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CEREBRAL BLOOD FLOW-BASED EVIDENCE FOR MECHANISMS OF LOW- VERSUS HIGH-FREQUENCY TRANSCUTANEOUS ELECTRIC ACUPOINT STIMULATION ANALGESIA: A PERFUSION FMRI STUDY IN HUMANS

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Abstract—Brain activities in response to acupuncture have been investigated in multiple studies; however, the neuromechanisms of low- and high-frequency transcutaneous electric acupoint stimulation (TEAS) analgesia are unclear. This work aimed to investigate how brain activity and the analgesic effect changed across 30-min low- versus high-frequency TEAS. Forty-six subjects received a 30-min 2, 100-Hz TEAS or mock TEAS (MTEAS) treatment on both behavior test and functional magnetic resonance imaging (fMRI) scan days. On the behavior test day, the pain thresholds and pain-related negative emotional feeling ratings were tested five times – at 4.5 min before treatment, at 10, 20, and 30 min during treatment and 4.5 min after the treatment. On the fMRI scan day, to match the time-points in the behavioral testing session, the cerebral blood flow (CBF) signals were collected and incorporated with five independent runs before, during and after the treatment, each lasting 4.5 min. The analgesic effect was observed in both the TEAS groups; the analgesic affect was not found in the MTEAS group. The effect started at 20 min during the treatment and was maintained until the after-treatment states. In both TEAS groups, the regional CBF revealed a trend of early activation with later inhibition; also, a positive correlation between analgesia and the regional CBF change was observed in the anterior insula in the early stage, whereas a negative relationship was found in the parahippocampal gyrus in the later stage. The TEAS analgesia was specifically associated with the default mode network and other cortical regions in the 2-Hz TEAS group, ventral striatum and dorsal anterior cingulate cortex in the 100-Hz TEAS group, respectively. These findings suggest that the mechanisms of low- and high-frequency TEAS analgesia are distinct and partially overlapped, and they verify the treatment time as a notable factor for acupuncture studies.

Key words: frequency, acupuncture analgesia, transcutaneous electric acupoint stimulation, cerebral blood flow.

INTRODUCTION

Acupuncture is gaining widespread popularity, and its analgesic effect has been consistently reported (Sim et al., 2002; Sun et al., 2008; Molsberger et al., 2010; Han, 2011). Compared to traditional manual acupuncture, electroacupuncture (EA) or transcutaneous electric acupoint stimulation (TEAS) has the advantage of the precision of stimulation parameters, high reproducibility of the therapeutic effects and significant reduction in labor. The popularity of these new modalities of acupuncture has increased. Different frequencies were used for EA and TEAS. Although both are effective for pain-relief (Han, 2011; Xiang et al., 2012), low- and high-frequency stimuli could treat specific dysfunctions. Low-frequency EA was specifically recommended for muscular atrophy (Liu, 1998), and high-frequency EA was specifically effective in treating spinal spasticity (Yuan et al., 1993). For treating drug abuse, EA/TEAS at high frequency was shown to reduce withdrawal syndrome better, whereas low frequency was preferred for the suppression of psychic dependence (Cui et al., 2008). In basic animal research, low- and high-frequency EA/TEAS could facilitate the release of endogenous opioid peptides, and the difference was that the former was mediated by the μ and δ opioid receptors, whereas the latter was mediated by the κ opioid receptor (Han, 2003). Investigations on its brain mechanisms had been limited in humans until the recent development of noninvasive neuroimaging techniques, especially functional magnetic resonance imaging (fMRI).

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Abbreviations: ANOVA, analysis of variance; ASL, arterial spin labeling; BOLD, blood oxygen-dependent level; CBF, cerebral blood flow; DMN, default mode network; EA, electroacupuncture; fMRI, functional magnetic resonance imaging; FOV, field of view; MTEAS, mock TEAS; ROI, region of interest; TEAS, transcutaneous electric acupoint stimulation; VAS, visual analog scale.

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Only a few studies have tried to investigate the brain activity changes induced by low- versus high-frequency EA/TEAS in humans. Zhang et al. first reported a significant correlation between the blood oxygen-dependent level (BOLD) signal intensity and the extent of analgesia in some brain areas induced by 2- vs. 100-Hz TEAS, by using a model including a 6-min block-designed fMRI scan as well as pain threshold tests before and after 30-min TEAS (Zhang et al., 2003a,b). Another study revealed that 2- and 100-Hz EA, especially 2 Hz, produced more widespread fMRI signal increase than manual acupuncture (Napadow et al., 2005). On the basis of the above research, the brain regional activations induced by EA/TEAS were only focused on short-period treatment, and the time dependency factor in the development of the treatment effect was considered only to a lesser degree. As far as we are aware, 20–45 min acupuncture treatment has been conventionally used in clinical practice (Sim et al., 2002; Cui et al., 2008; Ahsin et al., 2009; Molsberger et al., 2010; Unterrainer et al., 2010), and a relatively long treatment period showed a better analgesic effect than a short treatment period (Ulett et al., 1998; Cheing et al., 2003). In a 31-min EA block-designed fMRI study, the results of the first- and last-3 blocks were different (Napadow et al., 2009). More recently, we found that the cerebral blood flow (CBF) decreased globally and in some areas, including the limbic region as well as in the somatosensory brain regions following a 30-min 2-Hz TEAS; this finding is different from the somatosensory-activation results in most short-period acupuncture research (Wu et al., 2002; Zhang et al., 2003a,b; Bai et al., 2009; Hui et al., 2010). These studies implied that the brain activities in response to short- and long-period acupuncture might be different.

In this study, we addressed three research questions. How does the time course of brain activity change across a 30-min TEAS? Which brain region plays a critical role in analgesia at different treatment stages? Based on these two questions, are there any frequency-dependent findings that imply different analgesic mechanisms between low- vs. high-frequency TEAS? Arterial spin labeling (ASL) perfusion fMRI was employed in this work to measure the CBF in each time period; it has displayed excellent reproducibility over a long period of time and less between-subject variability compared to BOLD fMRI (Aguirre et al., 2002; Wang et al., 2003b; Wang et al., 2003a). A combined fMRI scanning and behavior-testing model was applied (Fig. 1) in this study, and the pain thresholds and time-matched CBF signals were collected before, during and after 30-min TEAS treatments.

EXPERIMENTAL PROCEDURES

Subjects

Fifty-two healthy, right-handed participants naïve to acupuncture (27 males, mean age 23 years, range 19–28) were enrolled in this experiment, and no female subject was having a menstrual period. All the subjects signed informed consent to the purposes, procedures and potential risks of this study and were free to withdraw from the experiment at any time. The research procedures were approved by the ethics committee of the Peking University, and the experiments were conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki), printed in the British Medical Journal (18 July 1964).

Experiment procedures

The subjects were recruited to participate in one behavioral testing session and one fMRI scanning session. The two sessions were set in random order and separated by a minimum of one week. The 52 subjects were randomized into one of three groups, receiving mock TEAS (MTEAS), 2-Hz TEAS or 100-Hz TEAS.

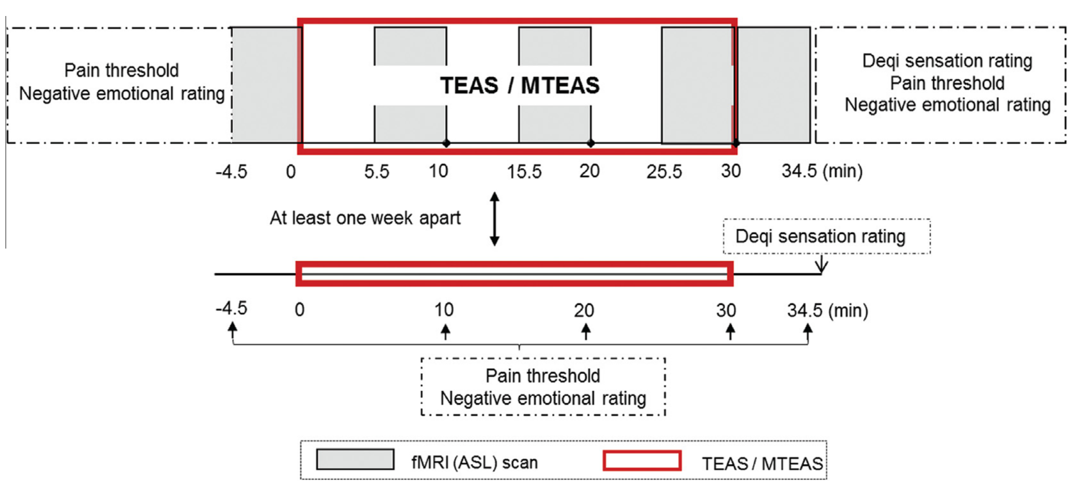

Fig. 1. Details of experimental procedure. The subjects received a 30-min TEAS/MTEAS treatment on both the fMRI scan day and the behavior test day. On the fMRI scan day, functional scanning incorporated with five independent runs, each lasting 4.5 min, as follows: beginning at 4.5 min before; 5.5, 15.5 and 25.5 min during; and immediately after the treatment. The pain threshold and the associated negative emotional rating were tested before and after the whole scans. On the behavior test day, pain thresholds and the negative emotional ratings were tested five times as follows: at 4.5 min before; 10, 20 and 30 min during; and 4.5 min after the treatment. Moreover, the feelings of *'deqi'* were collected on both days after the last pain threshold measurement.

