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Research report

Relations between brain network activation and analgesic effect induced by low vs. high frequency electrical acupoint stimulation in different subjects: a functional magnetic resonance imaging study

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Abstract

Two- or 100-Hz electrical acupoint stimulation (EAS) can induce analgesia via distinct central mechanisms. It has long been known that the extent of EAS analgesia showed tremendous difference among subjects. Functional MRI (fMRI) studies were performed to allocate the possible mechanisms underlying the frequency specificity as well as individual variability of EAS analgesia. In either frequencies, the averaged fMRI activation levels of bilateral secondary somatosensory area and insula, contralateral anterior cingulate cortex and thalamus were positively correlated with the EAS-induced analgesic effect across the subjects. In 2-Hz EAS group, positive correlations were observed in contralateral primary motor area, supplementary motor area, and ipsilateral superior temporal gyrus, while negative correlations were found in bilateral hippocampus. In 100-Hz EAS group, positive correlations were observed in contralateral inferior parietal lobule, ipsilateral anterior cingulate cortex, nucleus accumbens, and pons, while negative correlation was detected in contralateral amygdala. These results suggest that functional activities of certain brain areas might be correlated with the effect of EAS-induced analgesia, in a frequency-dependent dynamic. EAS-induced analgesia with low and high frequencies seems to be mediated by different, though overlapped, brain networks. The differential activations/de-activations in brain networks across subjects may provide a neurobiological explanation for the mechanisms of the induction and the individual variability of analgesic effect induced by EAS, or that of manual acupuncture as well.

Theme: Sensory systems

Topic: Pain modulation: anatomy and physiology

Keywords: fMRI; Acupoint nerve stimulation; Acupuncture analgesia; Frequency specificity; Anterior cingulate; Hippocampus

Abbreviations: ACC, anterior cingulate cortex; AFNI, analysis of functional neuroimaging; ARC, arcuate nucleus; BA, brodmann area; BOLD, blood oxygenation level dependent; EAS, electrical acupoint stimulation; EPI, echo-planar imaging; fMRI, functional magnetic resonance imaging; FOV, field of view; Hi, hippocampus; Ins, insula; MI, primary motor area; MRI, magnetic resonance imaging; MTG, middle temporal gyrus; NAc, nucleus accumbens; PBN, parabrachial nucleus; PET, positron emission tomography; ROI, regions of interest; S.E.M., standard error of the mean; SI, primary somatosensory area; SII, secondary somatosensory area; SMA, supplementary motor area; STG, superior temporal gyrus; TE, time of echo; Th, thalamus; THK, thickness/space; TR, time of repetition

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1. Introduction

Acupuncture has been used against highly diversified pain in the Orient for thousands of years. Recently, it has been recognized in the West as a useful procedure in complementary medicine, especially in the treatment of pain, under a milestone of the NIH Consensus in 1997 [1]. Many pivotal studies on mechanisms underlying acupuncture analgesia have contributed to this great progress. It is generally accepted that endogenous opioid system plays a key role in acupuncture analgesia [12,32,38].

Among the aforementioned studies, electrical acupoint stimulation (EAS) was widely used as a substitute for classical acupuncture. The electrical parameters (frequency, pulse width, intensity, etc.) have been optimized to produce analgesic effect similar as, if not stronger than, manual acupuncture. It was revealed that acupuncture analgesia could be induced by either low-frequency stimulation, such as 2 or 4 Hz, or high-frequency stimulation, such as 100 or 200 Hz. Studies into the mechanisms of EAS induced analgesia have shown that the central nervous system responded differently to peripheral electric stimulation of different frequencies. These differential effects on the brain are: (1) stimulation of 2 Hz mobilized enkephalin that act on mu- and delta-opioid receptors, while 100 Hz released dynorphin that bind with kappa receptor in both animals and human beings [18,21–23]; (2) lesion of arcuate nucleus (ARC) of hypothalamus abolished the analgesic effect induced by 2 Hz stimulation [45], while damaging the parabrachial nucleus (PBN) of the pons diminished the effect of 100 Hz [46] in rats; and (3) peripheral electric stimulation of 2- or 100-Hz could activate brain areas in specific pattern as shown with the expression of *c-fos* gene [18,19,32]. In detail, both low- and high-frequency EAS could produce fos-like immunoactivity in many brain areas such as ventral periaqueductal gray and amygdala. Arcuate nucleus of hypothalamus and medial geniculate of thalamus was more strongly activated by low-frequency EAS, while parabrachial nucleus of pons and rostral ventromedial medulla was more specifically related with high-frequency EAS. These findings strongly suggested that central nervous system might have frequency-specific response to peripheral electric stimulation. However, most of the invasive methodologies employed in these studies would be difficult to be applied in human beings.

The development of non-invasive brain imaging techniques, including positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), provided the possibility to address these problems [5,25,26,31,48,49]. Various brain regions were found to be activated or de-activated by acupuncture stimulation. For example, visual cortex could be activated by acupuncture on acupoints correlated with eye disorders [11]. Studies on acupuncture at acupoints with strong analgesic effect (such as Zusanli and Hegu) implied that the limbic system was involved during the stimulation [25,26,48]. Nevertheless, no brain imaging research has yet been performed to address the frequency specificity of EAS, and none has ever incorporated the behavioral index of acupuncture analgesia into the fMRI study. It has not been clear yet whether those activations were really related with acupuncture analgesia, since acupuncture in the same acupoints could also be applied for other disorders except pain. Hence, it remains to be explored whether these observed brain changes could really reflect the mechanisms of acupuncture- or EAS-induced analgesia.

The aim of the present study was to verify whether analgesic effect induced by electrical acupoint stimulation (EAS) of different frequencies was mediated by distinct central networks in human beings. Like in animal studies, although many brain areas were positively labeled with *c-fos* during EAS stimulation [19], only a few of them were indispensable for EAS analgesia via lesion investigation [45,46]. Thus, through the correlation method between the fMRI changes and the EAS-induced analgesic effect, we tried to uncover the specific brain areas for EAS analgesia in human beings.

