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Thirty minute transcutaneous electric acupoint stimulation modulates resting state brain activities: A perfusion and BOLD fMRI study

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A B S T R A C T

Increasing neuroimaging studies have focused on the sustained after effects of acupuncture, especially for the changes of brain activities in rest. However, short-period stimuli have mostly been chosen in these works. The present study aimed to investigate how the resting state brain activities in healthy subjects were modulated by relatively long-period (30 min) acupuncture, a widely used modality in clinical practice. Transcutaneous electric acupoint stimulation (TEAS) or intermittent minimal TEAS (MTEAS) were given for 30 min to 40 subjects. Functional MRI (fMRI) data were collected including the pre-stimulation resting state and the post-stimulation resting state, using dual-echo arterial spin labeling (ASL) techniques, representing both cerebral blood flow (CBF) signals and blood oxygen-dependent level (BOLD) signals simultaneously. Following 30 min TEAS, but not MTEAS, the mean global CBF decreased, and a significant decrease of regional CBF was observed in SI, insula, STG, MOG and IFG. Functional connectivity analysis showed more secure and spatially extended connectivity of both the DMN and SMN after 30 min TEAS. Our results implied that modulation of the regional brain activities and network connectivity induced by thirty minute TEAS may associate with the acupuncture-related therapeutic effects. Furthermore, the resting state regional CBF quantified by ASL perfusion fMRI may serve as a potential biomarker in future acupuncture studies.

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1. Introduction

Acupuncture has been used to treat various disorders in the Orient for thousands of years, and is gaining widespread popularity as an alternative and complementary treatment in the modern medicines (NIH Consensus Conference, 1998). It has been quite accepted that the treatment effects of acupuncture could last for a long period of time even hours after removing the needles (Mayer, 2000; Price et al., 1984). Recently, increasing neuroimaging studies have paid close attention not only to the immediate effect, but also to the sustained after effects.

Dhond et al. first reported that 5.5 min acupuncture could enhance the post-stimulation spatial extent of resting brain networks to other brain regions contributing to pain-relief, memory and affection (Dhond et al., 2008). Studies seeded at amygdala (Qin et al., 2008) and anterior insula (Bai et al., 2009) revealed that the intrinsic coherences in brain can be modulated by acupuncture for 1.5 min. In addition, some reports attempted to demonstrate the specificity of the acupoint in modulating the resting state brain activities (Bai et al., 2010a, 2010b; Feng et al., 2011; Liu et al., 2009; Qin et al., 2011; Ren et al., 2010). Recently, the temporal neural responses in wide brain networks following 1.5 min acupuncture were reported (Bai et al., 2010a, 2010b). Aside from fMRI study, positron emission tomography (PET) was used to study changes of the binding potential of μ-opioid receptors (MOR) in multiple pain and sensory processing regions induced by 5 min acupuncture in patients with fibromyalgia (Harris et al., 2009).

However, in all of the above-mentioned reports, short-period acupuncture (no more than 6 min) was used, which is different from the conventional clinical practice of using acupuncture for 20–45 min (Ahsin et al., 2009; Cheing et al., 2003; Cui et al., 2008; Lambert et al., 2009; Lin et al., 2002; Molsberger et al., 2010; Sim et al., 2002; Unterrainer et al., 2010). A study conducted in the early 1970s in our research group revealed a time–effect curve for the anti-nociceptive effect of acupuncture lasting for 50 min in healthy human beings. Skin pain threshold started to increase in 5–10 min, peaked at 20–40 min and maintained a plateau thereafter. After removing the needles, the pain threshold started to decrease with a half-life of 16 min (Research Group Of Acupuncture Anesthesia, 1973). Neuroimaging studies in humans exploring the analgesic effect of acupuncture often used a protocol with at least 25 min stimulation (Dougherty et al., 2008; Kong et al., 2009a, 2009b; Zhang et al., 2003a, 2003b). Clinical researches using acupuncture to treat drug addiction also mostly chose a 30 min stimulation (Cui et al., 2008; Lambert et al., 2009). In order to achieve the full expression of treatment effects, a period of no less than 20 min acupuncture or electroacupuncture (EA) treatment was suggested (Han, 2011; Mann, 1974; Mayer, 2000). In addition, a 31 min block-designed EA fMRI study revealed that the effects produced by latter blocks were significantly influenced by previous blocks (Napadow et al., 2009). All these studies proposed that short-period acupuncture may not fully model the clinical effect produced by longer period acupuncture intervention, and that relatively long-period acupuncture may produce different sustained after effects. A few previous reports have investigated the perfusion and cerebral metabolic changes following 15–25 min acupuncture using SPECT and PET (An et al., 2009; Newberg et al., 2005), yet the description on sustained after effects seemed inconsistent. It may be due to the small sample size, lack of control group, different acupuncture stimuli and various time periods of data collection.

One of the challenges in carrying out neuroimaging studies on sustained after effects of relatively long-period acupuncture stimulation arises from methodological limitation. Blood oxygen-dependent level (BOLD) functional magnetic resonance imaging (fMRI) has been mostly used to explore the mechanism of acupuncture, but it could not provide an absolute physiological index. PET can overcome this limitation, but the poor temporal and spatial resolutions make it hard to detect the functional connectivity changes.

To investigate the sustained after effects in resting state induced by 30-min transcutaneous electric acupoint stimulation (TEAS), the present study utilized arterial spin labeling (ASL) MRI technique, which provides a quantitative assessment of cerebral blood flow (CBF) at task-free resting states (Rao et al., 2007a, 2007b). It uses non-invasive magnetically labeled arterial blood water as an endogenous marker (Detre and Wang, 2002; Detre et al., 1992), and could collect not only CBF signals, but also BOLD signals simultaneously. The brain activities in rest before and after stimuli were measured, and the control of TEAS was an intermittent minimal TEAS (MTEAS). The CBF signals were used to detect the brain regional activation or deactivation induced by TEAS, and the BOLD signals were for investigating functional connectivity changes induced by TEAS. The latter was focused on the default mode network (DMN) and the sensorimotor network (SMN), as these networks all have been reported to be modulated by acupuncture in former fMRI studies (Dhond et al., 2008; Hui et al., 2009).

2. Results

2.1. *General results of experimental performance*

All 40 subjects finished the whole procedure in the experiment. All subjects reported a feeling of ‘*deqi*’, and no one felt sharp pain during the stimulation. The percentages of the subjects who reported ‘*deqi*’ feelings, including soreness, numbness, fullness, heaviness and dull pain, were varied between TEAS and MTEAS (Fig. 1A). Also, using two way ANOVA, a significant sensation intensity difference was revealed between the TEAS and the MTEAS groups (Fig. 1B). However, the mean intensities of all sensations showed no difference between the two groups (Fig. 1C). After each functional scanning run, no one reported that he or she slept during scanning, and no one felt uncomfortable in the scanner except a little noisy. In the functional data processing, no subject had head movements exceeding 1 mm on any axis or head rotation greater than 1°. All functional datasets were enrolled for the later analysis.

