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Research article

Brain plasticity following lumbar disc herniation treatment with spinal manipulation therapy based on resting-state functional magnetic resonance imaging

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ABSTRACT

As a prevalent spine disorder, Lumbar disc herniation (LDH) has been affecting more than 2 % of the worldwide population and is characterised by uncertain causes and recurring episodes. Studying the brain activity of patients could potentially provide insights into its pathogenesis and significantly enhance therapy. Therefore, we here examined brain function in patients under Spinal Manipulative Therapy (SMT). By analysing regional homogeneity (ReHo) at different frequency bands, we identified the discrepancies in brain activity between LDH patients and healthy people, highlighting the frequency dependence of spontaneous low-frequency oscillations among patients with LDH. Choosing seeds based on the peak ReHo differences helped to elucidate the functional connectivity alterations in the brain regions of LDH. Overall, this study showed that SMT significantly reduced pain, improved dysfunction, and partially rectified aberrant local consistency and functional connection in patients with LDH, not only offering insights into the pathophysiology of LDH from a neurological standpoint, but also providing inspiration for the development of new therapies based on neurobiology.

1. Introduction

Lumbar disc herniation (LDH) is a frequent underlying cause of persistent lower back pain. It occurs when the annulus fibrosus of the intervertebral disc in the lumbar region ruptures, causing the nucleus pulposus to protrude into the spinal canal. These protrusions can irritate the spinal nerve roots or cause compression, resulting in lower back pain and functional impairment [1]. Recent research has indicated that LDH not only affects the peripheral nervous system (PNS), but also influences the central nervous system (CNS),

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inducing alterations in brain structure and function [2–4]. Benefiting from non-invasive neuroimaging technique (rs-fMRI, Resting-state functional magnetic resonance imaging), people now can examine spontaneous brain activity, functional connections, and brain plasticity changes in disease states, and the effects of therapeutic interventions [5,6].

By focusing on alterations in spontaneous brain activity and functional connectivity, many studies have primarily examined how Spinal Manipulative Therapy (SMT) affect the brain function of people suffering from low back pain. Cerritelli et al. conducted a study to examine how manual techniques using osteopathic methods affect the brain's interoception, and found that SMT can decrease BOLD signals in particular regions of the brain, such as the bilateral insula (INS), anterior cingulate cortex (ACC), and left striatum [7]. Yang et al. conducted a study on the influence of SMT on patients with chronic low back pain. They found that SMT lead to improvements in regional homogeneity (ReHo) in certain areas of the brain, namely the left precuneus and left superior frontal gyrus [8]. Changes in activity within the default mode and salience networks were also observed [9,10]. While these results validate the impact of SMT on the functional brain activity of people with low back pain, it is still uncertain if these therapies can completely restore aberrant brain activity or functional connectivity to normal levels. Continued investigation into the changes in brain activity related to LDH and confirmation of the ability to reverse these changes is of great significance for clinical treatment.

Spontaneous low-frequency oscillations (LFOs) are commonly assessed to study the local brain activity. Zuo et al. [11] previously categorised LFOs into five bands: 0.1981–0.25 Hz (slow-2), 0.072–0.198 Hz (slow-3), 0.021–0.073 Hz (slow-4), 0.01–0.027 Hz (slow-5), and 0–0.01 Hz (slow-6). LFOs within the 0.01–0.073 Hz range (slow-4 and slow-5) typically indicate spontaneous neuronal activity in the grey matter [11].

Prior research has detected abnormal brain function across various frequency ranges in individuals with LDH by focusing on ALFF (low-frequency fluctuations) and fALFF (fractional ALFF). These data indicate that the irregularity of natural low-frequency oscillations in individuals with LDH is influenced by frequency [12]. We also calculated the regional homogeneity (ReHo) in patients with LDH within the conventional frequency band, to provide insights into the synchronisation of neuronal activity in local brain regions of LDH patients [13]. ReHo, an indicator of spontaneous brain activity, assesses the coherence of a specific voxel with a time series of neighbouring voxels (e.g. 7, 9, and 27 voxels), demonstrating high repeatability, sensitivity, and reliability [13–15].

This study aimed to enhance our understanding of abnormal brain activity in patients with LDH by analysing ReHo in various frequency bands, thereby providing valuable clinical evidence into the frequency-dependent abnormalities of spontaneous low-frequency oscillations in this patient population. Additionally, functional connections were calculated from seed points based on the peak points of multi-frequency differential brain regions to investigate potential changes in patients with LDH. This study further examined the effects of SMT on correcting abnormal changes in patients with LDH to elucidate the central response characteristics of effective SMT.

2. Materials and methods

2.1. Participants

This research was approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang Chinese Medical University (No. 2017-k-237-01), and was registered at the China Clinical Trial Registration Center (No. NCT03475095). Thirty LDH patients and a group of 30 healthy individuals with similar distributions in age, sex, and education volunteered to participate in this research. Written informed consent was obtained from all participants, and the study was conducted in accordance with the principles outlined in the Declaration of Helsinki. The research was conducted at the First Affiliated Hospital of Zhejiang Chinese Medical University and lasted for 10months (November 2020 to August 2021).

The diagnostic criteria for LDH involved referencing the relevant criteria outlined in the 'Surgery (7th Ed)' published by the People's Medical Publishing House. Diagnosis is typically based on the presence of lumbar disc herniation confirmed through MRI or CT imaging, along with two or more of the following accompanying symptoms: ① low back pain with radiating pain in the lower limbs extending to the calf or foot; ② a positive straight leg raising or strengthening test; ③ hypersensitivity/hyperesthesia in the affected nerve innervation areas of the lower limbs, potentially leading to muscle atrophy in long-standing cases; ④ decreased toe muscle strength and altered knee/Achilles tendon reflexes.

