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An fMRI study on the interaction and dissociation between expectation of pain relief and acupuncture treatment

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Abstract

It is well established that expectation can significantly modulate pain perception. In this study, we combined an expectancy manipulation model and fMRI to investigate how expectation can modulate acupuncture treatment. Forty-eight subjects completed the study. The analysis on two verum acupuncture groups with different expectancy levels indicates that expectancy can significantly influence acupuncture analgesia for experimental pain. Conditioning positive expectation can amplify acupuncture analgesia as detected by subjective pain sensory rating changes and objective fMRI signal changes in response to calibrated noxious stimuli. Diminished positive expectation appeared to inhibit acupuncture analgesia. This modulation effect is spatially specific, inducing analgesia exclusively in regions of the body where expectation is focused. Thus, expectation should be used as an important covariate in future studies evaluating acupuncture efficacy. In addition, we also observed dissociation between subjective reported analgesia and objective fMRI signal changes to calibrated pain on the analysis across all four groups. We hypothesize that as a peripheral-central modulation, acupuncture needle stimulation may inhibit incoming noxious stimuli; while as a top-down modulation, expectancy (placebo) may work through the emotional circuit.

Keywords

acupuncture; acupuncture analgesia; placebo; placebo analgesia; expectancy; expectancy manipulation; conditioning; pain; sham acupuncture needle

Introduction

It is well known that expectation can significantly modulate pain perception (Benedetti, 2008; Benedetti et al., 2006; Kong et al., 2007c). Recent advances in brain imaging have contributed to our mechanistic understanding of how expectancy (combined with placebo/sham treatment) can produce placebo analgesia and nocebo hyperalgesia effects (Bingel et al., 2006; Colloca et al., 2008; Craggs et al., 2007; Kong et al., 2008; Kong et al., 2006a; Lieberman et al., 2004; Petrovic et al., 2002; Price et al., 2007; Scott et al., 2007,, 2008; Wager et al., 2004; Wager et al., 2007; Zubieta et al., 2005). However, the interaction between expectancy and genuine treatment has rarely been studied.

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In one of the few studies on this topic performed in human subjects, Vokow and colleagues investigated how expectation can influence response to the stimulant drug methylphenidate in both cocaine abusers (Volkow et al., 2003) and non abusers (Volkow et al., 2006). They found expectation significantly modified both subjective report (Volkow et al., 2003) and pattern of brain activation in response to a challenge dose of methylphenidate (Volkow et al., 2006; Volkow et al., 2003).

To date, acupuncture has been studied in almost one thousand different randomized controlled trials (RCTs) (Ernst, 2006; Kaptchuk, 2000; Linde et al., 2001). In these trials, it is not uncommon for "placebo/sham acupuncture/minimal acupuncture" controls to induce positive therapeutic effects on the same order of magnitude as verum (genuine) acupuncture, with verum and sham groups usually demonstrating superiority and clinical benefits over wait list or standard of care controls (Brinkhaus et al., 2006; Haake et al., 2007; Kaptchuk, 2000,, 2002; Leibing et al., 2002; Linde et al., 2005; Melchart et al., 2005). In an attempt to find predictors of acupuncture response, Linde and colleagues (Linde et al., 2007) reanalyzed the results from several RCTs of acupuncture treatment for chronic pain and found that expectation of relief was the only factor that correctly predicted outcome.. Thus, it is important to elucidate the interaction between expectancy and acupuncture treatment in a well-controlled experimental setting.

One difficulty in studying expectancy is its large heterogeneity among healthy volunteers in part due to past treatment experience or knowledge that can condition responses. To overcome this challenge we have modified an expectancy/conditioning manipulation model used in other studies (De Pascalis et al., 2002; Kong et al., 2008; Kong et al., 2006a; Montgomery and Kirsch, 1997; Price et al., 1999; Voudouris et al., 1990; Wager et al., 2004) and applied it to a treatment that is relatively novel in this culture, acupuncture. Our group's previous studies (Kong et al., 2008; Kong et al., 2006a) that employ this paradigm demonstrate successful alteration of subjects' expectancies for acupuncture analgesia following experimental pain in a relatively short period of time.

In the current study, we combined the same expectancy/conditioning manipulation model and fMRI to investigate how expectancy can modulate acupuncture analgesia to calibrated noxious stimuli. In this experiment, subjects were randomized into four groups, receiving verum or sham acupuncture paired with either a high or low expectancy/conditioning manipulation. Then analgesic response to experimental heat pain applied on the right forearm was tested. In each group, we also included a within-subject control. Subjects were told that if they responded to the acupuncture treatment they would experience analgesia only on the treated (meridian) side of their arm, but *not* on the untreated (non-meridian) side of their arm (Figure 1). Forty-eight subjects (12 in each group) completed the experiment. In this manuscript, we will focus on the data from the two real (verum) acupuncture groups (High and Low Expectancy) to investigate the mechanism of how expectancy can modulate treatment effects using within-subject and between-subject comparisons. To facilitate the understanding of the data, we will also include results from the other groups as secondary endpoints of this study. Data from other groups have also been presented in a separate manuscript (Kong et al., 2009) with additional analyses still underway.

Material and Methods

Subjects

Seventy-seven healthy, right-handed subjects enrolled in this experiment, which was said to be a study of acupuncture analgesia. All subjects were naïve to acupuncture. Experiments were conducted with the written consent of each subject and approval by the Massachusetts General Hospital's Institutional Review Board. All subjects were debriefed at the end of the experiment.

Procedures for the Delivery and Assessment of Noxious Thermal Stimuli

Subjects were recruited to participate in two behavioral testing sessions and one fMRI scanning session. Each session was separated by a minimum of three days.

Calibrated thermal pain stimuli were delivered to the right medial aspect of the forearm using a TSA-2001 Thermal Sensory Analyzer with a 3×3 cm probe (Medoc Advanced Medical Systems, Rimat Yishai, Israel) running computerized visual analog scale software (COVAS). All stimuli were initiated from a baseline resting temperature of 32 °C and increased to a target temperature (determination of the target temperature is described below). Each stimulus was presented for 12 seconds, including a 2.5 second ramp up and ramp down, and the inter-stimulus interval ranged from 24–30 seconds.

Gracely Sensory and Affective scales (Gracely et al., 1978a; Gracely et al., 1978b) were used to measure subjective pain ratings. To ensure consistent pain administration, a 2×3 grid was drawn in marker along the palmar side of the forearm, with three boxes each on radial and ulnar sides. We placed the thermal probe in one box of the grid for each stimulus sequence (Figure 1).

