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Special Topic for 973 Program

Influence of different-distance mild moxibustion at Zusanli (ST 36) on functional brain imaging in healthy population

不同距离温和灸正常人足三里穴对脑功能成像的影响

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Abstract

Objective: To explore the beneficial regulatory effect of mild moxibustion from different distances at Zusanli (ST 36) of healthy population on the functions of temperature-related brain regions.

Methods: In 20 recruited healthy subjects, the change of the temperature-related brain regions induced by mild moxibustion from different distances at Zusanli (ST 36) was observed by functional magnetic resonance imaging (fMRI).

Results: In comparison of the values in amplitude of low-frequency fluctuation (fALFF) during and before moxibustion, it has been found that in moxibustion of 2 cm distance, fALFF value increased in the brain regions of the left anterior cingulated cortex and lateral surrounding cerebral regions, and fALFF value decreased in the cerebral regions of the peripheral cortex of the calcarine fissure; in moxibustion of 3 cm distance, fALFF value increased in the brain regions of the right and medial side and paracingulated gyrus, and fALFF value decreased in the cerebral zone of the left middle temporal gyrus; in moxibustion of 4 cm distance, fALFF value increased in the brain regions of the right and medial and paracingulated gyrus; and in moxibustion of 5 cm distance, fALFF value increased in the brain regions of the left hippocampus. In comparison of the value of regional homogeneity (ReHo), it has been found that in moxibustion of 2 cm distance, ReHo value increased in the cerebral zone of the posterior lobe of the right cerebellum, and ReHo value decreased in the cerebral zone of the right occipital lobe; in moxibustion of 3 cm distance, ReHo value increased in the brain regions of the left cerebellar posterior lobe and left frontal lobe, and ReHo value decreased in the cerebral zone of the right inferior temporal gyrus; in moxibustion of 4 cm distance, ReHo value increased in the brain regions of the right superior frontal gyrus and ReHo value decreased in the brain regions of the right parietal lobe and angular gyrus; in moxibustion of 5 cm distance, ReHo value increased in the cerebral zone of the right frontal lobe and ReHo value decreased in the cerebral zone of the right brainstem.

Conclusion: In moxibustion of 3 cm distance, the changes in the brain regions basically conform to the transmission route of body trunk temperature.

Keywords: Moxibustion Therapy; Moxa Stick Moxibustion; Point, Zusanli (ST 36); Research on Acupoints; Magnetic Resonance Imaging; Healthy Volunteers

【摘要】目的：探讨不同距离温和灸正常人足三里穴对温度相关脑区功能的良性调整作用。**方法：**纳入 20 例健康受试者，通过功能磁共振观察不同距离温和灸正常人足三里穴引起的温度相关脑功能区的变化。**结果：**比较艾灸过程中与艾灸前的低频振幅(fALFF)值，发现灸距 2 cm 时，左侧扣带回前部及侧面环绕脑区等脑区的 fALFF 值升高，距状裂周围皮层等脑区的 fALFF 值降低；灸距 3 cm 时，右侧内侧和旁扣带脑回等脑区的 fALFF 值升高，左侧颞中回等脑区的 fALFF 值降低；灸距 4 cm 时，右侧内侧和旁扣带脑回等脑区的 fALFF 值升高；灸距 5 cm 时，左侧海马等脑区的 fALFF 值升高。艾灸后与艾灸前局部一致性(ReHo)值比较，发现灸距 2 cm 时，右侧小脑后叶等脑区的 ReHo 值升高，右侧枕叶等脑区的 ReHo 值降低；灸距 3 cm 时，左侧小脑后叶、左侧额叶的 ReHo 值升高，右侧颞下回等脑区的 ReHo 值降低；灸距 4 cm 时，右侧额上回的 ReHo 值升高，右侧顶叶、角回等脑区的 ReHo 值降低；灸距 5 cm 时，右侧额叶等脑区的 ReHo 值升高，右侧脑干等脑区的 ReHo 值降低。**结论：**灸距为 3 cm 时脑区变化