Acute pain was induced by the potassium iontophoresis method (Research Group of Acupuncture Anesthesia, 1973; Xiang et al., 2012). The K^+ was carried by gradually increasing the anode current to the skin of the medial aspect of the right forearm. The current started from 0 mA and increased at a rate of 0.1 mA/s. It was terminated immediately when the subject reported that the stimulus was painful. The pain threshold was determined by the average of three consecutive trials with an inter-trial interval of 30 s. Immediately after the pain threshold measurement, the subjects were asked to rate the negative emotional feeling from the pain, using a visual analog scale (VAS) ranging from 0 (none) to 100 (max). During the pain threshold measurement period, the TEAS/MTEAS treatment was temporarily interrupted.

The subjects received 30-min TEAS/MTEAS treatments on the behavior test and fMRI scan days. On the behavior test day, the pain thresholds and pain-related negative emotional feeling ratings were tested five times as follows: at 4.5 min before; 10, 20 and 30 min during; and 4.5 min after the treatment. On the fMRI scan day, to match the time points in the behavioral testing session, functional scanning was incorporated with five independent runs before, during and after the treatment, each lasting 4.5 min, as follows: beginning at 4.5 min before; 5.5, 15.5 and 25.5 min during; and immediately after the treatment. During the scanning, the subjects lay supine on the scanner bed, with head immobilization with cushioned supports; they wore earplugs to suppress the scanner noise. The subjects were instructed to keep their eyes closed with their minds clear and to remain awake. The feelings of '*deqi*' were recorded in both sessions including soreness, numbness, fullness, heaviness and dull pain. The subjects were asked to rate the feeling they experienced during the treatment from 0 to 100 on the VAS (Fig. 1).

Acupoint stimulation

The skin electrodes were applied on the Li-4 acupoint (Hegu, on the dorsum of the first interosseous muscle) of the left hand and on the PC-8 acupoint (Laogong, on the volaris of the second interosseous muscle) of the same hand and connected to the output socket of the Hans acupoint nerve stimulator (HANS model LH-202H, Neuroscience Research Institute, Peking University, Beijing, China). The frequency was set at 2 or 100 Hz in the two TEAS groups. The intensity of the TEAS output in mA for each subject was individually adjusted to a maximal yet comfortable level. In this study, the current intensities used were 9–23 mA in the 2-Hz TEAS group and 8–16 mA in the 100-Hz TEAS group. This range of intensity is effective in relieving acute pain in human subjects and suppressing withdrawal syndromes in subjects with substance abuse (Zhang et al., 2003a,b; Cui et al., 2008; Lambert et al., 2011; Zhang et al., 2011). The MTEAS group was treated with a device named 'Mock HANS'. The appearance of the device is identical with the genuine HANS machine. The only difference is that the maximal output was cut off at 5 mA, although the digital display could be increased at each bottom push. The output was set at the "intermittent" mode, with 10 s on and 20 s off. As a result, the total electric current output was reduced by a factor of 9 (3:1 reduction in intensity and 3:1 reduction in the stimulation time period). MTEAS was reported to be physiologically ineffective (compared to TEAS) in studies using TEAS to reduce tobacco urges in dependent smokers (Lambert et al., 2011) and to increase the success rate for women undergoing embryo transfer (Zhang et al., 2011). In this study, the frequency of MTEAS was fixed at 2 Hz. Additionally, the TEAS/MTEAS treatment lasted 30 min in each session, and identical stimulation parameters were used for the same subject in the two sessions.

MRI data acquisition

The MRI experiments were performed on a GE 3 T whole body scanner with an 8-channel receive-only head coil. The functional data were acquired with an echo spiral-out pulsed ASL sequence (PICORE QUIPSS II) (Wong et al., 1997; Wang et al., 2003; Jiang et al., 2012). The acquisition parameters were as follows: TR = 3000 ms, TE = 3.1 ms, flip angles = 90°, field of view (FOV) = 230×230 mm^2 and inversion time = 1500 ms. Twelve axial sections, each 8.0 mm thick with 2.0 mm inter-slices, were collected to encompass the entire cerebrum and most of the cerebellum. In addition, at the end of the fMRI scanning session, high-resolution structural images of each subject were acquired using a three-dimensional gradient-echo T1-weighted sequence (TR/TE = 25/4 ms, FOV = 230×230 mm^2, slice thickness = 2.0 mm with no gap, in-plane resolution = 1×1 mm^2).

The behavior data analysis

Graph Pad Prism 5.0 software was used for the behavior data analyses. One-way ANOVA was used to compare the ages and CBF baselines among the three groups; two-way ANOVA with Bonferroni posttests was used for comparing the pain threshold and negative emotional feeling rating baselines as well as the '*deqi*' sensation intensities among the three groups between the two sessions. To investigate how the pain threshold and negative emotional feeling were modulated by TEAS/MTEAS, different statistical methods were used to process the data of the two sessions. The paired *t*-test was used on the data of the fMRI scanning session, and a repeated measure ANOVA with the Newman–Keuls multiple comparison test was used on the data of the behavioral testing session. The differences in the pain threshold change rate (% of baseline) among the three groups were explored using a two-way ANOVA. The threshold of significance for all the above comparisons was set at $P < 0.05$.

The fMRI data analysis

The SPM2 software (Wellcome Department of Cognitive Neurology, London, UK) based ASL data processing toolbox, the ASLtbx (Wang et al., 2008), and Graph Pad Prism 5.0 software were used for the fMRI data analysis.

To eliminate the inhomogeneous effects of magnetization, the first 10 volumes of each run were discarded, and then the remaining 80 volumes were realigned to the first one. The perfusion-weighted image series were then generated by pair-wise subtraction of the label and control images, and CBF quantification was used to generate the absolute CBF image series based on a single-compartment continuous ASL perfusion model by the ASLtbx (Liu and Wong, 2005; Jiang et al., 2012). For each run, the resulting CBF data contained 40 acquisitions with an effective TR of 6 s. After being co-registered with the anatomical images, the image data were further processed with spatial normalization based on the Montreal Neurological Institute (MNI) 152 standard space and resampled at $3 \times 3 \times 3$ mm. The datasets were then spatially smoothed with the 8-mm full-width at half maximum (FWHM) Gaussian Kernel. Finally, one mean CBF image was generated for each run. Each subject had five CBF images for time points as follows: the baseline (−4 to 0 min); 10 (6–10) min, 20 (16–20) min and 30 (26–30) min during treatment; and the after-treatment state (30.5–34.5 min).

The absolute global CBF was calculated and compared by repeated measure analysis of variance (ANOVA) with the Newman–Keuls multiple comparison test in each group; the global CBF change rates (% of baseline) among the three groups were compared using the two-way ANOVA test, and the threshold of significance was set at $P < 0.05$. To observe the TEAS/MTEAS effects on the brain regions at different periods, paired *t*-contrasts using voxel-wise general linear model (GLM) analysis were defined to compare the baseline with the states of the other periods in each group, including the baseline vs. 10 min, baseline vs. 20 min and baseline vs. 30 min during the treatment; and the baseline vs. after the treatment. The activated or de-activated clusters were identified at a significance level of the cluster-level corrected to $P < 0.05$. The results of the paired *t*-tests mentioned above were made into masks for the later correlation analysis.