Session 1—We used the first behavioral session to familiarize subjects with the rating scales and determine appropriate stimulus intensities using methods employed in our previous studies (Dougherty et al., 2008; Kong et al., 2005; Kong et al., 2008; Kong et al., 2006a; Kong et al., 2009; Kong et al., 2006b). In summary, temperatures eliciting subjective intensity ratings in the LOW pain range (~ 5; which indicates *weak* on the 0–20 Sensory Scale) and HIGH pain range (~ 15; *strong*) were selected for each individual using an ascending series of noxious stimuli (increasing by 1 °C per stimulus). We then applied a series of 8 noxious stimuli, 4 HIGH and 4 LOW pain stimuli, presented in random order, (Random Pain sequence indicated by the abbreviation RP) and a series of 6 identical HIGH pain noxious stimuli (Identical Pain sequence, indicated by IP) to the right arm. Temperatures were adjusted when necessary to ensure that each subject's subjective ratings of HIGH and LOW remained in the desired range and the final temperature settings were used in the following two sessions.

Session 2—We used Session 2 to manipulate subjects' expectancy to acupuncture treatment using a method modified from previous studies (De Pascalis et al., 2002; Kong et al., 2008; Kong et al., 2006a; Montgomery and Kirsch, 1997; Price et al., 1999; Voudouris et al., 1990; Wager et al., 2004).

At the start of Session 2, subjects were randomized into one of four arms of a 2×2 factorial design: verum or sham acupuncture paired with either high or low expectation manipulation.

At the beginning of Session 2, subjects were told that responses to acupuncture can be variable and that a given subject's response tends to remain consistent across sessions. Subjects then viewed a Traditional Chinese Medicine meridian diagram and were falsely told that, according to previous literature, acupuncture could only produce analgesia on the side of the arm where the meridian passed through but not on the other side of the arm. To balance the design, half the subjects were then shown accurate diagrams (real diagram) of the Large Intestine (LI) Meridian passing through the radial side of the right arm, while the other half viewed a modified diagram (fake diagram) showing the LI Meridian passing through the ulnar side of the arm. (For clarity, we use the terms meridian (real and fake) side and non-meridian (real and fake) side in the following.)

Next, RP sequences were administered to the bottom two boxes of the 2×3 grid and IP sequences were applied to the top four. To proceed in the study, subjects had to consistently rate the HIGH pain stimuli as being more painful (indicated by a higher score on the Sensory

scale) than the LOW pain stimuli when RP stimuli were applied to the right forearm. Additionally, subjects had to report approximately equivalent ratings (average pain rating difference less than 1.5) to IP stimuli on both the radial and ulnar sides of their arm.

Then, according to their randomization, subjects received either verum or sham electroacupuncture (see below). After treatment, subjects were told they would be receiving the same pre-treatment IP stimuli to test the analgesic effect of acupuncture. Subjects then completed an expectancy form indicating the maximum pain they had experienced during IP application and their expectations regarding how this pain level would change after receiving acupuncture treatment.

In actuality, we repeated pre-treatment IP sequences on both meridian and non-meridian sides only in low expectancy groups. In high expectancy groups, we surreptitiously decreased temperatures (delineated by decreased IP (dIP) on Figure 1) on the “meridian” side of the arm to elicit “faint to weak” (~5) ratings and give subjects an unmistakable experience of analgesia. On the “non-meridian” side, IP temperatures remained at pre-treatment HIGH levels to further impress the “positive effect” of acupuncture treatment. Subjects once again completed the expectancy form at the end of this session.

Session 3—Session 3 was performed in the fMRI scanner and subjects were told that Session 2 procedures would be repeated. In actuality, the same Session 2 procedures were performed only for the low expectancy groups. For high expectancy groups however, only one dIP was administered on the bottom of the meridian side of the right forearm arm to remind subjects of the analgesia they experienced in Session 2. Noxious stimuli for the remaining 5 boxes (the top 4 boxes and 1 bottom box on the non-meridian side receiving RP before treatment) were all delivered at the original IP.

The differences between pre- and post-treatment pain ratings and brain activation during these final IP sequences on the top four boxes constituted the primary outcomes of this study. Additionally, at the beginning of Session 3 and after the expectancy boost “reminder,” subjects were again required to complete the expectancy forms.

During the scanning of pain sequences, subjects were instructed to focus on a small black fixation cross in the center of a screen in front of them. The cross turned red to cue the onset and duration of each stimulus (12 seconds) and after a delay of 4, 6, or 8 seconds, turned black again.

Next, the Sensory Box Scale was displayed on the screen for 8 seconds and subjects used a pointer to indicate their subjective ratings.

Acupuncture administration

Identical verum or sham acupuncture was performed at Large Intestine 3 and 4 (LI 3 & 4) on the right hand by a licensed acupuncturist in Sessions 2 and 3.

For verum electroacupuncture, needles were inserted into the skin about 1.5 cm and adjusted until subjective *deqi* (Kong et al., 2007b), but no sharp pain, was evoked. Needles were then connected to an electroacupuncture device passing a 2 Hz current (OMS Medical Supplies IC-1107) (Kong et al., 2005), and intensity was gradually increased to the highest level subjects could tolerate without the sensation of sharp pain.

For sham electroacupuncture, specially-designed Streiberger sham acupuncture needles were placed on the surface of the skin and connected to a de-activated electroacupuncture device. The Streiberger placebo needle has been validated and used in many studies (Kleinhenz et al.,

1999; Kong et al., 2005; Kong et al., 2006a; McManus et al., 2007; Streitberger and Kleinhenz, 1998; White et al., 2003).

In total, verum or sham acupuncture treatment lasted approximately 25 minutes. Verum treatments were further broken down into three 6.5-minute (current ON), 1.5-minute (current OFF) blocks. After treatment, sensations evoked by verum and sham acupuncture were measured with the MGH (Massachusetts General Hospital) Acupuncture Sensation Scale (MASS), a rubric created by acupuncture researchers at MGH (Kong et al., 2005; Kong et al., 2007a; Kong et al., 2007b).

Behavioral Data Analysis

The primary endpoint of this manuscript is the modulation effect of expectancy on acupuncture treatment outcome, using data from the two verum acupuncture (Low and High Expectancy) groups, including the within-subject control ("non-meridian" side) and between-subject comparisons. However, to facilitate the interpretation of the data, we will present some related results from other groups.

In the high expectancy groups (VH and PH), meridian sides were always manipulated to make subjects expect acupuncture analgesia, and so we refer to the meridian side (real or fake) as the High Expectancy (HE) side. While we did not intentionally manipulate expectation to acupuncture effects on the meridian side in the Low Expectancy group (VL and PL), by adjusting post-treatment temperatures in Session 2, the consequence of our paradigm was to diminish expectation of relief (assuming subjects compared pain experience between meridian and non-meridian sides). Hence we refer to the meridian side (real or fake) as the Low Expectancy (LE) side in this group. Because subjects in both groups were told acupuncture would not produce any effects on the non-meridian side, we refer to the non-meridian side as the Control (C) side for both groups.

