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Effect of acupuncture treatment for patients with knee osteoarthritis on brain fluctuation amplitude and functional connectivity: a randomized three-armed fMRI study

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Abstract

Background In clinical practice, the overall effect of a treatment includes specific and nonspecific components. Although the analgesic mechanism of specific acupuncture effect has been studied extensively, the understanding of neural basis about nonspecific placebo effect is insufficient. We aimed to reveal the neurological signatures associated with both specific effect and nonspecific effect of acupuncture in chronic pain using the functional magnetic resonance imaging (fMRI).

Methods In this three-armed, randomized controlled trial, 90 patients with knee osteoarthritis (KOA) were divided into acupuncture group (AG), sham acupuncture group (SG), or waiting list group (WG), receiving 12 times of treatments during 4 weeks. NRS, WOMAC score, and fMRI data were collected before and after treatment. We assessed the amplitude of low frequency fluctuation (ALFF) and seed-based functional connectivity (FC) to investigate the brain functional bases of the nonspecific (SG vs. WG) and specific (AG vs. SG) effects of acupuncture.

Results Acupuncture could significantly reduce NRS score in KOA patients (overall $P < 0.001$). Neurally, significant ALFF changes in left dorsolateral prefrontal cortex (DLPFC) were observed in the AG (vs. SG) and SG (vs. WG; Voxel $P < 0.001$, Cluster $P < 0.001$), respectively. And the decreased ALFF values of DLPFC had a positive correlation with the NRS score changes in the SG (*spearman rho* = 0.592, $P = 0.002$). Moreover, FC between DLPFC and thalamus was increased in the AG compared to SG. And compared to WG, there was a decreased FC between DLPFC and cerebellum in the SG.

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Conclusion The spontaneous activity of the DLPFC was involved in both the specific and nonspecific acupuncture effects, whereas different DLPFC FCs related to the specific or nonspecific effects of acupuncture. Our findings contribute to the understanding about the neuroimaging evidence of the acupuncture effects.

Trial registration numbers Chinese Clinical Trial Registry (www.chictr.org.cn) ChiCTR1900025799. This study was registered on 9th September 2019.

Keywords Acupuncture, ALFF, Functional connectivity, Knee osteoarthritis, fMRI

Introduction

In clinical practice, an intervention acts through specific biological effect, placebo effect related to expectancy and conditioning, and nonspecific psychological effect [1–3]. The contributions of these therapeutic components vary with intervention types and clinical contexts [4]. As an important non-pharmaceutical therapy, acupuncture has shown satisfactory efficacy in large clinical randomized controlled trials (RCTs) for many diseases [5, 6], especially chronic pain [7, 8]. However, these trials mainly evaluated the specific effect of acupuncture with sham acupuncture as control, very few several arms design (including verum, placebo, and natural course) is available to give information about different components of the acupuncture effect and control the natural course of the disease. Meanwhile, acupuncture acts through complex neurological and physiological mechanisms [8, 9], the complexity and limited understanding of the neural basis responsible for the specific and nonspecific effects hinder the development of acupuncture treatments.

Neuroimaging may provide nuanced and objective neural markers of the treatment effects in RCTs, where active treatment and inert placebo treatment might be differentiated at the brain circuit level [10]. Some functional magnetic resonance imaging (fMRI) studies have shown that prefrontal cortex (PFC) plays a key role in the development of pain, and the connections between the PFC with the thalamus and amygdala are involved in the pain processing [11]. In particular, the dorsolateral PFC (DLPFC) is an important part in the regulation of chronic pain [12], which could be used as an intervention target for pain relief as shown in many rTMS/TDS-related studies [13–15]. Meanwhile, DLPFC has been reported to be involved in acupuncture analgesia [16]. The treatment of acupuncture combined with enhance expectation produce a stronger analgesic effect, which is associated with the regulation of DLPFC-nucleus accumbens related functional connectivity (FC) [17]. Moreover, connectivity between the DLPFC and temporoparietal junctions have also been reported to be involved in the neural processes of the placebo effect [18]. Placebo analgesia is the most well-studied placebo effect, and it is also thought to begin in the DLPFC which then triggers the brain's descending pain regulatory system and other pain regulatory pathways [19]. The DLPFC-precentral gyrus FC as a biometric could predict the response of a placebo pill to chronic pain [20]. However, it is unclear whether DLPFC or its related FC are involved in acupuncture-related placebo or other non-biological effects, and if so, what is the difference from the mechanics of specific effect that need to be evaluated by several-armed RCT.

To preferably evaluate the neurological basis related to the specific and nonspecific effect of acupuncture, we performed a three-armed randomized neuroimaging trial. Here, we randomized knee osteoarthritis (KOA) patients into 3 separate groups: acupuncture group (AG), sham acupuncture group (SG, without needle insertion and manipulation), and waiting list group (WG, no treatment during the trial). The specific effect was defined as the comparison between the AG and SG, in contrast, the nonspecific placebo effect of acupuncture was obtained between the SG and WG. We investigated the changes after 4 weeks of acupuncture and sham acupuncture treatments on KOA pain, and the amplitude of low frequency fluctuation (ALFF) and FC. The ALFF reflects the spontaneous neural activity of brain [21], and the FC reveals the functional interactions between different brain regions [22].

Materials and methods

Participants

We recruited patients with KOA who meet the diagnostic criteria of the American College of Rheumatology criteria [23] at Dongzhimen Hospital of Beijing University of Chinese Medicine. The key inclusion criteria were: (1) 45 to 65 years old; (2) knee pain lasts at least 6 months; (3) KL (Kellgren-Lawrence) grade II or III in recent 12 months; (4) numerical rating scale (NRS) ≥ 4 in the past week; (5) right-handedness. The key exclusion criteria: (1) history of knee replacement, waiting surgery, arthroscopy within 1 year or intra-articular injection within 6 months, and discomfort of knee joint caused by other reasons; (2) serious organic pathological changes, mental abnormalities, psychiatric or neurological disorders, or coagulation disorders; (3) MRI contraindications, such as claustrophobia; (4) received acupuncture or massage treatment in recent 1 month; (5) allergy to needles and alcohol or fear to acupuncture, and participated in other clinical studies in the past 3 months.