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基本符合躯体温度传导路径。
【关键词】灸法；艾条灸；穴，足三里；穴位研究；磁共振成像；健康受试者
【中图分类号】R245.8　　【文献标志码】A

Moxibustion is an important component of acupuncture therapy and its functional mechanism involves many factors, such as medicinal constituents, themogenetic effect, optical radiation effect and combustion products and aromatherapy of moxa[1]. Among them, the themogenetic effect is one of the important effective factors in moxibustion. The themogenetic effect in moxibustion is closely related to change of temperature. Studies have indicated that tolerable temperature of moxibustion is different in different-distance moxibustion[2-3]. Therefore, the appropriate temperature in moxibustion during the treatment is significantly important for clinical treatment, but its mechanism is still unknown. The resting state functional magnetic resonance imaging (rs-fMRI) does not involve complicated operation. It is easy in operation[4] and strong in repeatability, and is suitable for diagnosis of diseases and evaluation of the clinical effects of the therapeutic modalities. Nowadays, the study on acupuncture therapy by rs-fMRI is mainly focused on acupuncture mechanism, less on mechanism of moxibustion[5]. By selecting Zusanli (ST 36) as target acupoint, this study was designed to observe the images of cerebral functions of the subjects during the whole moxibustion of different distances at Zusanli (ST 36) of healthy population, to compare the changes of the brain regions before and after moxibustion, in a goal to explore the impact of moxibustion on the changes of temperature-related brain regions, in order to provide possible imaging foundation for moxibustion in the central regulatory mechanism.

1 Data and Methods

1.1 Subjects of study

Totally, 20 healthy volunteers at school were recruited in the study, 9 males and 11 females, with the average age of (26.9±2.6) years, average height of (163.0±7.3) cm, and average body weight of (54.6±8.6) kg. The volunteers did not have the history of traumatic injury in the head and mental disorders, without contraindications for MRI scanning. This study had been proved by the Ethic Committee of the First Affiliated Hospital of Hunan University of Chinese Medicine. Before study, the subjects were informed experimental process and signed the informed consents.

1.2 Main materials and instruments

Taiyi moxa rolls (specification: 18 mm × 200 mm, Nanyang Lüying Moxa Grass Biological Products Co., Ltd., China); HDxt 3.0T magnetic resonance imaging instrument (GE, USA).

1.3 Study design

The changes of resting brain functions of the healthy adults were determined before and after mild moxibustion at Zusanli (ST 36) by functional magnetic resonance imaging (fMRI). First, the subjects lied down prone on the magnetic resonance detection equipment for scanning the brain by magnetic resonance. The head coil was used in scanning, with the foamed plastics to fix the head of the subjects to reduce head motion, and cotton balls and earplug to reduce noise. The subjects were told to get familiar to the environment first, and then lie down in prone, with the eyes closed to keep conscious, try not to think, and reduce motion of the body. After starting operation, first the resting scan was given for 3 min. Afterward, left Zusanli (ST 36) was applied with mild moxibustion for 3 min, with the acupoint localized in reference to *Nomenclature and Location of Acupuncture Points* (GB/T 12346-2006)[6]. After moxibustion, the resting scan was given for 10 min. The whole process is a sequence. During this process, scan was given continuously. After the scan finished, the subjects were changed and the above operation was repeated. Totally, the experiment lasted for 4 d, and the distances of moxibustion changed every day (2 cm on the first day, 1 cm added every day, 5 cm on the last day) and the rest items were kept same.

1.4 rs-fMRI scanning sequences

1.4.1 Structure image

3D-BRAVO sequential scanning of structure image was adopted. The scanning parameters were as follows: repeat time (TR)/echo time (TE)＝7.8 ms/3.0 ms, field of view (FOV)＝240 mm× 240 mm, layer thickness＝1.0 mm, interval between layers＝0 mm, matrix＝256 ×256, resolution ratio＝1, flip angle (FA)＝15 °, band-width＝41.67 Hz, and scanning time＝163 s.