Region of interest (ROI) analysis was conducted to calculate the quantitative regional CBF changes and evaluate whether regional CBF changes were correlated with the analgesic effect induced by TEAS. The ROIs were determined by the results of the above-mentioned paired *t*-test in each group. Each ROI was centered in the highest *t* value's location with an isotropic 4.5-min radius, and the mean absolute CBF change value of each ROI was calculated. One-way ANOVA with Newman–Keuls multiple comparison tests was used to compare the absolute CBF change in the specific ROI among the three groups, with a threshold of $P < 0.05$. Correlation analyses were performed between the pain threshold changes and the regional CBF change values in the ROIs within the masks (based on the paired *t*-test mentioned above) at every specific time-period within each group. A linear regression test was used, with the threshold set at $P < 0.05$. Voxel-based regression was also utilized for the regions outside the masks, and a stricter threshold was set to the cluster level corrected to $P < 0.05$.

RESULTS

General results of the experimental performance

Three subjects did not finish the five runs on the fMRI scan day for the following reasons: one could not tolerate TEAS, one had hyperalgesia with a pain threshold lower than 0.4 mA, and one withdrew. Thus, 46 of the 52 consenting volunteers completed the study and were used for the data analysis, including 16 for the MTEAS, 15 for the 2-Hz TEAS and 15 for the 100-Hz TEAS. All the subjects reported a feeling of '*deqi*', and there was no significant '*deqi*' sensation difference among the three groups or between the different test days, suggesting that the blinding of the three groups was successful. In the functional data processing, no subject had head movements exceeding 1 mm on any axis or head rotation greater than 1°. All the functional datasets were enrolled for later analysis.

Results of analgesic effects induced by TEAS/MTEAS

No significant difference was revealed in the baseline level of nociception or negative emotional rating among the three groups or between the different test days. On the fMRI scan day, the pain threshold and negative emotional rating were tested only twice, i.e., before and after the 30-min TEAS/MTEAS treatment. The pain threshold increased significantly following the 30-min 2-Hz ($t = 4.747$, $P < 0.001$) and 100-Hz ($t = 3.749$, $P = 0.002$) TEAS; the pain threshold did not increase significantly in the MTEAS ($t = 0.662$, $P > 0.05$) treatment group (Fig. 2A). On the behavior test day, the pain threshold and negative emotional rating were tested five times before, during and after the treatment. As shown in Fig. 2B, the pain threshold showed a significant increase at 30 min (post hoc $t = 7.825$, $P < 0.001$) during the 2-Hz TEAS ($F_{(4,56)} = 8.885$, $P < 0.001$) and at 20 min (post hoc $t = 4.067$, $P < 0.05$) during the 100-Hz TEAS ($F_{(4,56)} = 3.947$, $P = 0.007$), which lasted until the after-treatment states. Taking the rate of the pain threshold change as a parameter of comparison among the different time-points, both TEAS groups showed a significant increase at 20 min (post hoc $t = 3.846$, $P < 0.05$ for the 2-Hz TEAS; post hoc $t = 4.296$, $P < 0.05$ for the 100-Hz TEAS) during the treatment, lasting to the after-treatment states ($F_{(4,56)} = 8.474$, $P < 0.001$ for the 2-Hz TEAS; $F_{(4,56)} = 4.491$, $P = 0.003$ for the 100-Hz TEAS) (Fig. 2B). No significant changes in the pain threshold among the five time-points were found in the MTEAS group (all $P > 0.05$) (Fig. 2B). When comparing the pain threshold change rate among the three groups, the 2 Hz ($F_{(1,58)} = 16.63$, $P < 0.001$ for the fMRI scan day; $F_{(1,145)} = 16.29$, $P < 0.001$ for the behavior test day) and 100 Hz ($F_{(1,58)} = 9.62$, $P < 0.01$ for the fMRI scan day; $F_{(1,145)} = 7.86$, $P < 0.01$ for the behavior test day) TEAS produced more analgesic effects than did the MTEAS, and there

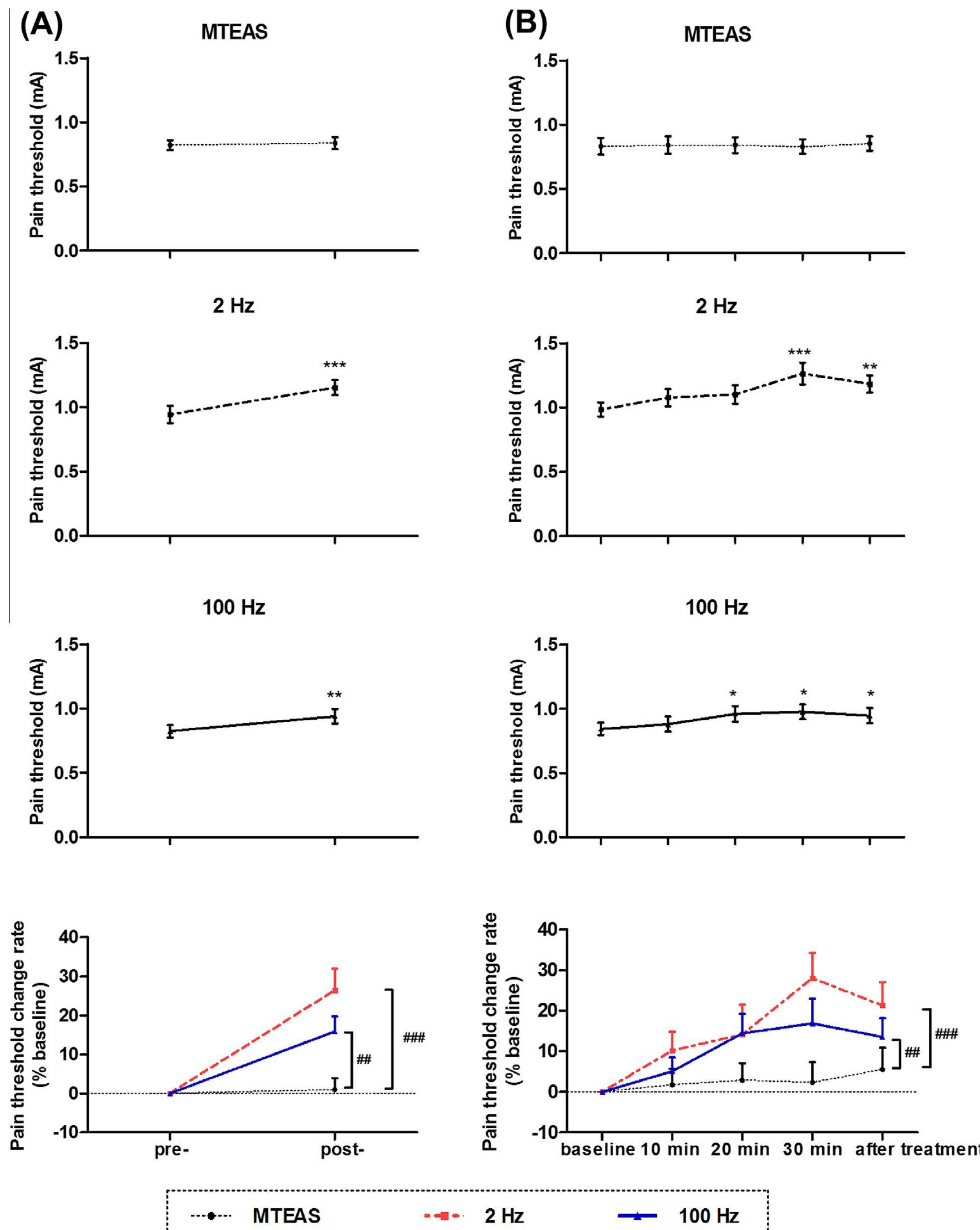

Fig. 2. The pain threshold changes by TEAS/MTEAS treatment on the fMRI scan day (A) and the behavior test day (B). The analgesic effect could be observed in both the 2- and 100-Hz TEAS groups, but not in the MTEAS group. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, compared to pre- (A) or baseline (B). Additionally, the pain threshold change rate showed more increases in the two TEAS groups than MTEAS. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, compared to MTEAS.

was no difference between the two TEAS groups ($P > 0.05$) (Fig. 2). No significant changes were found in the groups in the negative emotional rating (all $P > 0.05$).

