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Original Research Articles

Characterizing Heat-Sensitization Responses in Suspended Moxibustion with High-Density EEG

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Abstract

Objective. We have reported "heat-sensitization" responses during suspended moxibustion, whose occurrence is associated with significantly better therapeutic effects. The present study aimed to characterize the electrophysiological features of this interesting phenomenon with high-density electroencephalography (EEG).

Methods. We performed EEG recording in a group of patients with chronic low back pain before, during, and after moxibustion treatment at DU3.

Results. 12 out of 25 subjects experienced strong heat-sensitization during moxibustion, which was accompanied by increased power spectral densities (PSDs) at the theta, alpha, and beta frequency bands. The scalp topographies of averaged power indicated that the theta and beta PSD changes were most obvious in fronto–central regions, whereas those of the alpha band were more global. In addition, nonsensitized and sensitized groups showed distinct activity patterns, with heat-sensitization inducing increased phase coherence at the theta and beta ranges.

Conclusions. These data were the first objective evidence of heat-sensitization responses during suspended moxibustion, which were characterized by widespread oscillatory changes in scalp EEG.

Key Words. Moxibustion; Heat-sensitization; Chronic Low Back Pain; Electroencephalography; Acupuncture

Introduction

Acupuncture and moxibustion have been used to treat a variety of diseases in China for over 2,000 years. Unlike acupuncture which uses mechanical stimulation, moxibustion is primarily a type of thermal stimulation with burning dried plant materials (*Artimisia moxa*). It can be applied either with a small amount of burning *moxa* directly on the skin (direct moxibustion) or with heat generated from burning *moxa* 3–5 cm away from the skin surface (suspended or indirect moxibustion, supplementary Figure S1). Moxibustion exerts antinociceptive, anti-inflammatory, and immunomodulatory effects in humans, and gains increasing popularity from both clinicians and researchers worldwide [1–7].

We previously reported a "heat-sensitization" phenomenon during suspended moxibustion [1]. That is, patients often become sensitized to moxibustion stimulation at certain locations on the body. They experience strong warmth or heat spreading around the stimulating site or penetrating into the body, which quite frequently is accompanied by pleasant feelings. These locations are not always acupoints anatomically, but may change within a certain range centered by specific acupoints during the progression of disease. Each disease has a specific set of such sensitized acupoints, and such phenomenon is not

Table 1 Clinical characteristics of recruited CLBP patients

	Nonsensitized Group	Sensitized Group	*P*
Number of patients	13	12	>0.05
Gender: M/F	8/5	6/6	>0.05
Age: mean in years (SE)	42.7 (3.4)	47.3 (4.0)	>0.05
Duration of pain: mean in months (SE)	45.9 (17.4)	44.2 (11.0)	>0.05

CLBP = chronic low back pain.

commonly seen in healthy volunteers. For example, patients with chronic low back pain (CLBP) frequently showed heat-sensitization around Yaoyangguan (DU3) areas [2–4]. Recent randomized controlled trials revealed that patients with heat-sensitization responses during moxibustion had significantly better clinical outcomes than those without [1–3,5], and that performing moxibustion on these functionally sensitized locations yields better therapeutic effects than the anatomically defined but nonsensitized acupoints [3]. These studies suggest a potential link between the sensitization responses induced by moxibustion and its modulatory effects on pain and inflammation. However, there are no objective evidences for the presence of these responses, nor information about their biological mechanisms.

The noninvasive feature of electroencephalography (EEG) allows its application in conscious human subjects. Oscillatory and coherent activities recorded from scalp EEG reflect intra- and inter-regional interactions in the brain and their characteristic changes have been identified in a number of physiological and pathological conditions [8–10]. The strong perceptual and emotional responses during heat-sensitization in moxibustion almost definitely implicate cortical activities. In the present study, we performed high-density EEG recording in a group of patients with CLBP before, during, and after moxibustion treatment and studied EEG changes associated with the heat-sensitization responses.

Methods

Subjects and Ethics Statement

Twenty-five right-handed Chinese patients suffering from CLBP were recruited to receive moxibustion treatment in the Affiliated Hospital of Jiangxi University of TCM (Table 1). The diagnosis of CLBP was in accordance with previous guidelines [11]. These patients aged between 18 and 60 years and none had a history of major medical illness, head trauma, neuropsychiatric disorders, evidence of root damages, or other diseases that would affect brain activities. A previous study had shown that quantitative EEG of such patients was comparable with healthy volunteers [12]. The experimental procedures were approved by the Local Ethical Committee on Human Studies based in Jiangxi University of TCM and in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All patients were given written informed consent before the experiment.