We used Session 3 pre- and post- treatment pain sensory intensity rating differences in response to administration of the IP sequence to test our hypothesis. The data of the four groups were analyzed via a $2 \times 2 \times 2$ mixed model analysis of variance (ANOVA), in which what participants were told (meridian side or non-meridian) was a within-subject factor, and what they received (verum acupuncture or placebo acupuncture) and how their expectancy was manipulated (HE and LE) were between subject factors. Fisher's Least Significant Difference (LSD) tests were applied for post-hoc comparisons.

fMRI Data Acquisition and Analysis

Brain imaging was performed with a 3-axis gradient head coil in a 3 Tesla Siemens MRI System equipped for echo planar imaging. At the midpoint of the study, an MRI scanner upgrade replaced the 3 Tesla head-only Siemens Allegra MRI System with a 3 Tesla whole-body Siemens TIM Trio MRI System, but scanning parameters remained consistent across the two systems. Comparable numbers of subjects from each group were distributed across the two scanner systems: 6–8 subjects per group were tested on the old scanner and 4–6 subjects per group were tested on the new scanner.

Thirty axial slices (4 mm thick with 1 mm skip) parallel to the anterior and posterior commissure covering the whole brain were imaged with 2000 ms TR, 40 ms TE, 90° flip angle and 3.13×3.13 mm in-plane spatial resolution. A high-resolution 3D MPRAGE sequence for anatomic localization was also collected.

Pre-processing and statistical analysis were performed using SPM2 software (Wellcome Department of Cognitive Neurology). Pre-processing included motion correction, normalization to the MNI stereotactic space, and spatial smoothing with an 8 mm Gaussian

kernel. Then for each individual, HIGH pain minus LOW pain for RP sequence, and the fMRI signal differences between pre- and post-treatment (pre- minus post-treatment) during IP administration on the HE or LE side and the Control side were calculated for each subject using a general linear model (GLM). Low-frequency noise was removed with a high-pass filter applied with default values (128s) to the fMRI time series at each voxel.

Group analysis was performed using a random-effects model. We first compared all pre-treatment HIGH pain with LOW pain on both HE/LE and Control sides in four groups when the RP were applied as outlined previously (Kong et al., 2008; Kong et al., 2006a; Kong et al., 2009; Wager et al., 2004). This yielded a mask of pain-intensity associated brain regions with which we could test for the acupuncture effects in the following analysis. According to previous imaging studies on placebo analgesia evoked by expectation and pain modulation (Benedetti et al., 2005; Kong et al., 2008; Kong et al., 2006a; Kong et al., 2009; Kong et al., 2007c; Petrovic and Ingvar, 2002; Wager et al., 2004), brain regions including medial prefrontal cortex (MPFC), dorsal lateral prefrontal cortex (DLPFC) and orbital prefrontal cortex (OPFC) play an important role in pain modulation. We added these to our *a priori* regions of interest (ROIs). In addition, given the important role of rostral ACC (rACC) and amygdala in modulation of anxiety and emotions such as fear evoked by pain (Critchley, 2004; Kalisch et al., 2005; Kong et al., 2007c; Shin et al., 2004; Shin et al., 2005; Vogt, 2005), we also included the two regions in the ROIs.

Then, we performed the following calculations with and without the mask described above, by: 1) comparing pre- and post-treatment fMRI signal change (pre- minus post-treatment) differences between VH and VL groups on HE/LE and Control sides separately using a two-sample t-test, and 2) comparing pre- and post- fMRI signal change differences between the HE/LE and Control sides within the same groups with a paired t-test.

In addition, ANOVA models were also used to analyze pre- minus post-treatment fMRI signal changes on the meridian side among the four groups to estimate the main effects of treatment mode (verum VS placebo), expectancy level (high VS low) and their interaction. We used only the meridian side for the analysis because we only measured the subjects' expectancy levels on the meridian side. More specifically, the brain networks involved with the treatment modes (verum VS placebo) were calculated by comparing pre- and post-treatment fMRI signal change differences during pain administration between *verum acupuncture with low and high expectancy groups* and *placebo acupuncture with low and high expectancy groups*. The brain networks involved with expectancy level (high VS low) were calculated by comparing pre- and post-treatment fMRI signal change differences during pain administration between *verum and placebo acupuncture with high expectancy groups* and *verum and placebo acupuncture with low expectancy groups* The brain regions involved in the interaction between treatment modes and expectancy level were calculated by comparing fMRI signal changes during the pain administration between *verum acupuncture with low expectancy groups and placebo acupuncture with high expectancy groups* and *verum acupuncture with high expectancy groups and placebo acupuncture with low expectancy groups.*

The threshold of activation within the mask (pre-treatment HIGH pain > LOW pain) and the pre-defined ROIs (rACC, MPFC, DLPFC and OPFC) were set at voxel-wise $p<0.005$ uncorrected with 20 contiguous voxels. A threshold of voxel-wise $p<0.001$uncorrected with 20 contiguous voxels and cluster $p<0.05$ corrected was used to detect activation in other regions.

Results

Subjects

Forty-eight of seventy-seven consenting volunteers completed the study and were used for data analysis (average age 26.4 ± 4.9; 24 males). Twelve subjects did not fit the criteria for continued inclusion in the study (average ratings for HIGH pain were not greater than average ratings for LOW pain, or IP ratings on the radial and ulnar sides of their right arm were not approximately equivalent), eleven voluntarily withdrew, two could not tolerate the heat pain in Session 1, one could not tolerate electroacupuncture, and one could not tolerate the scanning session. Data from two subjects completing the study was also excluded because of poor quality (head movement within each functional run exceeded 2 mm).

Subjective Ratings of Pain and Expectancy

Average pain ratings—We used Session 3 pre- and post- treatment pain sensory intensity rating differences in response to administration of the IP sequence to test our hypothesis. Table 1 and Figure 2 presents subjects' pre- and post-treatment IP ratings on the HE/LE and Control sides for the four groups.

The data of the four groups were analyzed via a 2 × 2 × 2 mixed model analysis of variance (ANOVA), in which what participants were informed (meridian side or non-meridian) was a within-subject factor, and treatment mode (verum acupuncture or placebo acupuncture) and manipulated expectancy (HE and LE) were between subject factors. The ANOVA revealed a significant main effect on what participants were informed, F (1,44) = 16.73, $p < .001$. Participants reported greater pain reduction on the "meridian" side than on the "non-meridian" side of the arm. There was also a significant interaction between what they were told (meridian or non-meridian) and expectancy (HE or LE), F (1,44) = 10.86, $p <.002$. There were no other significant main effects or interactions. Most important, the effect of acupuncture (verum versus placebo) did not approach significance, F (1,44) = 0.30, $p > .58$, nor did any of the interactions involving acupuncture approach significance.

The result from the mixed-model ANOVA indicated a significant main effect on what participants was informed, in order to investigate whether this effect differs across the four groups, we performed a one-way ANOVA analysis of the within-subject differences between meridian and non-meridian sides. The null hypothesis is rejected (F(3,44) = 11.21, P=0.02), and therefore Fisher's Least Significant Difference approach for post-hoc t-tests protected by a significant ANOVA was applied. We conclude that the four pairings of a HE group (verum or placebo acupuncture with high expectancy) with a LE group (verum or placebo acupuncture with low expectancy) are all statistically significantly different (P=0.03 for HE placebo group VS LE placebo group, P=0.02 for HE placebo group VS LE verum acupuncture group, P=0.03 for HE verum acupuncture group VS LE placebo group, P=0.02 HE verum acupuncture group VS LE verum acupuncture group). In addition, the differences between meridian and non-meridian sides are statistically significantly different in two high expectancy groups (P=0.0005 for HE placebo group; P=0.0007 for HE verum acupuncture group), but not significant in other low expectancy groups (P=0.57 for LE placebo group, P=0.82 for LE verum acupuncture group).