The inclusion criteria. ① Right-handed; ② Meeting the specified diagnostic criteria for LDH; ③ Age between 20 and 60 years old; ④ Visual Analogue Scale (VAS) score not lower than 30/100; ⑤ Chinese Short Form Oswestry Disability Index Questionnaire (C-SFODI) score not lower than 20 %; ⑥ Never received pain treatment for at least 30 days prior to enrolment.

The exclusion criteria. ① History of spinal surgery or severe spinal trauma; ② Bone tuberculosis, tumours, severe osteoporosis, or other orthopaedic diseases; ③ Presence of serious medical or mental illnesses, such as cardiovascular, cerebrovascular, blood, or digestive system disorders; ④ Pregnant or breastfeeding; ⑤ Autoimmune diseases, allergic conditions, acute or chronic infections; ⑥ Contraindications to fMRI, such as internal metal implants or claustrophobia; ⑦ MRI findings indicating free nucleus pulpous or Cauda equine syndrome; ⑧ Visual impairment or vestibular dysfunction.

The inclusion criteria for healthy controls (HCs). ① Right-handed; ② Twenty to sixty years old; ③ Never experienced any symptoms of lower back or leg pain, numbness, or soreness; ④ No pain-related treatment for more than 1 month prior to enrolment.

2.2. Sample size Calculation

There is currently no universally agreed upon method for determining the sample size in the field of fMRI research [16–18]. Previous research has indicated that reliable results can be achieved with group sizes of 15 individuals or more, with appropriate

corrections [19,20]. This study meticulously accounted for factors such as head movement bias, noise during data collection, and subject dropout during the intervention treatment. Finally, 30 patients with LDH and 30 HCs were enrolled.

2.3. Research Design

The HCs underwent a single fMRI scan upon enrolment. Patients with LDH underwent their initial functional magnetic resonance scan after completing the VAS and C-SFODI scales. They then completed a second assessment of the VAS and C-SFODI scales, along with another fMRI scan, after receiving six SMTs.

2.4. Spinal manipulative therapy

Physician requirements were as follows: SMT should be administered by a physician with over 5 years of clinical experience, medical qualifications, and expertise in specific spinal manipulation techniques for the treatment of patients with LDH. Maintaining stability in terms of strength, direction, and frequency during treatment was also deemed crucial.

Environmental and equipment requirements include the use of a multifunctional massage table and the adjustment of the angle and height for patient comfort. Standard treatment towels and disposable sheets from the First Affiliated Hospital of Zhejiang University of Chinese Medicine were used. Operating techniques involve soft tissue release methods such as stroking, kneading, and elbow pushing to relax the waist muscles, as well as joint adjustment techniques such as oblique pulling to correct spinal joint disorders (Fig. 1). The goal of SMT was to alleviate pain and enhance waist function. Each patient received six manual treatments thrice a week, with each session lasting 25 min.

2.5. Observation indicators

The clinical evaluation indicators were as follows: ① The internationally recognised VAS was used to assess the patient's pain level; ② The Chinese Short Form Oswestry Disability Index Questionnaire (C-SFODI) was used to evaluate the patient's functional limitations. The magnetic resonance imaging indicators were as follows: ① ReHo, an indicator of spontaneous brain activity that assesses the synchronicity of a time series between a specific voxel and neighbouring voxels (e.g. 7, 9, and 27 voxels) [14]; ② Seed-based functional connectivity (FC): by computing the correlation coefficient of the blood oxygen level-dependent time series between a specific seed

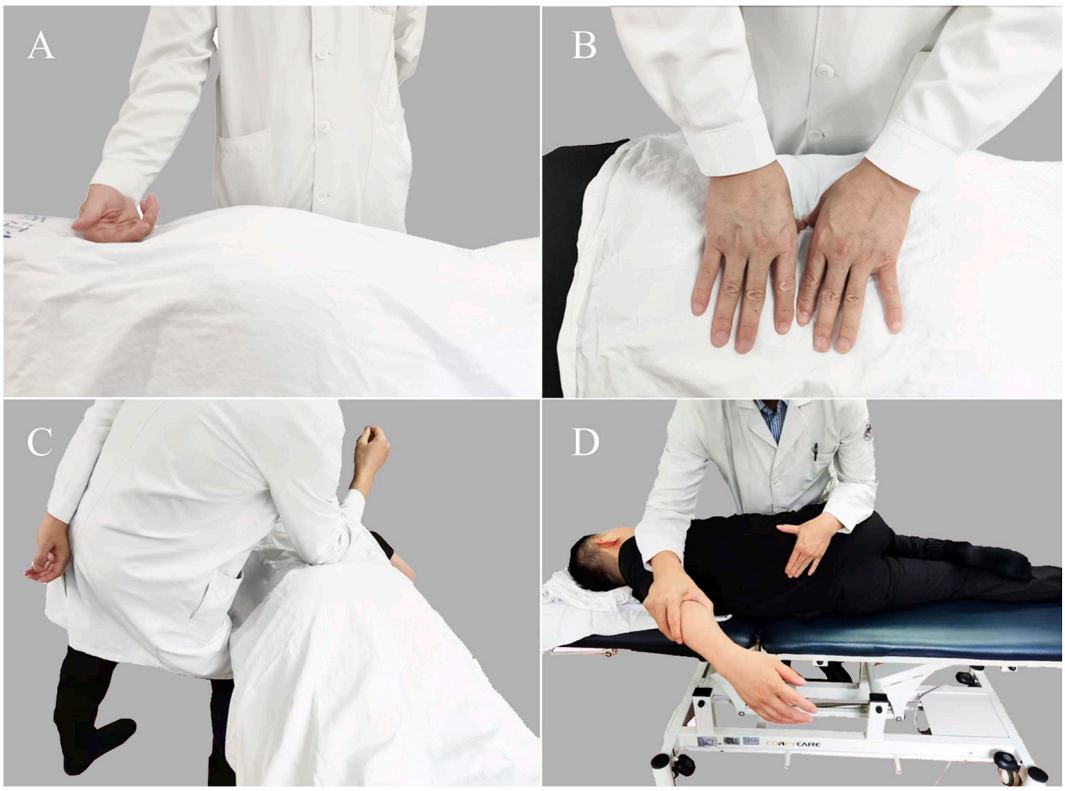

Fig. 1. Spinal Manipulation Therapy (SMT) performed by rolling (A), kneading (B), pushing (C), and the pulling and rotating (D) to relax the muscles in the lower back area for the purpose of alleviating pain and improving lumbar function.

point and all other brain voxels, the strength of functional connectivity in each brain region can be determined [21].