Moxibustion and Grouping

The patients took a comfortable prone position in a quiet and dimly lit room. An experienced clinician performed suspended moxibustion with two *moxa* sticks over Yaoyangguan (DU3), which locates between the fourth and fifth lumbar vertebra. The *moxa* sticks were fixed 3–5 cm above the skin surface to get a skin surface temperature of 41°C (supplementary Figure S1). The treatment lasted 30 minutes. Patients who experienced strong warmth or heat penetrating into the body, spreading around the stimulating site, or conducting along the spine [1] fell into the heat-sensitized group (N = 12), whereas those experiencing only mild local warmth entered the nonsensitized group (N = 13) (Figure 1).

EEG Recording and Preprocessing

Spontaneous resting EEG was recorded via a 128 channel EGI (Electrical Geodesics, Inc.) system. Patients were requested to keep relaxed but alert with eyes closed. The central electrode Cz served as the reference during

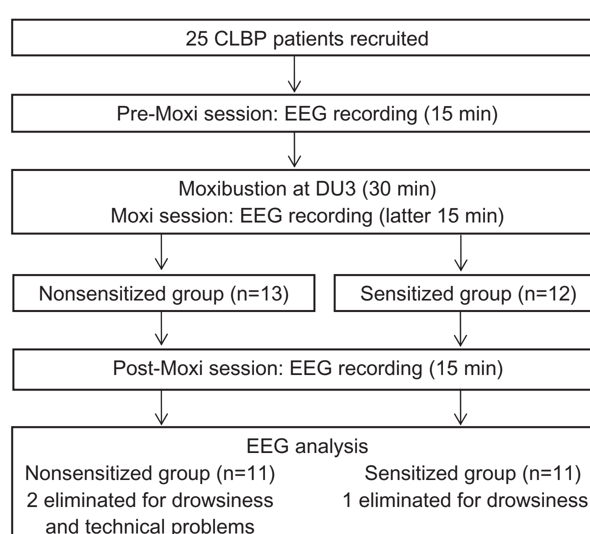

Figure 1 Experimental flowchart.

recording which was re-referenced to the averaged ear lobe off-line. The sample frequency was 1,000 Hz with a notch filter at 50 Hz. The electrode impedances were under 50 kΩ. Three sessions(15 minutes each) of resting EEG were recorded for each patient: one before moxibustion (pre-Moxi session), one during the last 15 minutes of moxibustion (Moxi session), and one immediately after moxibustion (post-Moxi session).

EEG data were resampled at 250 Hz with a band pass filtered off-line in the frequency range of 1–100 Hz for removing linear trends. The middle 5 minutes of each session was chosen for further analysis. Conspicuous artifacts were eliminated by visual inspection on time series and data exceeding ±80 μV in any channel were rejected by manual, remaining data: 252.67 ± 42.69 seconds per subject in a range of 144.92–299.63 seconds. Due to equipment problems, data from two of the original 128 channels were off-line replaced by the average data from the neighboring channels. In addition, 38 out-line channels were excluded because of their vulnerability against muscle activities or eye movements, leaving a total of 90 channels for further off-line analysis (supplementary Figure S2).

Power Spectral Densities (PSDs)

Data were analyzed off-line in Matlab interface (The Mathworks, Natick, MA) using Matlab and EEGLAB (http://www.sccn.Ucsd.edu/eeglab/index.html). Spectra power analysis used the EEGLAB spectopo method (with a window length of 512 points, fast Fourier transform (fft) length of 1,024 points, non-overlap) to estimate and plot PSDs of EEGs per frequency. To compare spectral parameters, all P values are two sided from nonparametric Wilcoxon tests. To summarize the data, we first averaged the log-transformed spectra of the 90 scalp electrodes for each subject (spectra from all electrodes had similar shapes and scales). The EEG data were further divided into five different bands: delta (δ, 1–4 Hz), theta (θ, 4–8 Hz), alpha 1 (α1, 8–10 Hz), alpha 2 (α2, 10–13 Hz) and beta (β, 13–30 Hz), and the PSDs of each band were estimated again using Welch's averaged modified periodogram method (MATLAB function: window length: 256, fft length: 256, overlap 50%).

Phase Coherence Analysis

To calculate the correlation coefficient among signals from regions of interest, magnitude squared coherence (MSCxy) in the theta, alpha, and beta frequency bands was measured. The coherence (value lies between 0 and 1) was a function of the averaged PSD (Pxx and Pyy) and the averaged cross PSD (Pxy) of x and y signals. Mathematically, MSCxy is equal to Pxy normalized by Pxx and Pyy, namely, MSCxy = |Pxy|2/(Pxx × Pyy). MSCxy equals 0 means that the two signals have no linear relationship at a given frequency. However, when two signals are completely phase-locked and have a constant amplitude ratio, MSCxy = 1, which means the two signals are in a good phase synchronization. Here we chose 18 out of 90 channels according to the standard 10–20 system, including Fp1/Fp2, Fz/F3/F4/F7/F8, C3/C4, T3/T4, Pz/P3/P4, T5/T6, O1/O2 (Cz, as the reference in recording session, was excluded here). The coherence spectra of frequency bands for all pairs of the 18 electrodes were calculated with a window length of 2 seconds, fft length of 1 second, and 50% overlap. The correlation coefficients of phase coherence of each electrode pair were compared with paired *t*-tests.