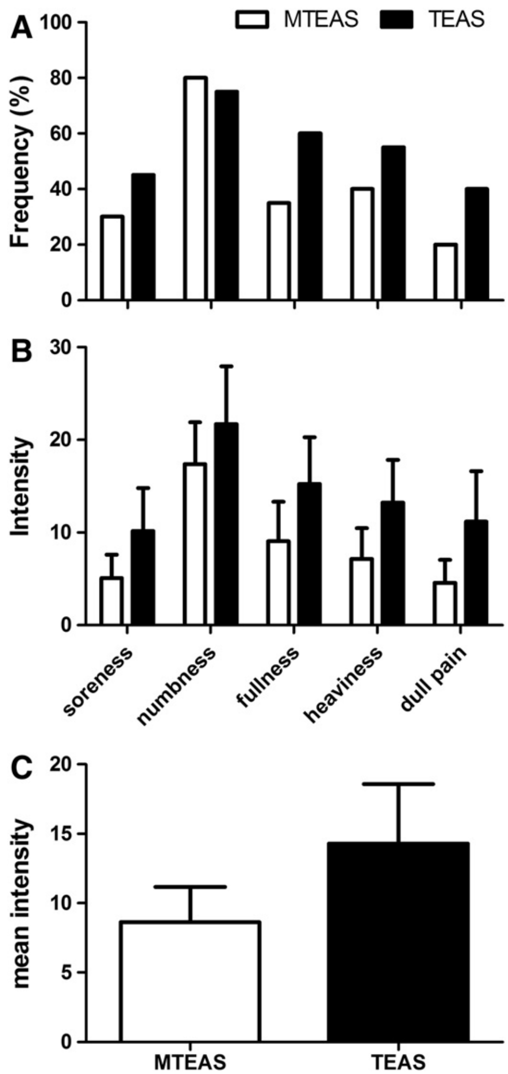

Fig. 1 – Reports of '*deqi*' sensations. A) The percentage of subjects who reported having experienced the feelings of '*deqi*'. B) The intensity of reported sensations measured by an average score (mean ± SEM), testing by VAS from 0 to 100. A significant sensation intensity difference was revealed between the TEAS and the MTEAS groups, using two way ANOVA, threshold at $p<0.05$. C) The mean intensity of all sensations, unpaired *t*-test was used, threshold at $p<0.05$, and there was no difference between the two groups.

2.2. *Results of global quantitative CBF*

Individual quantitative CBF maps for all resting runs were of high quality. For instance, the mean quantitative CBF image from a representative subject is illustrated in Fig. 2. The mean global CBF values of all subjects (mean ± SEM, in ml/100 g/min) were 66.65 ± 2.68, 64.44 ± 2.36, 63.84 ± 2.41 and 63.17 ± 2.57 for the pre-TEAS rest, the post-TEAS rest, the pre-MTEAS rest and the post-MTEAS rest, respectively. There was no global difference between the pre-TEAS rest and the pre-MTEAS rest or between the pre-MTEAS rest and the post-MTEAS rest (all $p>0.4$) (Figs. 3A and B). However, the mean globe CBF was decreased following 30 min TEAS ($t=3.59$, $p<0.01$) (Fig. 3C).

2.3. *Functional CBF results of TEAS and MTEAS effects*

Compared to the pre-TEAS rest, the post-TEAS rest showed decreased CBF in right primary somatosensory cortex (SI), insula, superior temporal gyrus (STG) and parahippocampal gyrus (parahipp G), left middle occipital gyrus (MOG), inferior frontal gyrus (IFG) and cerebellum, and bilateral cuneus (Table 1 and Fig. 4). However, there was no CBF change following 30 min MTEAS. Absolute CBF analyses of the functional ROIs were based on the above mentioned regions. In all the ROIs, absolute CBF showed significant decrease after 30 min TEAS (all $p<0.001$) as compared with that recorded before (Fig. 5A). There was no difference of the absolute rCBF (all $p>0.05$) between the pre-MTEAS rest and the post-MTEAS rest (Fig. 5A). In addition, there was no rCBF difference between the pre-MTEAS rest and the pre-TEAS rest in all above ROIs (all $p>0.05$, unpaired t-tests).

2.4. *Results of ROI comparisons between the effects of TEAS and MTEAS*

Absolute CBF change analysis revealed that rCBF decreased for 5.06 (SEM = 0.78), 5.88 (SEM = 1.18), 8.78 (SEM = 1.28), 8.57 (SEM = 1.39), and 4.58 (SEM = 1.04) ml/100 g/min in right SI, insula and STG, left MOG and IFG, respectively, which were significantly more decreased than in MTEAS (all $p<0.05$) (Fig. 5B). These 5 regions also showed a significant increase in CBF change rate with TEAS other than MTEAS (all $p<0.05$), and the rates of decrease were 7.86% (SEM = 1.13%), 6.74% (SEM = 1.43%), 10.08% (SEM = 1.63%), 10.59% (SEM = 1.71%) and 5.53% (SEM = 1.12%), respectively (Fig. 5C). The left cerebellum showed more decreased absolute CBF (7.92 ± 1.55 ml/100 g/min) in TEAS group as compared to MTEAS group (Fig. 5B), and the left cuneus following 30 min TEAS showed more decreased CBF change rate (12.50 ± 2.81%) than 30 min MTEAS treatment (Fig. 5C).

2.5. *Results of the resting state connectivity analysis*

The DMN group maps in the four different resting states demonstrated consistently robust spatial distribution with the DMN template, including bilateral posterior cingulate, precuneus, medial prefrontal cortex (mPFC), middle temporal lobe (MTL) and inferior parietal lobule (IPL) (the DMN group map for each state showed in Fig. 6A). When comparing connectivity maps after 30 min TEAS with that before, increased connectivity of this network was observed in some brain regions, including left IPL, precuneus, STG and middle temporal gyrus (MTG), and right pre-supplementary motor area (pre-SMA) (Table 2 and Fig. 7A). In MTEAS group, following 30 min MTEAS, left STG and right SI showed increased connectivity with the DMN, meanwhile, decreased connectivity with this network was shown in mPFC and precuneus (Table 2 and Fig. 7B). As compared to the MTEAS group, DMN showed more increased connectivity with IPL after 30 min TEAS (Table 2).

The SMN group maps for the four resting states included bilateral pre- and post-central gyrus, supplemental motor area (SMA) and SII (the SMN group map for each state showed in Fig. 6B), which were also consistently robust spatial