2.6. fMRI data Acquisition

The fMRI was performed using a 3T scanner (Verio, Siemens AG, Erlangen, Germany, Table 1) with a 12-channel head coil. The participants were given instructions to keep eyes closed, refrain from engaging in any thoughts, and stay awake during the entire scan.

Quality control measures were implemented to guarantee the dependability and consistency of the fMRI results. These included scans performed by a senior imaging technician trained by Siemens. Additionally, all image data were reviewed on-site by two senior neuroimaging specialists to prevent distortion of the results from organic brain lesions, or artefacts caused by head movements. Subjects with specific conditions in their MRI data were either excluded or scheduled for re-scanning.

2.7. Magnetic resonance data preprocessing

RESTplus V1.25 software was applied for rs-fMRI data preprocessing following several steps: ① discarding the data of the initial 10 time points, ② correcting time layers, ③ correcting head motion, ④ standardising spatial data, ⑤ removing linear trends, ⑥ regressing the association Variable [22], and ⑦ applying band-pass filtering (0.01–0.08 Hz, 0.027–0.073 Hz, and 0.01–0.027 Hz) to eliminate low-frequency drift and physiological high-frequency noise. In this stage, three patients and two healthy individuals were excluded.

2.8. Regional homogeneity analysis

Kendall coefficient of concordance (KCC) was used to quantify the similarity between a voxel and its 27 adjacent voxels, followed by t applying Fisher's Z transformation for the final statistical analysis to determine the regional functional integration of spontaneous neuronal activity. To enhance the signal-to-noise ratio, all images underwent spatial smoothing by using an isotropic Gaussian kernel with a half-maximum width of 6 mm.

2.9. Functional connectivity analysis based on brain regions of interest

Brain areas with significant inconsistence in ReHo values between patients and HCs were identified as regions of interest (ROIs). Seed points were placed based on the peak points of the ROIs, and the average blood oxygen level-dependent (BOLD) signals were extracted from each ROI series. Pearson's correlation coefficients were calculated between the average time series of each ROI and the time series of each voxel (excluding voxels within the seed points). To improve data normality, we further obtained z-functional connectivity maps using Fisher's r-to-z transformation for further statistical comparisons.

2.10. Statistics

Demographic and clinical variables were analysed using the statistical software SPSS 22.0 (IBM, NY, USA). Student's t-tests and Pearson's chi-square test were applied to compare demographic and clinical characteristics between patients with LDH and HCs. A two-sample *t*-test was conducted to compare the mean regional homogeneity (mReHo) and functional connectivity (zFC) in patients with LDH and HCs. Multiple comparison correction was applied using a Gaussian random field (GRF) (voxel level, $P < 0.05$; cluster level, $P < 0.05$). To assess the impact of spinal manipulation, brain masks were created based on abnormal brain regions identified in group comparisons between patients with LDH and healthy subjects. The mReHo and zFC values within these brain masks for each group were extracted, and a two-sample *t*-test was applied to compare LDH patient pretreatment with healthy subjects, while a paired *t*-test was applied to compare LDH patients pre- and post-SMT.

Table 1
Parameters for MRI.

	slices	MS	FOV (mm × mm)	TR (ms)	TE (ms)	FA (°)	ST (mm)	Gap	VS	VN
F MRI	43 interleaved	64 × 64	220 × 220	2000	30	90	3.2	0	3.4 × 3.4 × 3.2	230
S MRI	176	256 × 256	256 × 256	8100	3.1	8	1	0	1 × 1 × 1	–

Abbreviation.
F MRI: Functional MRI.
S MRI: Structural MRI.
MS: matrix size.
FOV: field of view.
TR: repetition time.
TE: echo time.
FA: flip angle.
ST: slice thickness.
VS: voxel size.
VN: total number of volumes.

3. Results

Thirty patients with LDH and 30 HCs were enrolled. All participants adhered to the experimental protocol, provided baseline information, completed the scales, and underwent brain functional magnetic resonance imaging. The patients received six SMT sessions without any complications.

3.1. Demographic and clinical characteristics

There were no statistical differences in age ($P = 0.8552$), education ($P = 0.5691$), and sex distribution ($P = 0.8638$) between the two groups of participants (Table 2). After six times of SMT, the Visual Analogue Scale and C-SFODI scores of patients significantly decreased($P < 0.0001$).

3.2. Variations in ReHo

The ReHO value of the left orbital part of the middle frontal gyrus (LO-MFG) of patients with LDH in the conventional frequency band was much smaller than that of HCs (Table 3, Fig. 2A), as well as the Slow-5 frequency band (Table 3, Fig. 2B). Additionally, in the Slow-4 frequency band (Table 3, Fig. 2C), patients with LDH exhibited a significant increase in the ReHO value of the left cerebellum at _4_5.

3.3. ROI-based functional connectivity differences between LDH patients and HCs

In the present study, we examined the differences in ReHO between patients with LDH and HCs, using them as regions of interest (Table 4). Whole-brain functional connectivity based on seed points was compared between the groups. Specifically, we compared the abnormal brain areas identified in the conventional frequency band (ROI 1, MNI coordinates: −30, 48, −6) and the slow-5 frequency band (ROI 2, MNI coordinates: −33, 51, −6). Our results showed that when using the LO-MFG as the seed point, the functional connection between this region and the bilateral orbital inferior frontal gyri was weaker among patients with LDH than in HCs (Table 5, Fig. 3A; Fig. 3B).

