Objectives: Our study aimed to investigate the sustained effects of sham (SHAM) and verum acupuncture (ACUP) into the post-stimulus resting state. Methods: In contrast to previous studies, in order to define the changes in resting state induced by acupuncture, changes were evaluated with a multi-method approach by using regional homogeneity (ReHo) and amplitude of low frequency fluctuation (ALFF). Twelve healthy participants received SHAM and ACUP stimulation right GB34 (Yanglingquan) and the neural changes between post- and pre-stimulation were detected. Results: The following results were found: in both ReHo and ALFF, the significant foci of left and right middle frontal gyrus, left medial frontal gyrus, left superior frontal gyrus, and right posterior cingulate cortex, areas that are known as a default mode network, showed increased connectivity. In addition, in ReHo, but not in ALFF, brain activation changes in the insula, anterior cingulate cortex, and the thalamus, which are associated with acupuncture pain modulation, were found. Conclusions: In this study, results obtained by using ReHo and ALFF, showed that acupuncture can modulate the post-stimulus resting state and that ReHo, but not ALFF, can also detect the neural changes that were induced by the acupuncture stimulations. Although more future studies with ReHo and ALFF will be needed before any firm conclusions can be drawn, our study shows that particularly ReHo could be an interesting method for future clinical neuroimaging studies on acupuncture.

Key words: fMRI, Regional homogeneity, Amplitude of low frequency fluctuation, Acupuncture, Rest, Post effect

Introduction

Acupuncture is an ancient therapeutic technique that is increasingly important as a modality of complementary medicine in Western countries\(^1,2\). As a result, numerous researchers have studied the effects of acupuncture\(^3-5\). The develop
opment of imaging techniques, such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), has provided new tools for researchers to investigate the physiological changes during acupuncture stimulations in humans. Particularly, fMRI is often used since it is a non-invasive technique that can demonstrate the neural changes induced by acupuncture\(^6\,7\). In recent years, acupuncture researchers have studied the so called "post-effect" of acupuncture with fMRI. This means that they were not just studying the direct neural responses, but defining the neural changes after acupuncture stimulations. Some clinical reports have indicated that acupuncture efficacy is sustained for changes after acupuncture stimulations. Fortunately, with fMRI, the functional changes of resting state connectivity can be evaluated\(^10\,11\), moreover, the post-effect of acupuncture stimulations can also be detected with this resting state connectivity\(^12\,14\).

A review of the literature suggests that resting state connectivity in a default mode network using independent component analysis (ICA), which has been commonly used for depicting functional connectivity within a default mode network in resting-state fMRI studies\(^15\), can be changed after receiving acupuncture stimulation\(^7\,13\,16\). Posterior cingulate cortex (PCC) and precuneus strongly interacted with other brain areas during the pre- and post-stimulation state\(^14\) and there was an increased default mode network with pain (anterior cingulate cortex (ACC), periaqueductal gray), affective (amygdala, ACC), and memory (hippocampal formation, middle temporal gyrus) related brain regions\(^15\). Study using ReHo also showed the modulation of the medial frontal gyrus, anterior cingulate cortex, precuneus and posterior cingulate cortex which were reported as default mode network induced by acupuncture\(^17\). In this fMRI study on acupuncture, in line with previous research, the post-effect of acupuncture was studied.

To investigate the sustained effects of acupuncture into post-stimulus rest: changes of regional homogeneity (ReHo), which measures the temporal similarity of voxels within a given cluster in a voxel-wise fashion\(^18\), and amplitude of low frequency fluctuation (ALFF)\(^19\,20\), which quantifies the strength of the fluctuations in each voxel, on “resting-state networks” were evaluated.

ReHo and ALFF are also methods to detect the resting state network, and many studies have already been done\(^19\,22\), however, to the knowledge of the authors, a comparison on neural changes induced by acupuncture using these methods, has not been demonstrated yet.

This study aimed to investigate the changes in resting state networks after sham (SHAM) and verum acupuncture (ACUP) with ReHo and ALFF. It was hypothesized that acupuncture would modulate post-stimulus rest in a multi-method approach. Moreover, it was expected that both ReHo and ALFF are suitable methods in order to create a deeper insight in the changes in the resting-state brain network induced by acupuncture. Finally, interest was raised in the question: which method (ReHo or ALFF) is most suitable for future clinical neuroimaging research on acupuncture, for instance, for studies on pain modulation\(^7\)?

To address these questions, the following hypothesis was formulated: If acupuncture can modulate resting state connectivity in a default mode network using ICA, then it can also modulate resting state connectivity in a default mode network using ReHo and ALFF.

**Materials and Methods**

1. **Target population**

Twelve healthy participants entered our study, six were males and six were females, and their ages ranged from 35 to 71 (mean age 56.4±9.8). They were without any neurological or psychiatric history and were right-handed as verified by the Edinburgh Handedness Inventory\(^23\), their mean score was 100% (SD=0%). All participants provided written, informed consent prior to participation in this experiment, which was performed according to the Declaration of Helsinki and was approved by the institutional