Group results of quantitative CBF

The mean global CBF values showed no significant difference among the three groups. Further comparisons revealed that the mean global CBF values decreased from 20 min (post hoc $t = 5.448$, $P < 0.01$, compared to 10 min) during the treatment and were maintained until the after-treatment state in the 2-Hz TEAS group ($F_{(4,56)} = 25.66$, $P < 0.001$) and not in the 100-Hz TEAS and MTEAS groups ($P > 0.05$). There was no difference in the global CBF change rate among the three groups ($P > 0.05$) (Fig. 3).

Compared to the CBF image of the baseline level in each group, specific regional CBF changes were found in the treatment periods in the 2- and 100-Hz TEAS groups; they were not in found in the MTEAS group. In the 2-Hz TEAS group, an increase in the regional CBF was displayed at 10 min of treatment in the right primary somatosensory/motor areas and the middle temporal gyrus. Subsequently, the regional CBF turned to a trend of deactivation, including the right occipital lobe and the bilateral thalamus at 20 min and additional brain areas

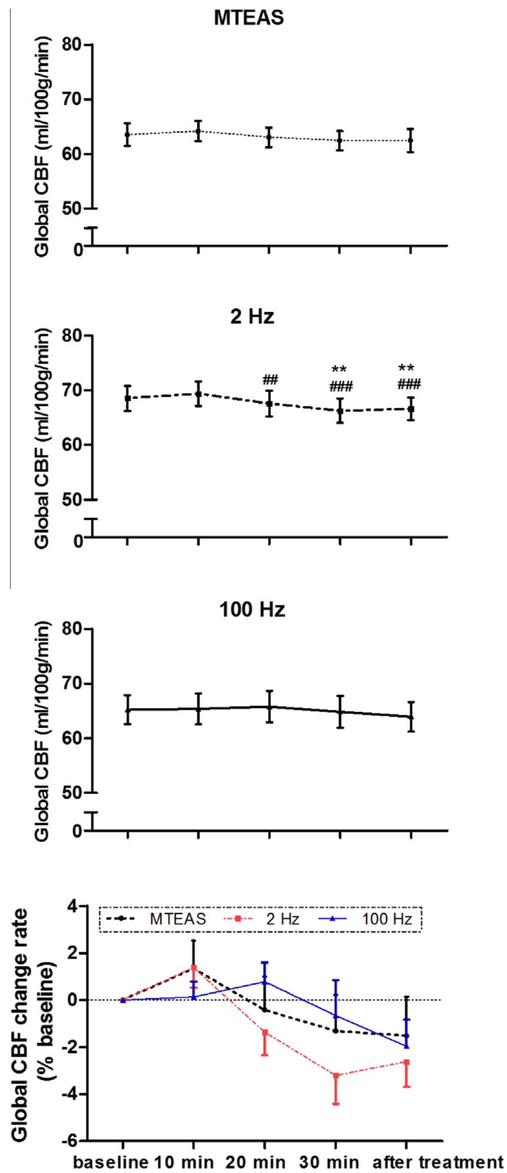

Fig. 3. Comparisons of mean global CBF value. The mean global CBF value showed significant decrease across time in the 2-Hz TEAS group, but not in the MTEAS and 100-Hz TEAS groups. Repeated measure ANOVA with Newman–Keuls multiple comparison test was used. $^{**}P < 0.01$, compared to baseline; $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, compared to 10 min. In addition, the global CBF change rate showed no difference among the three groups, with a two-way ANOVA test.

at 30 min, including the bilateral posterior cingulate cortex/precuneus, the insula and the superior temporal gyrus, the left parahippocampal gyrus, the thalamus and the inferior parietal lobe. The identical trend continued after the end of the TEAS treatment in the left superior temporal gyrus, the parahippocampal gyrus, the thalamus, the inferior parietal lobe and the secondary somatosensory area (Figs. 4–7A and Table 1). Similar trends occurred in the 100-Hz group. The CBF increased at 10 min (the right premotor and the bilateral dorsal anterior cingulate cortex) as well as at 20 min (the fusiform gyrus and the hypothalamus) and turned to deactivation in the after-treatment state (the right transverse temporal gyrus, the left inferior temporal gyrus and the occipital lobe) (Figs. 4 and 5B, Fig. 7B and Table 2).

The absolute CBF changes in the above regions were calculated and compared among the three groups. In the 10-min treatment state, the 2- and 100-Hz TEAS increased the regional CBF in the primary somatosensory/premotor area, which increased more significantly than it did by the MTEAS ($F_{(2,43)} = 4.058$, $P = 0.024$), whereas greater increases of the CBF in the dorsal anterior cingulate cortex were observed in the 100-Hz group than in the 2-Hz TEAS group ($F_{(2,43)} = 3.742$, $P = 0.032$) (Fig. 4C). In the 20-min treatment, the 100-Hz TEAS-induced CBF changes in the fusiform gyrus ($F_{(2,43)} = 6.483$, $P = 0.004$) and the hypothalamus ($F_{(2,43)} = 6.929$, $P = 0.003$) showed a significantly greater increase than in the MTEAS and 2-Hz TEAS groups (Fig. 5C). For the 30-min treatment period, the 2-Hz TEAS decreased the CBF in the posterior cingulate cortex/precuneus ($F_{(2,43)} = 3.492$, $P = 0.039$), the insula ($F_{(2,43)} = 3.270$, $P = 0.048$), the inferior parietal lobule ($F_{(2,43)} = 3.704$, $P = 0.033$) and the parahippocampal gyrus ($F_{(2,43)} = 3.492$, $P = 0.040$), which showed greater decreased CBF than in the MTEAS group (Fig. 6B). For the after-treatment state, greater decreases of the CBF were observed in the secondary somatosensory area ($F_{(2,43)} = 5.442$, $P = 0.008$) in both the TEAS groups, compared to the MTEAS group. The 2-Hz TEAS specifically induced a greater CBF decrease in the inferior parietal lobule ($F_{(2,43)} = 3.528$, $P = 0.038$) and the superior temporal gyrus ($F_{(2,43)} = 4.393$, $P = 0.018$) than did the MTEAS; the CBF change in the superior temporal gyrus showed a greater decrease than did the 100-Hz TEAS. The 100-Hz TEAS induced greater CBF decrease in the inferior temporal gyrus than did the MTEAS and 2-Hz TEAS ($F_{(2,43)} = 6.007$, $P = 0.005$); a greater CBF could be observed in the occipital lobe ($F_{(2,43)} = 4.185$, $P = 0.022$), compared to the MTEAS group (Fig. 7C).

Relationship between the analgesic effects and the regional CBF changes induced by TEAS

Considering the tentative correlation between the change of pain sensitivity and the regional CBF, there is a general positive correlation trend in the early period (10–20 min of treatment) and a negative correlation in the later period (20–30 min, and the after-treatment stage). This trend was markedly distinct in the 2-Hz TEAS group and to a less marked extent in the 100-Hz TEAS group.

In the 2-Hz TEAS group, a positive correlation was revealed at 20 min in the brain areas such as the posterior cingulate cortex, the inferior parietal lobe and the anterior insula; significant negative correlations were found at 30 min (in the middle temporal gyrus, the occipital lobe, the posterior cingulate cortex and the parahippocampal gyrus) and in the after-treatment period (the middle temporal gyrus, the parahippocampal gyrus and the insula) (Fig. 8).