1.4.2 Functional image

BOLD-fMRI scanning was adopted by sequences of gradient echo and single-shot echo-planar imaging (EPI). The scanning parameters were as follows: TR/TE＝2 000 ms/30 ms, FA＝90 °, layer thickness＝5.0 mm, interval between layers＝1 mm, voxel size＝3 mm× 3 mm×5 mm, FOV＝240 mm × 240 mm, matrix＝256×256, and scanning time＝980 s.

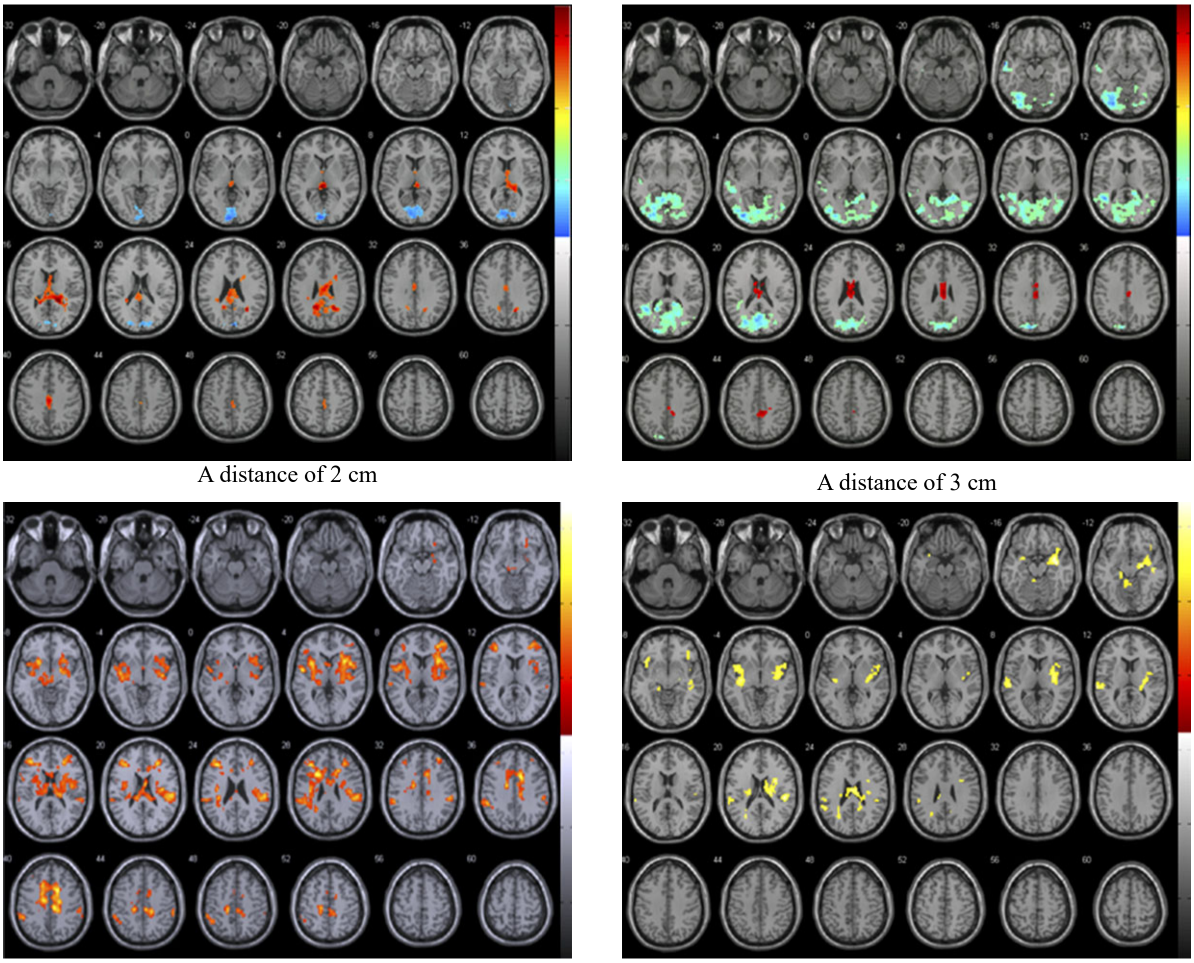
1.5 Pretreatment and statistical analysis of fMRI

MRIcron and SPM were used for pretreatment of data. The data pretreated before and during moxibustion were selected, and 'the specification of the first level' in SPM was used to process the specification and estimation of the experimental model for every subject. In the specification of the models, the previous parameter of head motion was added as regressor into the model design. In the examination of SPM results, the statistical comparison parameter was established to obtain the statistical comparison file as the initial data for group analysis.