2. Materials and methods

2.1. Subjects

Forty-eight healthy and right-handed subjects (23 males and 25 females), aged 21–36 years, were volunteered in the experiment. Twenty-four of them were firstly recruited and randomly allocated into two groups receiving either 2- or 100-Hz EAS (gender was equally represented in both groups). Another 24 subjects were subsequently recruited and four of them (two males and two females) received control stimulation (minimal-EAS). The remaining 20 subjects entered the supplementary behavioral study on the repeatability of the EAS analgesic effect. In compliance with guidelines of human experiments from local ethical committee of Peking University, each of the subjects had provided informed consent with the adequate understanding of the purpose and procedure of the study. All subjects were free to withdraw from the experiment at any time. None had a history of psychiatric or neurological disorder. None was in pain or distress at the time of the study. No subject, except one, had ever experienced acupuncture or EAS therapy before.

2.2. Administration of electrical acupoint stimulation

A pair of skin electrodes were placed on acupoints (specific points in the body with high sensitivity to acupuncture treatment) ST36 (or Zusanli, located 5 cm below the lateral flank of the knee joint) and SP6 (or Sanyinjiao, 5 cm above the medial tarsal of the ankle joint) of the left leg. Han's acupoint nerve stimulator (LH-202H,

Huawei Co. Ltd., Beijing) was used to deliver the stimulation. The proper intensities of EAS (constant current output) for each of the individual volunteers were tested before the start of formal experiments. For 2- and 100-Hz groups, the intensity was adjusted to a maximal but comfortable level, usually ranging from 8 to 15 mA (averaged at 11.25 mA). For minimal-EAS group, the intensity was just above the detectable threshold, i.e. 3 or 4 mA in all of the four subjects. No noxious or any unpleasant feeling was allowed. The frequency of stimulation was set at either 2 Hz (square wave with width of 0.6 ms) or 100 Hz (square wave with width of 0.2 ms). The minimal-EAS group and the remaining 20 subjects in the behavioral study received 2-Hz stimulation.

2.3. Image data acquisition

All MRI experiments were performed on a 1.9 T whole body MRI scanner (Prestige, GE/Elscint Ltd., Haifa, Israel) with a standard head coil. 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 2.9×2.9 mm, and TR/TE/flip angle at 3000 ms/45 ms/90°. The field of view (FOV) was 373×212 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 to fill 120 time points over 6 min. For anatomical images, a 3D gradient-echo T1-weighted sequence (TR/TE 25/4 ms; FOV 220×220 mm^2; THK 2.0/0.0 mm, Matrix 220×220; resolution: 1×1 mm^2) was selected and the images were used for Talairach transformation and functional mapping during data analysis later. Another set of 20 spin-echo T1-Weighted images (TR/TE 750/12 ms, FOV 220×220 mm^2; THK 6.0/0.0 mm; Matrix 220×220; resolution 1×1 mm^2) with same position of fMRI acquisition was obtained for image registration.

In the 6-min fMRI scanning, EAS was given within the 2nd, 4th and 6th min, each lasting 1 min. The 1st, 3rd, and 5th min served as the control or resting phases without any stimulation. This block design was diagramed with fine lines in Figs. 2b and 3b.

2.4. Assessment of individual responsiveness to EAS analgesia via noxious radiant heat stimuli

Normally as long as acupuncture analgesia is concerned, one question was often raised: how well does the subject respond to acupuncture analgesia? Hence we measured the analgesic effect of EAS on each of the subjects in a separate session 24 h to 7 days after fMRI scanning. A total of 3 min intermittent EAS stimulation is adequate for fMRI signal contrasting. However, it is too short to induce satisfactory analgesia and to differentiate behavioral responsiveness among subjects. The parameters (location, intensity, and frequency) of EAS were exactly the same as those during fMRI recording. The only difference was that the time of the stimulation was prolonged to 30 min according to the experience of acupuncture practice.

Pain threshold was determined by timing the latency of the foot withdrawal from the noxious thermal irradiation applied at dorsum of the foot. Focused light from a 12.5 W projection bulb was applied, with the strength of the light adjusted so that the pain threshold before stimulation fell into a range of 4–6 s. Every subject received three successive tests before, and another three tests after the 30-min EAS session. The increase of the averaged latencies after stimulation was calculated and expressed in percentage as the index of analgesic effect of EAS in each individual.

2.5. Supplementary study on the repeatability of EAS analgesia

The prerequisite to correlate the behavioral data acquired 1–7 days after fMRI scanning with fMRI changes was that behavioral data in the same subject was stable, at least in a short period. Although a good repeatability of EAS analgesic effect had been reported in rats [42], it has not yet been formally studied in human beings. Thus, we performed a supplementary experiment to address this question. Another group of 20 subjects (8 males and 12 females, age 26.4±4.4, mean±S.D.) participated in this supplementary experiment. On the first day of the experiment, pain threshold was determined and EAS was delivered exactly as described above. Three days later, the same procedure was repeated on each subject and again calculated the index of analgesic effect in percentage. Linear regression was done between the indices of the first and the second trial. The repeatability of EAS analgesia was shown with a regression line slope and regression coefficient close to one.

2.6. Data management and statistical analysis

Data from four subjects were excluded in our further analysis due to their relatively severe head motion during imaging; hence the result was consisted of four, nine and 11 subjects for minimal-, 2- and 100-Hz EAS investigation, respectively. Analysis of functional neuroimaging (AFNI) Software [13] was used in the data processing. For each of the remaining 24 subjects, motion correction was made in the first place. Then we calculated the mean value at each time-point across voxels of all brain regions, to obtain a time-course of the averaged signal, by which a detrending process with the algorithms of linear least squares was performed to achieve a sensible signal-to-noise ratio [34]. Afterwards, functional images were registered with the anatomical MR images. These image loci were then transformed into Talairach space [43].

