

Simultaneous CBF and BOLD Mapping of High Frequency Acupuncture Induced Brain Activity

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Introduction: Acupuncture, which was developed in Asia thousands of years ago, has long been used for drug addiction treatment, stroke rehabilitation, asthma and chronic pain relief, etc [1]. While more studies are still needed to better understand its biological underpinning. In the present study, the transcutaneous electrical nerve stimulation (TENS), one form of electrical acupoint stimulation (EAS), was applied to investigate acupuncture mechanisms [2,3,4]. The cerebral blood flow (CBF) and blood oxygenation level dependent (BOLD) provide similar but distinct measures of brain activity with their own advantages. Given the higher sensitivity of BOLD and higher spatial specificity and reproducibility of CBF [5,6], the two techniques are expected to be somewhat complementary. Some previous studies have indicated that both low frequency and high frequency acupuncture can produce analgesia, and peripheral electric stimulation with different frequencies can affect the central nervous system differently, even though some overlapped brain networks were found to be involved [2,7]. In many acupuncture studies, the acupoint LI4 (*Hegu*) has been selected because it is used very frequently for analgesia, sedation as well as relieving stress and depression [8,9]. For this acupoint, the case of low frequency acupuncture, by either manual or electrical stimulation, has been investigated in a number of BOLD or CBF fMRI studies. However, the stimulation with high frequency at LI4 has been scarcely studied by fMRI. With all of these considerations, we investigate brain activities induced by high frequency EAS at LI4, with a technique combining CBF and BOLD acquisitions to yield complementary information with minimal-EAS as a control method.

Materials and Methods: *Subjects:* Forty right-handed subjects (20 males and 20 females, age 22–30 years) participated in this study and all subjects were EAS naive. Twenty participants (10 males and 10 females) received EAS and the remaining 20 subjects (10 males and 10 females) received control stimulation (minimal-EAS). The average age of the EAS group was 25.9 and that of the minimal-EAS group was 26.4 years old (two tailed t-test, $p = 0.5364$).

Electroacupuncture: Electroacupuncture was delivered with an MRI-compatible Hans 200 electric acupoint stimulation device (Nanjing Gensun Medical Technology Co. Ltd., Nanjing, China). EAS/minimal-EAS at 100 Hz was administered with a pair of adhesive skin electrodes placed on the LI4 (*Hegu*) acupoint of left hand. The intensity was adjusted to a maximal but comfortable level for EAS [2,4], with the intensity ranged from 7 to 15 mA with an average of 9.6 mA. For participants receiving minimal-EAS, the intensity was set to a level just above the detectable threshold (3 to 4 mA,)[2]. Each fMRI scan lasted 6.3 minutes comprised of alternating 0.9-minute blocks of rest (A) and EAS/minimal-EAS (B) with the rest block first, i.e. A-B-A-B-A-B-A, resulting in 2.7-minute EAS/minimal-EAS and 3.6-minute rest. The acupuncture sensations (*deqi*, a mixture sensation including numbness, tingling, fullness, and dull ache with little or no pain) [8] experienced by the subjects during the stimulation were assessed immediately after the scanning using a questionnaire.

Image Acquisition: All MRI experiments were performed on a General Electric 3T Signa system (GE Medical Systems, Waukesha, WI) with a standard head coil. Functional data were acquired using a double readout spiral-out sequence with simultaneous Gradient-echo CBF and BOLD acquisitions, at short and long TEs, respectively [10,11]. Both readouts utilized slice thickness / gap (THK) of 8.0 / 2.0 mm with 3.6 x 3.6 mm² in-plane resolution, a 230 mm² field of view (FOV) with a 64 x 64 acquisition matrix, a repetition time (TR) of 3000 ms and a 90° flip angle. Following inversion, the time of the saturation pulse was 700 ms with an 800 ms delay between saturation and excitation. CBF/BOLD readouts were acquired at TEs of 3.1/30 ms, respectively, covering 10-12 axial slices of the whole brain.