Furthermore, when considering the left Cerebelum_4_5 (MNI coordinates: 9, −30, −36) that abnormal brain area found in the slow-4 frequency band as the seed point, we observed enhanced functional connections within the left Cerebelum_4_5 and between the left Cerebelum_4_5 and the right precuneus in LDH patients compared to healthy subjects (Table 5, Fig. 3C).

3.4. Evaluation of SMT

Evaluation of the SMT effect based on the difference in ReHO between patients with LDH and HCs was subsequently performed. The ReHO values of the LO-MFG, a key brain region that differs between two groups in the conventional frequency band, were extracted and compared between the groups. The results, as presented in Table 6 and Fig. 4A, indicate that following SMT, the initially decreased ReHO value in patients with LDH ($P < 0.001$) significantly increased ($P = 0.076$) to a level approaching that of healthy subjects ($P = 0.005$).

A similar trend was observed for the ReHO values in the slow-5 frequency band (Table 6 and Fig. 4B). Specifically, after SMT, the ReHO values of patients with LDH with initially reduced activity in the LO-MFG ($P < 0.001$) increased ($P = 0.166$), approaching the levels observed in HCs ($P = 0.011$).

Furthermore, the ReHO values of the left cerebellum _4_5, another brain area that differed between two groups in the slow-4 frequency band, were compared between the two groups (Table 6, Fig. 4C). Patients with LDH exhibited significantly elevated

Table 2
Demographic characteristics of the LDH patients and HC groups.

	LDH	HCs	*P*-value
Participants	27	28	–
Gender (male\female)	(17\10)	(17\11)	0.8638[a]
Age (year)	32.2 ± 9.5	31.8 ± 8.1	0.8552[b]
Years of Education (year)	16.04 ± 1.93	16.36 ± 2.20	0.5691[b]
VAS scores (LDH-pre\pos)	(5.6 ± 2.1\1.7 ± 1.1)	–	<0.0001[c]
C-SFODI scores (LDH-pre\pos)	(26.9 ± 8.1\18.7 ± 5.4)	–	<0.0001[c]

Abbreviation.
LDH: Lumbar disc herniation.
HCs: healthy controls.
VAS: Visual Analogue Scale.
C-SFODI: the Chinese Short Form Oswestry Disability Index Questionnaire.
[a] $\chi 2$ test.
[b] Two sample *t*-test.
[c] Paired *t*-test between LDH patients before and after SMT.

Table 3
Significantly different regions in ReHo between LDH-pre patients and HCs.

Band	Brain area	Voxel size	Peak(MNI,x,y,z)	Peak *T* value
0.01–0.08HZ	LO-MFG	727	−30,48,-6	−4.0725
0.01–0.027HZ	LO-MFG	843	−33,51,-6	−4.7288
0.027–0.073HZ	Cerebelum_4_5_L	596	9,-30,-36	4.2693

Abbreviation.
ReHo: regional homogeneity.
LDH-pre: Lumbar disc herniation patients before SMT treatment.
HCs: healthy controls.
LO-MFG: the left orbital part middle frontal gyrus.
MNI: Montreal Neurological Institute.

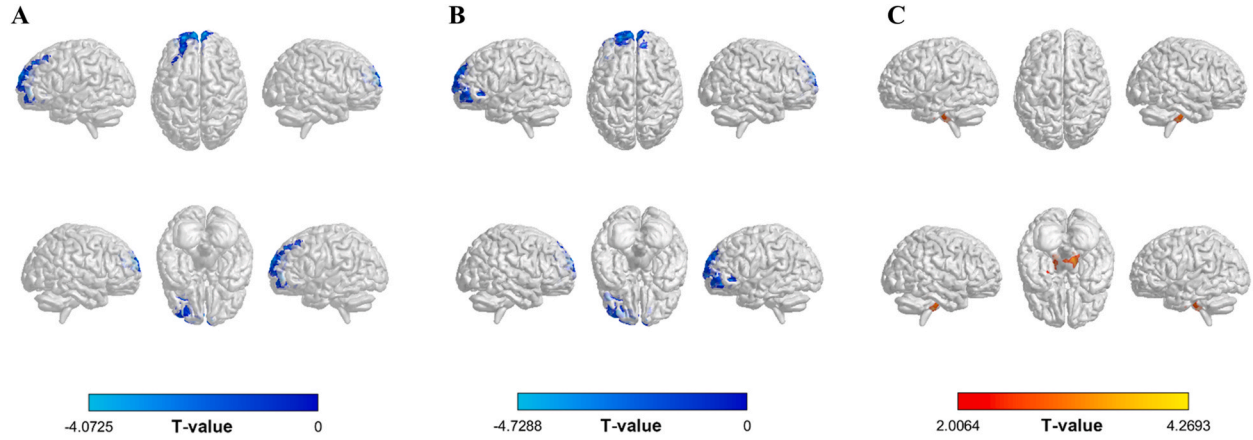

Fig. 2. Significantly different regions in ReHo between LDH-pre and HCs using two-sample t-tests. A. Result in the conventional band. LDH-pre patients showed decreased ReHo values in the left orbital part middle frontal gyrus (LO-MFG, MNI coordinate: −30 48 -6) compared with HCs. B. Result in the slow-5 band. LDH-pre patients showed decreased ReHo values in the left orbital part middle frontal gyrus (LO-MFG, MNI coordinate: −33 51 -6) compared with HCs. C. Result in the slow-4 band. LDH-pre patients showed increased ReHo values in the left Cerebelum_4_5 (MNI coordinate: 9, −30, −36) compared with HCs. (GRF correction, voxel $p < 0.05$ and cluster $p < 0.05$). Color bar indicates the t score.

