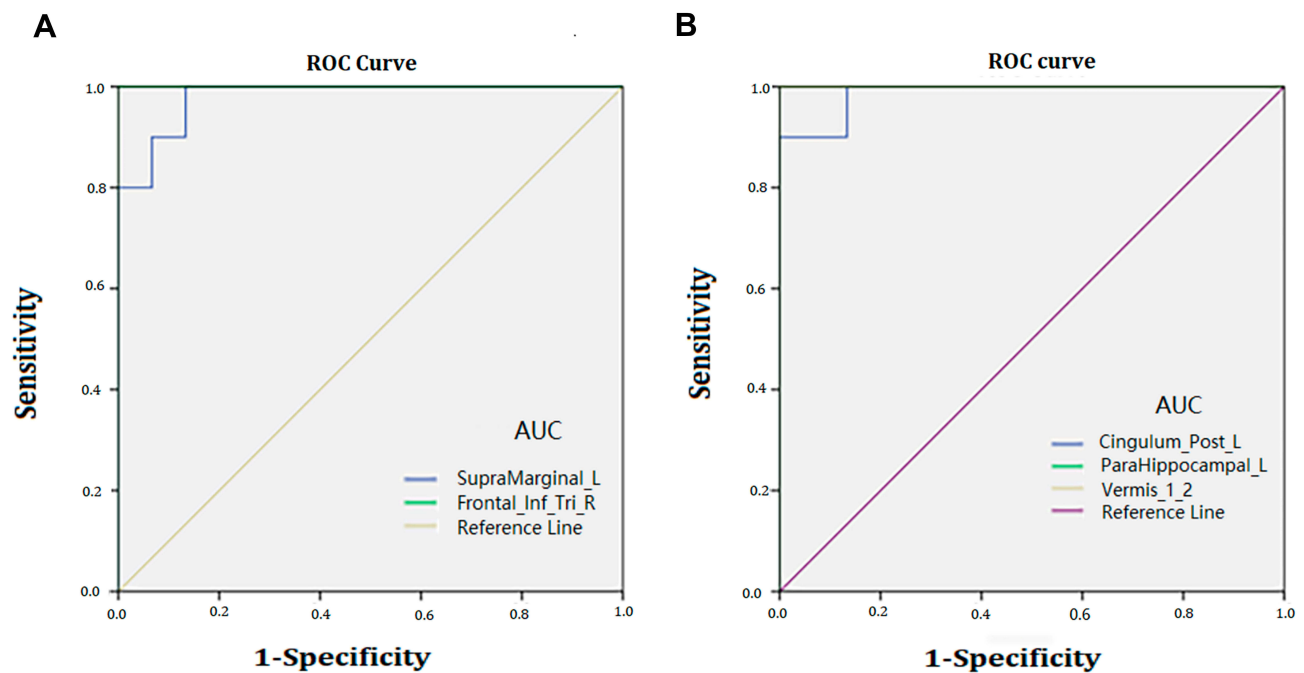


Figure 4 ROC curve analysis of the mean ALFF values for altered brain regions. **(A)** The area under the ROC curve was 0.980 ($P<0.001$; 95% CI=0.937–1.000) for SupraMarginal_L, Frontal_Inf_Tri_R 1.000 ($P<0.001$; 95% CI=1.000–1.000). **(B)** The area under the ROC curve was 0.987 ($P<0.001$; 95% CI=0.952–1.000) for Cingulum_Post_L; ParaHippocampal_L 1.000 ($P<0.001$; 95% CI=1.000–1.000); Vermis_1_2 1.000 ($P<0.001$; 95% CI=1.000–1.000).

Notes: **(A)** The area under the ROC curve were 0.980, ($p<0.001$; 95% CI: 0.937–1.000) for SupraMarginal_L, Frontal_Inf_Tri_R 1.000 ($p<0.001$; 95% CI: 1.000–1.000). **(B)** The area under the ROC curve were 0.987 ($p<0.001$; 95% CI: 0.952–1.000) for Cingulum_Post_L; ParaHippocampal_L 1.000 ($p<0.001$; 95% CI: 1.000–1.000); Vermis_1_2 1.000 ($p<0.001$; 95% CI: 1.000–1.000).

Abbreviations: ALFF, amplitude of low-frequency fluctuation; AUC, area under the curve; ROC, receiver operating characteristic; L, left; R, right; Cingulum_Post_L, left posterior cingulate cortex; Frontal_Inf_Tri_R, right trigonometric inferior frontal gyrus.

