Modulation of cold pain in human brain by electric acupoint stimulation: evidence from fMRI

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The purpose of this study is to investigate the modulation of pain responses in the human brain by electric acupoint stimulation (EAS). Eight healthy subjects were enrolled; each received real or mock EAS treatment in separate sessions. Cool (18°C) and cold (2°C) stimuli were delivered, during which functional magnetic resonance imaging scans were performed, before and after treatment. Real EAS specifically increased the pain-specific activation in bilateral secondary somatosensory area, medial prefrontal cortex, and Brodmann area (BA) 32, while it decreased the activation in contralateral primary somatosensory area, BA7, and BA24. We suggest that EAS may induce an analgesic effect via modulation of both the sensory and the emotional aspect of pain processing. *NeuroReport* 14:1591–1596 © 2003 Lippincott Williams & Wilkins.

Key words: Acupuncture; Analgesia; Anterior cingulate cortex; fMRI; Pain; Somatosensory area

INTRODUCTION

Acupuncture therapy has been used in Oriental countries for many centuries, and pain is the very first disorder that was recommended for treatment with acupuncture by the NIH 1997 consensus [1]. The best acceptance of acupuncture analgesia is based on numerous reports that the release of endogenous opioids could be accelerated by acupuncture stimulation [2–4]. However, the neural substrate of acupuncture analgesia in the brain is still far from clear, although some lesion studies in animals contributed a bit to this field [5,6]. The evidence in human beings was very slight until the development of brain imaging techniques such as fMRI.

Activations and deactivations in cortex and subcortical structures during acupuncture stimulation have been reported recently [7–12], emphasizing the key role of limbic system (hypothalamus, hippocampus, amygdale, nucleus accumbens, and anterior cingulate) in manual acupuncture or electric acupoint stimulation (EAS) actions. Our previous fMRI work also indicated that there was a linear relationship between the effect of EAS-induced analgesia and the activation/deactivation levels in similar areas (to be published elsewhere). However, all the aforementioned studies addressed the brain responses to acupuncture stimulation itself. Though authors of all these papers tried to relate these responses with analgesia, the specificity of these studies is still dubious since the acupoints tested most often (such as ST36 and LI4) could also be used for treating many other diseases, according to the traditional theory of acupuncture.

In the current study, we tried to investigate the direct modulation of human brain pain responses by electric acupoint stimulation. We hypothesized that cold pain-induced activation measured by fMRI would be changed (inhibited or enhanced) after EAS treatment, which might contribute to mechanisms underlying acupuncture analgesia in human beings.

MATERIALS AND METHODS

Subjects: Eight healthy right-handed college students (gender balanced), aged 25 ± 3.0 years (mean $\pm$ s.d.), were enrolled in the current study. None had a history of neurological illness, head injury, or substance abuse. None had a history of chronic pain or long-term use of analgesics. The study was approved by the local ethic committee. Understanding and written informed consent was obtained from all subjects.

Thermal stimuli and experimental protocol: All subjects underwent two scanning sessions (one electric acupoint stimulation session, and one mock-EAS session, order balanced) 8 days apart. Each session consisted of two repetitive functional cold-pain experiments: before and after EAS. Thermal stimuli were delivered to the thenar eminence of the left hand by an fMRI-compatible Peltier-type thermal

stimulator (TSA-II, MEDOC, Israel) with a $3 \times 3\,mm^2$ thermode. The stimuli were habituated in each subject with a test protocol before the formal experiment. No subject reported any additional pressure by the thermode other than the light contact. During the fMRI scanning, the thermal stimulation task consisted of three kinds of temperatures: A, 32°C (baseline, lasted 15 s); B, 18°C (cool, non-painful, lasted 45 s); and C, 2°C (cold, painful, lasted 45 s). The block design was either A-B-A-C-A-B-A-C-A-B-A-C-A or A-C-A-B-A-C-A-B-A-C-A-B-A, balanced among all subjects.

Immediately after MRI scanning all subjects were asked to rate the averaged extent of pain intensity and distress before and after electric acupoint stimulation, respectively. The eleven-point (0–10) rating scale was used, with 5 as the threshold for pain or distress. In addition, they were required to retrospectively rate the extent of anxiety during the whole experiment with a questionnaire proposed by Spielberger [13].