2 Results

2.1 Brain activities during mild moxibustion of different distances comparing with those before mild moxibustion

In the differences of the values in factional amplitude of low frequency fluctuation (fALFF), it was found that in moxibustion of 2 cm distance, fALFF value increased compared with that before moxibustion in the brain regions of the left anterior cingulated cortex and lateral surrounding cerebral regions of the subjects, and fALFF value decreased compared with that before moxibustion in the cerebral regions of the peripheral cortex of the calcarine fissure and right cuneus ($P<0.01$, $K>18$). In moxibustion of 3 cm distance, fALFF value increased compared with that before moxibustion in the brain regions of the right and medial side and paracingulated gyrus ($P<0.01$, $K>18$). In moxibustion of 4 cm distance, fALFF value increased compared with that before moxibustion in the brain regions of the right and medial and paracingulated gyrus ($P<0.01$, $K>18$). In moxibustion of 5 cm distance, fALFF value increased compared with that before moxibustion in the brain regions of the left hippocampus ($P<0.01$, $K>18$). Please see the details in Figure 1 and Table 1.



A distance of 2 cm

A distance of 3 cm

A distance of 4 cm

A distance of 5 cm

Figure 1. Changes of brain activities during mild moxibustion

Note: Red color and yellow color represent the activated brain areas, and blue color represents the inhibited brain areas

Table 1. Changes of brain activities during mild moxibustion

Distance	Brain area	Voxel	MNI coordinate of peak point			T value[1)]
			X	Y	Z	
2 cm	Peripheral cortex of the calcarine fissure	333	0	–93	3	–2.73
	Right cuneus	333	6	–87	24	–2.67
	Left anterior cingulated cortex and lateral surrounding cerebral regions	745	–3	–33	0	3.92
	Right thalamus	745	16	–29	10	3.09
3 cm	Left middle temporal gyrus	3 597	–57	–3	–15	–4.23
	Right, medial and paracingulated gyrus	243	12	–36	42	3.10
4 cm	Right, medial and paracingulated gyrus	3 689	18	–30	42	6.24
5 cm	Left hippocampus	134	36	–6	–15	5.98

Note: 1) T value indicates the activation (feedback) of the relative brain area, positive value indicates positive activation (feedback), negative value indicates negative activation (feedback)

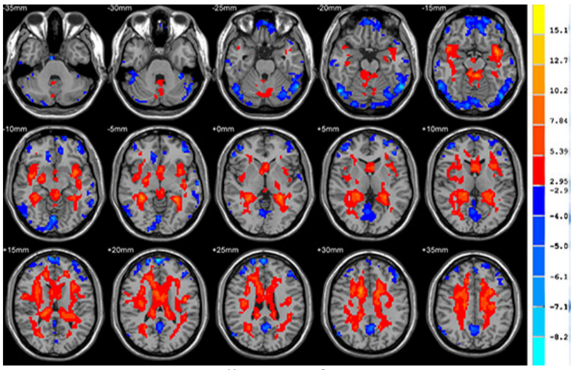
2.2 Changes of brain activities after mild moxibustion of different distances

In the differences of the values of regional homogeneity (ReHo), it was found that in moxibustion of 2 cm distance, ReHo value increased compared with before moxibustion in the cerebral zone of the posterior lobe of the right cerebellum of the subjects, and ReHo value decreased compared with before moxibustion in the cerebral zone of the right occipital lobe ($P<0.01$, $K>18$). In moxibustion of 3 cm distance, ReHo value increased compared with before moxibustion in the brain regions of the left cerebellar posterior lobe and left frontal lobe, and ReHo value decreased compared with before moxibustion in the cerebral zone of the right inferior temporal gyrus ($P<0.01$, $K>18$). In moxibustion of 4 cm distance, ReHo value increased compared with before moxibustion in the brain regions of the right superior temporal gyrus and ReHo value decreased compared with before moxibustion in the brain regions of the right parietal lobe and angular gyrus ($P<0.01$, $K>18$). In moxibustion of 5 cm distance, ReHo value increased compared with before moxibustion in the cerebral zone of the right frontal lobe and ReHo value decreased compared with before moxibustion in the cerebral zone of the right brainstem ($P<0.01$, $K>18$). Please see the details in Table 2 and Figure 2.