Functional images were resampled and blurred into 3×3×3 mm^3 voxels. The first 3 of the 120 time points were discarded due to a problem of the T1 equilibrium (stability) of the image system. The remaining 117 time-point curves were cross-correlated with ideal curve adjusted for the hemodynamic delay effects. Voxels with correlation coefficient exceeded 0.2390 ($P<0.01$) was reserved and clustered at the threshold of 4 voxels so that the corrected significance in whole brain is less than 0.05, as estimated by a Monte Carlo simulation. FMRI data were visually inspected to ensure that each reserved activation was actually within the brain, and the localization of the activated brain regions was confirmed by an experienced neuro-radiologist. Time-course graphs from each responded area were also constructed to confirm that the change occurred during the stimulation (see Figs. 1b and 2b, heavy lines).

Averaging within group is the most frequently used technique in group analysis. However, it may ignore some important information such as inter-subject variability. Hence in our study, we adopted an alternative method for the group analysis of 2- and 100-Hz group. First, brain regions activated or de-activated in at least five subjects (at close to half of the subjects in each group) were selected as regions of interest (ROI, defined according to Talairach and Tournoux human brain atlas [43]). Overall, 13 and 16 ROIs were defined in the 2- and 100-Hz group, respectively. Secondly, averaged correlation coefficient was calculated as the activation level of each ROI. Finally, linear correlation analysis was performed in each ROI between the activation level and the change of pain threshold (i.e. analgesic effect) across all subjects in the group (see Figs. 1c and 2c). The threshold for significant correlation is $P<0.05$. Since secondary somatosensory area (SII) and insula were generally difficult to separate both in space and function, they were regards as one ROI.

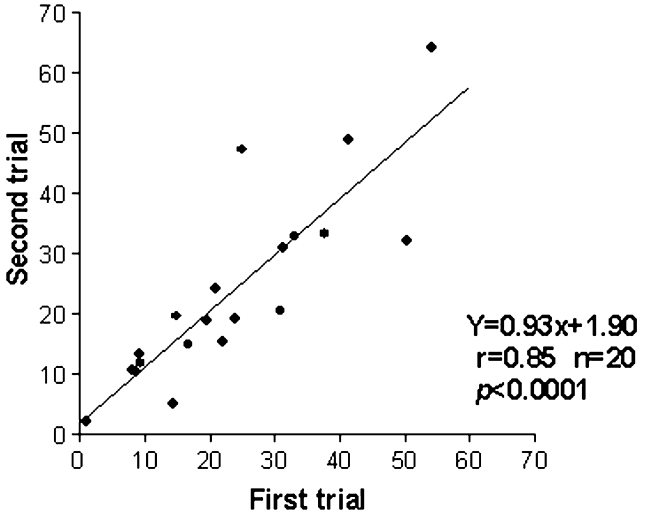

Fig. 1. Repeatability of the electric acupoint stimulation induced analgesic effect in human beings. Each dot represents data from one subject. The increase of the averaged latencies after stimulation was calculated and expressed in percentage as the index of analgesic effect.

We noticed that there was little variability within the minimal-EAS group, both in the fMRI and behavioral data. Therefore, functional data from four subjects of the minimal-EAS group were simply averaged after the Gaussian blur. Then the averaged time series were cross-correlated with the same ideal curve and then cut and clustered at the same thresholds as experimental groups.

3. Results

3.1. General responses to EAS analgesia

The change of pain threshold after EAS administration varied from subject to subject. It was in the range of 4.13 to 88.15% (mean±S.E.M.: 43.74±9.61%, $n=9$) in 2 Hz group, 3.67 to 35.25% (mean±S.E.M.: 21.24±3.29%, $n=11$) in 100 Hz group, and −1.33 to 2.13% (mean±S.E.M.: 1.02±0.81%, $n=4$) in control group, respectively. Student's *t*-test showed that the analgesic effect of 2-Hz EAS was slightly but significantly superior to that of 100-Hz ($P=0.0275$, $n=20$). However, both 2- and 100-Hz induced EAS better analgesic effect than minimal-EAS ($P<0.05$). Except for those of the control group, all subjects experienced soreness and numbness to different degrees around the stimulated sites, which are normal responses both in EAS and the traditional manual acupuncture treatment. Muscle twitching near the stimulation site would be observed only with 2 Hz EAS.

The repeatability of the EAS induced analgesia was demonstrated in Fig. 1. Good repeatability existed in the 20 subjects participated in the supplementary study. Regression analysis came out with a line slope of 0.93 and a regression coefficient of 0.85 ($P<0.0001$). Combined with our previous observations of the good repeatability of EA analgesia in rats [42], this supplementary behavioral result in human beings made it reasonable to correlate the analgesic effect with the fMRI signal across subjects, even though they were collected in different days.

3.2. Functional brain mapping of 2-Hz EAS

The brain areas activated or de-activated by 2-Hz EAS of each subject are summarized in Table 1, and some examples are shown in Fig. 2a. Notably, the contralateral primary somatosensory areas (SI) and ipsilateral middle temporal gyrus (MTG) were activated in all nine subjects. Bilateral secondary somatosensory area and insula (SII/Ins), contralateral caudal anterior cingulate cortex (ACC, BA24), contralateral superior parietal lobule (BA40), and ipsilateral superior temporal gyrus (STG) were activated in 8/9 subjects. Other areas such as contralateral motor cortex (MI) and supplementary motor area (SMA) were activated in 7/9 subjects. Contra- and ipsi-lateral thalamus