Electric acupoint stimulation: After the before-EAS experiment, each subject received real or mock EAS treatment. For both treatments, the stimulation sites were acupoints ST36 and SP6 on the left leg, and the stimulation frequency was 2 Hz with square wave width of 0.6 ms. The stimulation intensity for real EAS was adjusted to a maximal but comfortable level, ranging from 8 to 16 mA (10.5 ± 1.1 mA, mean $\pm$ s.e.m.). For mock EAS, the intensity was set at the sensation threshold (3.9 ± 0.1 mA). Both EAS and mock EAS lasted 30 min and the intensity of real EAS stepwise increased by 1 mA every 10 min. Based on our experience in acupuncture research [14] and literature review [7–12,15], acupuncture-induced analgesia does not show strong acupoint specificity. Thus, an alternative position may generate similar analgesic effect. This renders the alternative position control unsuitable to reveal mechanism of acupuncture analgesia. Hence we selected mock EAS (same point with minimal intensity) as the control treatment, because it is similar to the real EAS (suitable to serve as a placebo), quantitatively discriminatable, and also easy to explain the result (only one stimulation parameter was changed).

Imaging data acquisition: All MRI experiments were performed on a 1.9 T. whole body MRI scanner (Prestige, GE/Elscint Ltd., Haifa, Israel). For the fMRI images, a gradient echo planar imaging (EPI) T2*-weighted sequence based on blood oxygenation level dependent (BOLD) effect was employed. The slice thickness/space (THK) was set at 6.0/0.0 mm, in-plane resolution at 2.9 mm $\times$ 2.9 mm, and TR/TE/flip angle at 3000 ms/45 ms/90°. The field of view (FOV) was $373 \times 212\,mm^2$, and the acquisition matrix was 128×72. A complete set of 20 continuous axial sections covering the whole brain including cerebellum was obtained repeatedly every 3 s; hence there were 139 volumes for each fMRI scan. For anatomical images, a 3D gradient-echo T1-weighted sequence (TR/TE 25/6 ms; FOV 220 $\times$ 220 mm²; THK 2.0/0.0 mm, matrix 220 $\times$ 220; resolution 1 $\times$ 1 mm²) was employed and the axial images were acquired after the before-EAS experiment.

Data analysis: Image processing and statistical analysis was carried out using SPM99 [16]. The first three volumes were discarded to eliminate nonequilibrium effects of magnetization. The left 136 volumes were realigned to the first one. A mean image was created from the realigned volumes and was used for the derivation of normalization parameters, according to which all volumes were spatially normalized (resampling every 3 mm) later. Finally, Gausian smoothing was applied to the realigned and normalized BOLD-contrast images with an isotropic kernel (FWHM = 7 mm). Statistical analysis at individual and group level was introduced subsequently. At the individual level, volumes were analyzed using a general linear model with box-car functions convolved with a hemodynamic response function to each block. After the fMRI model estimation, three *t*-contrasts were defined as cool–baseline, pain–baseline and pain–cool. We paid more attention to the pain–cool contrast, which is a specific response to pain. Each contrast had a statistical parametric map of the *t*-statistic for result display.

In the group analysis, mean statistical parameter mapping was produced by one-sample *t*-test on a voxel-by-voxel basis. In all contrasts (cool–baseline, pain–baseline and pain–cool), no significant difference was found between the pre-treatment groups of real and mock EAS. Therefore, 16 sets of data, two from each of the subjects, were pooled together for the calculation of the averaged results. Voxel-by-voxel paired *t*-test was used to compare the changes before and after EAS treatment for the pain–cool contrast. The *p* threshold was set to 0.01 (uncorrected) for both individual and group analysis. The minimal cluster requirement was 10 voxels, hence the overall significance of the results (alpha) is < 0.05 calculated with a program (AlphaSim) proposed by Douglas Ward (http://afni.nimh. nih.gov/afni/AFNI_Help/AlphaSim.html).

All activation foci were superimposed on the standard T1 template images, and a utility compatible with SPM proposed by Sergey Pakhomov (http://www.ihb.spb.ru/ ~pet_lab/TSU/TSUMain.html) were used to locate those foci. Finally, the results were carefully examined by an experienced neuroradiologist (Z.J.).

Region-of-interest (ROI) delineation: Six ROIs were selected for analysis based on the preliminary examination of the results. Anatomical structures and Brodmann areas were designated according to coordinates from the brain atlas implemented in the program used for area localization mentioned above. The peak *t*-values for each ROI from each individual subject were extracted by SPM as the index to reflect spatially different activations and compared using the paired *t*-test between before EAS and after EAS conditions.