In Sessions 2 and 3, subjects were periodically asked to rate the highest pain level they had experienced during the IP sequences and then predict how intense this pain would be after acupuncture treatment. The difference between these two ratings, reported in Table 2, comprised subjects' expectancy of pain relief. To test the difference, we fit a fixed effect ANOVA model with treatment mode, expectancy and interaction as factors. Overall, there were no significant expectancy differences between any of the four groups at the beginning of

Session 2, F (3,44), p = 0.2. At the end of Session 2, the same analysis showed that the groups were highly significantly different, F (3,44) = 19.8, p<0.0001. The difference among the groups is entirely due to the expectancy factor; subjects in the high expectancy groups indicated significantly greater levels of expected pain relief than low expectancy groups (t = 5.1 p < 0.0001), and this strong expectancy difference among different groups was maintained for the remainder of the experiment.

For the all 48 subjects, stimulus temperatures and corresponding subjective sensory ratings (mean ± SD) were 48.4 ± 1.2 °C and 14.3 ± 1.8 for HIGH pain; 45.5 ± 1.5 °C and 4.8 ± 2.4 for LOW pain. As expected, temperature (p = 1.3E-31) and pain intensity ratings (p = 1.0E-28) differ significantly between the two stimulus intensity levels. There were no significant temperature differences between the two groups.

Subjective acupuncture sensation evoked by verum and sham acupuncture— Subjects in the study endorsed multiple descriptors to characterize their subjective experience of acupuncture. Results are shown in Table 3. The test the difference, we fit a fixed effect ANOVA model with treatment mode, expectancy and interaction as factors on average of MASS ratings.

The results showed that the groups were significantly different, F (3,44) = 7.9, p=0.0002. The different among the groups is entirely due to the treatment mode (verum vs placebo); subjects in the verum groups indicated significantly greater mass rating than low expectancy groups (t = 3.2 p < 0.002). There is no interaction (p = 0.8).

fMRI Results—To elucidate the brain regions correlating with pain intensity, we calculated a contrast between all pre-treatment HIGH and LOW pain (HIGH pain > LOW pain) of 48 subjects in four groups. The comparison yielded significant activations (voxel-wise $p<0.05$corrected with 10 contiguous voxels) in the entire predicted network of pain sensitive regions, including bilateral insular/opercular cortices, anterior cingulate cortex/medial prefrontal cortex (ACC/MPFC), secondary somatosensory cortex (SII), thalamus, periaqueductal grey (PAG), medulla, cerebellum, and left SI/M1 (contralateral). These results are consistent with previous studies (Kong et al., 2008; Kong et al., 2006a; Kong et al., 2009; Kong et al., 2006b; Wager et al., 2004) and we used these regions to comprise a mask for the following analysis.

Secondly, we performed a between-group comparison on pre- and post-treatment fMRI signal differences between the VH and VL groups on meridian (HE/LE) and non-meridian (Control) sides separately (Table 4 and Figure 3). The results showed that on the meridian side, verum acupuncture in the VH group produced significantly greater fMRI signal decreases than verum acupuncture in the VL group in brain regions including bilateral rACC/MPFC, MPFC, left DLPFC and OPFC. No region was above threshold for the opposite comparison. On the non-meridian side, no region was above threshold for the comparison of VH > VL. The only region above threshold for the opposite comparison, VL > VH was a small area in the left subgenual cortex.

The result of meridian side (HE/LE) and Control side comparisons within each of the VH and VL groups are shown in Table 5. In the VH group, identical noxious stimuli delivered to the HE side produced more signal decreases after treatment than those delivered to the Control side in left M1/S1, while noxious stimuli delivered to the Control side produced a greater signal decrease in right middle frontal gyrus (BA 10) than stimuli delivered to the HE side. In the VL group, no region above the threshold was observed for either contrast.

Then, we compared fMRI signal changes between pre- and post-treatment on both meridian (HE/LE) and non-meridian (Control) sides in both groups (Table 6). The results showed fMRI signal during calibrated pain administration exhibiting the greatest reduction after acupuncture treatment on HE side in VH group. In that contrast an extensive network of brain regions showed significantly smaller fMRI signal responses to the calibrated noxious stimuli after treatment (pre > post) including the left insula, dorsal ACC, S1/M1, paracentral lobule, superior temporal gyrus, lentiform nucleus, lateral OPFC, and right cerebellum. The opposite comparison (post > pre, indicating greater fMRI signal change in response to the painful stimuli) was significant only in superior frontal gyrus. In the VL group on the meridian (LE) side, the contrast of pre > post showed no region above the threshold, while the opposite contrast (post > pre) showed a significant difference in left MPFC/rACC and right MPFC; in the VL group on the control side, the comparison of pre > post indicated no region above the threshold, while the opposite comparison showed a difference in bilateral DLPFC/pre-motor cortex.

Finally, we performed an ANOVA analysis on the meridian sides of the four groups and the result are shown in Table 7 and Figure 4 (a matching ANOVA on the subjective pain rating changes were also performed, the results showed a significant main effect for expectancy level (high > low), F (2, 43) = 5.32, p<0.008; but not for treatment mode (verum VS sham acupuncture), p=0.8; nor interaction, p=0.37). The results showed the brain regions involved in the main effects of treatment mode (i.e., verum acupuncture (pre-post) – placebo acupuncture (pre-post)) include activations in bilateral PAG, thalamus; left insula/superior temporal gyrus/parahippocampus, pons/medulla oblongata, superior prefrontal gyrus and inferior frontal cortex(45)/insula; right OPFC/ambiens/amygdala and lentiform nucleus/insula. No voxels showed activation above the threshold when the opposite calculation was performed (e.g. placebo acupuncture with high and low expectancy (pre-post) - verum acupuncture with high and low expectancy (pre-post)).

The brain regions involved in the main effects of expectancy (e.g. verum and placebo acupuncture with high expectancy (pre-post) – verum and placebo acupuncture with low expectancy (pre-post)) included activations in bilateral MPFC/rostral ACC, precentral gyrus and MPFC/paracentral lobule, left M1/S1, posterior insula/operculum, lentiform nucleus, and superior frontal gyrus; right amygdala. Again, no voxels surpassed the threshold when the opposite calculation was performed. When the interaction effect between acupuncture and expectancy were calculated, brain regions showing a significant difference include bilateral inferior frontal gyrus and left medial frontal gyrus (Table 7 and Figure 4).