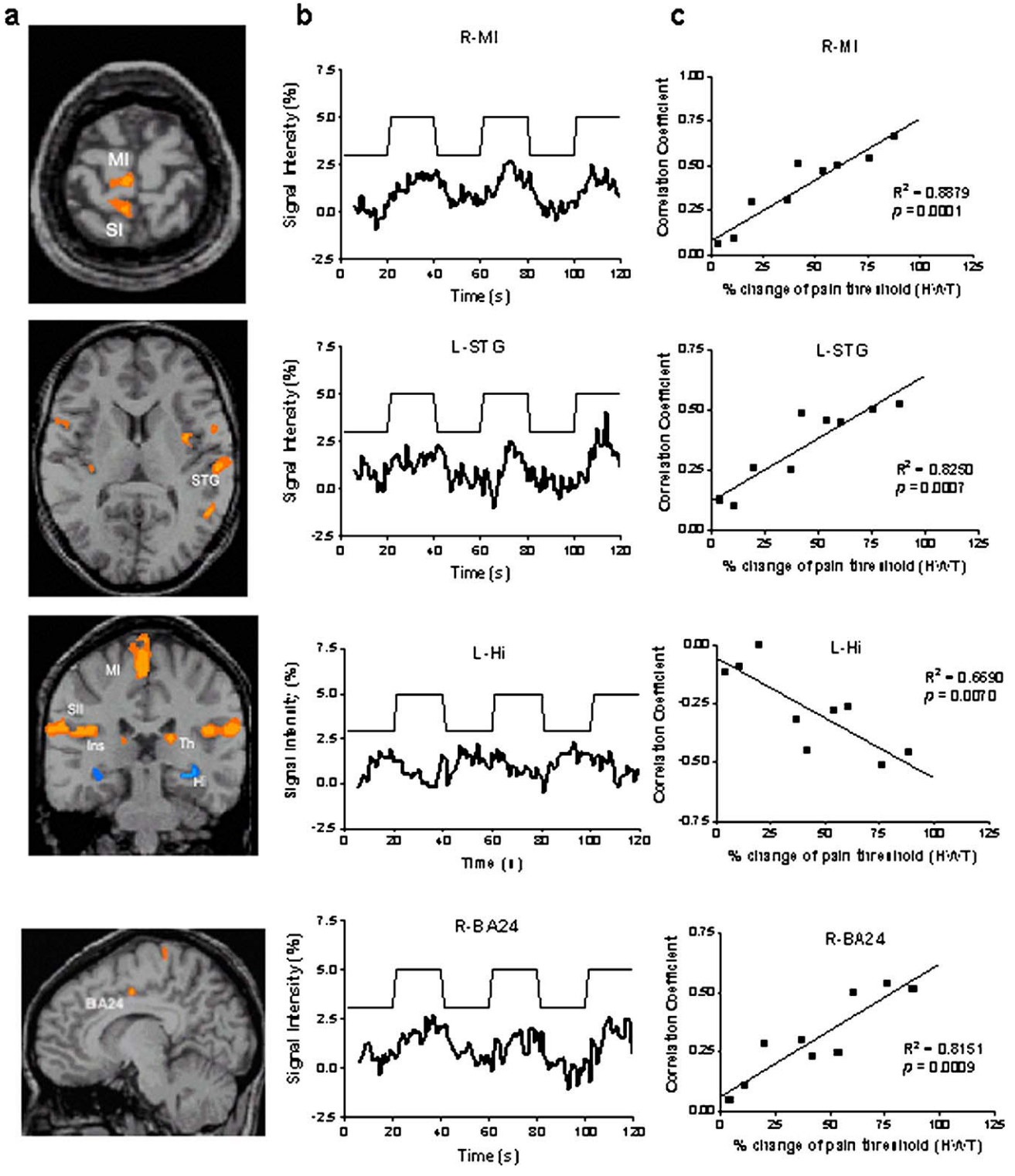

Fig. 2. Functional imaging induced by 2-Hz EAS. (a) Statistical mappings on the corresponding anatomical images in subject 1 of this group. BA24, Brodmman area 24 (caudal anterior cingulate); Hi, hippocampus; Ins, insular gyrus; MI, primary motor area; SI, primary somatosensory area; SII, secondary somatosensory area; STG, superior temporal gyrus; Th, thalamus. (b) Time course extracted from one of ROI in the left image. Heavy lines in the graphs showed the time-courses of the fMRI signal. Light lines were stimulation curves for comparison. Signal increasing or decreasing lagged behind the stimulation for a few seconds. (c) Scattered group plot of the individual change of pain threshold (representing analgesic effect) against the averaged activation levels of fMRI in the specified ROI.

Table 1
Individual fMRI reactions to 2 Hz EAS

Brain areas	Mean talairach coordinate			Mean correlation coefficient in each subject								
	R–L	P–A	I–S	1	2	3	4	5	6	7	8	9
L-Hi	33	−28	−6	−0.4570	−0.5112	−0.2643	−0.2820	−0.4535	−0.3204	0	−0.0935	−0.1155
L-MTG	52	−50	11	0.4738	0.5021	0.4605	0.4425	0.4705	0.2543	0.4333	0.1114	0.4413
L-SII/Ins	56	−22	19	0.5553	0.5407	0.4671	0.2950	0.5172	0.2598	0.2967	0.1197	0.1109
L-STG	57	−23	13	0.5252	0.5042	0.4485	0.4569	0.4855	0.2497	0.2612	0.1002	0.1280
L-Th	16	−26	13	0.2957	0.4901	0	0.4532	0.2576	0	0.2653	0.0839	0.0563
R-BA24	−5	−14	38	0.5166	0.5342	0.5023	0.2441	0.2337	0.2977	0.2825	0.1063	0.0448
R-BA40	−46	−30	28	0.5031	0.4769	0.5010	0.4522	0.4867	0	0.2672	0.0308	0.3236
R-Hi	−34	−31	−6	−0.4828	−0.5178	−0.2762	−0.4452	−0.2964	−0.2392	−0.2417	−0.0166	−0.1781
R-MI	−3	−26	56	0.6615	0.5395	0.4986	0.4651	0.5111	0.3055	0.2927	0.0864	0.0537
R-SI	−7	−39	66	0.5372	0.5406	0.4982	0.4576	0.5498	0.4597	0.4905	0.2864	0.5003
R-SII/Ins	−57	−23	19	0.6082	0.5075	0.5082	0.2845	0.5135	0.2821	0.4688	0.1517	0.0986
R-SMA	−5	−11	59	0.5561	0.4754	0.4786	0.4710	0.4464	0.4615	0.2722	0.0415	0.0700
R-Th	−17	−26	13	0.4962	0.4719	0.1578	0.4531	0.3159	0.2323	0.2813	0.1234	0.0087
Change of pain threshold (%)				88.1	76.2	60.6	54.0	42.2	37.2	20.1	11.1	4.1