RESULTS
Because of the habituation to the thermal stimuli and MRI scanning before the experiment, there was no apparent anxiety during the experiment for all subjects, as suggested by the average anxiety score of 31.6 ± 1.2 ($n = 16$), which is within the normal range for the high school students (39.45 ± 9.74) [13].

Analgesic effect of EAS: As indicated in Fig. 1, electric acupoint stimulation induced significant suppression of both the intensity and distress of pain ($p < 0.01$). On the contrary, mock EAS showed no significant analgesic effect in either component of pain.

Cool and pain sensation induced BOLD-contrast before EAS treatment: The surface-rendered maps of the averaged activation foci of the before-EAS experiments are shown in Fig. 2a. In the comparison of cool (18°C) *vs* baseline, bilateral secondary somatosensory area and insula (SII/Ins), lateral prefrontal cortex (LPFC, BA10, 46, 45, 44), orbital frontal cortex (BA11, 47), anterior cingulate cortex (ACC, BA 32, 24), posterior cingulate (BA31), thalamus, midbrain, and ipsilateral parietal superior lobule (BA7) were activated. While areas activated by the comparison of 2°C *vs* baseline include bilateral SII/Ins, LPFC, orbital frontal, medial prefrontal cortex (MPFC, BA6, 8, 9), BA32, thalamus, head of caudate, putamen, midbrain, and ipsilateral parietal BA7. The pain-specific response based on the contrast between 2°C *vs* 18°C is related with the activation in contralateral primary somatosensory and motor area (SI/MI), bilateral SII, MPFC, BA32, midbrain, pons, cerebellum, ipsilateral BA7 and middle temporal gyrus (BA37). In summary, patterns of cool sensation and cold pain evoked activation were inconsistent with previous reports [17–19].
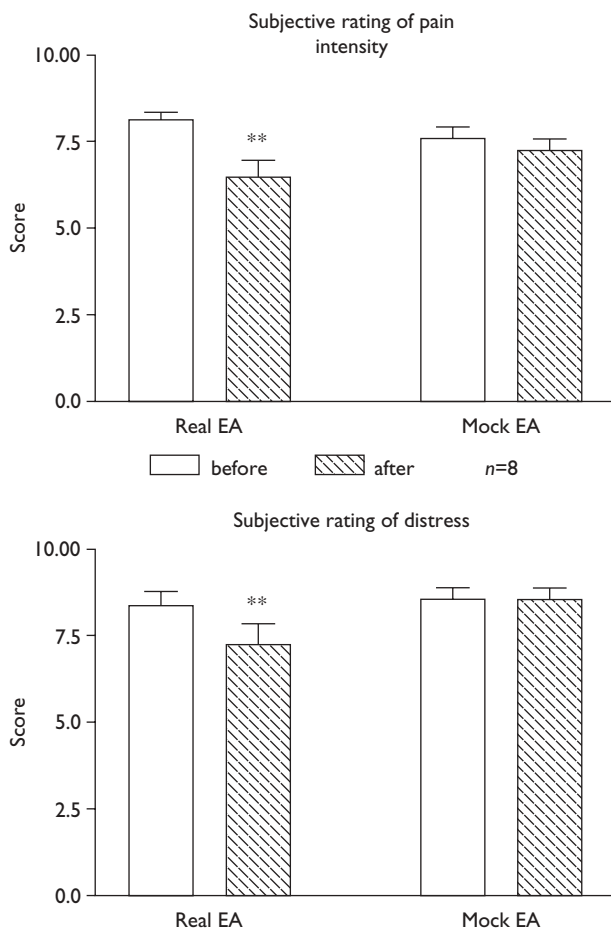
Modulation effect of electric acupoint stimulation on the cerebral response to pain: We took the pain *vs* cold *t*-contrast as the index of pain response, and performed a voxel-by-voxel paired *t*-test between the pain response before and after EAS treatment. Areas demonstrated increased or decreased responses were identified as shown in Fig. 2b. Activations in the following brain areas increased after real EAS treatment: (1) bilateral motor and premotor cortex (BA4, 6), SII, rostral ACC (BA32/24), orbital frontal cortex, MPFC, paracentral lobules, thalamus, midbrain, pons and cerebellum; (2) ipsilateral SI, LPFC, posterior temporal lobe (BA21, 22, 37), and putamen. Moreover, the range of increased activation in ipsilateral SII is larger than that of contralateral. On the other hand, the following areas showed an attenuated pain activation after EAS: (1) contralateral SI, LPFC, and inferior temporal (BA20, 37); (2) bilateral caudal ACC (BA24), parietal BA7, medial cuneus, midbrain, pons, and cerebellum; (3) ipsilateral parietal BA 39, and occipital BA19. It should be pointed out that although temporal lobe, ACC, cerebellum, midbrain, and pons were involved in both positive and negative modulation of EAS, different sub-areas were related with different effects, no obvious overlap was found between them. Among all areas with a uni-directional modulation by EAS, those specific for pain sensation (contralateral SI/MI, bilateral SII, MPFC, ipsilateral BA7) reasonably bear more significance and will be selected as regions of interest (ROI) for analysis later.