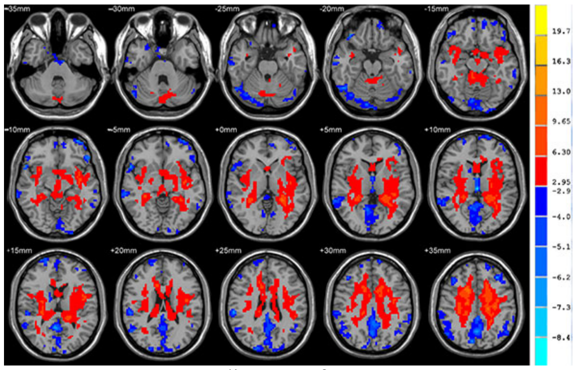
Table 2. Changes of brain activities after mild moxibustion

Distance	Brain area	Voxel	MNI coordinate of peak point			T value[1)]
			X	Y	Z	
2 cm	Right occipital lobe	2755	54	–66	–21	–8.9436
	Left parietal lobe	44	–39	–69	54	–4.7391
	Right posterior cerebellar lobe	28	6	–75	–42	5.0691
3 cm	Right inferior temporal gyrus	82	54	0	–42	–7.0061
	Left superior parietal lobe	34	–36	–69	48	–6.0629
	Left posterior cerebellar lobe	142	–9	–72	–27	5.164
	Left frontal lobe	8 959	–15	9	45	23.0468
4 cm	Right parietal lobe, angular gyrus	94	42	–75	36	–6.6075
	Right frontal lobe	18	51	6	54	–4.4187
	Right superior frontal gyrus	7 416	12	21	48	11.0756
5 cm	Right brainstem	152	6	–21	–45	–6.6359
	Left parietal lobe, angular gyrus	347	–48	–63	33	–6.712
	Right frontal lobe, white matter	10 042	21	–18	36	16.922

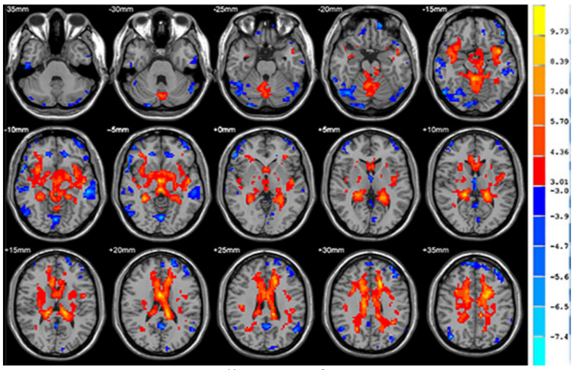
Note: 1) T value indicates the activation (feedback) of the relative brain area, positive value indicates positive activation (feedback), negative value indicates negative activation (feedback)



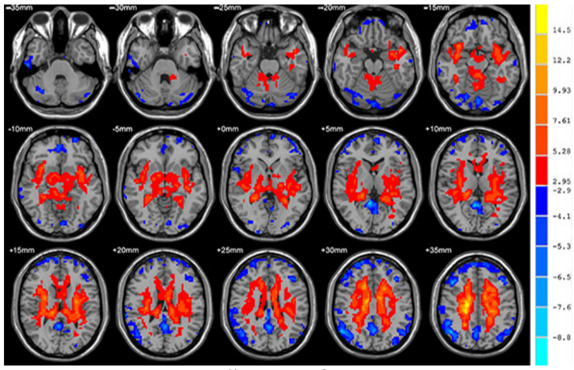
A distance of 2 cm



A distance of 3 cm



A distance of 4 cm



A distance of 5 cm

Figure 2. Changes of brain activities after mild moxibustion

Note: Red color and yellow color represent the activated brain areas, and blue color represents the inhibited brain areas