The recruitment process started since September 2019 and continued until July 2021.This study has been approved by the ethics committee of Dongzhimen Hospital Affiliated with Beijing University of Chinese Medicine (DZMEC-KY-2017-53-02), registered in Chinese Clinical Trial Registry (ChiCTR1900025799). All participants provided written informed consent according to the Declaration of Helsinki after study procedures were explained to them thoroughly.

Study design

Eligible patients were randomly assigned to acupuncture group (AG), sham-acupuncture group (SG), or waiting list group (WG) in a ratio of 1:1:1. The randomization sequence was generated using SAS software (version 9.3) by an independent researcher. Acupuncturists were not blinded to the allocation for successfully performing acupuncture treatment. However, they were not allowed to discuss the intervention type with patients. The patients, MRI operators, outcome evaluators, and statisticians were all blinded to group assignment. Each participant will receive 2 fMRI scans to explore the brain basis of the specific and nonspecific acupuncture effects (Fig. 1A).

Sample size

There is currently no precise standard for sample size calculation in MRI research. According to previous MRI studies related to chronic pain, differences in brain function indicators can be detected in a sample size range of 13 to 20 participates [8, 20, 24, 25]. In addition, power analysis performed in G*Power (version 3.1), with $\alpha = 0.05$ and power = 0.8, showed that 26 participants per group are needed to detect relatively large anticipated effects (0.8) for group differences on ALFF and FC metrics. Considering the impact of patients' dropout and excessive head movement during the MRI scanning, 30 patients were recruited for each group.

Clinical assessment

The primary outcome was the response rate, which was defined as the proportion of patients with a decrease of more than 2 points on the NRS at week 4 compared with baseline [26]. NRS is a line divided into 10 segments. The degree of pain is assessed by 0–10 scores, and 0 score represents pain-free and a 10 score represents the worst pain. The secondary outcomes were as follows: (1) Western Ontario and McMaster Osteoarthritis Index (WOMAC) scale was used to evaluate the degree of knee pain, joint function and stiffness of the patients. The

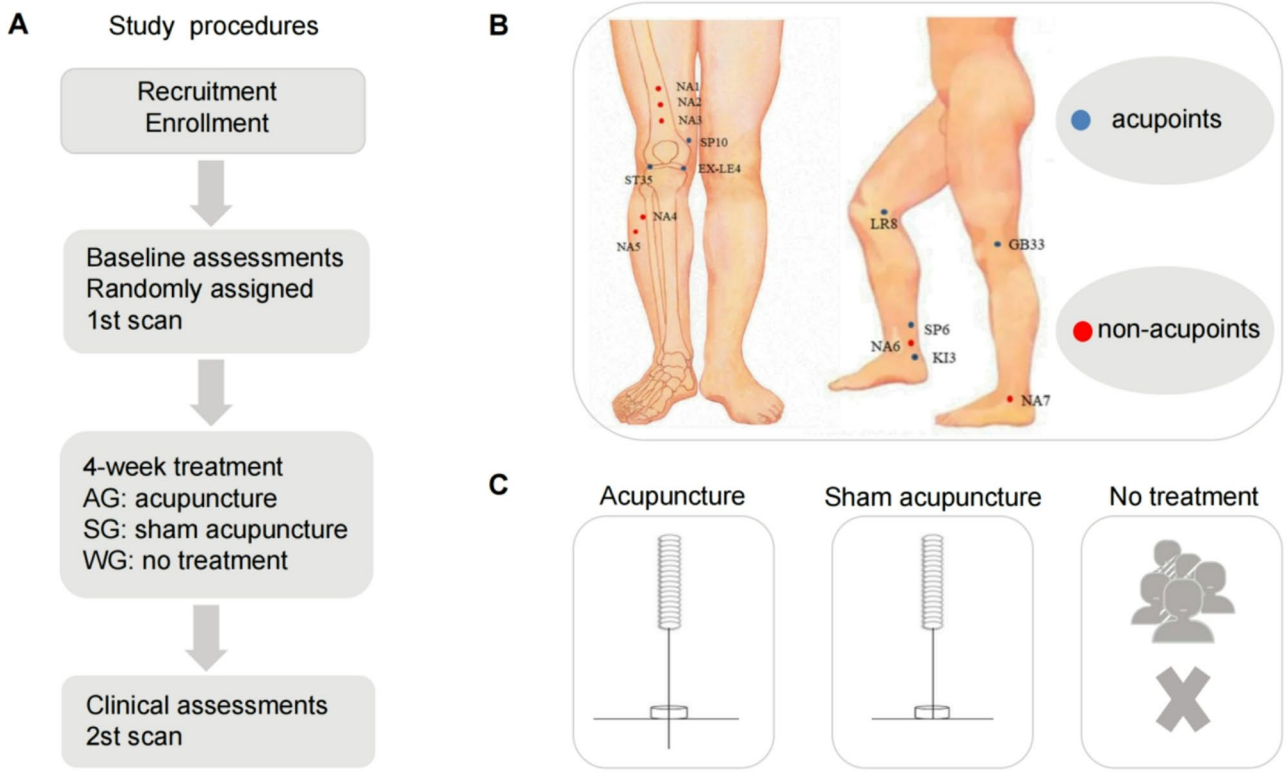

Fig. 1 Study design. **A**, The procedures in the present study. **B**, The location of acupoints and non-acupoints. Red circles: location of non-acupoints. Blue circles: location of acupoints. **C**, The picture of acupunctures, sham acupuncture and no treatment during the trial in WG. Abbreviation: ST35, Dubi; EX-LE4, Neixiyan; LR8, Ququan; GB33, Xiyangguan; SP10, Xuehai; SP6, Sanyinjiao; KI3, Taixi; NA, non-acupoint; AG: acupuncture group; SG: sham acupuncture group; WG: waiting list group

severity of knee joints increased with the higher scores of WOMAC. (2) State-Trait Anxiety Scale-State Anxiety Subscale (STAI-S) was used to evaluate the severity of current anxiety symptoms in patients with KOA. Patients filled out the WOMAC scale and the STAI-S scale at weeks 0, 2, and 4.