Mock EAS produced much less influence on cold pain *vs* cool contrast. Only activations in bilateral posterior cingulate and SII are found to be increased, while activations in contralateral medial occipital cortex were decreased after mock EAS. The spatial extent of increased response in bilateral SII was much smaller than that of real EAS.

Individual analysis of ROI: For each of the ROIs, the paired *t*-test was used to compare the maximum *t*-value in the specific region of each subject before and after real/mock EAS (Fig. 3). The results in contralateral SI, bilateral SII and MPFC, and ipsilateral parietal BA7 confirmed the voxel-by-voxel analysis results. Because of the complex involvement of ACC, midbrain, cerebellum and temporal lobe in both positive and negative modulation of EAS, no individual ROI analysis was performed in these regions.

DISCUSSION

The study of acupuncture analgesia is helpful not only for the investigation of its principles, but for the pain mechanisms as well. Published works up to date only observed the acupuncture stimulation induced brain activity with fMRI, but not its anti-nociceptive effect. The current study is the first imaging report indicating that in human beings electric acupoint stimulation could modulate the pain-induced activation in the brain. In the pain-specific regions identified by the comparison between painful

Fig. I. Analgesic effect induced by real and mock electric acupoint stimulation. Both sensory (pain intensity) and emotional (distress) dimension of pain were evaluated. **$p < 0.01$, compared with the scores before treatment.
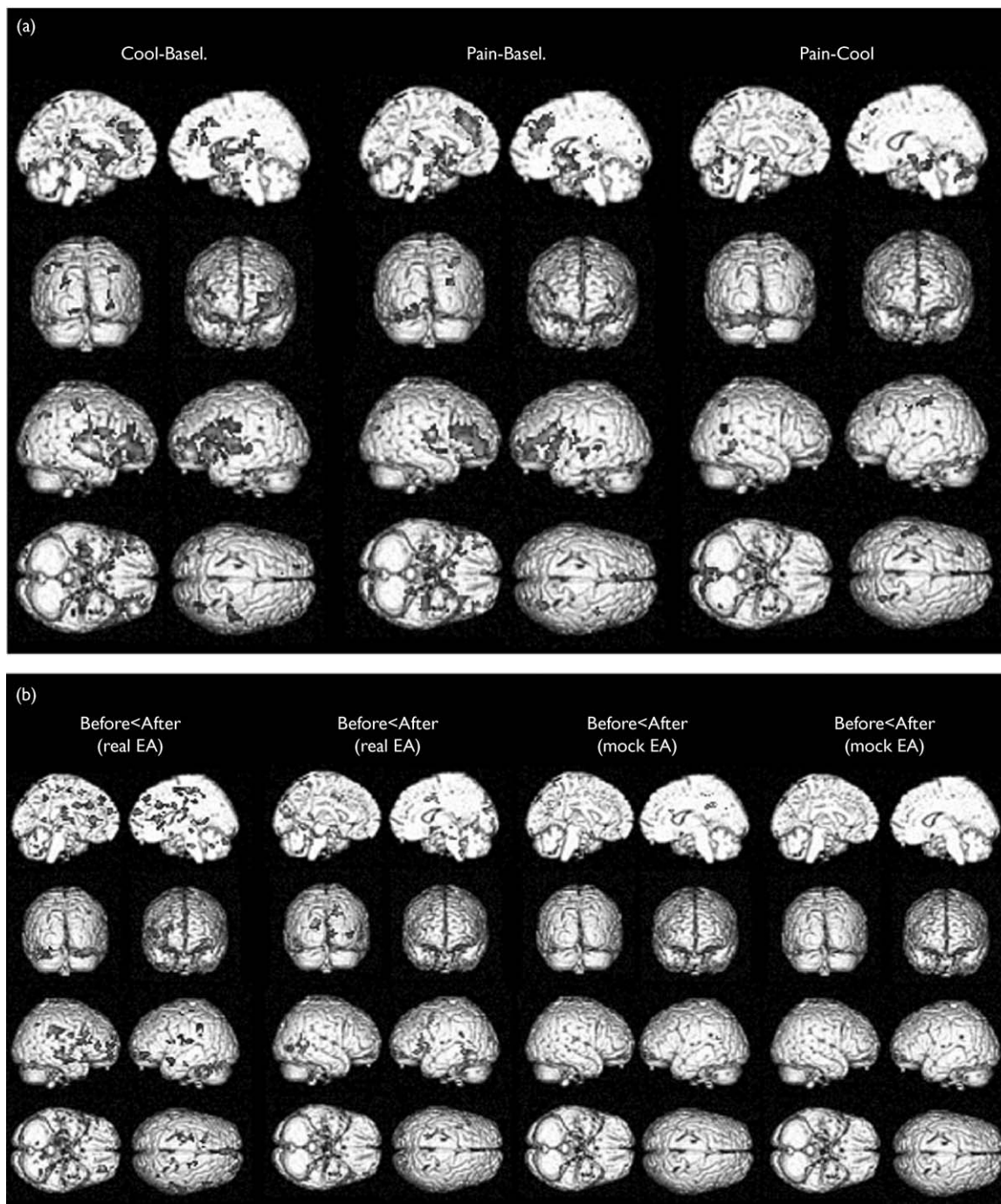
Fig. 2. (a) Functional brain mapping of cool and pain stimulation before electric acupoint stimulation; and (b) paired *t*-test between parameters of pain–cool contrast before and after electric acupoint stimulation. The statistical parameter mapping were superimposed on a rendered TI template. The threshold for activation is *p* < 0.0I (uncorrected) with a minimal cluster requirement of I0 voxels. Note that radiological convention (L is R) was employed.