Table 4
The names of the ROIs for FC analysis.

		Coordinate (x,y,z)	Radius (mm)
ROI 1	LO-MFG	(-30, 48, −6)	6
ROI 2	LO-MFG	(-33, 51, −6)	6
ROI 3	Cerebelum_4_5_L	(9,-30,-36)	6

Abbreviation.
ROI: regions of interest.
LO-MFG: the left orbital part middle frontal gyrus.

ReHO values post SMT, with initially high values ($P < 0.001$) decreasing markedly ($P = 0.004$) and nearing those of healthy individuals ($P = 0.049$).

The effect of spinal massage was evaluated through comparison of the differences in functional connectivity between patients with LDH and HCs, as well as the zFC values of the bilateral inferior frontal gyrus of the orbit, which showed abnormal functional connections to ROI 1, were extracted for comparison in each group. The results, presented in Table 7 and Fig. 5A1, revealed that the zFC value of patients with LDH with an initially decreased right orbital inferior frontal gyrus($P = 0.002$) increased ($P = 0.476$) following SMT, approaching the levels observed in HCs ($P = 0.019$). Conversely, the zFC value of the left orbital inferior frontal gyrus, which was abnormally decreased in patients with LDH ($P = 0.002$), showed a further decrease ($P = 0.218$), accentuating the difference from healthy subjects ($P < 0.001$) (Table 7, Fig. 5A2).

The zFC values of the bilateral orbital inferior frontal gyrus, the brain region with abnormal functional connections to ROI 2, were extracted and compared across groups. Following SMT, the zFC values of the abnormally decreased bilateral orbital inferior frontal gyrus of patients with LDH increased, approaching the levels observed in healthy individuals, although the difference was not statistically significant (Table 7 and Fig. 5B).

Following the SMT, the zFC values of brain areas with abnormal functional connections between each group and ROI 3 were

Table 5
sFC in conventional frequency band (LDH_pre vs HCs).

ROI	Brain regions	Cluster size	MNI coordinates			*t*-value
			X	Y	Z	
ROI 1	Frontal_Inf_Orb_R	507	6	33	3	−3.8655
	Frontal_Inf_Orb_L	515	−30	39	−6	−3.2968
ROI 2	Frontal_Inf_Orb_L	585	−27	−9	−9	−3.5085
	Frontal_Inf_Orb_R	592	6	33	3	−4.9958
ROI 3	Cerebelum_4_5_L	776	9	−30	−39	4.278
	Precuneus_R	1286	18	−60	27	4.1874

GRF correction, voxel $p < 0.05$, cluster $p < 0.05$.
Abbreviation.
sFC: static functional connectivity.
ROI: regions of interest.
LDH-pre: Lumbar disc herniation patients before SMT treatment.
HCs: healthy controls.
Frontal_Inf_Orb: the orbital part inferior frontal gyrus.
MNI: Montreal Neurological Institute.

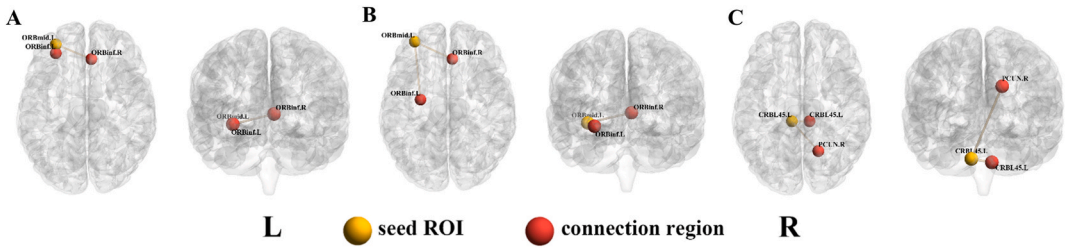

Fig. 3. Brain regions showing group differences between LDH_pre and HCs in static FC value. A.the ROI 1(Frontal_Mid_Orb_L, MNI: 6, 33, 3) as seed. B. the ROI 2 (Frontal_Mid_Orb_L,MNI: 30,39,-6) as seed. C. the ROI 3(Cerebelum_4_5_L, MNI: 9, −30, −36) as seed. ORBmid.L = Frontal_Mid_Orb_L; ORBinf.R = Frontal_Inf_Orb_R; ORBinf.L = Frontal_Inf_Orb_L. CRBL45.L = Cerebelum_4_5_L; PCUN.R = Precuneus_R (voxel $p < 0.05$, cluster $p < 0.05$, GRF correction).

Table 6
Group differences of ReHo.

Band	Brain regions	Contrast	t-value	*P*-value
0.01–0.08 Hz	LO-MFG (MNI:6,33,3)	LDH_pos vs HC	−2.938	0.005**
		LDH_pre vs HC	−4.317	<0.00***
		LDH_pos vs LDH_pre	1.848	0.076
0.01–0.027 Hz	LO-MFG (MNI: 30,39,-6)	LDH_pos vs HC	−2.634	0.011*
		LDH_pre vs HC	−4.876	<0.001***
		LDH_pos vs LDH_pre	1.426	0.166
0.027–0.073 Hz	Cerebelum_4_5_L (MNI: 9,-30,-36)	LDH_pos vs HC	2.027	0.049*
		LDH_pre vs HC	4.042	<0.001***
		LDH_pos vs LDH_pre	−3.118	0.004**

Abbreviation.
ReHo: regional homogeneity.
LDH-pre: Lumbar disc herniation patients before SMT treatment.
LDH-pos: Lumbar disc herniation patients after SMT treatment.
HCs: healthy controls.
LO-MFG: the left orbital part middle frontal gyrus.
MNI: Montreal Neurological Institute.
* Indicates significance level at $P < 0.05$, ** Indicates significance level at $P < 0.01$, *** Indicates significance level at $P < 0.001$.

extracted for comparison. The abnormally elevated zFC value of the left cerebellum_4_5 in patients with LDH ($P < 0.001$) decreased ($P = 0.078$) post-treatment, approaching the level observed in healthy individuals ($P = 0.018$) (Table 7 and Fig. 5C1). Similarly, the abnormally elevated zFC value of the right precuneus in patients with LDH ($P < 0.001$) decreased ($P = 0.101$) after SMT, reverting to the level observed in healthy subjects ($P = 0.075$) (Table 7, Fig. 5C2).