Results

Heat-Sensitization Responses During Moxibustion

With 30 minutes moxibustion at DU3, 12 out of 25 CLBP patients experienced moderate to strong heat-sensitization, whereas 13 reported only local warmth. They fell into the sensitized and nonsensitized groups, respectively. The general information of these patients did not differ between groups (Table 1).

Increased PSDs at Theta, Alpha1 and Beta Bands During Heat-Sensitization

To determine the EEG correlates of heat-sensitization during moxibustion, PSDs of the pre-Moxi, Moxi, and post-Moxi sessions were analyzed. In the nonsensitized group where patients experienced only local warmth, no differences of averaged PSDs were found at any frequency bands between the three sessions ($P > 0.05$, Wilcoxon tests, Figure 2A and C). In contrast, heat-sensitization responses in the sensitized group during moxibustion were accompanied by significant increases of PSDs in the theta, alpha1, and beta bands ($P < 0.05$, Wilcoxon tests, Figure 2B and D). The PSD increase in the beta range persisted to the post-moxibustion phase. Direct comparisons of PSDs of 90 electrodes at each frequency points from 1.2 to 30 Hz also indicated significantly more changes in the sensitized group (Figure 3A–D, and supplementary Figure S2).

The scalp topographies of average PSDs indicated that changes in the theta and beta bands were strongest in frontal–central regions, whereas those in the alpha1 band were more global (Figure 3E).

Increased Phase Coherence at Theta and Beta Bands During Heat-Sensitization

We next examined the phase coherence for theta, alpha, and beta frequency bands between pre-Moxi and Moxi sessions and between pre-Moxi and post-Moxi sessions. For all frequency bands, relatively few changes were detected in the nonsensitized group, whereas many electrode pairs showed significantly increased phase coherence between the pre-Moxi and Moxi sessions in the sensitized group. For the theta band (Figure 4), coherence between electrodes at the central–frontal regions significantly increased during heat-sensitization, which was in sharp contrast to that of the nonsensitized patients. But these changes were not present in the post-Moxi phase.