versus cool stimuli, bilateral SII, and MPFC showed stronger response to cold pain stimulation after EAS treatment, while responses in contralateral SI and ipsilateral BA7 decreased after EAS. The modulation effect in anterior cingulate, midbrain, pons, cerebellum, and posterior temporal lobe is more complex in that different sub-areas of those regions showed different (positive or negative) modulation effect by EAS.

Among the aforementioned brain areas, midbrain and pons have been proposed to be involved in EAS analgesia in our previous animal studies [5,6]. However, the involvement of other areas, especially the cortical areas such as SI,
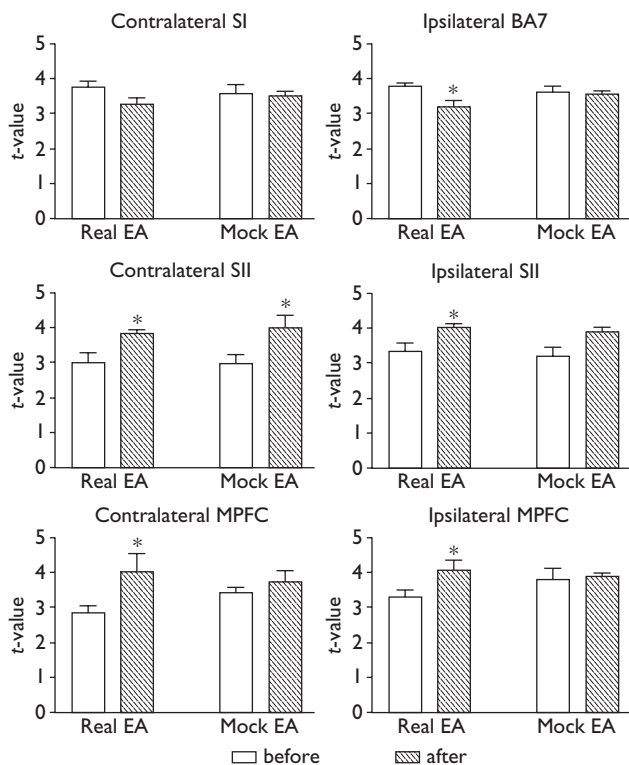
Fig. 3. Individual analysis of regions of interest (ROI). The values on each Y-axis represent the averaged peak *t*-value in the specific region. *$p < 0.05$. Note that in ipsilateral SII of mock EAS treatment and contralateral SI of real EAS treatment, the change is marginally significant ($p = 0.072$ and 0.086, respectively). SI: primary somatosensory area; SII: secondary somatosensory area; MPFC: medial prefrontal cortex; BA7: Brodmann area 7, parietal superior lobule.