Discussion

In this study, we combined an expectancy manipulation procedure and fMRI to investigate how expectancy can influence the analgesia effect produced by verum acupuncture. Our results showed that although electroacupuncture treatment in VH and VL groups both received identical electroacupuncture treatment and produced comparable magnitudes of acupuncture sensations as measured by MASS, analgesia effect was significantly modulated by expectancy. Positive expectation can significantly enhance acupuncture analgesia effects as evidenced by decreased subjective pain ratings as well as objective fMRI signal changes occurring during application of calibrated noxious stimuli. Brain regions including bilateral rACC/MPFC, left DOPFC and DLPFC are involved in this process. Further analysis across four groups indicated that expectancy can significantly modulate the subjective pain rating changes, and there are no significant differences between the verum and sham acupuncture treatment in analgesia effect by subjective pain rating changes. Nevertheless, the fMRI analysis has indicated significant greater fMRI signal reduction to calibrated pain stimuli in pain sensory associated brain regions comparing verum acupuncture and placebo acupuncture.

To explore the potential response predictors of acupuncture treatment, Linde and colleagues (Linde et al., 2007) combined data from four acupuncture RCTs on chronic pain ailments, including migraine, tension-type headache, chronic low back pain, and knee osteoarthritis. They found that positive expectation and attitude towards acupuncture treatment could predict positive outcomes independent of whether or not treatments were real or sham. Consistent with this finding, we found that expectation can significantly modulate the analgesia effect of electroacupuncture stimulation to calibrated noxious stimuli in healthy subjects. In a previous study, Benedetti (Benedetti et al., 1999) and colleagues investigated expectation of analgesia on four different parts of the body and the role of endogenous opioids in the process. They found analgesia to only occur on the regions of the body where subjects were told a powerful local anesthetic was applied (placebo cream in actuality), but not on the other untreated regions of the body. Interestingly, Benedetti et al. also found that local placebo analgesia effect could be completely blocked by an intravenous infusion of the opioid antagonist naloxone, indicating that placebo-activated opioids can selectively act on the part of the body where expectancy is directed. Consistent with this finding, our study showed that acupuncture analgesia to be selectively elicited from one area of the forearm (HE side) but not the adjacent area (Control side), demonstrating the power of expectancy to modulate the spatial specificity of acupuncture efficacy.

The primary aim of our experiment, as addressed in this manuscript, was to investigate the modulatory effect of expectancy on electroacupuncture treatment. We explicitly manipulated subjects' expectation of acupuncture effect, either enhancing it (VH) or diminishing it (VL). Thus, the lack of analgesia effect in the VL group by no means implies that acupuncture does not work, but rather indicates the importance of expectation in the treatment process. In fact, we believe this finding is consistent with the essence of Traditional Chinese Medicine (Liu, 2008). For instance, the oldest canonical classic of Chinese medicine, the *Yellow Emperor's Inner Classic* (*Huang Di Nei Jing*) written in the first century BCE, states that, "if a patient does not consent to therapy [acupuncture] with positive engagement, the physician should not proceed as the therapy will not succeed" (*SuWen* Chapter 11). We believe this sentence is emphasizing the power of mind in acupuncture treatment, and as such, our study provides experimental evidence of this Traditional Chinese Medicine tenet.

It worth pointing out the expectancy modulation effect is not unique to acupuncture. In a previous study, Volkow and colleagues (Volkow et al., 2003) investigated how expectation influenced response to the stimulant drug methylphenidate in 25 cocaine abusers. They found 50% larger increases in metabolism when methylphenidate was expected as opposed to when it was not. These differences were significant in cerebellum (vermis) and thalamus.

Methylphenidate-induced increases in self-reports of "high" were also approximately 50% greater when subjects expected the drug as opposed to when they did not. This result demonstrates expectancy's amplifying and reinforcing effects of methylphenidate in brain. In a subsequent manuscript (Volkow et al., 2006) performed in a healthy, non drug abusing cohort, a similar but milder amplified effect of expectation was also observed. The authors further suggest that state of expectation should be considered as a variable when evaluating drug effects. In our study, the pre- and post-treatment fMRI signal change comparison in meridian and non-meridian sides across the two groups showed the greatest fMRI signal decrease during administration of calibrated pain stimuli to the HE side of VH group. These decreases occurred in pain sensory encoding regions included in our *a priori* mask including left insula, dorsal ACC, S1/M1, paracentral lobule, superior temporal gyrus, lentiform nucleus, and right cerebellum. In addition, a significant reduction of subjective pain sensation (pre- minus post-) in this group was observed when compared with the Control side. In the VL group, the pre- and post-treatment difference was found in the brain regions involved in affective and cognitive components/modulation of pain, such as rACC, MPFC and DLPFC (Kong et al., 2007c). For

both groups, we deliberately made subjects believe that acupuncture would not produce analgesia on the Control side, and thus assume this negative expectation somehow blunted the acupuncture analgesia effect. The activity in brain regions including rACC, MPFC and DLPFC observed in this group may be involved in this process.

In this study, behavioral analysis on subjective pain rating changes across four groups showed there is no significant difference on analgesia effect produced by verum acupuncture treatment and sham acupuncture treatment; this result is consistent with clinical studies that showed "placebo/sham acupuncture/minimal acupuncture" controls can produce similar therapeutic effects as verum (genuine) acupuncture (Brinkhaus et al., 2006; Haake et al., 2007; Kaptchuk, 2000,, 2002; Leibing et al., 2002; Linde et al., 2005; Melchart et al., 2005). Nevertheless, fMRI analysis showed that, compared with placebo acupuncture, verum acupuncture produced more fMRI signal decrease to calibrated pain (e.g. verum acupuncture with high and low expectancy (pre-post) – placebo acupuncture with high and low expectancy (pre-post)) in brain regions including bilateral periaqueductal gray (PAG), thalamus, left insula/superior temporal gyrus/parahippocampus, pons/medulla oblongata, left superior prefrontal gyrus and inferior frontal cortex(45)/insula, right orbital prefrontal/gyrus ambiens and lentiform nucleus/insula. Most of these brain regions were within the mask of high pain minus low pain, indicating that this modulation occurred in the same brain regions that subserve pain sensory intensity encoding. This specific brain response reduction is consistent with the interpretation that acupuncture treatment suppresses pain perception by a more peripheral site of action involving inhibition of ascending nociceptive information (Han, 2003; Melzack, 1989; Pomeranz, 1997; Zhang et al., 2003a; Zhang et al., 2003b). This result is also consistent with a previous paper based on analysis of data from this experiment (Kong et al., 2009) comparing the verum acupuncture high expectancy group to the sham acupuncture high expectancy group, where we reported that although magnitudes of subjective acupuncture analgesia and placebo analgesia were similar, fMRI analysis showed that verum, but not sham, acupuncture could significantly inhibit the brain response to calibrated pain stimuli, as indicated by fMRI signal decreases in left insula, putamen, and superior temporal gyrus.