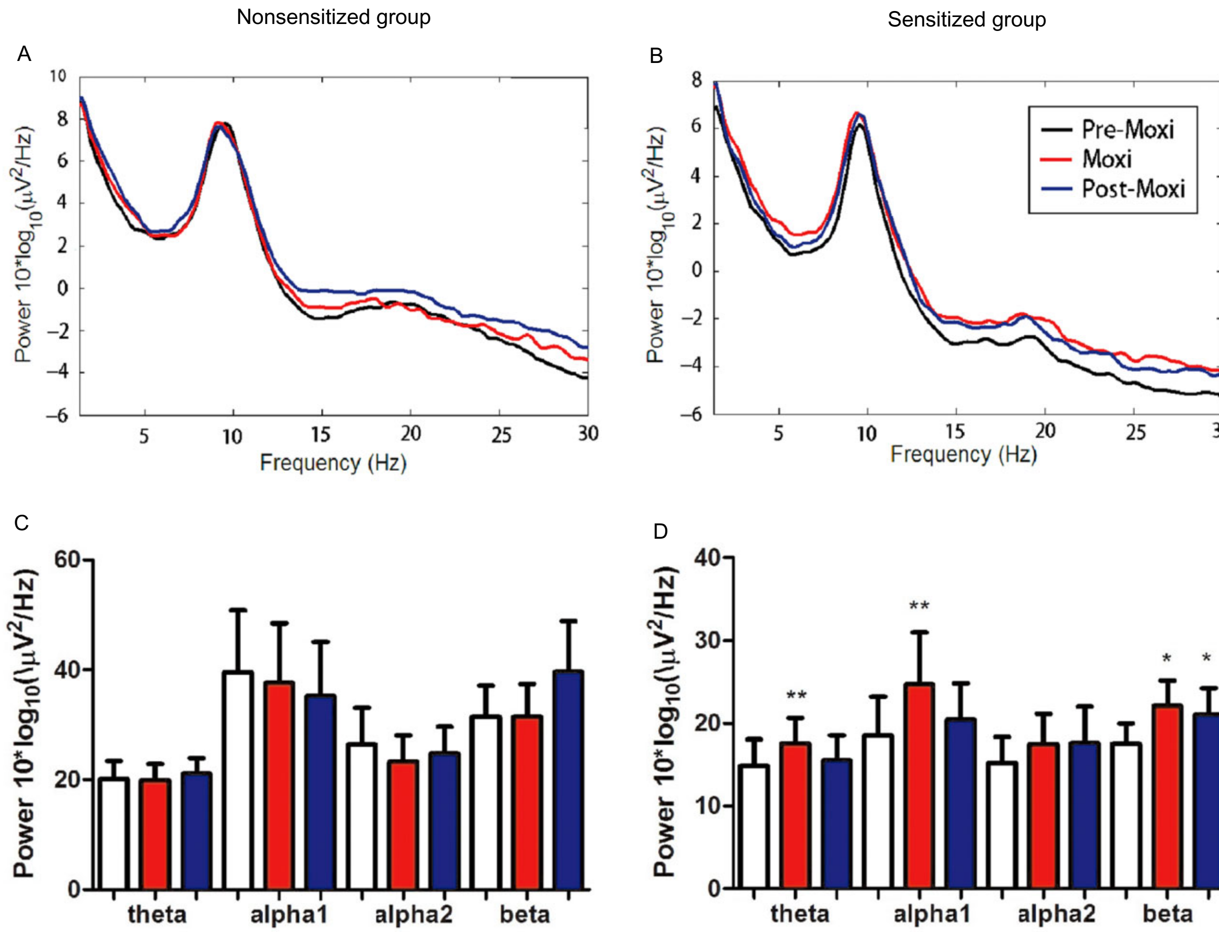

Figure 2 Average PSDs of 90 channels before (pre-Moxi session, black), during (Moxi session, red) and after (post-Moxi session, blue) moxibustion. No changes were detected at any frequency bands in the nonsensitized group (A and C). In the sensitized group (B and D), PSDs of theta, lower alpha, and beta bands significantly increased during moxibustion. Changes in the beta band persisted after moxibustion. $^{*}P < 0.05$, $^{**}P < 0.01$, two-sided Wilcoxon tests.

No obvious changes in the alpha band were present during moxibustion, regardless of the occurrence or absence of heat-sensitization (Figure 5). Phase coherence changes at the beta band were more global during heat-sensitization, and persisted even after the end of moxibustion (Figure 6). The clear difference between the nonsensitized and sensitized groups indicated distinct brain activity patterns between the two states, and implied potential inter-regional cross-talks during the heat-sensitization of moxibustion.

Discussion

The present study was designed to characterize moxibustion-induced heat-sensitization responses with EEG. Rhythmical activities of different frequency ranges recorded from EEG have their specific behavioral or cognitive correlates. For example, beta oscillations considered as an index of cortical arousal and beta synchronization between temporal and parietal cortexes appear during multimodal semantic processing, whereas long range frontal–parietal interactions during cognition induce theta and alpha oscillations [8–10]. Here we showed significant increases of the PSDs of theta, alpha, and beta bands in the central–frontal–parietal areas during heat-sensitization. Moreover, heat-sensitization was accompanied with increased phase coherence, indicating strong interactions between these regions. These changes were not observed in patients without heat-sensitization responses.

As far as we knew, the present study was the first EEG study of moxibustion. An interesting finding was that significant EEG changes were not observed in all subjects, but only in a proportion of patients with heat-sensitization responses. These changes provided objective indications of the subjective perceptions. One clear factor affecting