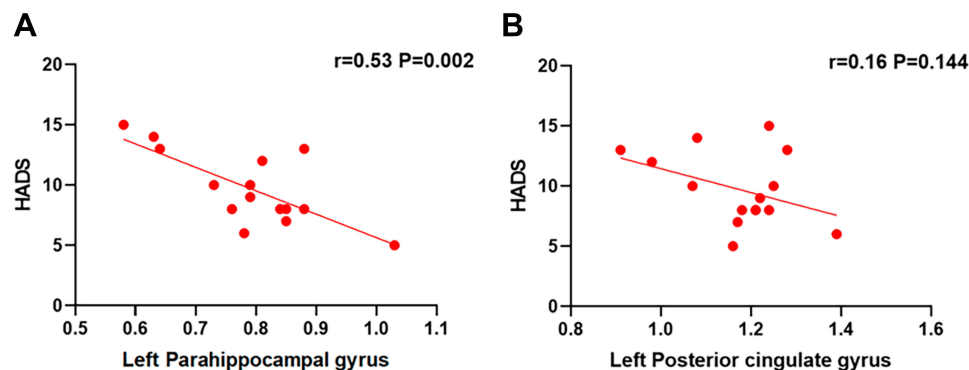


Figure 5 Correlations between the HADS and ALFF values of left parahippocampal gyrus and left posterior cingulate cortex. The results shown in **(A)** and **(B)** both represent a negative correlation with the HADS and ALFF values in left parahippocampal gyrus and left posterior cingulate cortex, respectively.

Notes: The results shown in **(A)** and **(B)** both represented negative correlation with the HADS and ALFF values in left parahippocampal gyrus and left posterior cingulate cortex.

Abbreviations: HADS, Hospital Anxiety and Depression Scale; ALFF, amplitude of low-frequency fluctuation.

IFG participates in inhibition and attention.²³ And a previous study showed lower ALFF values can be detected in the right IFG region of severe myopia patients.²⁴ And as we recorded the similar tendency of neuronal activities, it could be concluded that visual deficiency still occurred even for patients who took Lasik surgery. Thus, the Lasik surgery just partially corrected visual deficiency rather than fully compensated the visual impairment.

Analysis of Higher ALFF Values in the POL Group

The areas that showed higher ALFF values after Lasik were the left parahippocampal gyrus (PHG), cerebellar vermis, and left posterior cingulate cortex (Figure 6).

The PHG, comprising part of the medial temporal lobe, is located between the hippocampus and fusiform cortex.²⁵

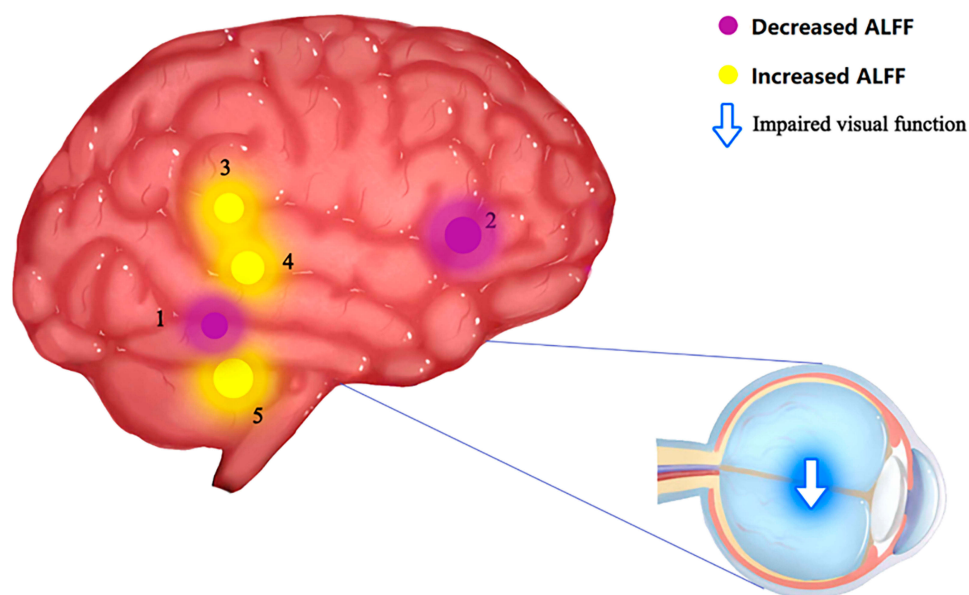


Figure 6 Significant differences in spontaneous brain activity between the PRL and POL groups. Different brain regions that were observed: 1) SupraMarginal_L; 2) Frontal_Inf_Tri_R; 3) Cingulum_Post_L; 4) ParaHippocampal_L; and 5) Vermis_I_2. The yellow areas present brain areas with increased ALFF values, and the purple areas present brain areas with decreased ALFF values.