3 Discussion

Zusanli (ST 36) is the He-Sea point of the Stomach Meridian, the lower He-Sea point of the stomach, acts to strengthen the spleen, dissolve phlegm, harmonize the stomach, descend counter-flow, regulate the intestines, remove stagnation, clear away heat and activate blood, thus supporting the body constitution and also dissipating the pathogens. It is one of the important tonic acupoints and can produce specific therapeutic effects for diseases in six hollow organs. Modern studies have indicated that acupuncture at Zusanli (ST 36) is effective for many systems of the human body, including regulating the functions of the stomach and intestines, enhancing the working ability of cerebral cortex, increasing white blood cell count, adjusting blood pressure, heart rate and improving cardiac functions[7]. Currently, the studies on Zusanli (ST 36) are numerous, including location of acupoint, microstructure, clinical functional mechanism, change and metabonomics in regional cerebral blood flow[8-9]. These studies also provide references to further understanding the relationship between Zusanli (ST 36) and brain functions. Because the subjects need to expose the upper body in the magnetic resonance equipment during the whole experiment, the acupoint for moxibustion is selected from the lower body. Zusanli (ST 36) is a commonly used acupoint in clinical treatment, is beneficial to experimental operation in its location, it is taken as target acupoint in experiment.

Because functional magnetic resonance technology is featured by many advantages of no radiation, non-invasive scanning, repeatability, easy location, high spatial resolution and time resolution, and no pollution, it has been extensively used in the recent years and has become an important means to observe the functional changes of the brain *in vivo*[10-11]. In comparing resting magnetic resonance with other magnetic resonance methods, because the subjects do not need to perform a task, and there is no intervention from other environmental factors, beneficial to the researchers in the design and execution of experiment, it has been extensively applied. In the field of acupuncture, it has been applied in the studies on the relationship between the needling sensation and nerves and on the relationship between Zusanli (ST 36), Hegu (LI 4), Taichong (LR 3), Quchi (LI 11) and brain, and in acupuncture anesthesia, depression and drug withdrawal[12-15].

In comparison of the differences of fALFF values during moxibustion in different distances and before moxibustion, it is found out that in moxibustion of 2-5 cm distance, fALFF values decrease in some experiments in the brain regions that regulate and control the vision, such as the surrounding cortex of calcarine fissure and left lingual gyrus, perhaps related to the light changes in experiment. In moxibustion of 2 cm distance, fALFF values increased in the brain regions of the anterior cingulated cortex and thalamus, but did not present in moxibustion of 3-5 cm distance. It has been shown in the relevant studies that the conduction pathways of pain were divided into two pathways of emotions and feelings, in which the pathway of emotion was composed of several brain regions of the nuclear group at the medial side of thalamus, anterior cingulated cortex, insular cortex, and anterior frontal lobe[16], and in which the anterior cingulated cortex accepted the reaction of painful sense from the nuclear group at the medial side of thalamus, and further processed and participated in the regulation and control of pain[17]. Therefore, the findings indicate that moxibustion in a distance of 2 cm may induce burning pain to the subjects, so as to induce the activity of the cerebral zone in charge of the conduction pathway of pain sense in the brain, and elevate fALFF values in the corresponding regions.

In comparison of the ReHo values after moxibustion of different distances and before moxibustion (delayed effect of moxibustion), it has been found that in the beginning period, the reaction is comparatively strong in the zones of the right hemisphere of the brain, presenting comparative quick change. But, in the analysis of the results after different distances of moxibustion, it is found out that it presented the delayed effect in a comparative longer period, more persistent and stronger than the left hemisphere of the brain. The brain is also called cerebrum, a high-level nervous center to generate feelings and achieve the advanced brain energy[18]. The strong reaction in this site may be a process that mild moxibustion stimulates the organism to show stress, and integrate and regulate information. As for experience of temperature, the reaction of the corresponding brain zone is not so remarkable during moxibustion, but ReHo values all change obviously in the corresponding brain zone (parietal lobe), in comparison of those after and before moxibustion in various groups, indicating that it needs a certain period of time to transmit the thermal sense near Zusanli (ST 36) to the brain. The decrease of the corresponding values indicates that the organism may gradually adapt and generate tolerance to temperature of moxibustion.