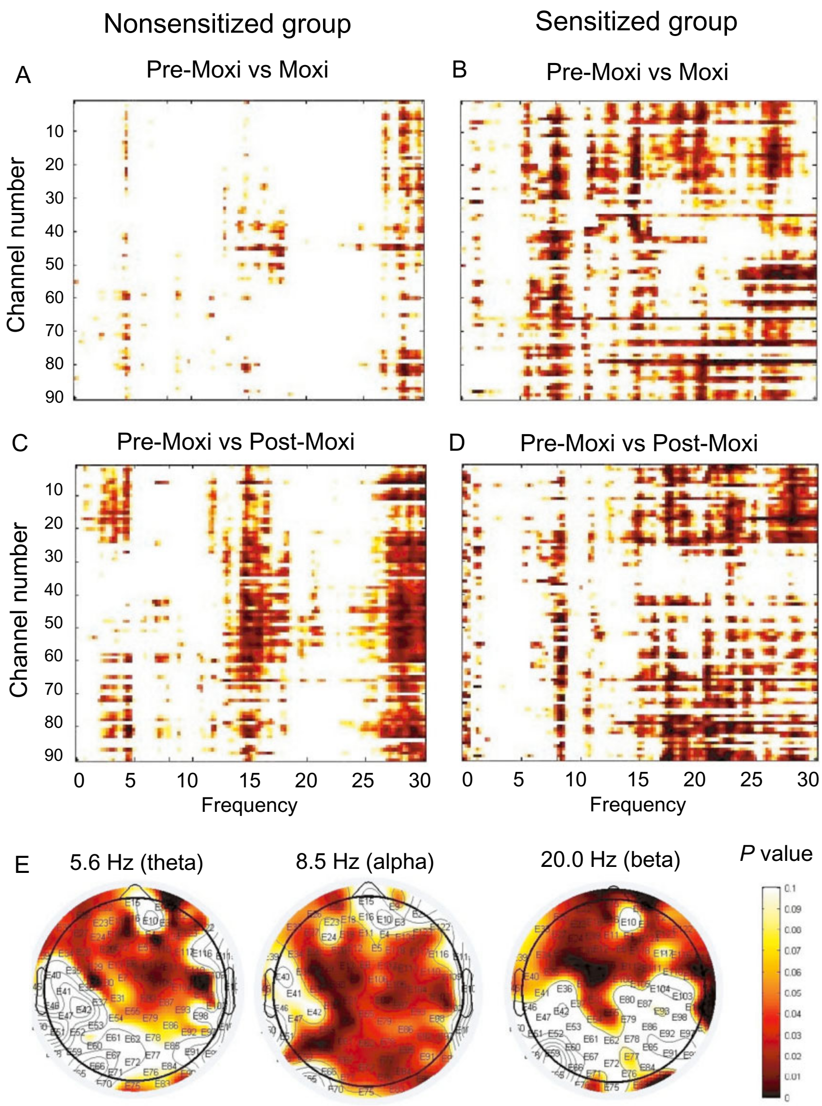

Figure 3 Statistical comparison of PSDs at each frequency points. Shown were the *P* values from Wilcoxon tests between pre-Moxi and Moxi sessions of nonsensitized (A) and sensitized (B) groups, and those between pre-Moxi and post-Moxi sessions of nonsensitized (C) and sensitized (D) groups. (E) The scalp topographies of PSD differences between pre-Moxi and Moxi sessions at 5.6 Hz (theta, left), 8.5 Hz (alpha, middle), and 20.0 Hz (beta, right) of the sensitized group. Changes at the theta and beta ranges were most obvious in the frontal–central regions, whereas changes at the alpha1 range were more global. No changes were detected in the nonsensitized group.

the occurrence of heat-sensitization is the state of the subject. Heat-sensitization could be induced in no more than 30% of healthy volunteers, but 50–70% under morbid states [1]. In the present study, heat-sensitization at DU3 appeared in 12 out of 25 CLBP patients. However, it is needed to note that failure to induce heat-sensitization at DU3 did not exclude the presence of such responses in other regions of the body. So the grouping in the present study only served for EEG characterization of heat-sensitization but could not reflect the presence or absence of such responses in each patient *per se*. It is still unclear why this phenomenon occurs in some patients but not others. Clinical data from the present study did not support gender, age, and pain duration as crucial factors, consistent with results from our previous studies [2–6].

It is interesting to compare current findings from moxibustion with those on acupuncture, which had been more intensively studied for years. Previous neuroimaging studies have shown that deqi sensation of acupuncture is associated with the deactivation of a limbic–paralimbic–neocortical network and activation of somatosensory brain regions [13]. Data from EEG recording during acupuncture were not consistent across studies due to different experimental designs and stimulating protocols, but all revealed widespread electrophysiological changes [14–20]. Sakai et al. [14] showed that acupuncture nonspecifically increased power of all spectral bands except the gamma band. Zhang et al. [15] and Tanaka et al. [16] reported similar results and further showed that these electrophysiological changes were correlated with analgesic effects. These results were comparable with our data from moxibustion, indicating some overlapping mechanisms under both situations, for example, widespread cortical and subcortical interactions. But they bear significant differences as well. The deqi response in acupuncture includes a variety of sensations such as aching, pressure, soreness, heaviness, fullness, warmth, cooling,