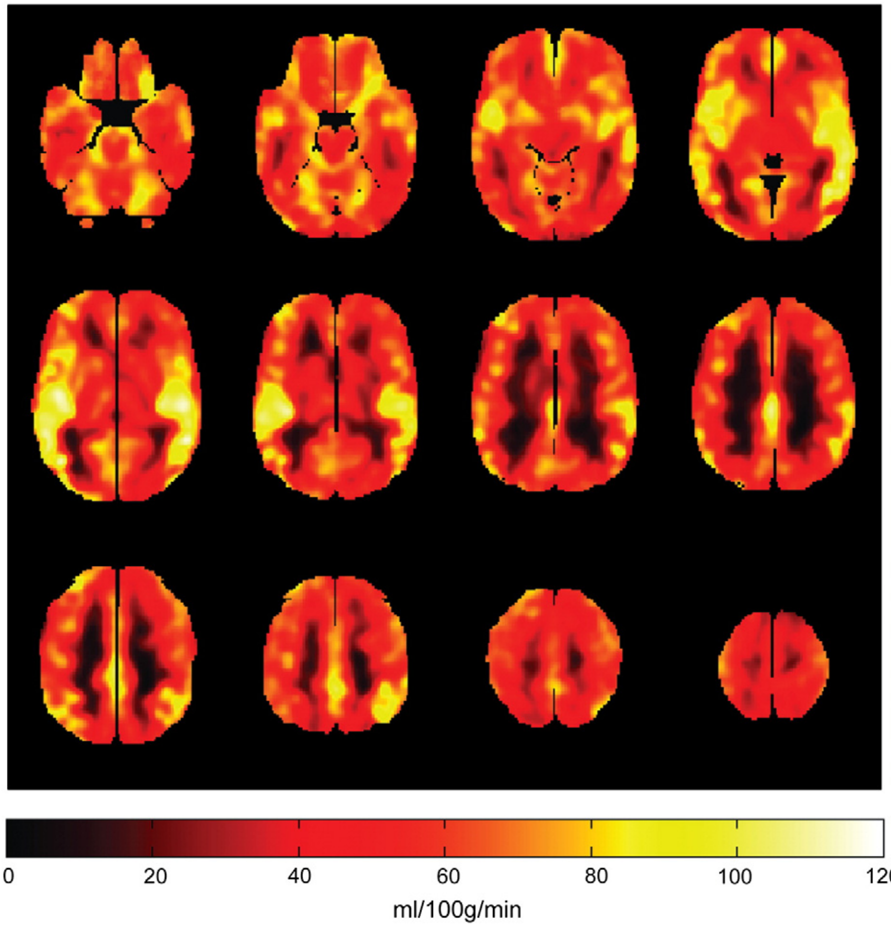

Fig. 2 – Quantitative CBF maps from a representative subject.

distributed with predefined template (Fig. 6B). After 30 min TEAS, increased connectivity of the SMN was observed with precuneus and cerebellum (Table 3 and Fig. 8A). In MTEAS group, only dorsolateral prefrontal cortex (DLPFC) showed decreased connectivity with the SMN following 30 min MTEAS (Table 3 and Fig. 8B). There was no difference in SMN connectivity changes between the two groups.

3. Discussion

The purpose of the present study was to assess how the resting state brain activities were modulated by relatively long-period (30-min) TEAS. The major findings of this study were that (1) the mean globe CBF was decreased after 30 min TEAS but not MTEAS; (2) compared with MTEAS, brain regions in right SI, insula and STG, left MOG and IFG were more deactivated by 30 min TEAS; (3) Following 30 min TEAS, but not MTEAS, there was more secure and spatially extended connectivity of the DMN and the SMN. To our knowledge, this is the first report on the study of acupuncture mechanisms using perfusion fMRI.

3.1. *TEAS was used in lieu of manual acupuncture*

Traditional acupuncture uses manual needling at specific acupoints to achieve a therapeutic effect. One of the difficulties encountered in this procedure was how to standardize the manipulation in order to make the results of the study reproducible. The finding that acupuncture-induced analgesic effect could be totally abolished by prior infiltration of the local anesthetic procaine deep into the acupoint, suggested the importance of neural conduction for acupuncture mechanism (Research Group Of Acupuncture Anesthesia, 1973). A natural consequence of this important finding was the use of electrical stimulation for nervous tissue instead of mechanical stimulation. The electrical pulses can be applied either on the stainless needle inserted into the acupoint (EA) or on the skin of the acupoints via self-sticky electrodes (TEAS). In a thorough study for the comparison of the analgesic effect produced by EA and TEAS in rats, Wang et al. revealed that TEAS can produce an analgesic effect comparable to, if not more potent than EA. Both EA- and TEAS-induced analgesic effects could be blocked by the opioid antagonist naloxone in a dose-dependent and frequency-dependent manner (Wang et al., 1992). The priorities of EA over manual needling are the

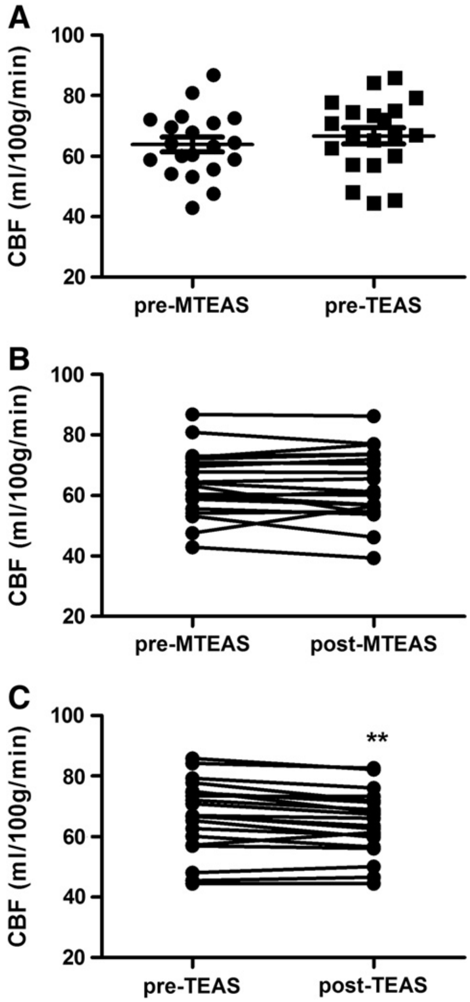

Fig. 3 – The mean global CBF value comparisons: (A) there was no global CBF difference between the pre-MTEAS and the pre-TEAS, using unpaired *t*-test; (B) the mean global CBF did not change following 30 min MTEAS, using paired *t*-test; (C) after 30 min TEAS, the mean globe CBF was decreased as compared to the pre-TEAS, **, $p<0.01$, paired *t*-test.

Table 1 – Brain areas showing significant deactivation (CBF decreases) after 30 min TEAS (pre->post-TEAS resting state, paired *t*-test).

Regions (BA)	Side	t value	Coordinate (MNI) x	y	z	Number of voxels in cluster
SI (2)	R	7.72	58	−32	48	49
STG (22)	R	6.93	60	−16	2	92
MOG (37)	L	6.85	−46	−68	−10	108
Cuneus (18)	L	6.53	−12	−104	4	33
	R	5.31	14	−92	2	14
Insula	R	5.68	38	16	2	33
Cerebellum	L	5.43	−36	−70	−28	37
IFG (44)	L	5.38	−54	4	16	12
Parahipp G (19)	R	4.98	26	−48	−6	10

Note. BA, Brodmann area; L, left; R, right; SI, primary somatosensory cortex; IFG, inferior frontal gyrus; STG, superior temporal gyrus; MOG, middle occipital gyrus; Parahipp G, parahippocampal gyrus. The threshold was set to FDR corrected, $p<0.05$ with at least 10 continuous voxels.

precision of the stimulation parameters, the high reproducibility of the therapeutic effects and the great saving of man power, while the priorities of TEAS over EA, on top of the previously mentioned ones, are the non-invasiveness of the procedure, hence its high acceptability by patients.