Studies[16-17] have indicated that anterior cingulated cortex, insular cortex, frontal cortex and thalamic cortex are the component parts of the nervous center in the visceral sensory area of brain functions. The anterior frontal cortex feels pain, and the insular cortex deals with the information of visceral sensory changes. As a transfer station of the nerves, the thalamus accepts the afferent fibers from the spinal cord and conducts the information to the high-level center. In comparison of the results after moxibustion of different distances, ReHo values change in varying degrees in the brain zone of the corresponding visceral sensory area, indicating that Zusanli (ST 36), its capability to treat visceral diseases, may be related to the regulation and control of the activities in the corresponding brain areas. The differences in ReHo values of the corresponding brain areas under different distances of moxibustion also indicate that different moxibustion temperatures are related to different therapeutic effects. The effect of moxibustion on brain functions is due to joint response of several brain zones. In the study on the changes of resting brain functions before and after moxibustion by applying thermal moxibustion to left Dubi (ST 35) of the patients sick with knee osteoarthritis under fMRI technology, Luo Q, *et al*[18] found that fALFF values increased in the areas of the right brain, outer nuclear, left cerebellum, left brain and white matters, and fALFF values decreased in the anterior central gyrus, frontal lobe and occipital lobe; ReHo values increased in the brain zones of thalamus, outer nuclear and parietal lobe, and ReHo values decreased in the right brain, left brain and frontal lobe. These finding indicate that the changes of the brain zones, induced by thermal moxibustion, basically accord with the conduction pathway of painful and warm sense in the body, and the regulation of the brain functions by thermal moxibustion may be achieved jointly by the network composed of several brain zones. The results from this study indicate that in moxibustion of 3 cm distance, fALFF values increase compared with before moxibustion in the brain zones of the right, medial and paracingulated gyrus, fALFF values decrease compared with before moxibustion in the brain zones of the left temporal gyrus. ReHo values increase compared with before moxibustion in the left posterior cerebellar lobe, and left frontal lobe, and ReHo values decrease compared with before moxibustion in the brain zone of the right inferior temporal gyrus. The changes basically accord with the conduction pathway of the temperature in the body. The activity of the sensory system of the body and relevant brain zones (such as thalamus, a center to transmit information) may be the objective foundation of propagated sensation of the meridians. Clinical study[3] have shown that in moxibustion of 3 cm distance at Zusanli (ST 36), the skin temperature the subjects could tolerate is (40.73±2.93) ℃, and it is believed that this temperature could be considered as the ultimate temperature to prevent burning clinically. In the further studies[19-20], some scholars pointed out that moxibustion temperature higher than 40 ℃ is an effective therapeutic temperature, and in distance of 3 cm, moxibustion temperature is between 61.0-40.6 ℃ [(61.0±3.6) ℃ within first minute, (40.6±2.0) ℃ within tenth minute], able to generate the warm and hot effect and also to avoid burning the skin. Those findings indicate that in moxibustion of 3 cm distance, the changes in the brain zones basically accord with the conduction pathway of body temperature, helping people to have a comfortable sensation, and also promoting the propagated sensation of the meridians, producing warm and heating effect, and providing a scientific foundation for clinical application of moxibustion of 3 cm distance.

In conclusion, the changes in the brain zones in moxibustion of 3 cm distance basically accord with the conduction pathway of body temperature. But, the sample size is small in this study, and the study of big sample size cannot be performed. Moreover, the results of magnetic resonance test are related to influence from various factors of spiritual and mental activities of the subjects. Therefore, the final results of the study could be influenced by subjective factors and need to be further verified.

Conflict of Interest

There was no potential conflict of interest in this article.

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Statement of Informed Consent

Informed consent was obtained from all individual participants included in this study.

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