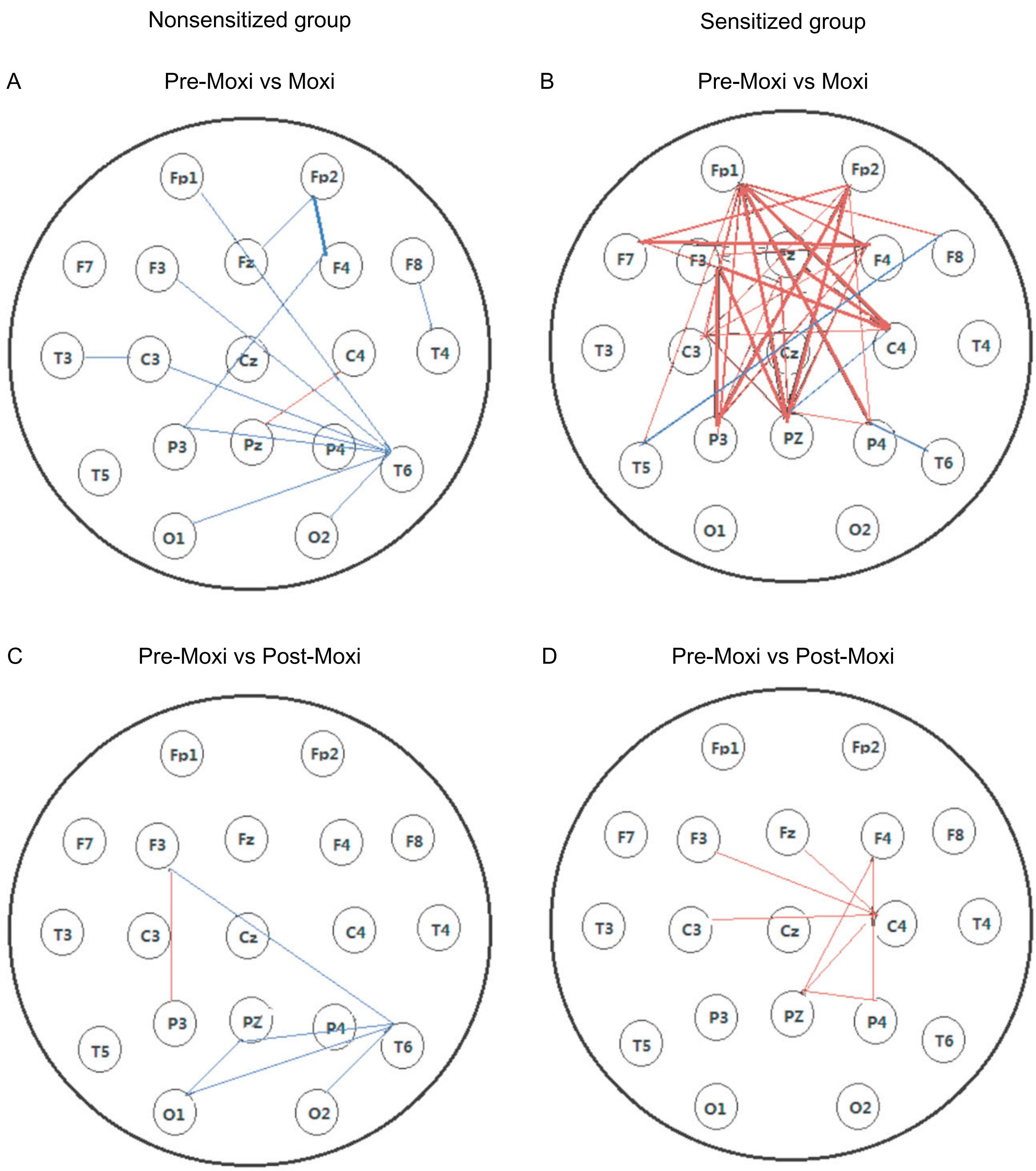

Figure 4 Phase coherence changes at the theta frequency range of nonsensitized (A and C) and sensitized (B and D) groups. Significant changes were observed in the sensitized group between pre-Moxi and Moxi sessions (B). Blue lines indicated coherence decrease and red indicated increase. Line thickness indicated the level of statistical significance (thin lines: $P < 0.05$; thick lines: $P < 0.01$; paired t tests).