3.2. *The design of a placebo group for acupuncture-like procedure*

Another difficulty in the research on acupuncture is how to rule out the psychological effects, since the complicated ritual of acupuncture would inevitably produce a strong psychological effect. The most widely used “mock needle” was the “Streitberger needle”, using touching of the needle tip on skin rather than piercing the skin (Streitberger and Kleinhenz, 1998). For mock EA, one can use shallow piercing of the needle through the skin without connecting to the electric stimulator (Ahsin et al., 2009; Lin et al., 2002; Sun et al., 2008; Wu et al., 2002). In the latter case no current is delivered to the body hence no feeling of electricity can be perceived to convince the subject. In our study, we used a mock stimulator with the same appearance, using the same skin electrodes applied on the acupoints. All the subjects admit that they did feel a ‘*deqi*’ sensation (the intensity of stimulation was limited to 5 mA) come and go (10 s on and 20 s off, in order to reduce the total time of stimulation by 2/3). Previous study has shown that this intermittent minimal stimulation did not produce any physiological effect in reducing the urge to smoke as the verum TEAS did (Lambert et al., 2009). Another point one may like to make is that unlike the procedure of leaving the needle in situ without movement (needle staying) which may not produce substantial physiological effect (Hamza et al., 1999; Zhao, 2008), the TEAS and MTEAS used in the present study are continuously functioning for the whole 30 min stimulation.

3.3. *A decrease of CBF after 30 min TEAS*

Our data clearly showed that continuous submaximal 2 Hz TEAS, but not MTEAS at 2 acupoints located at the left hand for 30 min did significantly decrease the mean global CBF in resting state in healthy human beings. We then analyzed the effects of TEAS and MTEAS on specific brain regions. The predominant TEAS effect was a decrease in rCBF, representing an inactivation rather than activation of the brain tissue.

Among the brain regions that showed a marked decrease in CBF (Table 1, Figs. 4 and 5), SI and insula are the most frequently reported brain regions activated by acute pain stimulation in previous studies (Borsook and Becerra, 2006; Tracey, 2005; Tracey, 2008). They were associated with localization and early qualitative characterization of somatosensory stimuli (Apkarian et al., 2005; Rainville et al., 1997). It was interesting to note that contralateral SI and insula were all deactivated after 30 min TEAS, and the rCBF in these regions was even more decreased after TEAS than that in MTEAS session. Similarly, the activation of the above regions

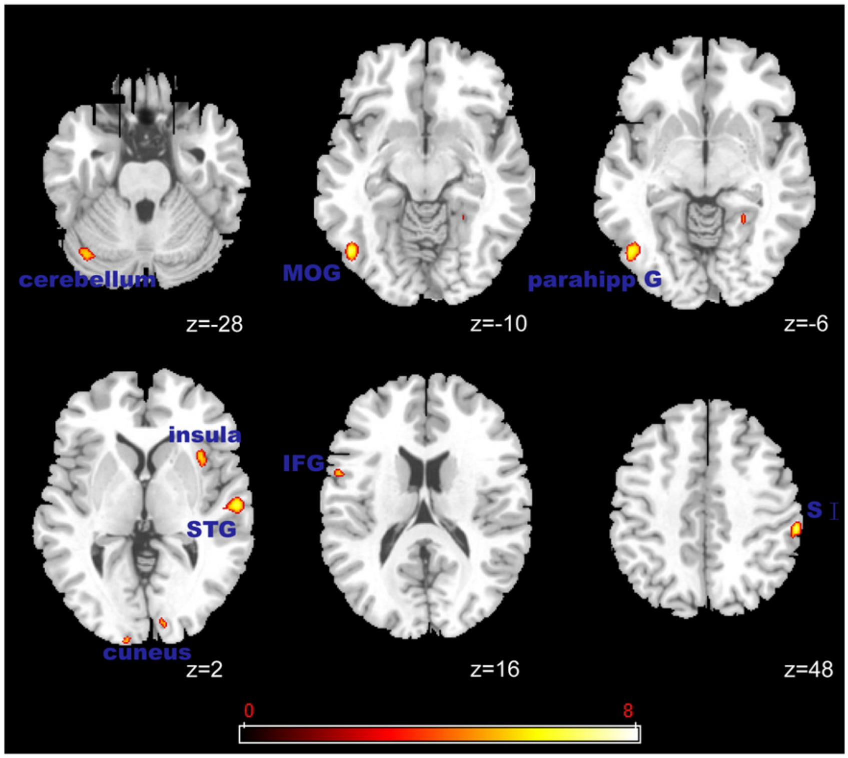

Fig. 4 – CBF group results from 20 subjects in TEAS group were shown in axial sections. Brain areas associated with the comparison of the pre- versus the post-TEAS resting state's CBF value: blood flow was decreased in SI, insula, STG, IFG, MOG, cuneus, cerebellum and parahipp G after 30 min TEAS. The threshold of display was set to FDR corrected, $p<0.05$ with at least 10 continuous voxels.

was revealed to be attenuated after 25–30 min TEAS or EA treatment in a cold pain research in humans (Zhang et al., 2003a, 2003b), a heat pain research in humans (Kong et al., 2009a, 2009b), and a radiant heat study in rats using multi-channel recording technique (Wang et al., 2004). Thus, we speculated that inhibition of the pain-processing areas may be involved in mediating the analgesic effect induced by relatively long-period acupuncture-like stimulation.

Aside from modulating in pain processing, the right fronto-insula cortex has been shown to be a key region in the salience network, which initiates dynamic switching between the DMN and the central-executive network (Sridharan et al., 2008). In our work, besides SI and right anterior insula, we also found a decreased rCBF in MOG in BA 37, STG in BA 22 and IFG in BA 44, which were defined as visual area, auditory and motor speech areas. In addition, parahipp G was revealed a deactivation following 30 min TEAS. Similarly, Hui's group has done a lot of work and showed that acupuncture stimulation, when associated with '*deqi*' sensation, evokes deactivation of a limbic–paralimbic–neocortical network (Hui et al., 2010). Moreover, the above findings were compatible with those of Napadow et al. using BOLD signal changes as an index (Napadow et al., 2009). They used a 31 min block designed EA stimulation, and found a linearly decreasing time-variant activation in sensorimotor brain regions, meanwhile the limbic regions showed activation in early blocks and deactivation in latter blocks. Also, recent researches revealed that acupuncture had a potential for ameliorating insomnia, which was probably due to neurochemical modulatory activity involving serotonin, dopamine and endogenous opioids (Sarris and Byrne, 2011). The inactivated resting state we observed might also imply an abirritative effect induced by acupuncture.

Additionally, CBF in insula showed no significant difference but a trend of increase ($p=0.066$) (see Fig. 5A) after 30 min MTEAS. This trend could be supported by Bai et al.'s report. They found an activation of insula induced by a brief (1.5 min) acupuncture stimulation (Bai et al., 2010a, 2010b). These findings suggested that the effect of acupuncture may have been differently dependent on the intensity and/or the duration of the stimulation.