Mean stereotactic coordiates (mm) of peak voxels are listed according to the atlas of Talairach and Tournoux. R–L, right vs. left; P–A, posterior vs. anterior; I–S, inferior vs. superior. R, right-sided; L, left-sided; BA, Brodmann Area; Hi, hippocampus; Ins, insula; MI, primary motor area; MTG, medial temporal gyrus; SI, primary somatosensory area; SII, secondary somatosensory area; SMA, supplementary motor area; STG, superior temporal gyrus; Th, thalamus. ROIs were listed in an alphabetical order according to their abbreviated names.

was activated in 6 and 5 out of 9 subjects, respectively. The only brain site exhibiting de-activation was shown in bilateral hippocampus (Hi) in 7/9 subjects. SI and MI activations were confined to areas relevant to the somatosensory localization of the leg area of ST36 and SP6.

3.3. Functional brain mapping of 100-Hz EAS

The brain areas activated or de-activated by 100-Hz EAS of each subject are summarized in Table 2 and shown in Fig. 3a, selectively. Firstly, contralateral SI and bilateral SII/Ins were activated, whereas the contralateral hippocampus was de-activated in all 11 subjects. Secondly, contralateral BA40 and pons were activated and ipsilateral amygdala and hippocampus were de-activated in 10/11 subjects. The ipsilateral SI was activated and contralateral amygdala de-activated in 9/11 subjects. Contralateral nucleus accumbens (NAc), ipsilateral caudal ACC (BA24), and bilateral thalamus were activated in 8/11 subjects. Only 5/11 subjects positively responded to 100-Hz EAS in the area of ipsilateral NAc.

3.4. Functional brain mapping of minimal-EAS

In all subjects of the minimal-EAS group, SII was activated on both hemispheres. Three out of four subjects

Table 2
Individual fMRI reactions to 100-Hz EAS

Brain areas	Mean talairach coordinate			Subject number										
	R–L	P–A	I–S	1	2	3	4	5	6	7	8	9	10	11
L-Amy	25	−3	−20	−0.4720	−0.5679	−0.4149	0.2180	−0.4726	−0.3243	−0.5001	−0.2415	−0.4836	−0.3124	−0.4397
L-BA24	7	−8	43	0.5227	0.4934	0.3358	0.2272	0.3464	0.3252	0.3103	0.2102	0.2013	0.2267	0.3364
L-HI	28	−13	−15	−0.4887	−0.5475	−0.3363	−0.3283	0.2150	−0.3330	−0.3380	−0.3329	−0.3076	−0.3355	−0.5244
L-NAc	12	13	−2	0.3457	0.2328	0.2209	0.3305	0.3354	0.3536	0.2295	0.3152	0.2287	0	0.0808
L-SI	10	−36	53	0.4695	0.5021	0.5000	0.4471	0.2274	0.4479	0.4838	0.2246	0.4683	0.4503	0.4845
L-SII/INS	51	−9	16	0.5052	0.5132	0.5039	0.4783	0.4880	0.4649	0.4537	0.4491	0.4655	0.3382	0.4462
L-TH	8	−22	3	0.4730	0.4931	0.3201	0.2191	0.3512	0.4251	0.4842	0.2118	0.3421	0.2357	0.3440
PONS	−8	−22	−25	0.5050	0.4639	0.4410	0.3598	0.3460	0.3460	0.4807	0.3275	0.2219	0.2416	0.3220
R-Amy	−27	−1	−21	−0.4628	−0.4845	−0.5439	−0.3195	−0.5019	−0.3949	−0.5377	−0.3120	−0.3233	−0.2215	−0.2343
R-BA24	−1	−11	40	0.5308	0.4846	0.4975	0.2257	0.3318	0.4504	0.3364	0.0335	0.0627	0.2227	0.3385
R-BA40	−50	−31	30	0.5577	0.4875	0.5570	0.4454	0.4725	0.4273	0.4669	0.3462	0.5257	0.2184	0.2396
R-HI	−28	−16	−15	−0.4705	−0.5044	−0.3609	−0.3148	−0.4763	−0.4387	−0.3428	−0.3301	−0.4529	−0.3165	−0.3399
R-NAc	−10	10	−1	0.3533	0.0323	0.2440	0.3145	0.3703	0.3457	0.4439	0.2288	0.3315	0.3239	0
R-SI	−9	−48	61	0.4906	0.5422	0.5238	0.5147	0.4670	0.3386	0.4615	0.3189	0.4443	0.3530	0.5317
R-SII/INS	−45	−14	7	0.5433	0.5247	0.5029	0.4935	0.4847	0.4703	0.4694	0.4679	0.4606	0.4559	0.3372
R-TH	−8	−9	7	0.5145	0.4893	0.4349	0.2353	0.3222	0.3251	0.4571	0.2273	0.3247	0.2226	0.3227
Change of pain threshold (%)				35.3	33.2	30.4	30.3	24.7	22.4	20.0	16.5	11.9	5.3	3.7

Amy, amygdala; NAc, nucleus accumbens. Other abbreviations are the same as Table 1.