Acupuncture treatment

Each patient of the AG and SG received 12 sessions of 30 min treatment during 4 weeks, with 3 sessions per week. Participants in WG did not receive acupuncture within 4 weeks. After 4 weeks of observation, patients will receive 12 sessions of acupuncture treatment as free compensation. The registered acupuncturists with more than 5 years of experience performed intervention procedures.

The acupoints included dubi (ST35), neixiyan (EX-LE4), ququan (LR8), xiyangguan (GB33), xuehai (SP10), sanyinjiao (SP6), taixi (KI3), and an ashi point (the point where the patient feels most pain). The location of the acupoints and non-acupoints was shown in Fig. 1B. In the AG, the adhesive pad was applied on the skin of the acupoints, and then single-use acupuncture needles (0.25 × 40 mm or 0.25 × 25 mm, Guizhou Andi Pharmaceutical Machinery, Ltd., Guizhou, China) were inserted into acupoints through the adhesive pad (Fig. 1C). Acupuncturists manually stimulated the needles to achieve de qi. Patients in SG received non-insertive acupuncture treatment on non-acupoints using sham needles with blunt tips (0.25 × 40 mm or 0.25 × 25 mm, Hwato, China). Sham needles did not penetrate the skin and did not require the "De Qi" sensation (Fig. 1C).

MRI data acquisition

MRI images were obtained at a Siemens 3.0 T MRI scanner (Skyra, Siemens, Erlangen, Germany) using a standard head coil in the Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University. We used comfortable foam pads to minimize head motion and earplugs to reduce noise interference. Before starting scanning, we instructed patients to keep their eyes closed, stay awake, avoid engaging in any specific thoughts, and keep still. The resting-state functional MRI (rs-fMRI) was scanned using echo planar imaging (EPI) sequence: repetition time (TR) = 2,000 ms, echo time (TE) = 30 ms, field of view (FOV) = 224 mm × 224 mm, flip angle = 90°, slice spacing = 4.375 mm, axial slices = 32, in-plane resolution = 64 × 64, voxel size = 3.5 × 3.5 × 3.5 mm [3] and 240 volumes. High-resolution brain T1-weighted (T1w) MRI was obtained using a sagittal 3D magnetization-prepared rapid gradient echo (MPRAGE) sequence: TR/TE/inversion time = 2,530/2.98/1100 ms, flip angle = 7°, slice number = 192, matrix = 256 × 256, voxel size = 1 × 1 × 1mm^3, slice gap = 0 mm.

MRI data preprocessing

Functional MRI data preprocessing Functional MRI data were processed using the software MATLAB 2016 and the toolbox for Data Processing & Analysis for Brain Imaging (DPABI, version 6.1, http://rfmri.org/dpabi). For each participant's image data, we discarded the first 10 volumes because of signal equilibrium, a total of 230 volumes for each subject were processed with the slice timing, head motion correction, spatial normalization (re-sampled to 3 mm × 3 mm × 3 mm), removing linear trend and the nuisance signals, and spatial smoothing (6-mm FWHM). Of note, patients with head motion exceeding 2.5 mm were excluded.

Brain metrics analysis

ALFF analysis

The ALFF measures the gross power of oscillations within a certain frequency range, using the DPBAI software. The calculation procedure: (1) Fast Fourier Transform (FFT) was used to convert all voxels from the time domain to the frequency domain; (2) the ALFF of every voxel was calculated by averaging the square root of the power spectrum across 0.01 Hz to 0.08 Hz; and (3) the resulting ALFF was converted into z-scores by subtracting the mean and dividing by the global standard deviation for standardization purposes. A comparison of the ALFF maps among three groups was performed using ANCOVA analysis, with Gaussian Random Field Theory (GRF) correction (voxel-level $P < 0.001$ and cluster-level $P < 0.001$). Finally, we extracted the ALFF values for brain regions with significant results (post minus pretreatment), and the two sample t-test analysis was applied to explore between-group differences (AG vs. SG, SG vs. WG).

Seed-based functional connectivity analysis

Before FC analysis, band-pass filtering (0.01–0.08 Hz) was performed and the linear trend was removed. A seed-based analysis was applied to measure FC by extracting time series from the region of interest. The seed region was defined as a significantly different region that was identified by ALFF analysis among the three groups. Seed-based correlation was calculated between the regions and whole-brain voxels by Pearson's correlation analysis. The obtained FC maps were converted to z values using Fisher's r-to-z transformation to normalize the connectivity map of each individual. The ANCOVA analysis was applied for the intergroup analysis (AG vs. SG, SG vs. WG) and the GRF correction threshold was voxel level $P < 0.005$ and cluster level $P < 0.005$.

Clinical data statistical analysis

Besides the image-based statistics, statistical analyses of clinical characteristics were performed in SPSS v20.0.