Recently, investigators have begun to apply neuroimaging methods to obtain sensitive, quantitative, objective biomarkers in pharmacological studies testing new analgesic agents (Chizh et al., 2008; Schweinhardt et al., 2006). Thus we believe that rather than conclude that acupuncture is virtually ineffective in this experimental setting as indicated by subjective pain sensory ratings, we believe that the fMRI signal decrease in pain sensory intensity associated brain regions shown above actually indicates the inhibition of noxious information after verum acupuncture treatment.

One natural question to be asked is why we did not observe differences in reported pain ratings between verum and placebo acupuncture treatments, if verum acupuncture is supposed to inhibit incoming noxious stimulation information compared with sham acupuncture treatment. We speculate some level of bias in subjective pain ratings to account for this outcome (Kong et al., 2007c; Montgomery and Kirsch, 1997; Price et al., 1999). From the point of view of cognitive neuroscience, subjective pain rating is a complicated decision making process, which can be significantly biased by previous experience and expectation (Mesulam, 1998; Miller and Cohen, 2001). For this reason, we believe as a more objective means to study a subjective phenomenon, brain imaging can enhance our understanding of pain and pain modulation processes.

It is worth noting that it is not uncommon for studies to show a discrepancy between objective and subjective improvements from placebo treatments (de Jong et al., 1996; Feather et al., 1972; Kelley et al., 2009; Nickel, 1998; van Leeuwen et al., 2006). For instance, Fregni and colleagues (Fregni et al., 2006) investigated the acute effect of levodopa, placebo pill or sham

transcranial magnetic stimulation on patients with Parkinson's disease on three different occasions. They found that on objective outcomes, only the levodopa group improved, but on subjective outcomes, patients in the two different controls reported improvement equal to the levodopa.

In this study, we also found a brain network involved in expectancy, which includes bilateral MPFC/rostral ACC, precentral gyrus and MPFC/paracentral lobule, left M1/S1, posterior insula/operculum, lentiform nucleus, and superior frontal gyrus and right amygdala. This result is partly consistent with previous placebo analgesia studies (Bingel et al., 2006; Kong et al., 2006a; Petrovic et al., 2002; Price et al., 2007; Wager et al., 2004; Zubieta et al., 2005). Particularly, the involvement of rACC/MPFC in this process (more accurately, it is located at pACC) duplicated previous findings from our own group (Kong et al., 2006a) and others (Petrovic et al., 2002; Zubieta et al., 2005). It is known that pACC is linked to arousal caused by emotion/motivational process (Critchley, 2004), closely associated with the lateral and accessory basal nuclei of the amygdala, and is engaged in positively valenced events, predominantly activated during experiences of happiness (Vogt, 2005), and pain stimuli-induced anxiety modulation (Kalisch et al., 2005).

In a recent study, Sharot and colleagues (Sharot et al., 2007) investigated the neural mechanisms underlying a bias toward optimism. When imagining positive future events relative to negative ones, they found a specific relationship between an optimism bias and enhanced activation in the amygdala and rostral ACC. They further suggest that brain regions such as the amygdala and rostral ACC can track the subjective salience of stimuli, assess emotional, motivational and autobiographical information, and then regulate incoming signals accordingly. In this study, in addition to the rACC, we also did find involvement of the right amygdala. To further test the correlation between rACC and amygdala activity, we extracted rACC and amygdala peak voxel beta values and performed a correlation analysis. Consistent with a previous study (Sharot et al., 2007), we found a positive correlation between the two regions (p=0.003, r = 0.42). Our results support the view that expectancy regarding pain may exert its effects through top-down modulation of emotional responses (Kong et al., 2006a).

In summary, analysis on two verum acupuncture treatment with different expectancy levels showed that expectancy can significantly modulate the analgesia effect evoked by acupuncture treatment. Positive expectation can enhance acupuncture analgesia effects as evidenced by both decreased subjective pain ratings as well as objective fMRI signal changes during application of calibrated noxious stimuli. In an analogous fashion, minimizing positive expectation may be able to diminish acupuncture analgesia effect. Expectancy not only modulates the magnitude of acupuncture analgesia, but also its spatial specificity, inducing analgesia exclusively in regions of the body where expectation is focused. In addition, we observed dissociation between subjective reported analgesia and objective fMRI signal changes to calibrated pain on the analysis across four groups. As a peripheral-central modulation, acupuncture needle stimulation may inhibit incoming noxious stimuli; while as a top-down modulation, expectancy (placebo) may work through the emotional circuit. We believe this is the first brain imaging study to elucidate the brain mechanisms underlying the ability of positive expectation to influence the therapeutic effects of verum acupuncture treatment. Future studies testing this hypothesis in patient populations may significantly enhance our understanding of acupuncture. We believe our study holds importance for future clinical and mechanistic studies of alternative medicine beyond acupuncture.

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References

Benedetti F. Mechanisms of placebo and placebo-related effects across diseases and treatments. Annu Rev Pharmacol Toxicol 2008;48:33–60. [PubMed: 17666008]

Benedetti F, Amanzio M, Vighetti S, Asteggiano G. The biochemical and neuroendocrine bases of the hyperalgesic nocebo effect. J Neurosci 2006;26:12014–12022. [PubMed: 17108175]

Benedetti F, Arduino C, Amanzio M. Somatotopic activation of opioid systems by target-directed expectations of analgesia. J Neurosci 1999;19:3639–3648. [PubMed: 10212322]

Benedetti F, Mayberg HS, Wager TD, Stohler CS, Zubieta JK. Neurobiological mechanisms of the placebo effect. J Neurosci 2005;25:10390–10402. [PubMed: 16280578]

Bingel U, Lorenz J, Schoell E, Weiller C, Buchel C. Mechanisms of placebo analgesia: rACC recruitment of a subcortical antinociceptive network. Pain 2006;120:8–15. [PubMed: 16364549]

Brinkhaus B, Witt CM, Jena S, Linde K, Streng A, Wagenpfeil S, Irnich D, Walther HU, Melchart D, Willich SN. Acupuncture in patients with chronic low back pain: a randomized controlled trial. Arch Intern Med 2006;166:450–457. [PubMed: 16505266]

Chizh BA, Greenspan JD, Casey KL, Nemenov MI, Treede RD. Identifying biological markers of activity in human nociceptive pathways to facilitate analgesic drug development. Pain 2008;140:249–253. [PubMed: 18950938]

Colloca L, Tinazzi M, Recchia S, Le Pera D, Fiaschi A, Benedetti F, Valeriani M. Learning potentiates neurophysiological and behavioral placebo analgesic responses. Pain 2008;139:306–314. [PubMed: 18538928]

Craggs JG, Price DD, Verne GN, Perlstein WM, Robinson MM. Functional brain interactions that serve cognitive-affective processing during pain and placebo analgesia. Neuroimage 2007;38:720–729. [PubMed: 17904390]

Critchley HD. The human cortex responds to an interoceptive challenge. Proc Natl Acad Sci U S A 2004;101:6333–6334. [PubMed: 15096592]

de Jong PJ, van Baast R, Arntz A, Merckelbach H. The placebo effect in pain reduction: the influence of conditioning experiences and response expectancies. Int J Behav Med 1996;3:14–29. [PubMed: 16250764]