SII, MPFC, and anterior cingulate cortex, were rarely suggested in previous animal reports. Indirect evidence for the possible roles of these areas in acupuncture analgesia was suggested by brain imaging studies on acupuncture or electroacupuncture stimulation [7,8,10–12]. By the direct comparison of the pain responses before and after acupoint stimulation, we further confirmed the roles of the above cortex areas in acupuncture-induced analgesia. The differences of key sites of EAS analgesia in previous animal studies and the current human study might be due to the specialized development of human cortex. In a word, the results of the current fMRI study enriched the theory on the neural pathways of acupuncture analgesia.

The roles of primary somatosensory area (SI) and caudal anterior cingulate (BA24) for the sensory and affective encoding of pain, respectively, have been suggested recently [20–22]. Our results that activation in contralateral SI and bilateral caudal ACC (BA24) was attenuated by EAS, together with the decreased pain intensity (representing sensory-discriminative component of pain) and distress (representing affective component of pain) scores after EAS, confirmed this point. These results also provided a neural functional proof for the first time that acupoint stimulation could diminish both the sensory and the affective dimension of pain. Another interesting area involved is medial prefrontal cortex. This area is known to be related with

cognitive functions. The enhancement of pain response in this area by acupoint stimulation indicated that acupuncture might also exert its analgesic effect via higher cognitive functions. This may explain the fact that it is very hard to separate the effect of acupuncture from placebo in clinical studies [23].

Why did EAS exert both facilitating and inhibiting effects to the pain response in different brain areas? We suggested that a complete pain-induced response in the CNS contain both ascending conductive and descending modulatory circuits. However, because of the relatively low temporal resolution of a block-designed fMRI study, the serial activation of ascending and descending activities could not be separated. Considering the known function of EAS to mobilize endogenous analgesic circuit and to inhibit the ascending pain signal, we suppose that the areas with increased responses after EAS (i.e. bilateral SII, MPFC, rostral ACC, midbrain, pons, and cerebellum) might belong to the pain-modulatory (presumably descending) system, whereas those showed decreased responses after EAS (i.e. contralateral SI, ipsilateral parietal BA7, bilateral caudal ACC, midbrain, pons, and cerebellum) most possibly belong to the ascending pain-conductive system. Since these two systems are actually integrated into a central network, they might be interlaced in areas such as midbrain, pons, and cerebellum. Hence they displayed both positive and negative responses to EAS in the current study. It should be pointed out that we also observed the signal enhancement in rostral ACC (BA32/24), a sub-area different from caudal ACC (BA24) anatomically and functionally (which showed signal attenuation after EAS). We hypothesized that different sub-areas of ACC might participate differentially the ascending or descending system. Detailed analysis will be performed on this area in the future.

Evidence that might be able to support the above explanation is that, the increased responses happened more frequently in the side ipsilateral to the stimulation. This is in consistence with the contralateral-to-ipsilateral conducting order of pain signal. Hence, a pain signal would first ascend via the contralateral conductive pathway, and then activate the descending pain modulating pathway, which propagate bilaterally. Since the ipsilateral side holds more descending modulatory versus ascending conductive activity compared with the contralateral side, thus more enhanced responses should be observed ipsilaterally after EAS stimulation. This explanation need to be further tested by methods with higher temporal resolution, such as event-related fMRI and electrophysiological recording of neuronal activities in these areas.

The aforementioned explanation is somehow over simplified and not all brain areas seem to fit it. For example, bilateral SII was thought conventionally to be more involved in pain presentation rather than modulation. However, our result indicated that it belong to the pain modulation pathway. It could be that SII is somehow involved in both pathways, and its involvement into pain modulation was revealed more clearly with electric acupoint stimulation. On the other hand, the explanation for changes in ACC, midbrain, pons, and cerebellum is more difficult, and should be applied with caution in the future.

Another limitation of our current study is our design of mock-EAS control. Since the stimulation intensity is the only

variable parameter compared with real EAS, there might be some non-specific effects on brain activation associated with the intensity of stimulation in a dose-dependent fashion rather than analgesia-related. Further studies with more control groups and deeper analysis will be helpful to preclude the possibility of this non-specific effect.

In conclusion, our study identified for the first time the brain areas where pain-related responses were modified by electric acupoint stimulation. We have also confirmed the previous results on the brain activation induced by cool sensation and cold pain. These results may shed lights on solving the maze of how acupuncture might induce analgesia in human brain. It may also intensify our understanding about how the central nervous system process and modulate the perception of nociceptive signals.

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