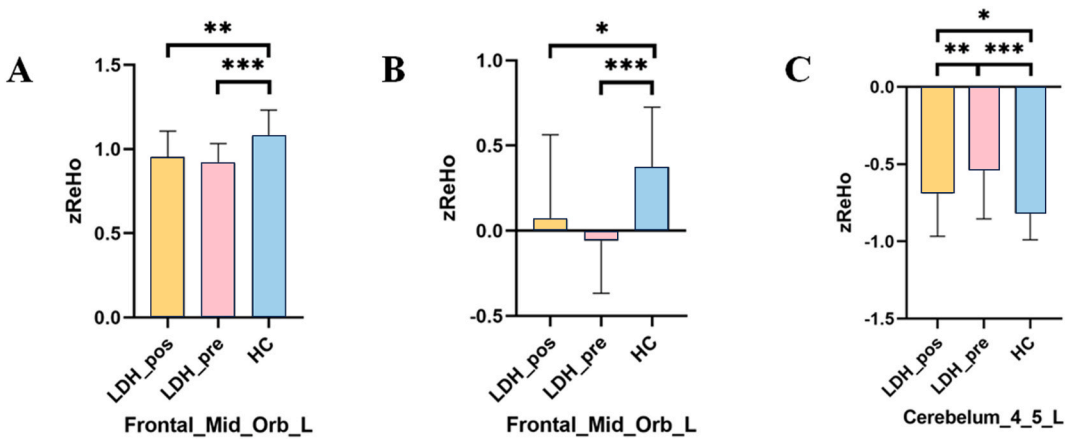

Fig. 4. Bar graph shows comparisons (LDH_pre vs HCs, LDH_post vs HCs and LDH_post vs LDH_pre) of average ReHo in regions that showed significant differences. A. significant differences in Frontal_Mid_Orb_L in 0.01–0.08 HZ. B. significant differences in Frontal_Mid_Orb_L in 0.01–0.027 HZ. C. significant differences in Cerebelum_4_5_L in 0.027–0.073 HZ. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 7
Group differences of zFC values from brain regions in conventional frequency band.

ROI	Brain regions	Contrast	t-value	P-value
ROI 1	Cluster 1	LDH_pos vs HC	−2.417	0.019*
LO-MFG (6,33,3)	Frontal_Inf_Orb_R (6,33,3)	LDH_pre vs HC	−3.194	0.002**
		LDH_pos vs LDH_pre	0.723	0.476
	Cluster 2	LDH_pos vs HC	−4.162	<0.001***
	Frontal_Inf_Orb_L (−30,39,-6)	LDH_pre vs HC	−3.279	0.002**
		LDH_pos vs LDH_pre	−1.262	0.218
ROI 2	Cluster 1	LDH_pos vs HC	−2.583	0.013*
LO-MFG (30,39,-6)	Frontal_Inf_Orb_L (−27,-9,-9)	LDH_pre vs HC	−3.229	0.002**
		LDH_pos vs LDH_pre	−0.600	0.554
	Cluster 2	LDH_pos vs HC	−2.708	0.009**
	Frontal_Inf_Orb_R (6,33, 3)	LDH_pre vs HC	−4.042	<0.001***
		LDH_pos vs LDH_pre	1.142	0.264
ROI 3	Cluster 1	LDH_pos vs HC	2.45	0.018*
Cerebelum_4_5_L (9,-30,-36)	Cerebelum_4_5_L (9,-30,-39)	LDH_pre vs HC	3.946	<0.001***
		LDH_pos vs LDH_pre	−1.838	0.078
	Cluster 2	LDH_pos vs HC	1.817	0.075
	Precuneus_R (18,-60,27)	LDH_pre vs HC	3.985	<0.001***
		LDH_pos vs LDH_pre	−1.702	0.101

Abbreviation.
sFC: static functional connectivity.
ROI: regions of interest.
LDH-pre: Lumbar disc herniation patients before SMT treatment.
HCs: healthy controls.
Frontal_Inf_Orb: the orbital part inferior frontal gyrus.
MNI: Montreal Neurological Institute.
* Indicates significance level at $P < 0.05$, ** Indicates significance level at $P < 0.01$, *** Indicates significance level at $P < 0.001$.

4. Discussion

Overall, this work tried to figure out the frequency-specific characteristics of changes in ReHO among LDH patients based on previous research. Additionally, this study sought to determine whether there are any functional connectivity changes in patients with LDH based on local differential brain areas. We further examined whether SMT can address abnormal changes in patients with LDH and identify the central response characteristics of effective SMT.

These findings indicate that patients with LDH exhibit local spontaneous brain activity changes compared with HCs. These changes included weakened ReHO in the LO-MFG, enhanced ReHO in the left cerebellum_4_5, and frequency-specific alterations in the spontaneous brain activity. Furthermore, our analysis revealed weakened functional connectivity between the LO-MFG and the bilateral orbital inferior frontal gyri, as well as enhanced functional connectivity within the left cerebellum_4_5, between the left cerebellum_4_5 and the right precuneus.