3.4. *Changes in functional connectivity of the resting state network*

In the present study, there was more secure and spatially extended connectivity of both the DMN and SMN following 30 min TEAS, but not MTEAS. The increased DMN connectivity associated with brain regions including left IPL, precuneus, STG and middle MTG, and right pre-SMA. In SMN, after 30 min TEAS, there was increased connectivity with precuneus and cerebellum. Similar results were reported by Dhond et al. (2008). It's

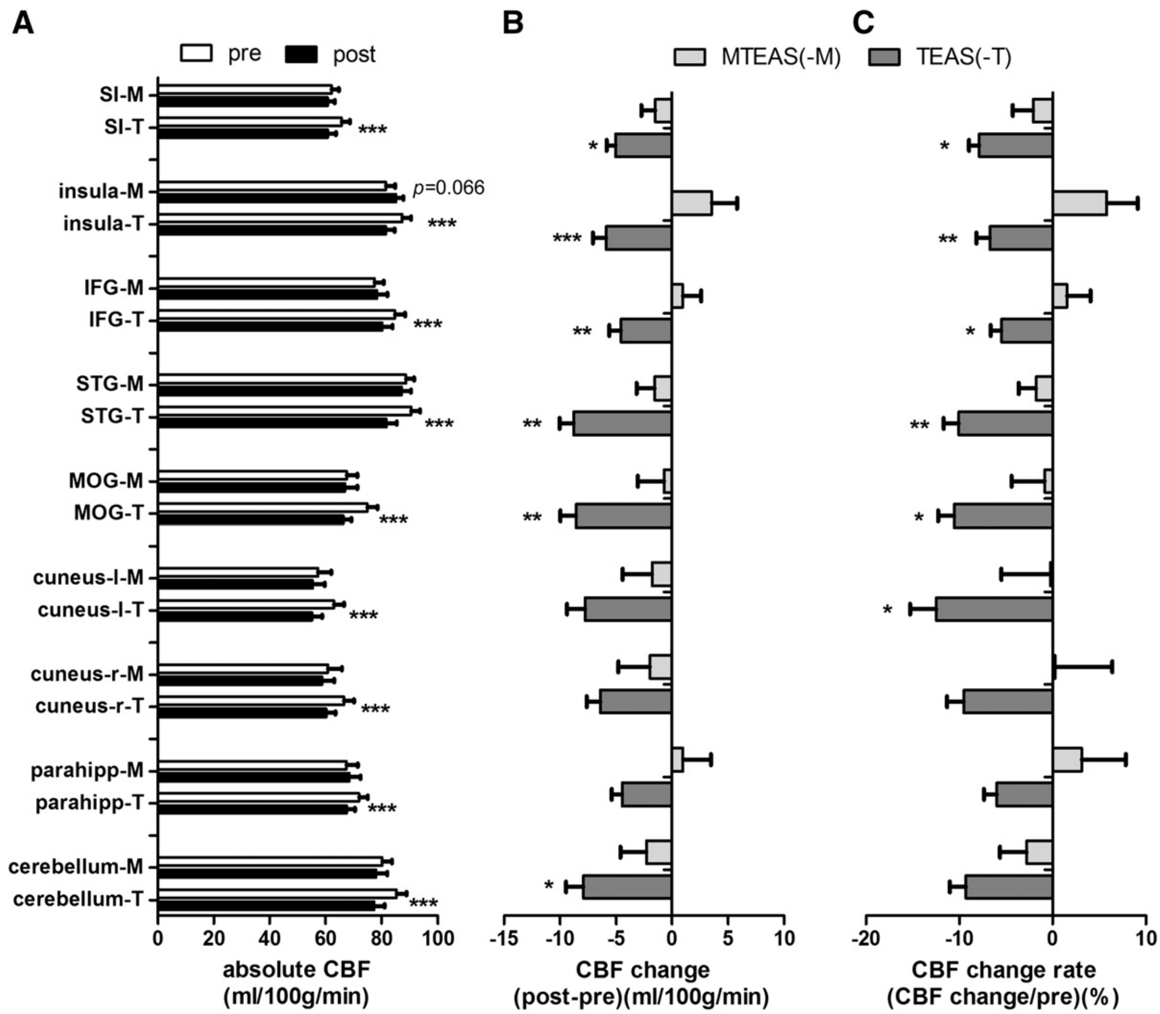

Fig. 5 – Group results from CBF ROI analysis for 30 min TEAS and MTEAS effect. For all the subjects: (A) absolute CBF comparisons between the pre- and the post-TEAS or MTEAS in each ROI, using paired *t*-test; (B) the comparisons of absolute CBF change (post−pre) between TEAS and MTEAS in each ROI, using unpaired *t*-test; (C) the comparisons of CBF change rate (((post−pre)/pre)×100%) between TEAS and MTEAS in each ROI, using unpaired *t*-test. *$p<0.05$; **$p<0.01$; ***$p<0.001$.

interesting to note that precuneus, one important component of DMN, showed increased functional connectivity with both DMN and SMN following 30 min TEAS. In addition, as compared to the MTEAS group, after 30 min TEAS, only IPL, another component of DMN, showed more increased connectivity with DMN. DMN is defined as the brain regions in concerted action to maintain the resting state of the brain (Raichle et al., 2001), so it may not be difficult to infer the DMN as the basic network to maintain the homeostasis. Baliki et al. revealed that the disruption of DMN might underlie the cognitive and behavioral impairments accompanying chronic pain (Baliki et al., 2008). A recent study revealed more connectivity between the periaqueductal gray (PAG) and the PCC during a 25 min EA as compared to sham EA (Zyloney et al., 2010), suggesting that the DMN may play a central role in acupuncture analgesia. Thus, it may give us an insight into the mechanisms of acupuncture's clinical effects. The treatment effects of acupuncture might be the result of modulating the dysfunction of the DMN's functional connectivity in diseased states.

3.5. *Limitation*

It should be noted that this study focused mainly on changes of resting state brain activities induced by relatively long-period TEAS in healthy subjects. Since neither pain stimulation nor patients were introduced here, the present study only gave us with some clues in exploring mechanisms of acupuncture treatment. Further studies should be conducted in healthy subjects in response to quantifiable noxious stimulus, or in patients with acupuncture effective diseases.

Although previous study has proved that the MTEAS cannot reduce the urge to smoke (Lambert et al., 2009), the concern in this study is different, and we did observe the connectivity alterations with ICA analysis and sub-thresholded changes in