Table 1 Characteristics of study participants at baseline

	AG (*n* = 27)	SG (*n* = 25)	WG (*n* = 22)
Female, no. (%)	23(85.19%)	17(68.00%)	15(68.18%)
Age(year)	63.00(57.00,65.00)	60.00(53.50,65.00)	54.50(49.00,62.00)
Pain duration(year)	6.00(2.00,10.00)	6.00(3.50,10.00)	3.50(2.00,5.00)
BMI (kg/m^2)	25.71(2.54)	24.75(2.97)	24.27(3.83)
Education(year)	9.00(9.00,15.00)	12.00(9.00,15.00)	12.00(9.00,15.25)
Affected knee, no. (%)			
Unilateral knee	4(14.81%)	5(20.00%)	7(31.82%)
Bilateral knees	23(85.19%)	20(80.00%)	15(68.18%)
History of acupuncture, no. (%)			
Yes	8(29.63%)	7(28.00%)	2(9.10%)
No	19(70.37%)	18(72.00%)	20(90.91%)

We used mean (standard deviation) if the measurements were normally distributed, and median (lower quartile to upper quartile) if the measurements were not normally distributed. AG: acupuncture group; SG: sham acupuncture group; WG: waiting list group; BMI: body mass index

Table 2 Clinical measurements changes in the three groups

Measurements	Group (*n*)	Pre_treatment	Post_treatment	ES	*P*
NRS	AG (27)$^{\#}$	6.00(5.00, 7.00)	3.00(2.00, 5.00)	0.733	**< 0.001**
	SG (25)$^{\#}$	5.00(4.50, 7.00)	4.00(2.50, 5.00)	0.643	**< 0.001**
	WG (22)$^{\#}$	5.00(4.00, 6.00)	4.82(1.71)	0.073	0.205
WOMAC	AG (27)$^{\#}$	19.00(15.00, 23.00)	12.56(5.60)	0.984	**< 0.001**
	SG (25)$^{\#}$	19.00(12.50, 26.50)	13.00(9.50, 20.00)	0.697	**0.002**
	WG (22)$^{\#\#}$	16.32(6.48)	15.59(6.97)	0.108	0.501
STAI-S	AG (27)$^{\#}$	30.00(28.00, 35.00)	30.00(26.00, 35.00)	0.266	0.183
	SG (25)$^{\#\#}$	32.36(6.47)	32.04(5.68)	0.052	0.746
	WG (22)$^{\#\#}$	31.77(6.02)	32.00(3.70)	-0.040	0.797

We used mean (standard deviation) if the measurements were normally distributed, and median (lower quartile to upper quartile) if the measurements were not normally distributed. #: Nonparametric tests: Wilcoxon-W; ##: paired t-test. AG: acupuncture group; SG: sham acupuncture group; WG: waiting list group; NRS: numeric rating scale; WOMAC: Westem Ontario and MoMaster Universities Osteoarthritis Index; STAI-S: State-trait anxiety inventory-State; ES: effect size. Effect sizes were depicted as Cohen's d and computed at https://www.psychometrica.de/effect_size.html

As for categorical variables (i.e., gender, response rate), we used the Chi-Square test to evaluate the differences among groups. The Shapiro-Wilk tests were conducted for all variables to ensure they were normally distributed. We used One-Way ANOVA analysis and Student's t test for normally distributed data. For the data that failed normality testing, the Kruskal-Wallis, Wilcoxon and Mann-Whitney nonparametric test were used to compare the group differences. To account for potential baseline imbalances, between-group comparisons of post-treatment outcomes were adjusted for baseline NRS scores using ANCOVA. Cohen's d was applied to express effect size (ES) between-group differences. The ES computed at https://www.psychometrica.de/effect_size.html. The Pearson correlation and Spearman correlation analyses were used to investigate the relationships between each altered ALFF and FC with the change (4-week minus baseline) of significant clinical measurement. Statistical significance level was set to $P < 0.05$.

Results

Patient characteristics

Of 154 KOA patients screened, 90 patients were enrolled in this study, who completed baseline clinical characteristics and the first fMRI scan. Three patients from the AG, four patients from the SG and six patients from the WG were discontinued from the study. One patient from the AG and two patients from the WG were excluded due to head motion (larger than 2.5 mm). Finally, 74 patients (27 in the AG, 25 in the SG, and 22 in the WG) finished the whole study and were included in the analysis [8]. The baseline demographic characteristics of all patients are summarized in Table 1.

Clinical measures

First, within-group comparisons showed that the acupuncture treatment had significant and greatest clinical measurement changes (Table 2). For the primary outcome, the response rate was 85.2% (23/27 patients) in the AG, meanwhile, the response rates were 52% (13/25 patients) in the SG, and 13.64% (3/22 patients) in the WG ($X^2 = 24.903$, $P < 0.001$; Table 3). For the secondary outcome, there were significant group differences among the three groups in the NRS ($H = 28.058$, $P < 0.001$; after controlling for baseline NRS, significant differences were still remained in group effects [$F = 17.286$, $P < 0.001$]; Table 3) and WOMAC score ($H = 10.193$, $P = 0.006$; Table 3). The post hoc analysis showed that the NRS score ($ES = 0.900$,

Table 3 Comparison of acupuncture effect on clinical measurements changes

	AG (*n* = 27)	SG (*n* = 25)	WG (*n* = 22)	Statistic	Overall *P*	AG vs. SG		SG vs. WG	
						ES	*P*	ES	*P*
Response, no. (%)	23 (85.2%)	13 (52%)	3 (13.64%)	24.903[a]	<0.001[*]	0.770[a]	0.010[*]	0.883[a]	0.006[*]
NRS score	-3.00 (-4.00, -2.00)	-2.00(-2.00, -1.00)	0.00 (-1.00,1.00)	17.286[b]	<0.001[*#]	0.900[e]	0.002[*]	1.064[e]	0.001[*]
WOMAC	-7.44 (9.51)	-7.20 (10.21)	-0.73 (4.98)	10.193[c]	0.006[*]	-0.025[f]	0.929	-0.822[f]	0.008[*]
STAI-S	-1.96 (7.46)	-0.32 (4.88)	0.23 (4.09)	0.979[d]	0.381	-0.259[f]	0.356	-0.121[f]	0.681