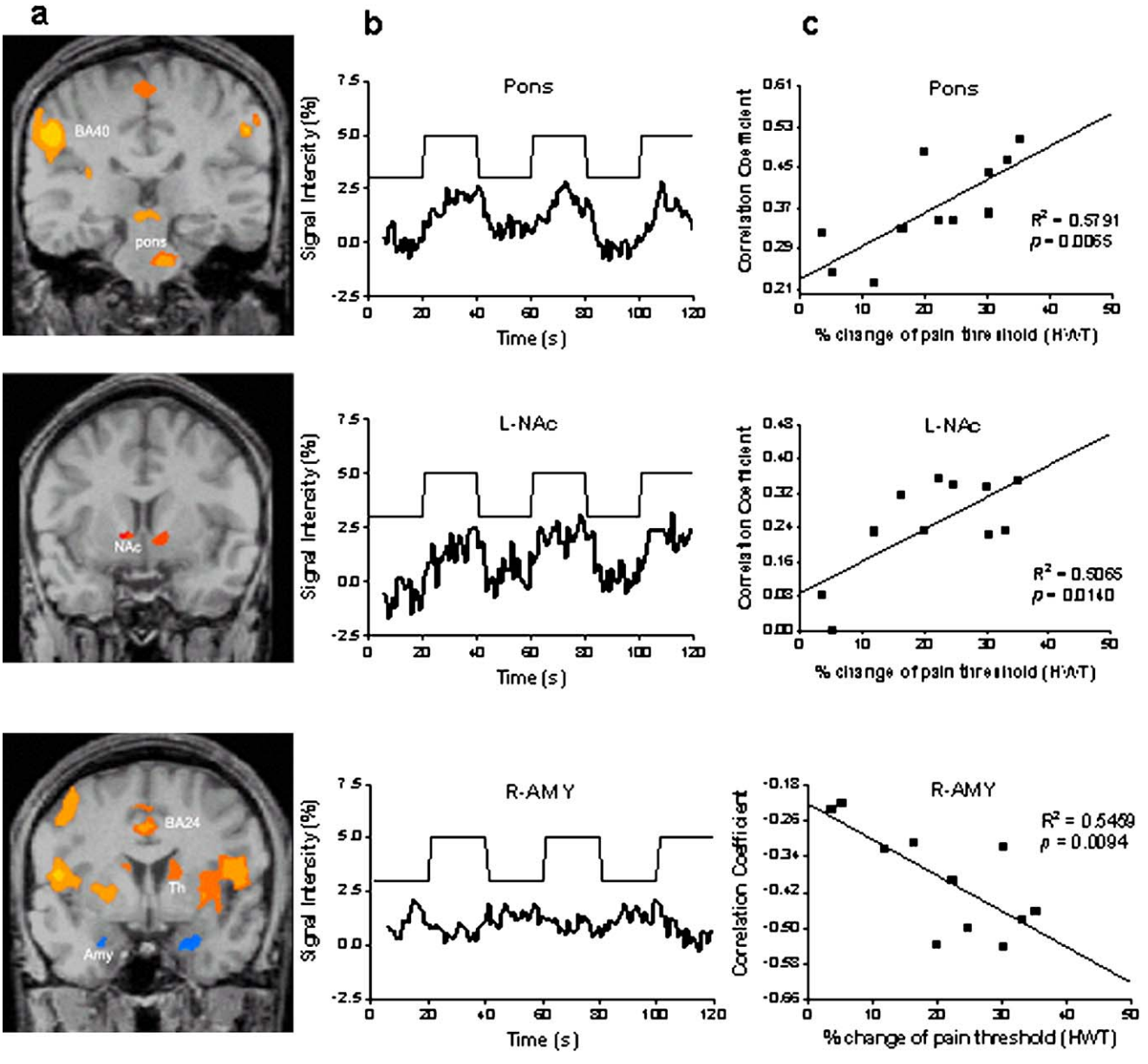

Fig. 3. Functional imaging induced by 100-Hz EAS. (a) Statistical mappings on the corresponding anatomical images in subject 2 of this group. Amy, amygdala; BA24, brodmann area 24 (anterior cingulate cortex); BA40, superior parietal lobule; NAc, nucleus accumbens; Th, thalamus. (b) Time course extracted from one of ROIs in the left image. Heavy and light lines in the graphs showed the time-courses of the fMRI signal and stimulation curves for comparison. Signal increasing or decreasing lagged behind the stimulation for a few seconds. (c) Scattered group plot of the individual change of pain threshold (representing analgesic effect) against the averaged activation levels of fMRI in the specified ROI.

showed contralateral SI activation. Fig. 4 showed the averaged result from all four subjects.

3.5. Correlation between the signal intensities and the analgesic effect

The result of linear correlation analysis between the averaged activation level of each ROI and the individual analgesic effect across all subjects is summarized in Table 3, and some examples are shown in Figs. 2c and 3c. The activation level in the areas of bilateral SII/Ins, contralateral BA 24 and thalamus, both in 2- and 100-Hz EAS groups, were found to be positively correlated with the analgesic effect. In 2-Hz EAS group, however, the positive correlations were observed in contralateral MI and SMA, ipsilateral STG, while negative correlations were found in bilateral hippocampus. In 100-Hz EAS group, positive correlations were observed in contralateral BA40, ipsilateral BA24, NAc, and pons, while negative correlation was detected in contralateral amygdala. Although bilateral SI and ipsilateral MTG were defined as ROIs according to the criteria mentioned above, no linear correlation between the