Similar findings were obtained in the 100-Hz TEAS group. The CBF changes in the anterior insula and the

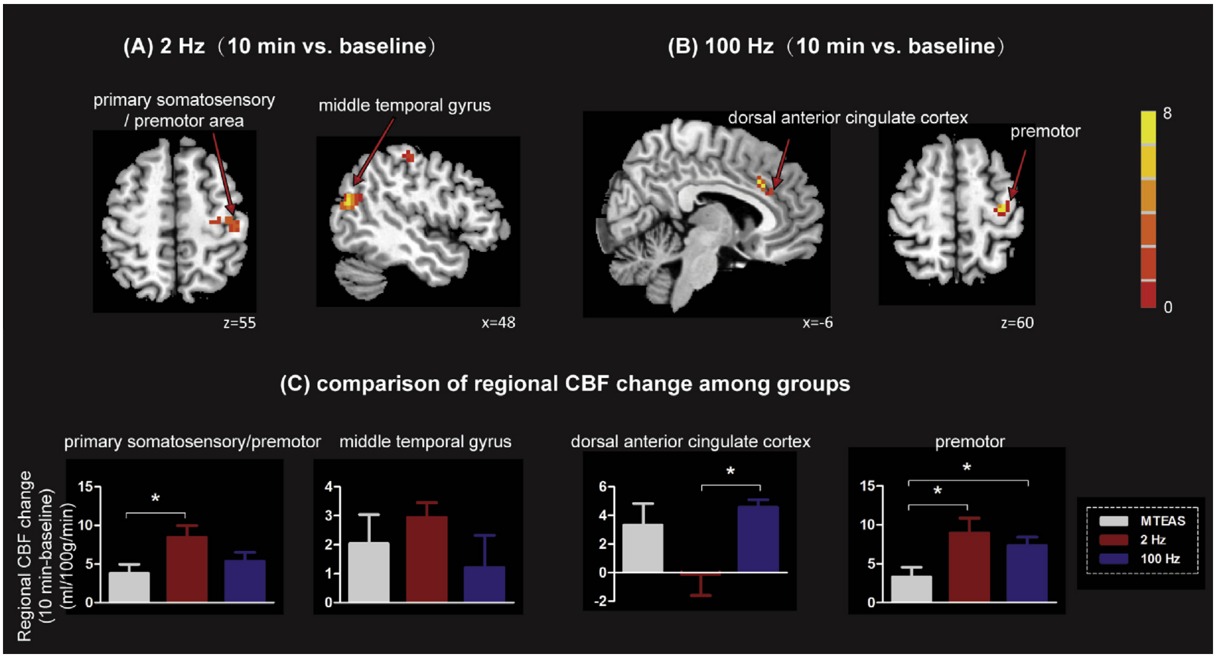

Fig. 4. Regional CBF changes in 10-min treatment. Brain regions associated with the CBF changes in the state of 10-min treatment against baseline, including the (A) 2-Hz and (B) 100-Hz TEAS groups. Using paired *t*-test. The threshold of display was set to cluster level corrected $P < 0.05$. Color bar indicates *T*-values. There was no region above the statistical threshold in the MTEAS group. Comparisons of the absolute rCBF changes (10 min-baseline) among the three groups were showed in (C), by using a one-way ANOVA with Newman–Keuls multiple comparison test, $^{*}P < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

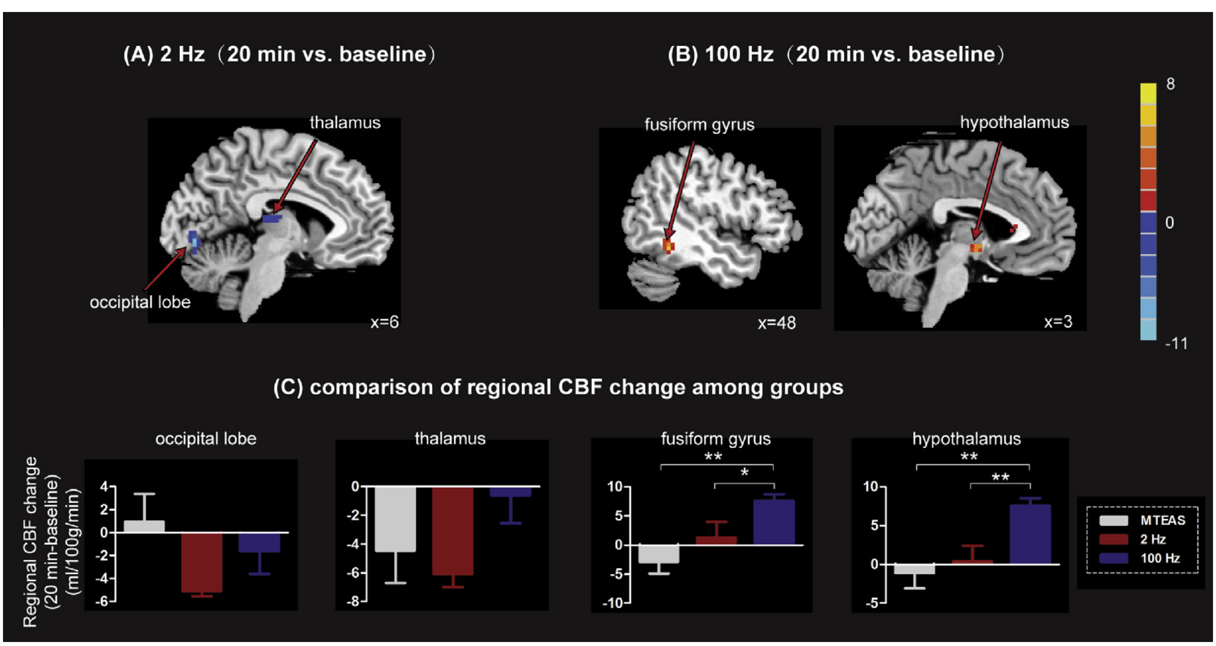

Fig. 5. Regional CBF changes in a 20-min treatment. Brain regions associated with the CBF changes in the state of a 20-min treatment against baseline, including the (A) 2-Hz and (B) 100-Hz TEAS groups. Using paired *t*-test. The threshold of display was set to cluster level corrected $P < 0.05$. Color bar indicates *T*-values. There was no region above the statistical threshold in the MTEAS group. Comparisons of the absolute rCBF changes (20 min-baseline) among the three groups were showed in (C), by using a one-way ANOVA with Newman–Keuls multiple comparison test, $^{*}P < 0.05$; $^{**}P < 0.01$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ventral striatum were positively correlated with the analgesic effect at the 20-min treatment period. A negative correlation was observed in the dorsal anterior cingulate cortex and the parahippocampal gyrus at the 30-min during treatment stage and the after-treatment state (Fig. 9).

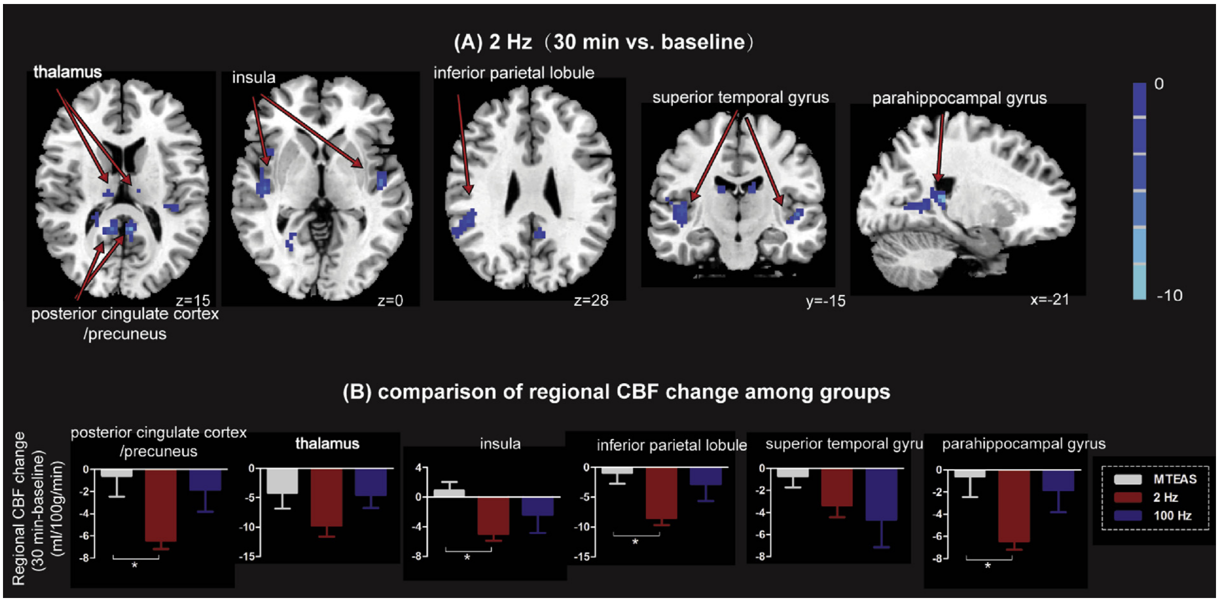

Fig. 6. Regional CBF changes in 30-min treatment. Brain regions associated with the CBF changes in the state of a 30-min treatment against baseline in the 2-Hz TEAS group. Using paired *t*-test. The threshold of display was set to cluster level corrected $P < 0.05$. There was no region above the statistical threshold in the MTEAS or 100-Hz TEAS groups. Comparisons of the absolute rCBF changes (30-min baseline) among the three groups were shown in (C), by using a one-way ANOVA with Newman–Keuls multiple comparison test, $^{*}P < 0.05$.