Notes: Different brain regions that were observed: (1) SupraMarginal_L; (2) Frontal_Inf_Tri_R; (3) Cingulum_Post_L; (4) ParaHippocampal_L; (5) Vermis_I_2. The yellow areas present brain areas with increased ALFF values. And the purple areas present brain areas with decreased ALFF values.

Abbreviations: ALFF, amplitude of low-frequency fluctuation; L, left; R, right; Cingulum_Post_L, left posterior cingulate cortex; Frontal_Inf_Tri_R, right trigonometric inferior frontal gyrus.

PHG holds a variety of important functions that make it a vital portion of our brain, including scene perception,^{26,28} spatial representation,^{29,31} emotional processing,^{32,35} and

episodic memory.²⁵ Besides, dysfunctions of PHG is believed to be related to diseases such as bipolar disorder³⁶ and schizophrenia.³⁷ A previous study showed a hypoactive

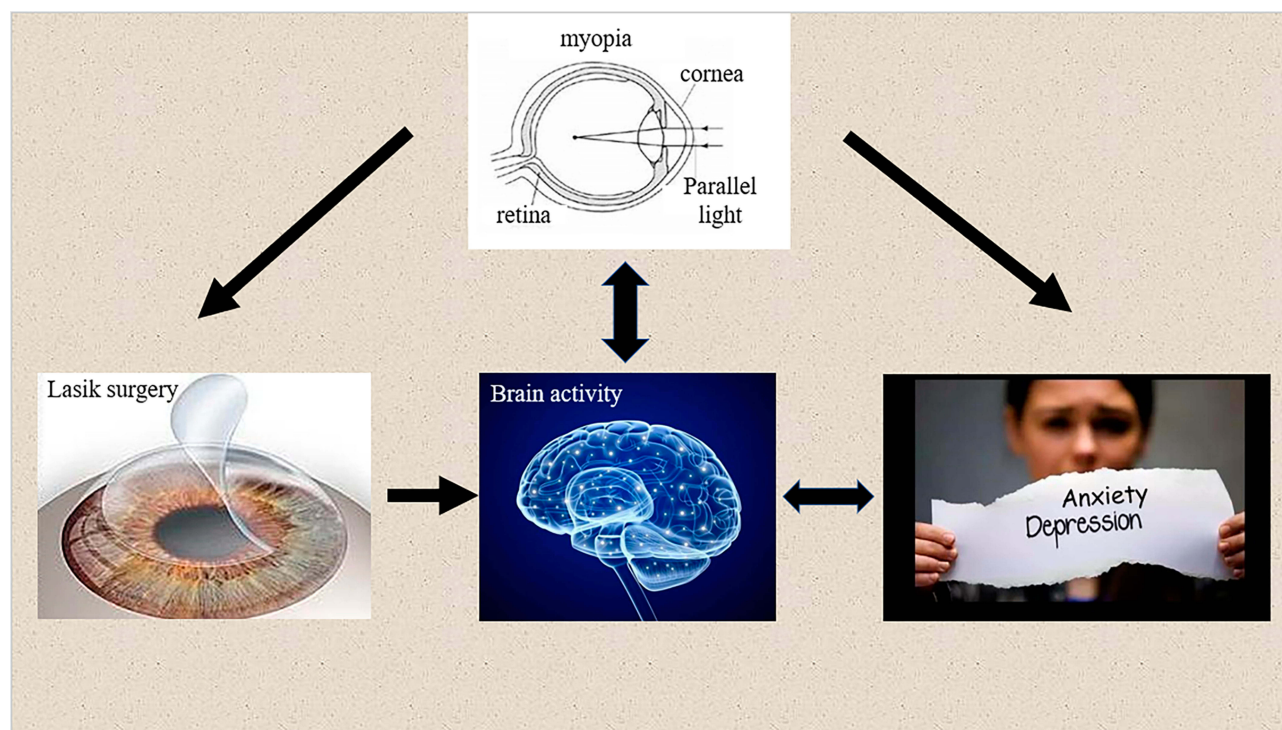


Figure 7 The relationship between changes in internal eye states and vision, brain activity, and emotional state.