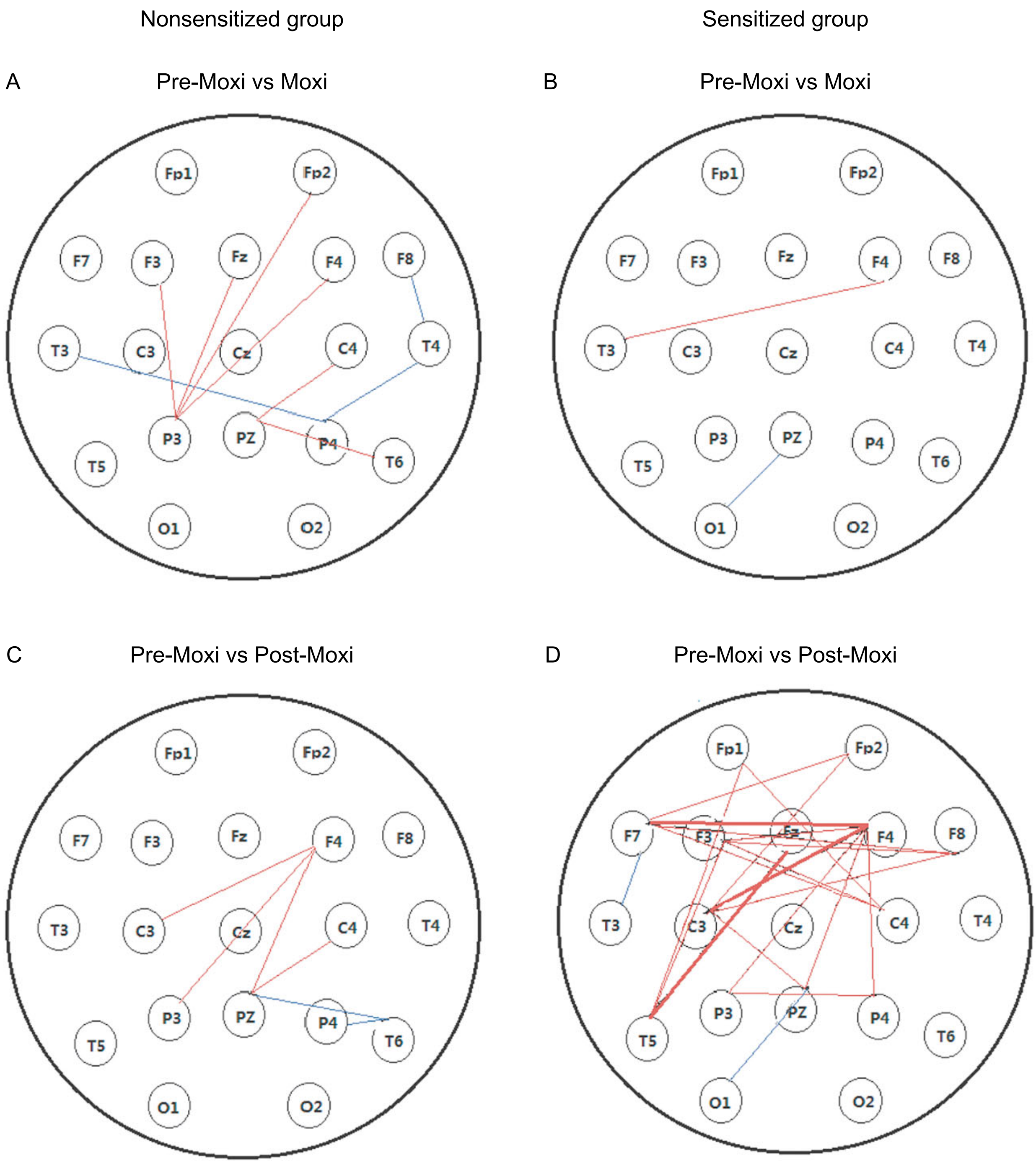

Figure 5 Phase coherence changes at the alpha frequency range of nonsensitized (A and C) and sensitized (B and D) groups. Blue lines indicated coherence decrease and red indicated increase. Line thickness indicated the level of statistical significance (thin lines: $P < 0.05$; thick lines: $P < 0.01$; paired t tests).

numbness, tingling, and dull pain [13], and is not disease specific. In contrast, the heat-sensitization in moxibustion is not commonly observed in healthy subjects and is mainly composed of strong warmth or heat spreading around the stimulating site or penetrating into the body [1]. The clear difference in induction (mechanical vs thermal stimulation) and sensory modalities implies distinct nature between them.