DISCUSSION

This study aimed to investigate the time-variant analgesic effect and brain activities in response to low- and high-frequency TEAS. TEAS analgesia was observed in the 2 and 100-Hz TEAS groups; it was not observed in the MTEAS group. The analgesic effect started at 20 min during the treatment and was maintained until the after-treatment states. The TEAS-induced CBF changes revealed a trend of early activation with later inhibition. In the TEAS groups, analgesia was positively associated with CBF changes in the anterior insula in the early stage, whereas a negative relationship between the analgesia and CBF changes was revealed in the parahippocampal gyrus in the later stage. The TEAS analgesia was specifically associated with the default mode network (DMN) and other cortical regions in the 2-Hz TEAS group and the ventral striatum and the dorsal anterior cingulate cortex in the 100-Hz TEAS group. To our knowledge, this work is the first study using perfusion fMRI to report on the mechanisms of acupuncture analgesia.

Treatment time is a notable factor for TEAS analgesia

In 1973, we first reported that manual acupuncture at Li-4 in normal human volunteers produced a significant increase in the pain threshold, with a curve of slow onset, followed by a slow decay after the termination of the stimulation. Such changes were not found in the control group (Research Group of Acupuncture Anesthesia, 1973). This basic finding was reproduced in this study in a completely different setting, using TEAS in lieu of manual needling and MTEAS as the control. Fig. 2 shows that a significant anti-nociceptive effect started at 20 min and lasted after its termination. A 30-min treatment period might be needed for the full expression of acupuncture analgesia, and no less than 20 min is required to achieve the treatment effect, which is in agreement with the conventional clinical practice of acupuncture (Cheing et al., 2003; Ahsin et al., 2009; Han, 2011) and essentially identical to the regularities of the time-effect process of acupuncture intervention (i.e., shorter or longer latency, the effect-increasing phase, the effect-exhibition plateau, and the decline phase) (Zhong et al., 2003).

Time-varied brain activities in response to TEAS

Exploring whether brain activities follow similar trends of time dependency with behavioral analgesia and whether there are any differences between low- and high-frequency TEAS is merited. As hypothesized, the brain activities varied among the different treatment periods, which could be summarized as a trend of early activation with later inhibition in the low- and high-frequency TEAS groups. This predominant TEAS effect is in agreement with our previous report, in which we found inactivation rather than activation of the brain regions following a 30-min 2-Hz TEAS treatment (Jiang et al., 2012). In the 10-min states during TEAS, the 2- and 100-Hz groups revealed regional CBF increases in the sensory-motor associated areas including the primary somatosensory/motor areas, the middle temporal gyrus, the dorsal anterior cingulate cortex and the premotor, which were mostly reported by other studies using short periods of acupuncture (Wu et al., 2002; Zhang et al., 2003a,b; Napadow et al., 2005; Bai et al., 2009; Hui et al., 2010). At 20 min in the 100-Hz group, increasing regional CBF was observed in the hypothalamus, an area that is hypothesized to play a critical role in acupuncture analgesia (Han, 2003). As the TEAS treatment continued, the activation pattern

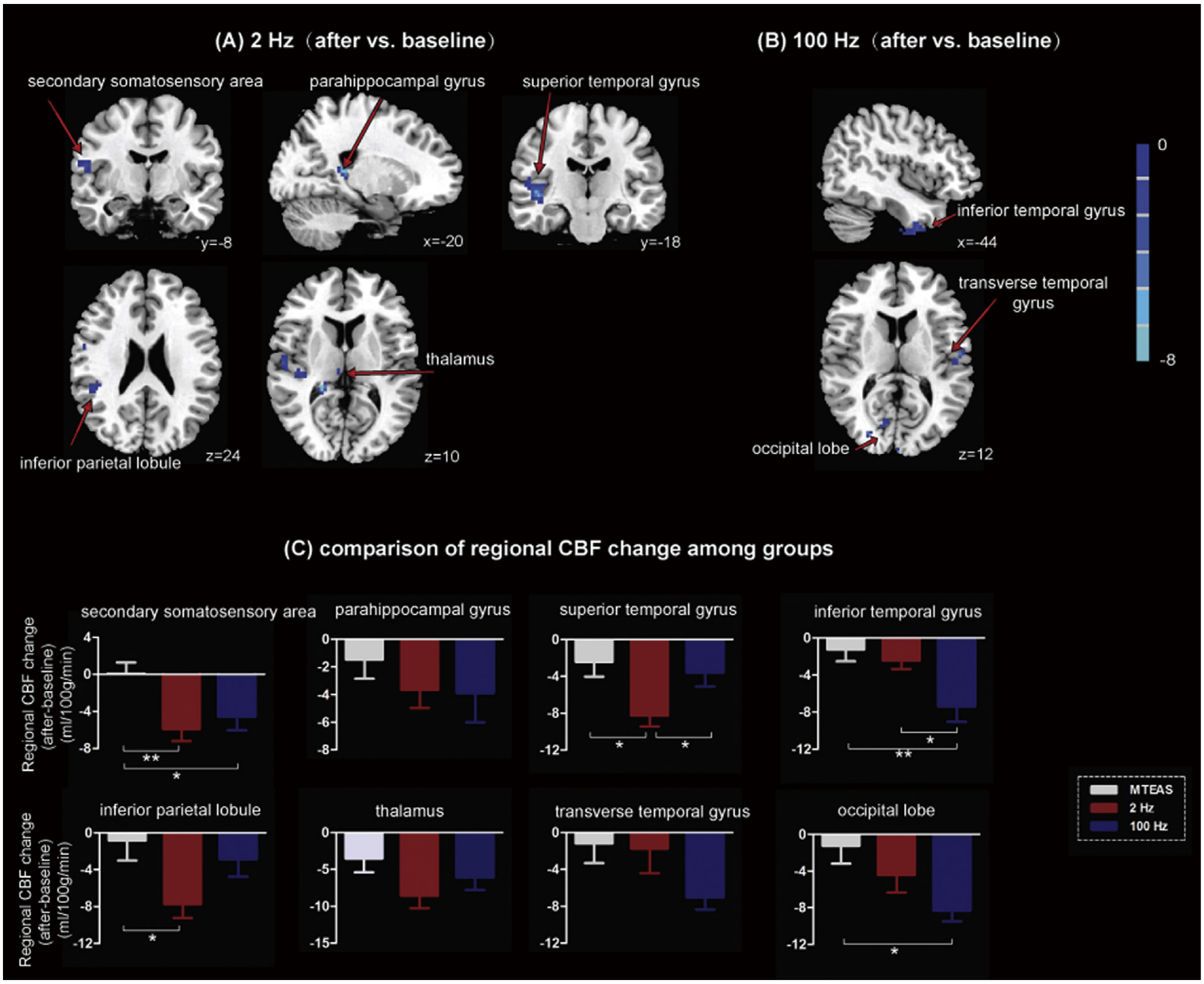

Fig. 7. Regional CBF changes after treatment. Brain regions associated with the CBF changes in the state of after-treatment against baseline, including the (A) 2-Hz and (B) 100-Hz TEAS groups. Using paired *t*-test. The threshold of display was set to cluster level corrected $P < 0.05$. Color bar indicates *T*-values. There was no region above the statistical threshold in the MTEAS group. Comparisons of the absolute rCBF changes (after-baseline) among the three groups were showed in (C), by using a one-way ANOVA with Newman–Keuls multiple comparison test, $^{*}P < 0.05$; $^{**}P < 0.01$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

was replaced by inhibition, starting at 20 min in the 2-Hz TEAS group and in the after-treatment state in the 100-Hz TEAS group. Decreased CBF was observed in the DMN system, sensory cortex (the insula and the secondary somatic sensory area), limbic area (the parahippocampal gyrus), the thalamus and the other cortical cortex (the temporal and the occipital lobe). This finding is similar to the findings of Napadow et al. in a study using BOLD signals as the index (Napadow et al., 2009). Using a 31-min block-designed EA stimulation, they found that the sensorimotor areas showed a linearly decreasing time-variant activation, whereas the limbic areas revealed early activation with later deactivation.