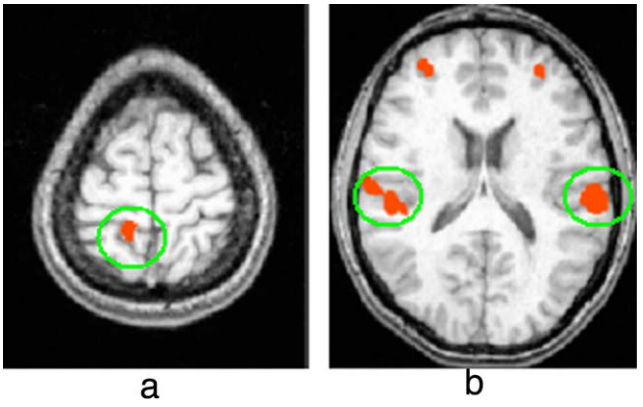

Fig. 4. Functional imaging induced by minimal-EAS. Statistical mapping of the averaged fMRI data across four subjects in minimal-EAS group was displayed. Green circles indicate (a) contralateral primary somatosensory area and (b) bilateral secondary somatosensory area.

activation level and the analgesic effect was found in these ROIs in either of groups.

4. Discussion

The apparent difference between the fMRI results of EAS and minimal-EAS indicated that the activation in the EAS group is specific to the acupuncture effect, i.e. due to a stimulation strong enough to induce those acupuncture-like feeling (soreness and numbness, etc.), rather than a somatosensory response. Furthermore, comparing the results of the present study with other pain imaging studies, most of the EAS-activated brain areas in this study were pain-related, which is in line with acupuncture studies of Biella et al. [5] and Wu et al. [49]. Moreover, the EAS-induced fMRI responses were similar to those induced by traditional acupuncture [26,48], although some differences existed. Interestingly, we all identified the de-activation in limbic system, such as hippocampus and amygdala, during acupuncture-like stimulation. This is rarely reported in the studies of pain imaging. Logically, brain areas that showed correlation with the analgesic effect of EAS (as summarized in Table 3) were more likely to directly mediate the effect of EAS-induced analgesia. Thus, our further discussion will be mostly focused on these areas.

Table 3
Correlated brain areas of EAS analgesia

Brain areas	2 Hz	100 Hz
R-SII/Ins	$+(R^2=0.6054)$	$+(R^2=0.7205)$
L-SII/Ins	$+(R^2=0.7665)$	$+(R^2=0.6230)$
R-Caudal ACC (BA24)	$+(R^2=0.8151)$	$+(R^2=0.3448)$*
R-Th	$+(R^2=0.6533)$	$+(R^2=0.3439)$*
R-MI	$+(R^2=0.8879)$	
R-SMA	$+(R^2=0.7871)$	
L-STG	$+(R^2=0.8250)$	
R-HI	$-(R^2=0.7577)$	
L-HI	$-(R^2=0.6693)$	
R-BA40		$+(R^2=0.6268)$
L-BA24		$+(R^2=0.3794)$
L-NAC		$+(R^2=0.5065)$
PONS		$+(R^2=0.5791)$
R-AMY		$-(R^2=0.5459)$

Linear correlation was performed between the averaged activation levels and the effect of EAS analgesia across subjects. +/−, Positive/negative correlation; blank, no significant correlation was found. R^2 represents the goodness of fit between the data and the regression line. * Marginally significant ($0.05<P<0.06$). Abbreviations are the same as listed in Table 1.

4.1. Shared network involved in EAS-induced analgesia of low and high frequencies

Our previous studies had revealed that both low and high frequency peripheral electric stimulation could induce analgesia with some distinct but overlapped mechanisms [18,47]. In the present study, the following brain areas were found to be involved in EAS analgesia of both frequencies, such as thalamus, bilateral SII/Ins, and contralateral BA24. The activations of thalamus during EAS of both frequencies were consistent with *c*-fos labeling study [19]. However, the labeling study revealed activations in many deep nuclei and few cortices, while our current fMRI study displayed activations in more cortex areas. This difference might be due to different subjects (human beings vs. rats) and techniques (fMRI vs. *c*-fos labeling) used.

SII/Ins is the most frequently reported area activated in pain studies [40]. The activity intensity of bilateral SII/Ins in our study was linearly correlated with the analgesic effect. This is in accordance with the well-known bilaterality of SII receptive fields in human and animals. The ascending sensory information is thought to be integrated into limbic system to generate the affective aspect through two neural pathways. One is the direct spino–thalamo–limbic pathway, and the other is the spino–thalamo–cortico–limbic pathway [41]. SII/Ins is an important cortical site involved in the latter route. Thus, the activation correlated with analgesic effect observed in SII/Ins and thalamus in the current study revealed that electrical acupoint stimulation might modulate the affective dimension of pain by modifying both the direct (thalamic) and indirect (SII/Ins) pathway of sensorial integration.

Area BA24 of caudal ACC has been repeatedly described to be activated during pain stimulation in PET and fMRI studies [7,8,10,16,27,39,40]. It is considered to play a complex pivotal role in the affective-motivational component of pain [41]. In addition, administration of analgesics such as fentanyl [2] and nitrous oxide inhalation [20] has also increased the regional cerebral blood flow of caudal ACC. In the current study, we also observed a similar activation, which correlated positively with the

analgesic effect. Thus, fentanyl injection, nitrous oxide inhalation, as well as EAS might all generate their analgesic effect by activating caudal–ACC–related pathway, presumably through modulation of the affective-motivational component of pain.

It should be noted that de-activation of the rostral part of ACC (BA32) has been reported during manual acupuncture [48] and electroacupuncture [49]. On the other hand, Hui et al. [26] reported the de-activation in the caudal part of ACC, in which area both Wu et al. [49] and our current study found signal increase during the stimulation. The difference might be due to the methodological difference (electroacupuncture vs. acupuncture) [31]. Though not defined rostral ACC as one ROI, we did observe the de-activation in this area in 3/9 and 3/11 subjects in low- and high-frequency EAS groups, respectively (data not shown). As in the pain study, the function of ACC in the acupuncture stimulation seemed also miscellaneous and complicated. It would be more reasonable and necessary to discuss the function of ACC according to sub areas. As far as our results were concerned, we believe that the caudal ACC is more important for acupuncture analgesia.