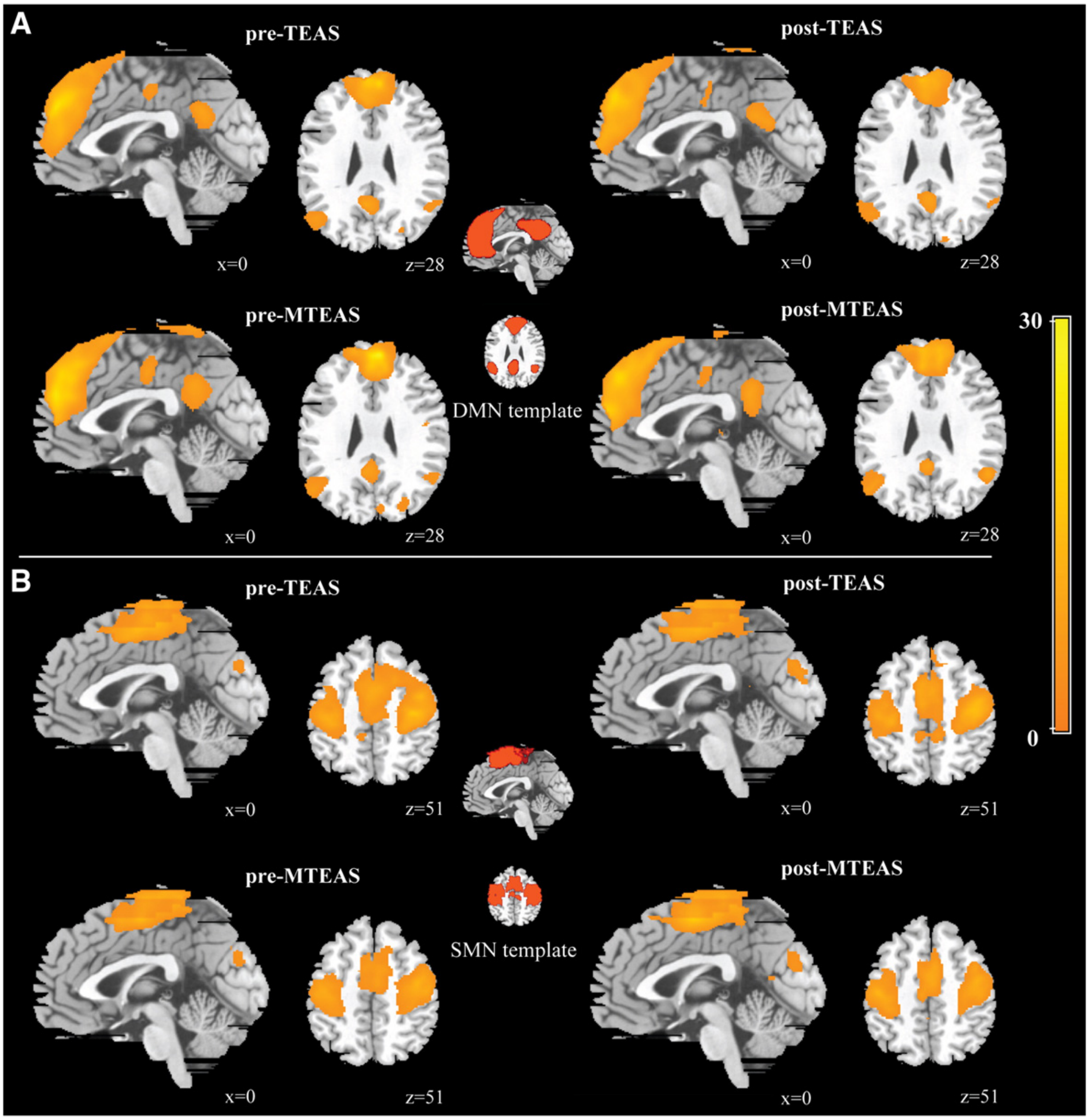

Fig. 6 – Group maps for the DMN and the SMN, before and after 30 min TEAS/MTEAS. The best-fit components were selected by using the templates of DMN and SMN shown in the middle line of the graph. The group results of (A) the DMN and (B) the SMN component decomposed by ICA in the pre-TEAS rest, the post-TEAS rest, the pre-MTEAS rest and the post-MTEAS rest. The threshold of one sample *t*-test was set as FDR corrected, $p<0.05$, with at least 15 continuous voxels in all group statistics.

rCBF induced by MTEAS. Thus, further studies are needed to consolidate the physiological effect of MTEAS on the brain function.

In terms of acupuncture-induced effect, PAG and hypothalamus are generally accepted to play an important role (Han, 2003; Mayer, 2000; Zhao, 2008). Unfortunately, we did not find changes in these regions. One possible explanation is the limited spatial resolution, which might lead to false negative results upon subcortical structures; another is that due to the partial brain coverage of the ASL imaging, regions at the inferior slices may not be consistently acquired (Wang et al., 2011). Further modification for sequence and parameters are needed in order to achieve a better spatial resolution.

4. Conclusion

Perfusion and BOLD fMRI were used to assess the resting state CBF and functional connectivity of the DMN and SMN modulated by TEAS lasting for 30 min in healthy subjects. Following 30 min TEAS, but not MTEAS, the mean global CBF decreased, and a significant decrease of rCBF was observed in SI, insula, STG, MOG and IFG. Functional connectivity analysis showed more secure and spatially extended connectivity of the DMN and SMN after 30 min TEAS. Modulation of these regional brain activities and brain network may relate to acupuncture analgesia as well as other acupuncture-associated therapeutic effects. Additionally,

Table 2 – Brain regions in the DMN modulated by 30 min TEAS or MTEAS.

Region (BA)	Side	z score	Coordinate (MNI)			Number of voxels in cluster
			x	y	z	
TEAS						
Pre > Post (no regions above threshold)						
Pre < Post						
IPL (40)	L	4.88	−66	−32	40	45
Precuneus (7)	L	3.98	−24	−60	48	92
Pre-SMA (6)	R	3.95	40	2	26	39
MTG (22)	L	3.46	−54	−46	0	17
STG (22)	L	3.30	−60	−58	8	24
MTEAS						
Pre > Post						
mPFC (9)	R	3.72	2	40	34	27
Precuneus (7)	R	3.57	10	−62	54	20
Pre < Post						
SI (1)	R	4.12	46	−26	62	79
STG (22)	L	3.76	−50	−42	10	29
TEAS vs. MTEAS (post TEAS–pre TEAS) vs. (post MTEAS–pre MTEAS)						
TEAS > MTEAS						
IPL (40)	L	4.81	−65	−32	40	44
TEAS < MTEAS (no regions above threshold)						

Note. BA, Brodmann area; L, left; R, right; SI, primary somatosensory cortex; STG, superior temporal gyrus; IPL, inferior parietal lobule; pre-SMA, pre-supplementary motor area; MTG, middle temporal gyrus; mPFC, medial prefrontal cortex. The threshold was set to voxel-wise $p<0.001$ uncorrected, with more than 15 continuous voxels.

perfusion fMRI may have a promising prospect for future brain imaging studies on acupuncture mechanisms.

5. Experimental procedures

5.1. *Subjects*

Forty healthy right-handed adult participants who were naïve to acupuncture (20 male, mean age 25 years, range 20 to 35) volunteered for the experiment. No subject had a history of psychiatric or neurological disorder. No female subject was in menstrual period. None was in pain or distress at the time of study. Prior to the commencement of the study procedures, all subjects signed informed consents regarding the purpose of the study, its procedure, its potential discomforts and risks, and were free to withdraw from the experiment at any time. All research procedures were approved by the local ethical committee of Peking University.

5.2. *Experiment procedures*

All of the 40 subjects were randomized into two groups. One group received 30 min TEAS and the other group with 30 min MTEAS. The period of resting state acquisition was 4.5 min, placed both before and after the stimulation (Fig. 9B). During the rest runs, subjects lay supine on the scanner bed, wearing ear plugs to suppress scanner noise and with the head immobilized by cushioned supports. They were instructed to keep their eyes closed and not to think of anything particular during the functional scans. After each run, subjects were asked whether they were sleeping during scanning.