The key regions associated with TEAS analgesia in different treatment stages

The relationship between TEAS analgesia and regional CBF changes showed a similar trend in the low- and high-frequency TEAS groups. Positive associations in the early stage followed by later negative relationships were observed during and after the treatment. Among the regions shown in Figs. 8 and 9, the CBF changes in the anterior insula and the parahippocampal gyrus were significantly linearly correlated with the analgesic effect in the 2- and 100-Hz groups, showing a positive relationship at the early stage and a negative correlation at the later stage, respectively. The anterior insula is generally hypothesized to be a key region in the salience network, which plays a role in initiating dynamic switching between the DMN and the central-executive networks (Sridharan et al., 2008). The anterior insula participates in poly-modal sensory integration that includes pain (Critchley et al., 2004; Craig, 2009; Lamm et al., 2011). Thus, the linear correlation between analgesia and CBF changes in the anterior insula indicated that TEAS might relieve pain by modifying the neural pathway of sensorial integration at the early stage. The hippocampus/parahippocampal gyrus, as a main component of the limbic system, is well documented to be involved in processing the affective

Table 1. Brain areas showing significant activation (CBF increases) and deactivation (CBF decreases) during and after 2-Hz TEAS treatment

Regions (BA)	Side	*t* value	Coordinate (MNI)			Number of voxels in cluster
			x	*y*	*z*	
10 min vs. baseline						
Primary somatosensory area/premotor(3,4)	R	5.16	45	−24	57	128
Middle temporal gyrus (39)	R	7.36	48	−69	18	132
20 min vs. baseline						
Occipital lobe (17)	R	−10.72	6	−81	−6	27
Thalamus	L, R	−6.28	−6	−21	12	103
30 min vs. baseline						
Posterior cingulate cortex/precuneus (29,31)	L	−5.13	−11	−47	12	91
	R	−8.26	6	−48	21	60
Insula	R	−7.01	36	−27	21	48
	L	−5.17	−42	13	−3	90
Superior temporal gyrus (22)	L	−6.54	−45	−18	3	73
	R	−6.62	48	−15	0	33
Parahippocampal gyrus	L	−4.8	−23	−48	7	23
Thalamus	L	−5.71	0	−24	12	25
Inferior parietal lobule (40)	L	−5.52	−51	−39	28	43
After−treatment vs. baseline						
Superior temporal gyrus (22)	L	−7.11	−45	−21	−3	64
Parahippocampal gyrus	L	−5.84	−20	−37	6	29
Thalamus	L	−4.61	−6	−21	10	23
Secondary somatosensory area (40)	L	−5.65	−58	−8	16	31
Inferior parietal lobule (40)	L	−5.11	−48	−39	24	23

Note: BA, Brodmann area; L, left; R, right. The threshold was set to cluster level corrected $P < 0.05$.

Table 2. Brain areas showing significant activation (CBF increases) and deactivation (CBF decreases) during and after 100-Hz TEAS treatment

Regions (BA)	Side	*t* value	Coordinate (MNI)			Number of voxels in cluster
			x	*y*	*z*	
10 min vs. baseline						
Dorsal anterior cingulate cortex (24)	L,R	6.43	−6	24	30	35
Premotor (4)	R	6.58	36	−18	60	32
20 min vs. baseline						
Fusiform gyrus (37)	R	6.25	48	−48	−15	25
Hypothalamus	R	6.07	3	−6	−9	26
30 min vs. baseline (No regions above threshold)						
After-treatment vs. baseline						
Inferior temporal gyrus (20)	L	−5.21	−42	0	−48	58
Occipital lobe (18,19)	L	−4.82	0	−75	9	151
Transverse temporal gyrus (42)	R	−5.2	57	−12	12	24

Note: BA, Brodmann area; L, left; R, right. The threshold was set to cluster level corrected $P < 0.05$.

and cognitive signals of pain (Chapman, 1996; Kakigi et al., 2000). Increasing evidence from Hui et al. showed that deactivation of the limbic-paralimbic-neocortical network would be evoked by acupuncture stimulation, when associated with '*deqi*' (Hui et al., 2010). Similarly, in a heat pain study in humans (Kong et al., 2009), the activation of this area was attenuated after 25 min of 2-Hz EA treatment. A previous report from our institution found that the decreased BOLD signal in the hippocampus induced by TEAS was associated with an analgesic effect (Zhang et al., 2003a,b). We hypothesized that TEAS might inhibit the affective and cognitive components of pain by inhibiting the pain signal in the limbic system areas such as the parahippocampal gyrus at a relatively later stage.

Frequency-specific effects in response to TEAS

This study facilitates the understanding of the differences between low- and high-frequency TEAS analgesia and brain activity changes. In our data, 2-Hz TEAS trended to be more effective, although there was no difference between the two TEAS groups in the analgesic effect. As shown in Fig. 2, 2-Hz TEAS produced more significant increases in the pain threshold than did the control (all $P < 0.001$) on both the test days; on the

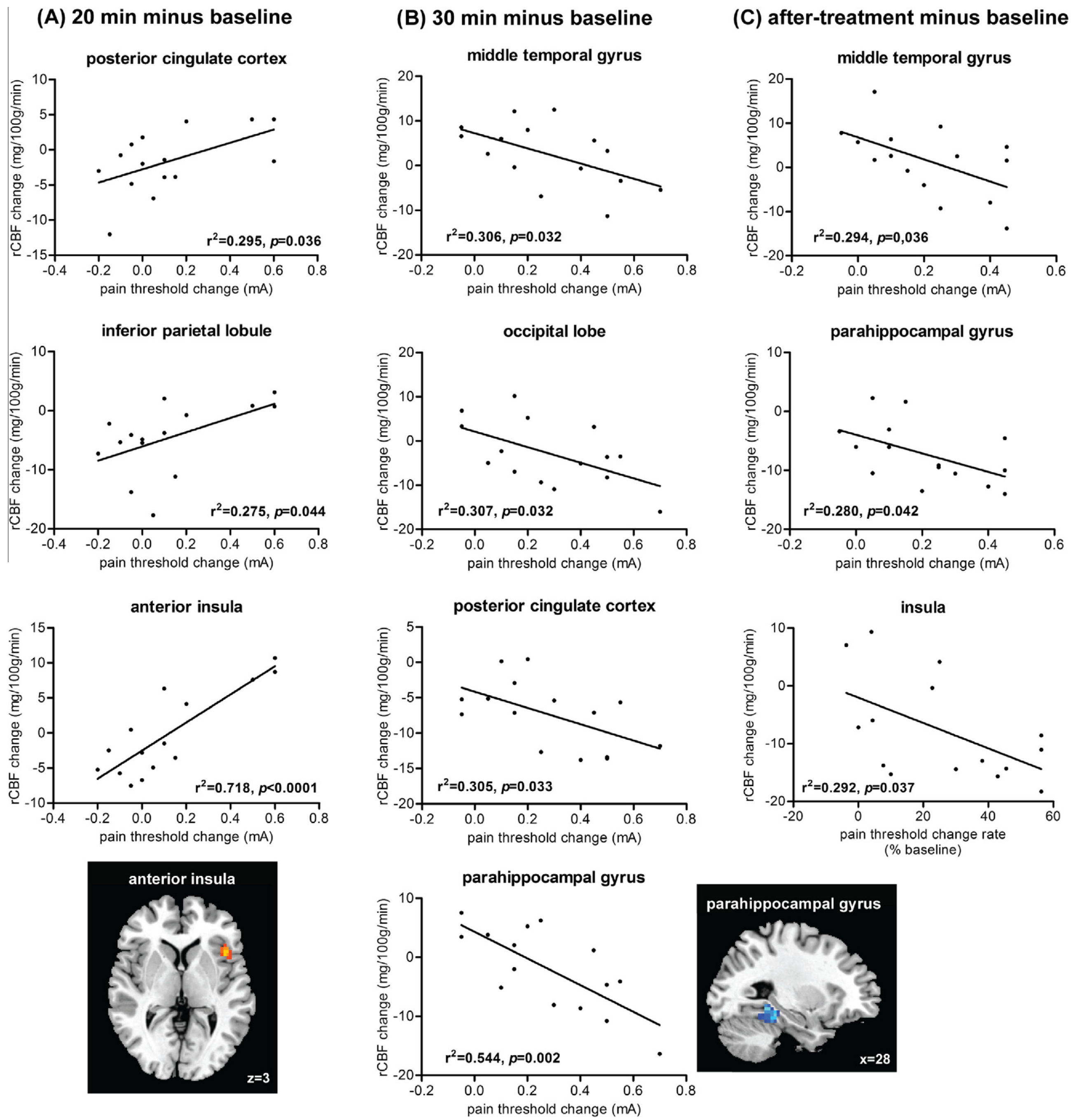

Fig. 8. Regional CBF (rCBF) analysis showing positive or negative associations of pain threshold change or change rate with rCBF change in a 2-Hz TEAS group at (A) 20 min, (B) 30 min, and (C) after-treatment state. Using linear regression test, threshold at $P < 0.05$. In addition, voxel-based regression was used for regions outside the mask, and the threshold of display was set to cluster level corrected $P < 0.05$, including the anterior insula in (A) and the parahippocampal gyrus in (B).