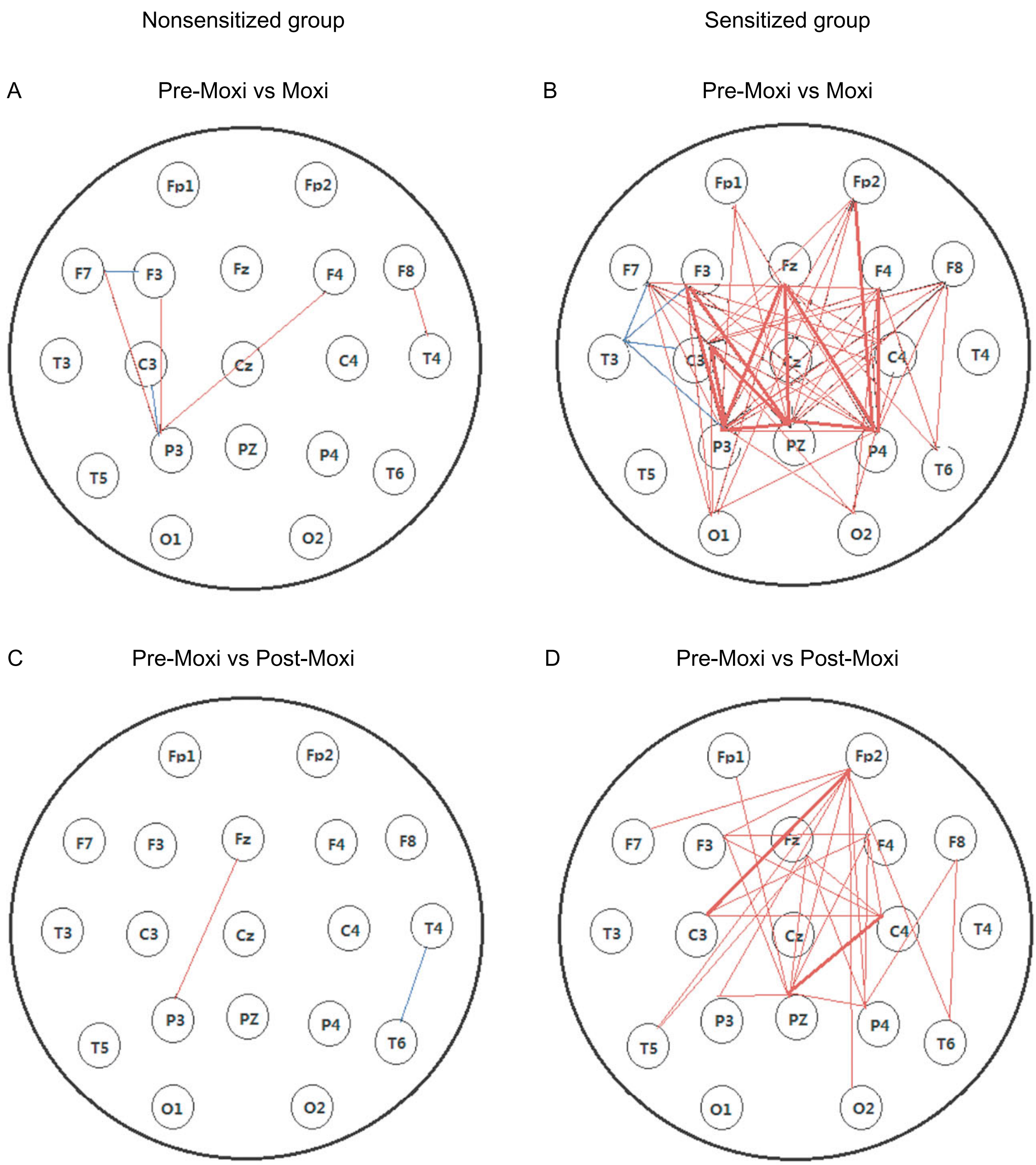

Figure 6 Phase coherence changes at the beta frequency range of nonsensitized (A and C) and sensitized (B and D) groups. Significant changes were observed in the sensitized group between pre-Moxi and Moxi sessions (B) and between pre-Moxi and post-Moxi sessions (D). Blue lines indicated coherence decrease and red indicated increase. Line thickness indicated the level of statistical significance (thin lines: $P < 0.05$; thick lines: $P < 0.01$; paired t tests).

What are the mechanisms of heat-sensitization? Most of the sensitized points sit around the pathological regions, but they can be detected at distant locations as well. So local, spinal, and supra-spinal mechanisms may all contribute. Furthermore, the dynamically changed sites of sensitization with disease progression do not support a pure structural explanation. Functional mechanisms such as peripheral or central sensitization may be involved. A number of pain conditions, such as myofascial pain syndrome, are characterized by the presence of "trigger points," points tender to applied stimuli. These trigger points share many similarities with heat-sensitized sites, suggesting some common pathophysiological basis [21]. It is known that moxibustion promotes the local release of neuropeptides such as substance P and calcitonin gene-related peptide [22,23]. Heat shock proteins are also induced in the local areas [24]. In the central nervous system, moxibustion modulates dopaminergic and serotonergic metabolism in reward-related regions [25,26]. This is consistent with our clinical observation that heat-sensitization during moxibustion is frequently accompanied with pleasant feelings [1].

A crucial clinically relevant question is the behavioral correlates of heat-sensitization and/or the corresponding EEG changes. Previous clinical trials indicated that the presence of heat-sensitization during moxibustion treatment is correlated with better therapeutic effects, not only in low back pain [2–4] but also in other diseases such as knee osteoarthritis [5,6] and persistent asthma [7]. Although increasing evidence confirms the therapeutic effects of moxibustion, very few studies have addressed the underlying mechanisms. This was not the aim of the present study, but it was rational to hypothesize that the significant EEG changes accompanying heat-sensitization reflected pain and inflammatory modulation. Pain itself significantly affects central neuroplasticity and EEG [12,15,20,27,28], and as discussed earlier, analgesic manipulations such as acupuncture induce widespread EEG changes. The increases of the PSDs of theta, alpha, and beta bands in patients with heat-sensitization were most obvious in the frontal–central regions. These areas include a number of pain-related cortices, including pre-frontal cortex and anterior cingutate cortex (ACC). ACC has been shown crucial for at least some types of acupuncture [29]. Considering the presence of pleasant feelings during heat-sensitization, cognitive and affective modulation would be one rational mechanism of moxibustion analgesia.

In conclusion, our pilot EEG data provided the first objective evidence of heat-sensitization responses during suspended moxibustion, which were accompanied with widespread oscillatory changes in scalp EEG. Further investigation is required to elucidate its behavioral correlates and the underlying mechanisms.

Acknowledgments

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's Website:

Figure S1 Suspended moxibustion was performed with burning *moxa* sticks suspended above the body. The exact distance was adjusted to reach a skin surface temperature of around 41°C.

Figure S2 Scalp localization of 90 out of 128 EEG channels included in data analysis.