4.2. Brain areas specifically correlated with analgesic effect of low-frequency EAS

The activation levels in MI and SMA were positively correlated with the 2-Hz EAS-induced analgesic effect. This leads to the prospect of integrating motor-related areas into the pain modulation network. Supporting evidence also came from anatomical and clinical studies. Motor-related areas including MI and SMA were connected with sensory system such as SI and thalamus via cortico–cortical and cortico–thalamic fibers [44]. Stimulation on motor cortex has been clinically applied for pain control [6]. This is the first report showing correlation between the activation of motor-related areas and 2-Hz, rather than 100-Hz induced analgesia. A possible explanation for this frequency specificity is that muscle twitching could be seen on the left leg during the low-, rather than high-frequency EAS stimulation. Hence, this passive muscle movement might somehow trigger the motor-related analgesic circuit, and generate the observed analgesic effect.

Interestingly, we have found obvious de-activation in bilateral hippocampus during 2 Hz EAS, which is consistent with studies by Wu et al. [48] and Hui et al. [26]. Again, the de-activation level was correlated linearly with the analgesic effect across individuals. Taking together, these results provide evidence for the involvement of hippocampus in the EAS analgesia.

Hippocampus is thought to be involved in processing the affective and cognitive signals of pain [9,29,35]. However, the accumulated data of PET and fMRI studies of pain have seldom demonstrated a clear hippocampus activation or de-activation. In contrast, our current study along with previous findings [26,48] consistently demonstrated the de-activation of hippocampus with acupoint stimulation. The fact that fMRI signals correlated with 2-Hz EAS analgesic effect brought more significant implication into the hippocampal de-activation. It has been proved that BOLD contrast could reflect more of the input processing of a given area [33]. Thus, the observed hippocampal de-activation might indicate a decrease of input signals into this area. Therefore, acupuncture or EAS might be able to inhibit the pain signal processing in hippocampus, which in turn inhibited the affective and cognitive component of pain sensation.

4.3. Brain areas specifically correlated with analgesic effect of high-frequency EAS

Areas specifically correlated with 100-Hz EAS analgesia included contralateral BA40, amygdala, ipsilateral BA24, NAc, and pons. Besides the well-known pain-related BA24, nucleus accumbens [15], amygdala [24], and pons [37] were also involved in the process of anti-nociception or analgesia. Recent fMRI study [3] confirmed that the reward circuitry including amygdala and NAc could be activated by noxious stimuli, hence in turn acted as parts of anti-nociceptive system.

A possible explanation for the specificity of the correlations between activation/de-activation level of the above areas and the analgesic effect of high-frequency, but not low-frequency, EAS might be built by relating these areas with dynorphin, an endogenous opioid peptide. Dynorphin could be specifically released by high-frequency stimulation and in turn activate the kappa-opioid receptor [21,32]. Studies have confirmed that both dynorphin/preprodynorphin [17,28,30,36] and kappa-opioid receptors [14,28,50] are heavily or moderately distributed in the above areas in rodents and primates. Thus the current observation that high-frequency EAS induced analgesia was correlated with fMRI response in the aforementioned brain areas might be due to systematic activation of the dynorphin-kappa receptor pathway for initiation of analgesic effect.

The role of pons in the high-frequency EAS analgesia has been demonstrated in previous animal studies. Lesion of parabrachial nucleus (PBN) of pons in rats could abolish the analgesic effect induced by 100-Hz EAS [46]. The fos-like immunoactivity was much stronger in PBN during 100-Hz EAS than that during 2-Hz EAS [19]. Recent data suggested that PBN of pons was involved in the spino-parabrachio-amygdaloid pathways in the affective emotional aspects of pain [4]. Our fMRI study again confirmed the important role of pons and amygdala in high-frequency EAS analgesia in human. These results again provided the evidence that high frequency EAS might exert its analgesic effect by modulating the affective component of pain sensation.

4.4. Limitations

It is still a controversy about how to set suitable control stimulation for acupuncture or EAS. The minimal-EAS (same acupoints with low intensity) used in the current study was only one of the putative controls. However, it could effectively exclude the placebo or expectancy effect in both the fMRI and behavioral parts of this study because it is similar to the real EAS (suitable to serve as a placebo), quantitative discriminable, and helpful for explanation of the result (only one stimulation parameter was changed). Similar control stimulation was also used by other acupuncture fMRI studies [26,48]. It should be pointed out that the four subjects in the control group were recruited subsequent to collection of the main cohort. A confirmative study with randomly allocated subjects in different groups should be done in the future.

There are several other limitations in the current experimental design. The most important one is the inconsistency between the stimulation time during the fMRI scanning and pain threshold evaluation (3 vs. 30 min), which might weaken the significance of the correlation observed between data from these two processes. However, this is unavoidable because the block design of fMRI study require brief stimulation period which is insufficient to induce analgesia alone. We considered that what happened during the first few minutes of stimulation might be the basis for its cumulative effect 30 min later. Thus, the correlation observed in the current study may still bear significance in the explanation of the mechanism of EAS induced analgesia, at least for the initiation part of this procedure.

4.5. Conclusions

EAS-induced analgesia with low and high frequencies were mediated by different, though overlapped (SII/Ins, thalamus, ACC) brain networks. Motor-related areas (MI and SMA) and hippocampus are more specifically involved in low-frequency EAS analgesia, while BA40, pons, NAc and amygdala are more important for high-frequency EAS analgesia. The differentially involved brain areas for low and high frequency EAS and among subjects might also provide a neurobiological explanation for the mechanisms of the induction and individual variability of EAS analgesia.

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