TEAS or MTEAS was performed at acupoints, LI-4 on the left hand (Hegu, on the dorsum of the first interosseous muscle) and PC-8 on the same hand (Laogong, on the volaris of the second interosseous muscle) (Fig. 9A). A pair of electrodes was placed on these two points. In TEAS group, Han's acupoint nerve stimulator (HANS model LH-202H, Neuroscience Research Center, Peking University, Beijing, China) was used to deliver the stimulation with a frequency of 2 Hz. The intensity of TEAS (constant current output) for each subject was adjusted to a maximal but comfortable level. This modality of stimulation has been shown to be effective in treating acute pain and substance abuse (Cui et al., 2008; Lambert et al., 2009; Zhang et al., 2003a, 2003b). In our research, the current intensities were 8–25 (18.9±5.1) mA. In MTEAS group, we used a new machine called "Mock HANS" with an appearance identical with the HANS LH-202H. The differences were that (1) the current output was fixed on 5 mA, just above the sensory threshold (3 or 4 mA) (Zhang et al., 2003a, 2003b), no matter what the display was showing, and (2) the output was intermittent, with 10 s on and 20 s off. MTEAS was reported not physiologically effective (same as the 0 Hz or null output group) in a research using TEAS to reduce tobacco urges in dependent smokers (Lambert et al., 2009). In both TEAS and MTEAS sessions, no noxious feelings were allowed. In addition, the feelings of '*deqi*' were collected, including soreness, numbness, fullness, heaviness, and dull pain. Subjects were asked to rate each sensation feeling they had experienced during the stimulus by using a visual analog scale (VAS) from 0 to 100. The reported percentage of subjects and the intensity of the feeling sensations were recorded respectively.

5.3. *MRI scanning procedure*

All MRI experiments were performed at a GE 3 T whole body scanner with an 8-channel receive-only head coil. CBF and BOLD data were acquired using a quantitative pulsed ASL sequence (PICORE QUIPSS II) (Wang et al., 2002, 2003; Wong et al., 1997) with a dual-echo single-shot spiral acquisition (TE1 = 3.1 ms and TE2 = 30 ms). The acquisition parameters were: TR = 3000 ms, flip angles = 90°, field of view (FOV) = 230 × 230 mm^2, matrix = 64 × 64, in-plane resolution = 2.9 × 2.9 mm^2, saturation pulse time = 700 ms, and delay time (between saturation and excitation) = 800 ms. Twelve axial sections, each 8.0 mm thick with 2.0 mm inter-slices, were collected to encompass the whole cerebrum and most of the cerebellum. After both functional imaging sessions, high-resolution structural information on each subject was acquired using a 3D FSPGR sequence for anatomical localization (TR/TE = 7.8/3.0 ms, flip angles = 20°, IR = 450 ms, FOV = 230 × 230 mm^2, slice thick = 2.0 mm with 1.0 mm overlap, in-plane resolution = 1 × 1 mm^2).

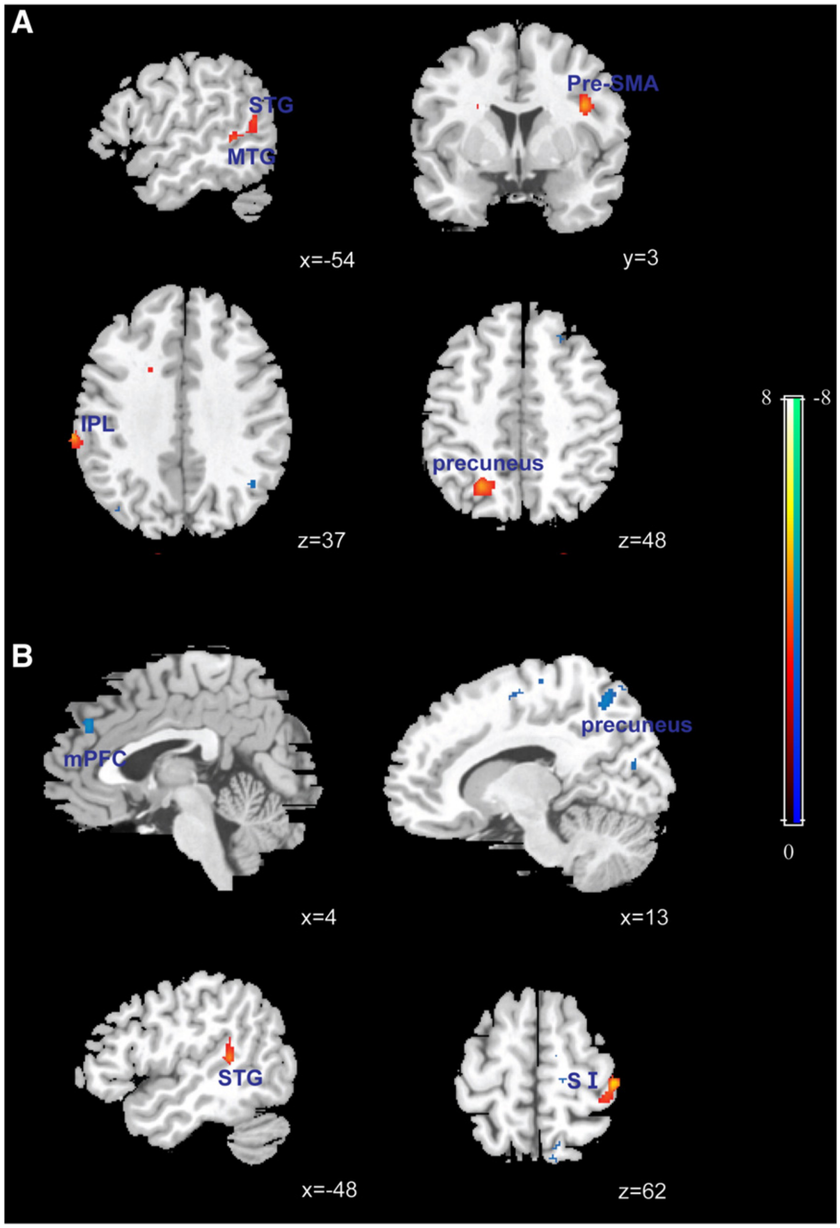

Fig. 7 – The changes in functional connectivity of the DMN after 30 min TEAS or MTEAS (after–before). (A) Following 30 min TEAS there was increased connectivity of the DMN with STG, MTG, pre-SMA, IPL and precuneus. (B) Changes in the DMN for 30 min MTEAS involved increased connectivity with STG and SI, and decreased connectivity with mPFC and precuneus. The threshold of display was set to voxel-wise $p<0.001$ uncorrected, with at least 15 continuous voxels.

5.4. *CBF data analysis*

A statistical parametric mapping software (SPM 2, Wellcome Department of Cognitive Neurology, London, UK) based ASL data processing toolbox, ASLtbx (Wang et al., 2008), and software Graph Pad Prism 5.0 were used for data analyses.

For each run's data, the first ten volumes were discarded to eliminate nonequilibrium effects of magnetization, and then the left 80 volumes were realigned to the first one. Perfusion-weighted image series were then generated by pairwise subtraction of the label and control images using sinc interpolation, followed by subtraction to suppress BOLD contaminations (Liu and Wong, 2005). Then, CBF quantification was used to generate absolute CBF image series based on a single-compartment continuous arterial spin-labeling perfusion model utilizing ASLtbx. Thus, the resulting CBF datasets 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 MNI152 standard space and resampled at 2 mm × 2 mm × 2 mm. The datasets were then spatially smoothed with 8 mm full-width at half maximum (FWHM) Gaussian kernel. One mean CBF image was generated for each run for each individual subject. Thus, each subject had two CBF images for the pre-TEAS rest and the post-TEAS rest, or the pre-MTEAS rest and the post-MTEAS rest, respectively.