The middle and inferior orbitofrontal gyri are components of the orbitofrontal cortex (OFC), and are crucial for emotional

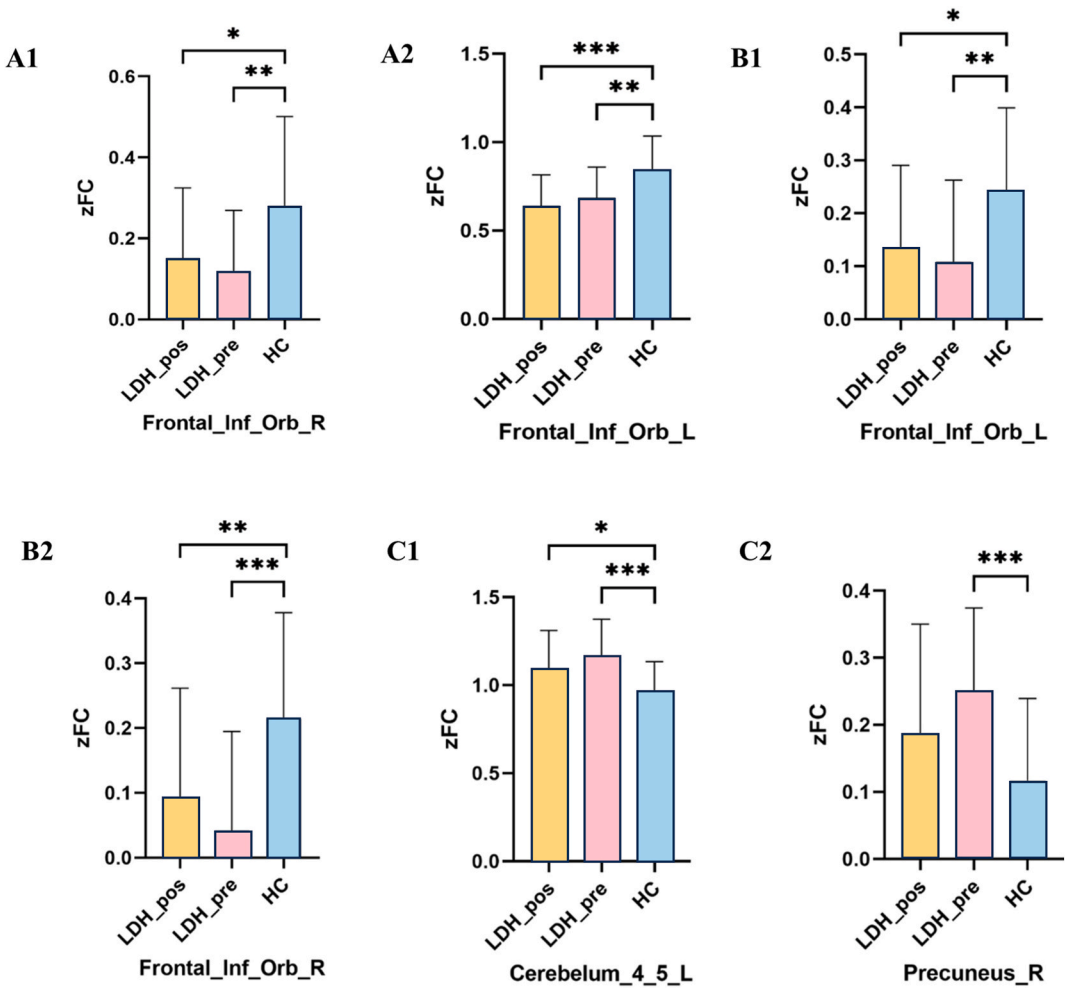

Fig. 5. Group differences of zFC values from brain regions in conventional frequency band. A1. The zsFC values of signal extracted from Frontal_Inf_Orb_R (6, 33, 3) of each participant. A2. The zsFC values of signal extracted from Frontal_Inf_Orb_L(-30, 39, −6) of each participant. B1. The zsFC values of signal extracted from Frontal_Inf_Orb_L (−27, −9, −9) of each participant. B2. The zsFC values of signal extracted from Frontal_Inf_Orb_R (6,33, 3) of each participant.C1. The zsFC values of signal extracted from Cerebelum_4_5_L (9, −30, −39) of each participant.C2. The zsFC values of signal extracted from Precuneus_R(18,-60,27) of each participant. The LDH patients and HCs were compared using two-sample t-tests. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

regulation, decision making, and behavioural control. Studies have indicated that the OFC is responsible for modulating emotional reactions, assessing the consequences of actions, and adjusting emotional responses [23]. This region is also closely linked to planning and decision-making processes, and facilitates the evaluation of different options to make optimal choices [24,25]. In addition, it aids in suppressing inappropriate behaviours and selecting more suitable responses [26]. Moreover, the OFC is associated with social behaviour and interpersonal interactions, including the ability to comprehend the emotions and intentions of others [27]. Although the specific impact of the OFC on LDH remains unclear, it is known to influence pain perception and emotional regulation, both of which could be significant factors in LDH. The OFC may be involved in the perception and processing of painful stimuli in LDH by interacting with alternative brain regions, such as the anterior cingulate cortex (ACC) and the limbic system [28]. Emotional factors have further been shown to influence pain perception and coping mechanisms [29]. Indeed, the OFC may affect a patient's pain experience and coping strategies by regulating emotional responses [30]. This study conducted a further analysis of the disparities in the Regional Homogeneity (ReHO) between patients with Lumbar Disc Herniation (LDH) and Healthy Controls (HCs) across different frequency ranges. The findings revealed that patients with LDH exhibited significantly decreased ReHo values in the LO-MFG in both the conventional and slow-5 frequency bands compared with healthy controls. Furthermore, analysis of functional connectivity between specific brain regions indicated a reduction in connectivity between the LO-MFG and inferior orbital frontal gyrus in patients with LDH. It has previously been hypothesised that chronic pain may disrupt spontaneous brain activity and internal functional connections within the OFC in patients with LDH, resulting in impaired pain perception and emotional detachment.