De Pascalis V, Chiaradia C, Carotenuto E. The contribution of suggestibility and expectation to placebo analgesia phenomenon in an experimental setting. Pain 2002;96:393–402. [PubMed: 11973014]

Dougherty DD, Kong J, Webb M, Bonab AA, Fischman AJ, Gollub RL. A combined [11C]diprenorphine PET study and fMRI study of acupuncture analgesia. Behav Brain Res 2008;193:63–68. [PubMed: 18562019]

Ernst E. Acupuncture--a critical analysis. J Intern Med 2006;259:125–137. [PubMed: 16420542]

Feather BW, Chapman CR, Fisher SB. The effect of a placebo on the perception of painful radiant heat stimuli. Psychosom Med 1972;34:290–294. [PubMed: 5074954]

Fregni F, Boggio PS, Bermpohl F, Maia F, Rigonatti SP, Barbosa ER, Pascual-Leone A. Immediate placebo effect in Parkinson's disease--is the subjective relief accompanied by objective improvement? Eur Neurol 2006;56:222–229. [PubMed: 17057382]

Gracely RH, McGrath PA, Dubner R. Ratio scales of sensory and affective verbal pain descriptors. Pain 1978a;5:5–18. [PubMed: 673440]

Gracely RH, McGrath PA, Dubner R. Validity and sensitivity of ratio scales of sensory and affective verbal pain descriptors: manipulation of affect by diazepam. Pain 1978b;5:19–29. [PubMed: 673439]

Haake M, Muller HH, Schade-Brittinger C, Basler HD, Schafer H, Maier C, Endres HG, Trampisch HJ, Molsberger A. German Acupuncture Trials (GERAC) for chronic low back pain: randomized, multicenter, blinded, parallel-group trial with 3 groups. Arch Intern Med 2007;167:1892–1898. [PubMed: 17893311]

Han JS. Acupuncture: neuropeptide release produced by electrical stimulation of different frequencies. Trends Neurosci 2003;26:17–22. [PubMed: 12495858]

Kalisch R, Wiech K, Critchley HD, Seymour B, O'Doherty JP, Oakley DA, Allen P, Dolan RJ. Anxiety reduction through detachment: subjective, physiological, and neural effects. J Cogn Neurosci 2005;17:874–883. [PubMed: 15969906]

Kaptchuk, TJ. Contemporary Books. McGraw-Hill; Chicago: 2000. The Web That Has No Weaver: Understanding Chinese Medicine.

Kaptchuk TJ. Acupuncture: theory, efficacy, and practice. Ann Intern Med 2002;136:374–383. [PubMed: 11874310]

Kelley JM, Boulos PR, Rubin PAD, Kaptchuk TJ. Mirror, mirror on the wall: Placebo effects that exist only in the eye of the beholder. J Eval Clin Pract 2009;15:292–298. [PubMed: 19335487]

Kleinhenz J, Streitberger K, Windeler J, Gussbacher A, Mavridis G, Martin E. Randomised clinical trial comparing the effects of acupuncture and a newly designed placebo needle in rotator cuff tendinitis. Pain 1999;83:235–241. [PubMed: 10534595]

Kong J, Fufa DT, Gerber AJ, Rosman IS, Vangel MG, Gracely RH, Gollub RL. Psychophysical outcomes from a randomized pilot study of manual, electro, and sham acupuncture treatment on experimentally induced thermal pain. J Pain 2005;6:55–64. [PubMed: 15629419]

Kong J, Gollub R, Huang T, Polich G, Napadow V, Hui K, Vangel M, Rosen B, Kaptchuk TJ. Acupuncture de qi, from qualitative history to quantitative measurement. J Altern Complement Med 2007a; 13:1059–1070. [PubMed: 18166116]

Kong J, Gollub RL, Polich G, Kirsch I, Laviolette P, Vangel M, Rosen B, Kaptchuk TJ. A functional magnetic resonance imaging study on the neural mechanisms of hyperalgesic nocebo effect. J Neurosci 2008;28:13354–13362. [PubMed: 19052227]

Kong J, Gollub RL, Rosman IS, Webb JM, Vangel MG, Kirsch I, Kaptchuk TJ. Brain activity associated with expectancy-enhanced placebo analgesia as measured by functional magnetic resonance imaging. J Neurosci 2006a;26:381–388. [PubMed: 16407533]

Kong J, Gollub RL, Webb JM, Kong JT, Vangel MG, Kwong K. Test-retest study of fMRI signal change evoked by electro-acupuncture stimulation. Neuroimage 2007b:1171–1181.

Kong J, Kaptachuk TJ, Polich G, Kirsch IV, Angel M, Zyloney C, Rosen B, Gollub R. Expectancy and treatment interactions: A dissociation between acupuncture analgesia and expectancy evoked placebo analgesia. Neuroimage 2009;45:940–949. [PubMed: 19159691]

Kong J, Kaptchuk TJ, Polich G, Kirsch I, Gollub RL. Placebo analgesia: findings from brain imaging studies and emerging hypotheses. Rev Neurosci 2007c;18:173–190. [PubMed: 18019605]

Kong J, White NS, Kwong KK, Vangel MG, Rosman IS, Gracely RH, Gollub RL. Using fMRI to dissociate sensory encoding from cognitive evaluation of heat pain intensity. Hum Brain Mapp 2006b;27:715–721. [PubMed: 16342273]

Leibing E, Leonhardt U, Koster G, Goerlitz A, Rosenfeldt JA, Hilgers R, Ramadori G. Acupuncture treatment of chronic low-back pain -- a randomized, blinded, placebo-controlled trial with 9-month follow-up. Pain 2002;96:189–196. [PubMed: 11932074]

Lieberman MD, Jarcho JM, Berman S, Naliboff BD, Suyenobu BY, Mandelkern M, Mayer EA. The neural correlates of placebo effects: a disruption account. Neuroimage 2004;22:447–455. [PubMed: 15110038]

Linde K, Streng A, Jurgens S, Hoppe A, Brinkhaus B, Witt C, Wagenpfeil S, Pfaffenrath V, Hammes MG, Weidenhammer W, Willich SN, Melchart D. Acupuncture for patients with migraine: a randomized controlled trial. JAMA 2005;293:2118–2125. [PubMed: 15870415]

Linde K, Vickers A, Hondras M, ter Riet G, Thormahlen J, Berman B, Melchart D. Systematic reviews of complementary therapies - an annotated bibliography. Part 1: acupuncture. BMC Complement Altern Med 2001;1:3. [PubMed: 11513758]

Linde K, Witt CM, Streng A, Weidenhammer W, Wagenpfeil S, Brinkhaus B, Willich SN, Melchart D. The impact of patient expectations on outcomes in four randomized controlled trials of acupuncture in patients with chronic pain. Pain 2007;128:264–271. [PubMed: 17257756]

Liu T. Acupuncture: What Underlies Needle Administration? Evid Based Complement Alternat Med. 200810.1093/ecam/nen1002

McManus CA, Schnyer RN, Kong J, Nguyen LT, Hyun Nam B, Goldman R, Stason WB, Kaptchuk TJ. Sham acupuncture devices - practical advice for researchers. Acupunct Med 2007;25:36–40. [PubMed: 17641566]

Melchart D, Streng A, Hoppe A, Brinkhaus B, Witt C, Wagenpfeil S, Pfaffenrath V, Hammes M, Hummelsberger J, Irnich D, Weidenhammer W, Willich SN, Linde K. Acupuncture in patients with tension-type headache: randomised controlled trial. Bmj 2005;331:376–382. [PubMed: 16055451]

Melzack, R. Folk medicine and the sensory modulation of pain. In: Wall, PD.; Melzack, R., editors. Textbook of Pain. Churchill Livingstone; Edinburgh: 1989. p. 1209-1223.