Data Analyses: For each subject, data were first motion corrected. *Sinc* interpolation of the ASL time course was performed to obtain time-matched control and label images, following by subtraction to suppress BOLD contamination [12]. Each subject's functional images were then coregistered with the corresponding anatomical images to facilitate transformation to Montreal Neurological Institute (MNI) space and resampling to isotropic 2 mm³ voxels. The data was spatially smoothed with a Gaussian kernel of 8 mm at full-width half-maximum and analyzed using a random effect approach subsequently. A general linear model (GLM) with hemodynamic response function was applied to the smoothed data to model MR signal responses of each experimental paradigm for all subjects individually. The data of BOLD were high-pass filtered with a cut-off of 240 s. Single subject contrast maps were then analyzed by a second-level analyses using two-sampled t test to obtain group comparisons between EAS and minimal-EAS. A combined threshold of $p < 0.001$ for single voxel with a minimum cluster size of 73 voxels, corresponding to a corrected p-value of 0.05 according to Monte Carlo simulation by the AlphaSim program was used for the generation of the comparison maps.

Results: Just detectable level of sensation and no *deqi* was experienced for all twenty subjects who underwent minimal-EAS. Varying degrees of *deqi* sensation such as tingling, fullness or numbness were experienced by all the subjects in the EAS group [8]. In the comparison of EAS versus Minimal-EAS, the right PMCC, caudal anterior cingulate cortex (ACC, BA24), thalamus, and left insula were activated in the CBF maps, while the activations in right SII, MI and inferior frontal gyrus were found in BOLD maps (Fig 1). Moreover, deactivations in right middle temporal gyrus and cuneus were found in CBF maps, and deactivations revealed by BOLD signals resided in bilateral rostral anterior cingulate cortex (rACC), frontal gyrus, ipsilateral cuneus, M1, parahippocampal gyrus, contralateral precuneus, occipital gyrus, and temporal gyrus.

Discussion: In the comparison of EAS and Minimal-EAS to examine the central effects elicited at LI4, deactivation in default mode network (such as middle temporal gyrus, cuneus and precuneus) was observed in both CBF and BOLD responses, and also in posterior cingulate cortex (PCC) as given in BOLD maps. Moreover, the activation in insula, thalamus, caudal anterior cingulate cortex (ACC, BA24) was only found in CBF, while activation in SII and deactivation in putamen, parahippocampal gyrus and rostral ACC (rACC) were solely observed in the BOLD responses. These different maps provided the complementary information of brain activation patterns, revealing the involvement of some limbic regions and the subcortical areas related to sense or pain. Also the response to hypothalamus was not presented in this study with high frequency, while demonstrated in low-frequency acupuncture [8,9]. This is consistent with the previous findings of hypothalamus' important role in mediating low but not high frequency EA analgesia [13], and may reflect the frequency related difference.

Conclusion: To our knowledge, this is the first study to examine brain responses to high frequency EAS and Minimal-EAS at LI4 acupoint by using CBF and BOLD contrasts simultaneously. The simultaneous use of both techniques provides complementary information for more comprehensive elucidation of the effects of EAS. The results suggest that the high frequency EAS at LI4 elicits central effects by modulating subcortical areas and limbic system.

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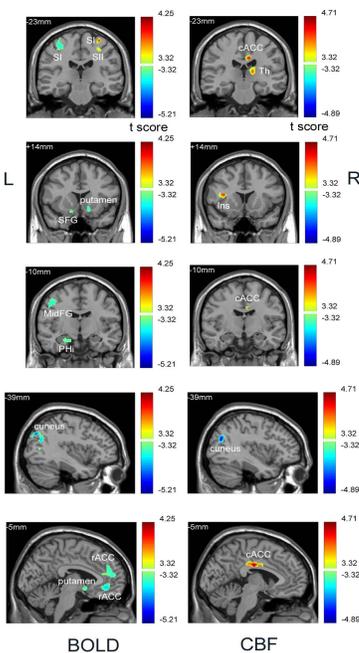


Fig1 Comparisons between EAS and minimal-EAS for BOLD (left column) and CBF (right column).