We used mean (standard deviation) if the measurements were normally distributed, and median (lower quartile to upper quartile) if the measurements were not normally distributed. NRS: numeric rating scale; WOMAC: Westem Ontario and MoMaster Universities Osteoarthritis Index; STAI-S: State-trait anxiety inventory-State; AG: acupuncture group; SG: sham acupuncture group; WG: waiting list group; ES: effect size. a: Chi-square test; b: ANCOVA; c: Kruskal-Wallis-H; d: One-Way ANOVA; e: Mann-Whitney-U; f: two sample t-test. #: *p*-values adjusted for baseline NRS. Effect sizes were depicted as Cohen's d and computed at https://www.psychometrica.de/effect_size.html. * $P < 0.05$

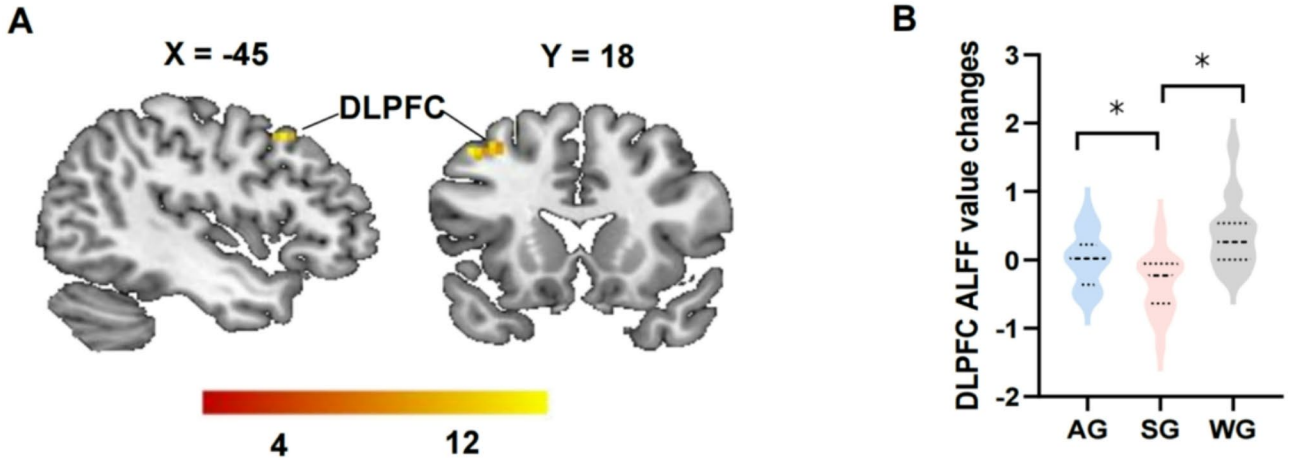

Fig. 2 ALFF changes among the three groups. **A**, After treatment, the ALFF analyses showed significant differences in the left DLPFC. **B**, The change of ALFF values in the DLPFC at each group. The value decreased in AG and SG, but increased in WG. Abbreviations: DLPFC, dorsolateral prefrontal cortexL; ALFF, amplitude of low-frequency fluctuation; AG: acupuncture group; SG: sham acupuncture group; WG: waiting list group; GRF corrected. Voxel $P < 0.001$, cluster $P < 0.001$

Table 4 Brain regions showed significant ALFF changes after treatment

Regions	Side	Peak MNI coordinates			Voxel Number	F value	AG vs. SG (ROI values)		SG vs. WG (ROI values)	
		X	Y	Z			ES	*P*	ES	*P*
DLPFC	Left	-45	18	48	23	14.989	0.880[a]	0.003*	-1.527[a]	< 0.001*

ALFF: amplitude of low-frequency fluctuation; DLPFC: dorsolateral prefrontal cortex; MNI: Montreal Neurological Institute; AG: acupuncture group; SG: sham acupuncture group; WG: waiting list group; ROI: region of interest; ES: effect size. F value is based on ANCOVA analysis, GRF corrected, voxel $P < 0.001$, cluster $P < 0.001$. ROI data are tested for post-treatment minus pre-treatment. a: two sample t-test; *: $P < 0.05$. Effect sizes were depicted as Cohen's d and computed at https://www.psychometrica.de/effect_size.html

$P = 0.002$) showed a significant group difference between AG and SG, yet there was no significant change in the WOMAC score (ES = -0.025, $P = 0.929$) between the two groups, and the SG had significantly lower NRS score ($ES = 1.064$, $P = 0.001$) and WOMAC score (ES = -0.822, $P = 0.008$) changes compared with WG. In addition, there was no significant group differences among the three groups in the STAI-S ($F = 0.979$, $P = 0.381$).

Neuroimaging outcomes

ALFF changes for specific and nonspecific effects of acupuncture

Significant differences among the three groups on the ALFF changes were observed in the left DLPFC (GRF corrected, voxel $p < 0.001$, cluster $p < 0.001$; Fig. 2A and Table 4). In post hoc analysis, the ALFF values was decreased in both AG and SG, with differences between groups ($ES = 0.880$, $P = 0.003$). Meanwhile, in the comparison between the SG and WG, left DLPFC showed significantly decreased ALFF values in the SG (ES = -1.527, $P < 0.001$) (Fig. 2B **and** Table 4). In addition, controlling for the influence of gender and duration, we found that the inter-group differences of DLPFC ALFF values still remained ($F = 12.263$, $P < 0.001$).