Table 3 Brain Regions with Altered ALFF Values and the Potential Impacts

Brain Regions	Experimental Result	Brain Function	Anticipated Results
SupraMarginal_L	Lasik-post<Lasik-pre	Melody processing, short-term memory, visual word recognition, emotional processing	Improved visual function
Frontal_Inf_Tri_R	Lasik-post<Lasik-pre	Language comprehension, inhibition, and attentional control	Hypoactivity of this area
ParaHippocampal_L	Lasik-post>Lasik-pre	Scene perception, spatial representation, emotional processing, episodic memory	Improved visual function, elevated level of visuospatial processing, improved emotional state
Vermis_I_2	Lasik-post>Lasik-pre	Balance and motor control, oculomotor processing	Hyperactivity of this area
Cingulum_Post_L	Lasik-post>Lasik-pre	Cognition, eye movement, visuospatial processing	Improved emotional state

Abbreviations: ALFF, amplitude of low-frequency fluctuation; L, left; R, right; Cingulum_Post_L, left posterior cingulate cortex; Frontal_Inf_Tri_R, right trigonometric inferior frontal gyrus.

state of PHG in severe myopia patients,²⁰ indicating severe visual dysfunctions could be related to neuronal activity alterations such as decreased PHG activities. And, the higher ALFF values recorded in our study may indicate compensatory recovery of PHG function after Lasik surgery. Patients thus should represent an elevated state of visuospatial processing. According to HADS results, we observed that patients with higher activities in the left parahippocampal gyrus showed less nervous emotional states, which indicated that patients had a more relaxed emotional state after Lasik surgery (Figure 7).

There is a longitudinally narrow structure running through the cerebellum, which is curled up like a worm and is called the vermis. The cerebellum is the center of balance and motor control.³⁸ Furthermore, it has also been confirmed to participate in oculomotor processing, in which the vermis has been believed to play a role.³⁹ Vermis activity was altered through visually guided saccades,⁴⁰ as well as in patients with convergence insufficiency, a prevalent binocular vision disorder.⁴¹ Higher ALFF values recorded in POL groups indicated hyperactivity of the vermis. And we speculated a better vision for patients who took the surgery.

The cingulate cortex is located at the medial aspect of the cerebral cortex,⁴² and the posterior cingulate cortex (PCC) forms part of the posteromedial cortex.⁴³ They comprise part of the limbic system.⁴² The posterior cingulate cortex is a highly active brain area, with the role of cognition,⁴³ eye movement,⁴⁴ and visuospatial processing.⁴⁴ The abnormal structure of the PCC is related to diseases such as Alzheimer's disease,⁴⁵ and the disordered neuronal activity in PCC can lead to depression, schizophrenia, and some other diseases.⁴⁶ We observed higher ALFF values in post-surgery

patients that indicated the hyperactivity of the PCC area in the brain. We speculated that the hyperactivity of PCC should be correlated to improved visual functions. And, based on the HADS, we observed that the more active the left posterior cingulate cortex was, the less anxious the patients felt, which may indicate that patients are in a better emotional state after Lasik surgery (Figure 7).

Conclusion

In summary, we demonstrated significant fluctuations of ALFF values in specific brain areas between the same patients before and after Lasik surgery. The altered ALFF values reflected the hyperactivity or hypoactivity of the brain areas, some of which may indicate better visual function for patients after Lasik; however, how the emotional state changed in patients cannot be determined with certainty (Table 3).

Inevitably, some limitations of our study exist. First, the small sample size may affect the reliability of the results. Second, the differences in vision between patients before the Lasik surgery may affect the recovery level after the surgery. Third, differences in the duration of myopia in patients may also add uncertainty to the final results.

Despite these limitations, the study still provided information on the altered activity in different brain areas. In future research, selecting patients based on more rigorous criteria could increase the accuracy of the results.

Ethics Approval and Consent to Participate

All procedures involving human participants were conducted in accordance with the ethical standards of the Ethical Committee of the First Affiliated Hospital of

Nanchang University and with the 1964 Declaration of Helsinki and its later amendments, or comparable ethical standards. The registration number is CDYFY 16021.

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Disclosure

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

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