Table 3 – Brain regions in the SMN modulated by 30 min TEAS or MTEAS.

Region (BA)	Side	z score	Coordinate (MNI)			Number of voxels in cluster
			x	y	z	
TEAS						
Pre > Post (no regions above threshold)						
Pre < Post						
Precuneus (7)	R	3.98	10	−40	48	42
		3.93	26	−68	34	22
Precuneus (19)	L	4.10	−24	−76	30	35
Cerebellum	L	3.33	−20	−36	−24	20
MTEAS						
Pre > Post						
DLPFC (9)	L	3.81	−34	22	52	18
Pre < Post (no regions above threshold)						
TEAS vs. MTEAS (post TEAS–pre TEAS) vs. (post MTEAS–pre MTEAS)						
TEAS > MTEAS (no regions above threshold)						
TEAS < MTEAS (no regions above threshold)						

Note. BA, Brodmann area; L, left; R, right; DLPFC, dorsolateral prefrontal cortex. The threshold was set to voxel-wise $p<0.001$ uncorrected, with more than 15 continuous voxels.

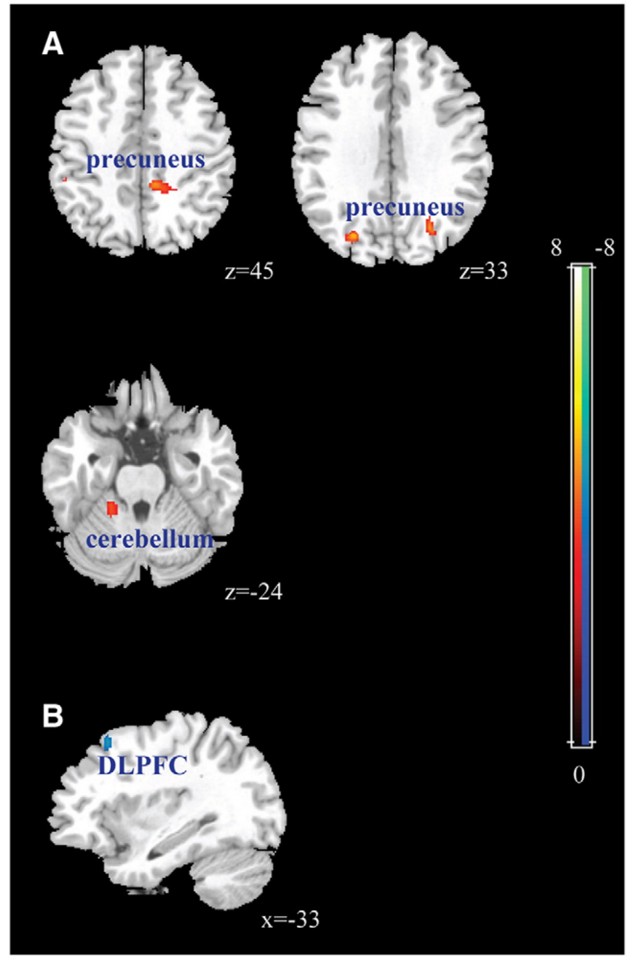

Fig. 8 – The changes in functional connectivity of the SMN after 30 min TEAS or MTEAS (after – before). (A) Following 30 min TEAS there was increased connectivity of the SMN with precuneus and cerebellum. (B) Changes in the SMN for 30 min MTEAS showed decreased connectivity with DLPFC. The threshold of display was set to voxel-wise $p<0.001$ uncorrected, with at least 15 continuous voxels.

Absolute global CBF was calculated and compared by paired *t*-test. Contrasts using voxel-wise general linear model (GLM) analysis were defined to compare the pre-TEAS rest and the post-TEAS rest (pre- vs. post-TEAS), also the pre-MTEAS rest and the post-MTEAS rest (pre- vs. post-MTEAS). The activated or deactivated clusters were identified at a significance level of FDR corrected, $p<0.05$ with cluster size no less than 10 voxels. ROI analysis was also conducted to calculate absolute (quantitative) regional CBF (rCBF) and to assess whether TEAS and MTEAS changed rCBF differentially. Each ROI was centered in the highest t value's location with isotropic 6 mm radius. Absolute CBF of the pre- and the post-TEAS or MTEAS was compared using paired *t*-test. Absolute CBF changes (post-minus pre-TEAS or MTEAS) and CBF change rates ((absolute CBF change/pre-TEAS or MTEAS) × 100%) were calculated, and unpaired *t*-tests were used to compare TEAS with MTEAS. The accepted level of statistical significance is $p<0.05$.

5.5. *BOLD data analysis*

BOLD imaging data processing and analyses were carried out with SPM 2 and Group ICA of the fMRI Toolbox (GIFT, http://icatb.sourceforge.net/). After discarding the first 10 volumes, head-motion correction, co-registration, normalization, and smoothing procedures were the same as the processing of CBF data. The smoothed data were then arranged into Group ICA, using the Informax ICA algorithm, the number of independent components was separated, which was estimated to be 25 by MDL criteria. The DMN or SMN component was identified by spatially sorting the entire components with the corresponding mask (Fig. 6) (Franco et al., 2009), and then the best-fit component for each subject was extracted from each individual run. One sample *t*-tests were used to examine the group results for the DMN and SMN maps (FDR corrected, $p<0.01$ with at least 15 continuous voxels). Paired *t*-tests were performed to determine differences in spatial extant between the DMN/SMN before versus after the TEAS/MTEAS runs, thresholded at voxel-wise $p<0.001$ uncorrected with 15 continuous voxels. Results of the above paired *t*-tests were made to mask for the later unpaired *t*-tests. Unpaired *t*-tests were used to compare between the two groups in different components, the threshold was set to $p<0.001$ uncorrected within the mask with at least 15 continuous voxels.

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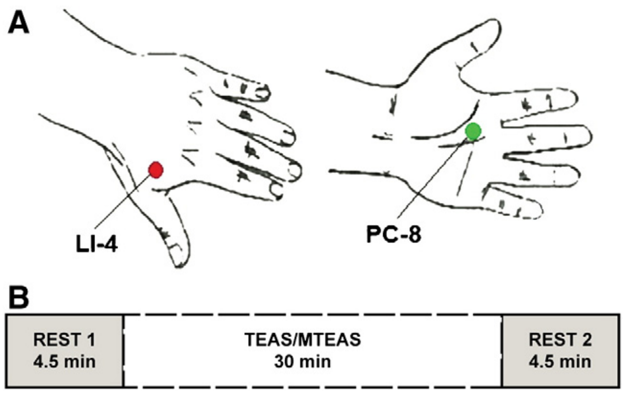

Fig. 9 – Experimental paradigm: (A) TEAS was performed at left LI-4 on the dorsum of the first interosseous muscle (Hegu, arrow pointing to red dot) and left PC-8 on the volaris of the second interosseous muscle (Laogong, arrow pointing to green dot); (B) functional scanning incorporated with two independent rest runs (REST 1, REST 2), each lasting 4.5 min, and the two runs were separated by a 30-min TEAS or MTEAS without scanning.

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