Correlation between altered ALFF and clinical symptoms

In the SG, correlation analyses revealed that the significantly decreased ALFF values of DLPFC had a positive correlation with the NRS score changes (*spearman rho* = 0.592, $P = 0.002$), however, no significant correlation

was found in the AG (*spearman rho* = -0.243, $P = 0.223$) and WG (*spearman rho* = 0.171, $P = 0.446$) (Fig. 3). In addition, we controlled for gender and duration for partial correlation analysis, and found the same correlation pattern between the ALFF values of DLPFC and the NRS score changes in the three groups (SG, [$r = 0.609$, $P = 0.002$]; AG, [r = -0.213, $P = 0.307$] and WG, [$r = 0.059$, $P = 0.804$]).

Functional connectivity changes after acupuncture treatment

First, we found that the FC between the DLPFC and thalamus had higher increase after treatment in the AG compared with SG ($ES = 0.935$, $P = 0.004$) (Fig. 4A-B and Table 5). Meanwhile, the FC between the DLPFC and cerebellum had higher decrease after 4 weeks in the SG compared with the WG (ES = -1.172, $P < 0.001$) (Fig. 4C-D **and** Table 5). In addition, controlling for the influence of gender and duration, we found that the intergroup differences of FC changes (FC of DLPFC-thalamus [$F = 13.400$, $P = 0.001$]; FC of DLPFC-cerebellum [$F = 11.030$, $P = 0.002$]) still remained.

Discussion

This study investigated the specific and nonspecific effects of acupuncture by three-armed randomized neuroimaging trial in patients with KOA. For clinical measurements, both acupuncture and sham acupuncture could reduce the NRS score, and the AG had a higher response rate. Acupuncture could significantly reduce WOMAC score, but there was no significant difference between the AG and the SG. Compared with the WG, both NRS score and WOMAC score decreased significantly in the SG. Second, fMRI analysis showed that acupuncture and sham acupuncture treatment both significantly decreased ALFF values in the DLPFC. Furthermore, the greater posttherapy decreases was associated with a greater reduction in NRS score in the SG. Third, the seed-based FC analysis found that acupuncture increased the FC between DLPFC and thalamus, and the sham acupuncture decreased the FC between DLPFC and cerebellum. Cumulatively, these results allow us to explicit the mechanistic path of specific and nonspecific effects in acupuncture treatment: both the specific and placebo effects of acupuncture modulated the activity of DLPFC. Whereas, the DLPFC-thalamus FC only respond to the specific effect, and the DLPFC-cerebellum FC only related to the nonspecific placebo effect.

For the primary outcome, there were significant group differences in response rate corresponding to both of the specific and nonspecific effects of acupuncture. Both acupuncture and sham acupuncture treatments could reduce NRS and WOMAC scores with much larger effect sizes than natural course (Table 2), suggesting that nonspecific components may also play an important role in the acupuncture analgesic process. However, there was no significant difference of the decreased WOMAC scores in the AG compared to the SG, in line with our previous large clinical trial that has found significant clinical differences in functional improvement at week 16 (2 months after treatment) but not at week 4 [7]. A meta-analysis also showed that significantly improved function in KOA patients was observed at 2 and 4.5 months after acupuncture compared to sham acupuncture, but not at other time points [27]. We speculate that pain relief mechanism may differ from functional improvement, the insignificant effect of acupuncture on functional improvement here may be due to the lack of long-term treatment and follow-up. Therefore, in this study, we focus on the neural mechanism of different components of acupuncture affecting analgesic effect and the relationship between them and pain improvement.

DLPFC is involved in the cognitive, affective, and sensory processing regulation of pain and plays an important role in the treatment of chronic pain [12, 13]. An fMRI study showed that exercise could regulate the cognitive

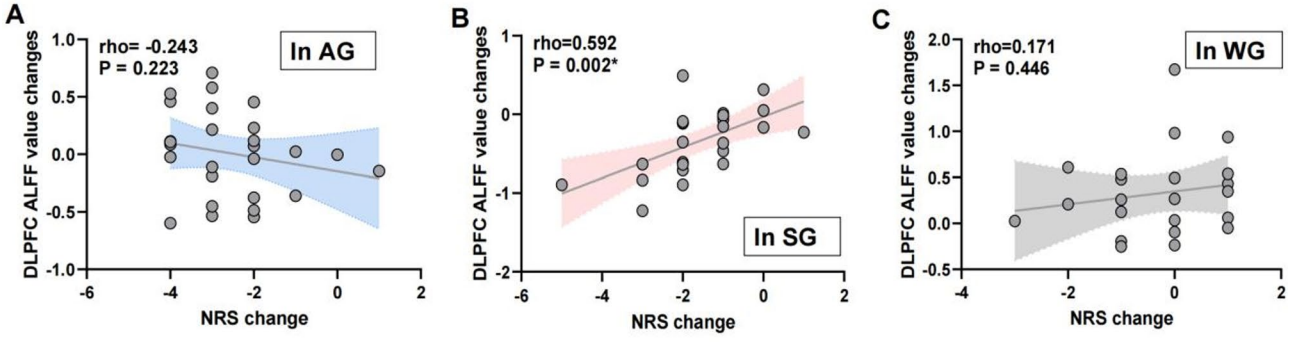

Fig. 3 The relationships between ALFF changes with clinical symptoms. **A**, The decreased NRS score had a positive correlation with the significant decreased ALFF values of DLPFC in SG (Spearman rho = 0.592, $p = 0.002$). **B-C**, No significant correlation was found in the AG (Spearman rho = -0.243, $p = 0.223$) and WG (Spearman rho = 0.171, $p = 0.446$). Abbreviations: DLPFC, dorsolateral prefrontal cortexL; ALFF, amplitude of low-frequency fluctuation; AG: acupuncture group; SG: sham acupuncture group; WG: waiting list group; NRS, numeric rating scale