The cerebellum, located behind the brainstem, primarily coordinates movement, balance, and posture, and plays a crucial role in sensorimotor circuits [31,32]. The middle and inferior cerebellar peduncles consist of afferent fibers of the cerebellum. These fibers

transmit information from the contralateral cerebellopontine and inferior olivary nuclei to the neocerebellum. On the other hand, efferent fibers form the superior cerebellar peduncle. These fibers relay information from the neocerebellum to the contralateral thalamus and cerebral cortex [33,34]. Various cerebellar subregions are vital to sensorimotor networks, and contribute to functions such as balance, motor function coordination, and planning. While early studies have focused on the role of the cerebellum in motor control [35,36], recent neuroanatomical, neuroimaging, and clinical research have shown the involvement of different cerebellar subregions and the vermis in learning, cognition, emotion, and behavior [37–39]. The precuneus, located in the prefrontal cortex, has been shown to be involved in various cognitive and emotional functions [40,41]. We observed an abnormal increase in the ReHo value of the left cerebellum 4_5 in patients with LDH, along with enhanced internal functional connections of the left cerebellum 4_5. This could be attributed to the diminished motor balance ability observed in patients with LDH, leading to a compensatory increase in local spontaneous brain activity and internal functional connections within the cerebellum. Additionally, due to the crucial role of the cerebellum in balance and motor control [42], it is possible that patients with LDH adopt forced postures to alleviate pain resulting from nerve root compression. Increased local spontaneous brain activity in the cerebellum and its functional connection to the precuneus may be associated with pain and pain-related emotions in patients with LDH. Moreover, this enhancement may contribute to the resistance to pain perception and pain-related emotions.

The two primary symptoms of LDH are pain and dysfunction [1]. Research has indicated that individuals with chronic pain, particularly those with lumbar disc herniation, are more susceptible to experiencing symptoms of anxiety and depression [43], which could be attributed to ongoing pain and functional limitations, leading to concerns regarding physical health and overall well-being. Prolonged physical discomfort may also affect an individual's mental state; conversely, anxiety and depression can exacerbate the perception of pain, creating a detrimental cycle [43,44]. In this context, fMRI studies on LDH have primarily focused on the brain regions associated with pain, motor function, and emotions. For example, Zhou et al. [45] demonstrated abnormal local spontaneous brain activity in the prefrontal cortex of patients with LDH compared to healthy controls. Further, both Koef et al. [46] and our previous research [12] observed heightened local spontaneous brain activity in the cerebellum of patients with LDH. These findings are consistent with the results of the present study. The ACC is a region representative of cortical pain that is predominantly involved in encoding emotional pain information [23]. Some studies have found that the spontaneous brain activity in the ACC increases in patients with LDH [46]. However, this study did not find any clear variations in spontaneous brain activity in the ACC of patients with LDH, which may be attributed to variations in the subject selection criteria and sample sizes. In addition, we found that spontaneous brain activity changes in the same brain area may be contradictory in different studies, which may be related to disease duration in patients with LDH. Short-term pain and dysfunction may lead to heightened local spontaneous brain activity, whereas chronic pain and functional impairment may result in reduced local spontaneous brain activity in LDH patients.

Future, SMT could correct the aforementioned abnormal changes in patients with LDH to some extent. Specifically, the intervention had a noticeable effect on the local consistency of the left cerebellum_4_5, suggesting that alterations in brain function due to LDH are reversible, and that SMT can help improve abnormal brain function changes in patients with LDH to a certain degree, potentially regulating cerebellar function. These findings offer a scientific basis for considering spinal manipulation as an effective intervention for treating LDH, which is consistent with our previous research results [12,13].

5. Conclusion

Overall, this study identified abnormal spontaneous brain activity and functional connectivity changes in multiple frequency bands among patients with LDH using ReHo analysis. Additionally, our analysis demonstrated the therapeutic effects of SMT. These findings indicate that patients with LDH exhibit alterations in local spontaneous brain activity and corresponding changes in functional connections that may vary across different frequency bands. Moreover, SMT was shown to partially normalise abnormal regional homogeneity and functional connections in patients with LDH. Specifically, changes in ReHO in the left cerebellum_4_5 could serve as a sensitive indicator of SMT effectiveness. Overall, this study offers preliminary clinical evidence to indicate the impact of SMT on brain activity, highlighting the need for further research in this area.

6. Limitations

This study had several limitations which should be mentioned. Firstly, we used only a small sample size due to strict inclusion and exclusion criteria. Future studies should consider using larger sample sizes to investigate abnormalities in spontaneous brain activity and functional connectivity among LDH patients. Further, this study focused on regional homogeneity across different frequency bands, observing differences in functional connections between brain regions. Subsequent research could employ more advanced analytical methods to enhance the understanding of the central brain response characteristics in LDH and the impact of SMT, while also expanding the sample size. Additionally, our findings indicate abnormal activity and functional connections in brain regions associated with pain, cognition, emotion, and movement in patients with LDH, supporting the intervention effect of SMT. However, further correlation analyses using relevant clinical indicators are required to explore the relationship between improved brain function and clinical outcomes. Moving forward, we aim to enhance our work and provide more robust evidence of the brain response characteristics of the SMT.

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Data availability statement

All necessary data was included in the manuscript. Other related data will be available upon request to Dr. Du.

CRediT authorship contribution statement

Hong-Gen Du: Writing – original draft, Project administration. **Ya Wen:** Writing – original draft, Formal analysis. **Jun-Xiang Dong:** Data curation. **Shao Chen:** Writing – review & editing, Validation. **Xin Jin:** Formal analysis. **Chen Liu:** Validation. **Dong-Ya Ling:** Data curation. **Li-Jiang Lv:** Writing – review & editing, Methodology.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Honggen Du reports financial support was provided by the National Natural Science Foundation of China. Ya Wen reports financial support was provided by Zhejiang Provincial Traditional Chinese Medicine Science and Technology Plan Project. Ya Wen reports financial support was provided by Natural Science Foundation of Zhejiang Province. Xin Jin reports financial support was provided by Natural Science Foundation of Zhejiang Province. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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