behavior test day, the comparisons in each time-period showed that only the 2-Hz TEAS and not the 100-Hz TEAS induced a more significant analgesic effect compared to MTEAS in the 30-min treatment period (post hoc $t = 3.934$, $P < 0.001$). For the brain activity changes, more obvious time-variant brain activity was observed in the 2-Hz TEAS group, showing a global CBF decrease from 20 min during the treatment (Fig. 3); the 100-Hz group did not show such a decrease. Compared to the 100-Hz TEAS group, the regional CBF deactivation appeared earlier and in more significant areas in the 2-Hz TEAS group, including the DMN and some cortical areas such as the temporal and the occipital lobes. The correlation analysis showed that the 2-Hz TEAS-induced anti-nociception was particularly associated with these areas (Fig. 8). The DMN is defined as the brain areas in concerted action to maintain the resting state of the brain (Raichle et al., 2001). The DMN, the basic network, plays an important role in maintaining homeostasis, and recent findings

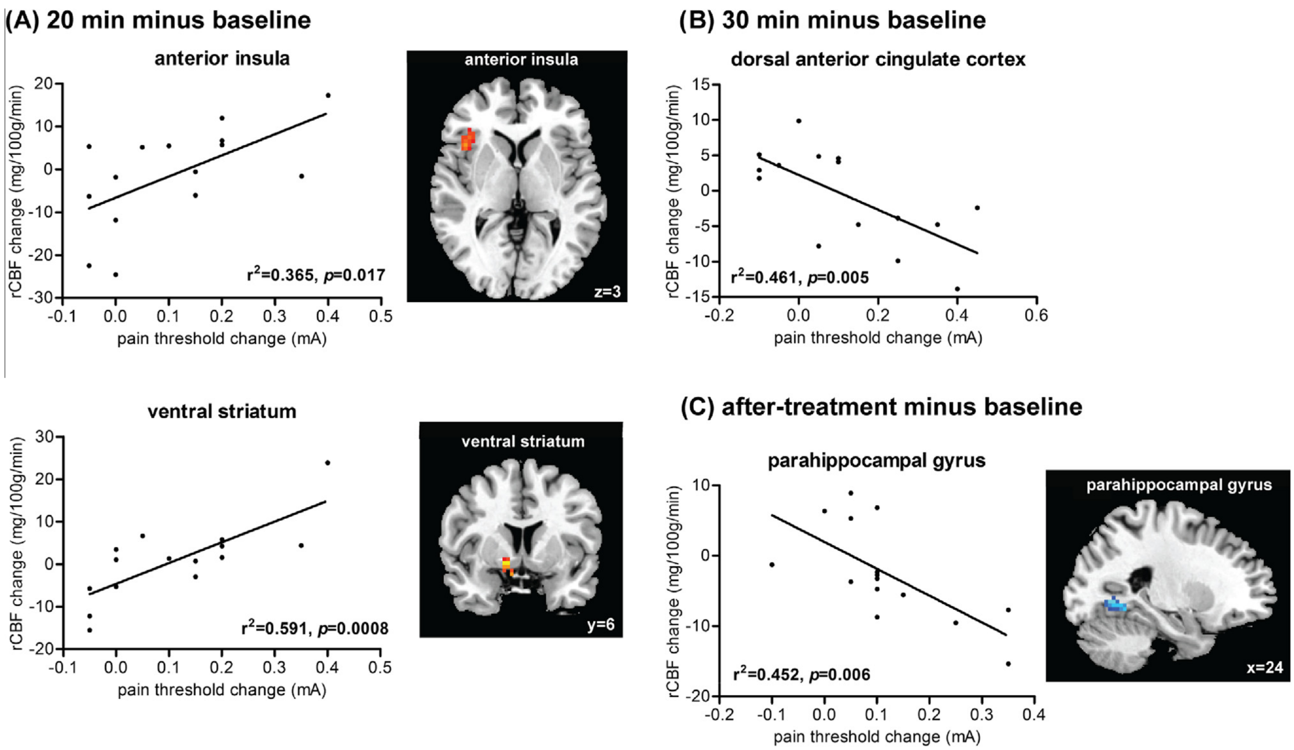

Fig. 9. Regional CBF (rCBF) analysis showing positive or negative associations of pain threshold change or change rate with rCBF change in the 100-Hz TEAS group at (A) 20 min, (B) 30 min and (C) after-treatment state. Using linear regression test, threshold at $P < 0.05$. In addition, voxel-based regression was used for regions outside the mask, the threshold of display was set to cluster level corrected $P < 0.05$, including the anterior insula and the ventral striatum in (A), and the parahippocampal gyrus in (C).

revealed that acupuncture has a potential for ameliorating insomnia (Sarris and Byrne, 2011). We hypothesized that 2-Hz TEAS treatment might play a beneficial role in sedation in addition to its well-known role in pain-relief. In the 100-Hz TEAS group, the correlation analysis revealed that the TEAS analgesia was specifically related to CBF changes in the ventral striatum and dorsal anterior cingulate cortex (Fig. 9). The dorsal anterior cingulate cortex was more activated in the 100-Hz TEAS than in the 2-Hz TEAS in the 10-min treatment (Fig. 4C). The ventral striatum, particularly the nucleus accumbens, is generally hypothesized to be associated with the processing of reward and positive stimuli and positive affect as well as anti-nociceptive processes (Gear et al., 1999; Villemure et al., 2012). Similar results from our previous work revealed that 100-Hz TEAS analgesia was associated with BOLD signal increases in the nucleus accumbens (Zhang et al., 2003a,b). The dorsal anterior cingulate cortex plays a complex pivotal role in the affective-motivational component of pain (Price, 2000). On the basis of the above, we verified the previously reported findings that high-frequency TEAS ameliorated pain by modulating the affective component of pain sensation (Zhang et al., 2003a,b).

Limitations

Acupuncture treatment effects could last for a long period of time, even hours after removing the needle (Price et al., 1984; Mayer, 2000), and our previous studies found the analgesic effect induced by acupuncture was attenuated with a half-life of 16 min (Research Group of Acupuncture Anesthesia, 1973; Ulett et al., 1998). In this study, we confirmed that the TEAS effect on the pain threshold change and brain activities was sustained; however, we only focused on the time-period immediately after the treatments. Future studies should concentrate on a relatively long period after treatment to discuss the sustained effect treatments. To ensure that the fMRI scanning states were stable and prevent interference from the behavior test, two sessions were utilized in this study. The two sessions were set in balanced order, and the periods of interest were carefully matched. The pain threshold baselines and '*deqi*' sensations showed no difference between the two session days. An analgesic effect was observed in the TEAS group on both session days, whereas the MTEAS showed no treatment effect. We cannot rule out contaminations from the between-days factor. Further modification of the experimental paradigm using real-time on-line rating during the scanning is required.

CONCLUSIONS

Our data demonstrated that the brain activities involved in low- and high-frequency TEAS analgesia were distinct with partial overlapping; the analgesic effect and brain activities in response to TEAS were time-variant. These results offer a new explanation for the clinical practice of acupuncture and suggest that treatment time is a notable factor in studies on the mechanisms of acupuncture.

CONTRIBUTOR

Yin Jiang: experimental design, subject recruit, data collection, data processing and analysis, paper writing.
Jing Liu: data collection, data processing and analysis, paper writing.
Junling Liu: experimental design and paper writing.
Jisheng Han: experimental design and paper writing.
Xiaoying Wang: data collection, data processing and analysis, paper writing.
Cailian Cui: experimental design, data analysis, paper writing.

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