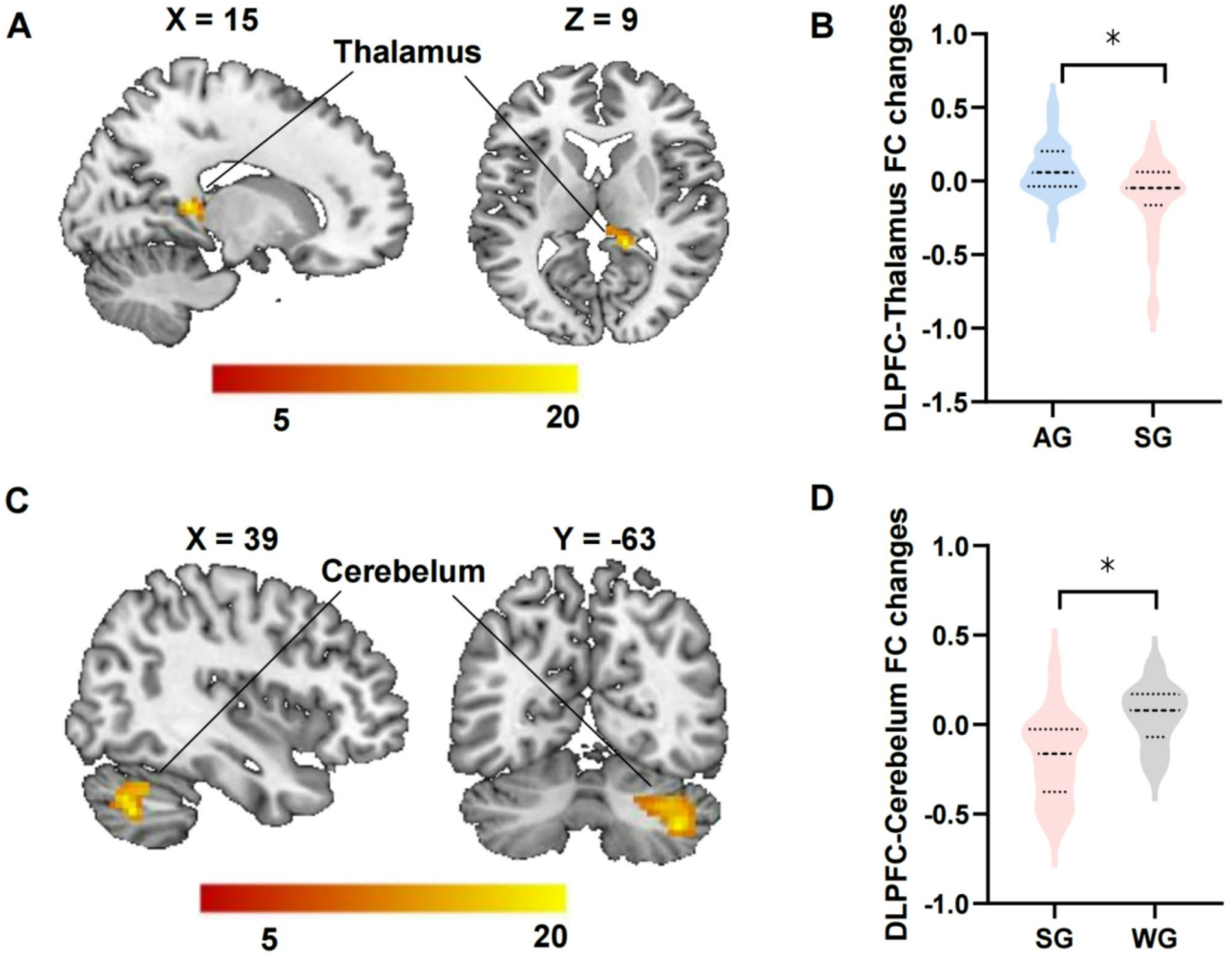

Fig. 4 DLPFC-based FC changes between groups. **A**, There were one increased FC in the AG compared with SG, the FC was between DLPFC and thalamus. **B**, The change of FC values which response to specific effect of acupuncture in AG and SG. **C**, Compared with WG, there was one decreased FC in SG. The FC was between DLPFC and cerebelum. **D**, The change of FC values which response to nonspecific effect of acupuncture in SG and WG. Abbreviations: DLPFC, dorsolateral prefrontal cortexL; FC, functional connectivity; AG, acupuncture group; SG, sham acupuncture group; WG, waiting list group; GRF corrected. Voxel $P < 0.005$, cluster $P < 0.005$

Table 5 Brain regions showed significant FC changes with DLPFC after treatment

Comparison	Regions	Side	Peak MNI coordinates			Voxel Number	F value	ES (FC values)	*P*
			X	Y	Z				
AG vs. SG	Thalamus	Right	15	-39	9	40	20.473	0.935[a]	0.004*
SG vs. WG	Cerebellum	Right	39	-63	-42	153	20.202	-1.172[b]	< 0.001*

DLPFC, dorsolateral prefrontal cortex; MNI: Montreal Neurological Institute; ANCOVA analysis, GRF corrected, voxel $P < 0.005$, cluster $P < 0.005$. AG: acupuncture group; SG: sham acupuncture group; WG: waiting list group; FC: functional connectivity; ES: effect size. FC data are tested for post-treatment minus pre-treatment. a: Mann-Whitney U; b: two sample t-test; *: $P < 0.05$. Effect sizes were depicted as Cohen's d and computed at https://www.psychometrica.de/effect_size.html

control pathways associated with DLPFC to exert the therapeutic effect of KOA pain [28]. Combined with previous research, the functional abnormalities of DLPFC could be reversed after acupuncture intervention [17, 22], our results suggested that acupuncture could modulate DLPFC activity to alleviate pain levels in KOA patients. Meanwhile, the DLPFC is reported to be a major brain region involved in the processing of the placebo effect [18, 29], and the function and biochemistry of DLPFC were related to the expression of placebo analgesia [19, 30]. Similarly, by setting up the WG, compared with SG, our study further found that decreased activity of DLPFC was associated with the analgesic effect of acupuncture placebo effect (NRS score decreased in the SG). In summary, we suspect that the two mechanisms involved in local brain activity changes of DLPFC (cognitive pathway of pain and placebo analgesia) have a coordinated

role in pain relief mediated by different components of acupuncture.

The thalamus is involved in the processing of the sensory and affective components of pain [31], and the abnormal changes in its functional metabolite and subcortical volume are associated with chronic musculoskeletal pain disorders (e.g., KOA) [32, 33]. Animal experiment showed that electroacupuncture could activate neurons in the thalamus to relieve pain [34], and some fMRI meta-analyses found that acupuncture stimulation could activate the thalamus [35], which is involved in the regulatory mechanism of acupuncture for musculoskeletal pain disorders [36]. Acupuncture could treat patients with chronic neck pain by regulating the FC of the dorsal raphe nucleus with the thalamus [37]. A recent fMRI study complemented previous brain pain matrix, suggesting that involvement of the DLPFC in the integration of dynamic FC with thalamus. The thalamus might transmit pain-related emotional and sensory information to the cerebral cortex via the DLPFC [38]. In this study, we found that specific components of acupuncture also modulate the FC between the DLPFC and thalamus in KOA patients, which might be involved in pain sensory and affective processing.

Otherwise, previous neuroimaging studies found abnormal altered of neural functional activity in the cerebellum in patients with KOA [39, 40]. The cerebellum is involved in motor control, cognition, emotion, and social behavior, and the neurotransmitters located in the cerebellum are also involved in responding to the environment [41]. At the cellular level, the prefrontal cortico-ponto-cerebellar pathway might play an important role in pain treatment in which the cerebellum is involved in cognitive pain regulation [42]. An earlier study showed that differences of fMRI signals in the cerebellum after placebo acupuncture treatment were associated with differences in subjective pain rating [43]. In the present study, sham acupuncture induced in changes in the FC of prefrontal cortex-cerebellum, suggesting that the placebo effect of acupuncture may be related to pain cognitive regulation and environmental response during sham intervention. Importantly, the DLPFC and thalamus are also key components of the cognitive control network, and the cerebellum is part of the sensorimotor network. Meanwhile, it is suggested that acupuncture could relieve pain by regulating specific networks (integral for sensory, affective, and cognitive processing) [44].

Some studies found that the immune pathway plays a promoting role in pain, and the neuroimmune interaction is involved in the pathogenesis of osteoarthritic pain [45, 46]. Continuous pain stimulation might lead to neuroinflammation, which is widespread in KOA, reshaping neural pathways and affecting the structure and function of the brain [47, 48]. An fMRI study showed that exercise could regulate immune indicators which is associated with the changes of DLPFC-related FC [28]. These suggested that the regulation of related immune pathways might also be the potential mechanism for pain relief in patients with KOA. However, in this study, there was no analysis involving the neuroimmune mechanism. In the future, we will combine neuroimmune and neuroimaging techniques to explore the potential mechanisms of acupuncture.

Our study included neuroimaging scans, a battery of questionnaires assessing pain characteristics, and a proper no treatment group to disentangle the specific effect of acupuncture from nonspecific effect. The analgesic regulatory mechanism of acupuncture's specific effect might involve multiple dimensions (cognitive, sensory, and affective) of the pain experience. The nonspecific component of acupuncture might be more related to cognitive regulation pathways to exert the main placebo effect. Acupuncture practitioners should pay attention to the nonspecific effect, such as friendly communication with patients in the daily clinical environment, which could help improve the overall therapeutic effect. The present findings must be interpreted in the light of several limitations. First, the correlation analyses were conducted with two-sided significance levels (alpha = 0.05) without corrections for multiple comparisons due to the small sample size and the exploratory nature of the study. The results of this study need to be interpreted with caution, and future studies need to expand the sample size to capture broader clinical heterogeneity and to verify the present conclusions. Second, nonspecific effect could occur not only in pain disease but also in different physiological systems in healthy volunteers. Similar studies are necessary to investigate the placebo effect of acupuncture in the treatment of many different clinical conditions. Third, follow-up and multiple time point fMRI data collection were not performed after acupuncture, and we speculate that the long-term effects of acupuncture may lead to lasting changes in brain structure and function. Future studies will focus on the neural mechanisms of long-term effects at multiple levels.

Conclusions

The present findings provide neural basis for the nonspecific effect of acupuncture and reveal the distinction and relation between the mechanism of specific effect. The DLPFC was involved in the regulation of pain cognitive pathways in specific effect of acupuncture, and plays a placebo analgesic role in nonspecific effect. The FC between the DLPFC and thalamus suggested that the specific effect of acupuncture involved multi-dimensional sensory and affective processing of pain. The FC between the cerebellum and DLPFC showed that pain cognitive

regulation and environmental response were important components in the nonspecific effect of acupuncture.

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Author contributions
All authors contributed to the manuscript, approved the final document, and agreed to be accountable for all aspects of the work. Experimental design: N.-Z, X.-Y. Wei, X. Wang, J.-F. Tu. Data acquisition: X.-Y. Wei, N.-Z, J.-L. Li, M.-M. Ren, J.-L. Liu, H. Zhou, C.-K Lee. Data analysis/interpretation: X.-Y. Wei, Z.-Y. Wang. Supervision and funding acquisition: C.-Z. Liu, J.-F. Tu, G.-X. Shi,. Manuscript preparation/revision: X.-Y. Wei, X. Wang. All authors reviewed the manuscript.

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Data availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
This study has been approved by the ethics committee of Dongzhimen Hospital Affiliated with Beijing University of Chinese Medicine (DZMEC-KY-2017-53-02). All participants provided written informed consent according to the Declaration of Helsinki after study procedures were explained to them thoroughly.
Our study adhered to CONSORT guidelines.

Competing interests
The authors declare no competing interests.

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