Mesulam MM. From sensation to cognition. Brain 1998;121:1013–1052. [PubMed: 9648540]

Miller EK, Cohen JD. An integrative theory of prefrontal cortex function. Annu Rev Neurosci 2001;24:167–202. [PubMed: 11283309]

Montgomery GH, Kirsch I. Classical conditioning and the placebo effect. Pain 1997;72:107–113. [PubMed: 9272794]

Nickel JC. Placebo therapy of benign prostatic hyperplasia: a 25-month study. Canadian PROSPECT Study Group. Br J Urol 1998;81:383–387. [PubMed: 9523656]

Petrovic P, Ingvar M. Imaging cognitive modulation of pain processing. Pain 2002;95:1–5. [PubMed: 11790461]

Petrovic P, Kalso E, Petersson KM, Ingvar M. Placebo and opioid analgesia-- imaging a shared neuronal network. Science 2002;295:1737–1740. [PubMed: 11834781]

Pomeranz, B. Scientific basis of acupuncture. In: Stux, G.; Pomeranz, B., editors. Basics of Acupuncture. Springer; Berlin: 1997. p. 6-72.

Price DD, Craggs J, Verne GN, Perlstein WM, Robinson ME. Placebo analgesia is accompanied by large reductions in pain-related brain activity in irritable bowel syndrome patients. Pain 2007;127:63–72. [PubMed: 16963184]

Price DD, Milling LS, Kirsch I, Duff A, Montgomery GH, Nicholls SS. An analysis of factors that contribute to the magnitude of placebo analgesia in an experimental paradigm. Pain 1999;83:147–156. [PubMed: 10534585]

Schweinhardt P, Bountra C, Tracey I. Pharmacological FMRI in the development of new analgesic compounds. NMR Biomed 2006;19:702–711. [PubMed: 16986120]

Scott DJ, Stohler CS, Egnatuk CM, Wang H, Koeppe RA, Zubieta JK. Individual differences in reward responding explain placebo-induced expectations and effects. Neuron 2007;55:325–336. [PubMed: 17640532]

Scott DJ, Stohler CS, Egnatuk CM, Wang H, Koeppe RA, Zubieta JK. Placebo and nocebo effects are defined by opposite opioid and dopaminergic responses. Arch Gen Psychiatry 2008;65:220–231. [PubMed: 18250260]

Sharot T, Riccardi AM, Raio CM, Phelps EA. Neural mechanisms mediating optimism bias. Nature 2007;450:102–105. [PubMed: 17960136]

Shin LM, Orr SP, Carson MA, Rauch SL, Macklin ML, Lasko NB, Peters PM, Metzger LJ, Dougherty DD, Cannistraro PA, Alpert NM, Fischman AJ, Pitman RK. Regional cerebral blood flow in the amygdala and medial prefrontal cortex during traumatic imagery in male and female Vietnam veterans with PTSD. Arch Gen Psychiatry 2004;61:168–176. [PubMed: 14757593]

Shin LM, Wright CI, Cannistraro PA, Wedig MM, McMullin K, Martis B, Macklin ML, Lasko NB, Cavanagh SR, Krangel TS, Orr SP, Pitman RK, Whalen PJ, Rauch SL. A functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic stress disorder. Arch Gen Psychiatry 2005;62:273–281. [PubMed: 15753240]

Streitberger K, Kleinhenz J. Introducing a placebo needle into acupuncture research. Lancet 1998;352:364–365. [PubMed: 9717924]

van Leeuwen JH, Castro R, Busse M, Bemelmans BL. The placebo effect in the pharmacologic treatment of patients with lower urinary tract symptoms. Eur Urol 2006;50:440–452. [PubMed: 16753253] discussion 453

Vogt BA. Pain and emotion interactions in subregions of the cingulate gyrus. Nat Rev Neurosci 2005;6:533–544. [PubMed: 15995724]

Volkow ND, Wang GJ, Ma Y, Fowler JS, Wong C, Jayne M, Telang F, Swanson JM. Effects of expectation on the brain metabolic responses to methylphenidate and to its placebo in non-drug abusing subjects. Neuroimage 2006;32:1782–1792. [PubMed: 16757181]

Volkow ND, Wang GJ, Ma Y, Fowler JS, Zhu W, Maynard L, Telang F, Vaska P, Ding YS, Wong C, Swanson JM. Expectation enhances the regional brain metabolic and the reinforcing effects of stimulants in cocaine abusers. J Neurosci 2003;23:11461–11468. [PubMed: 14673011]

Voudouris NJ, Peck CL, Coleman G. The role of conditioning and verbal expectancy in the placebo response. Pain 1990;43:121–128. [PubMed: 2277714]

Wager TD, Rilling JK, Smith EE, Sokolik A, Casey KL, Davidson RJ, Kosslyn SM, Rose RM, Cohen JD. Placebo-induced changes in FMRI in the anticipation and experience of pain. Science 2004;303:1162–1167. [PubMed: 14976306]

Wager TD, Scott DJ, Zubieta JK. Placebo effects on human mu-opioid activity during pain. Proc Natl Acad Sci U S A 2007;104:11056–11061. [PubMed: 17578917]

White P, Lewith G, Hopwood V, Prescott P. The placebo needle, is it a valid and convincing placebo for use in acupuncture trials? A randomised, single-blind, cross-over pilot trial. Pain 2003;106:401–409. [PubMed: 14659523]

Zhang WT, Jin Z, Cui GH, Zhang KL, Zhang L, Zeng YW, Luo F, Chen AC, Han JS. 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. Brain Res 2003a; 982:168–178. [PubMed: 12915252]

Zhang WT, Jin Z, Huang J, Zhang L, Zeng YW, Luo F, Chen AC, Han JS. Modulation of cold pain in human brain by electric acupoint stimulation: evidence from fMRI. Neuroreport 2003b;14:1591–1596. [PubMed: 14502082]

Zubieta JK, Bueller JA, Jackson LR, Scott DJ, Xu Y, Koeppe RA, Nichols TE, Stohler CS. Placebo effects mediated by endogenous opioid activity on mu-opioid receptors. J Neurosci 2005;25:7754–7762. [PubMed: 16120776]

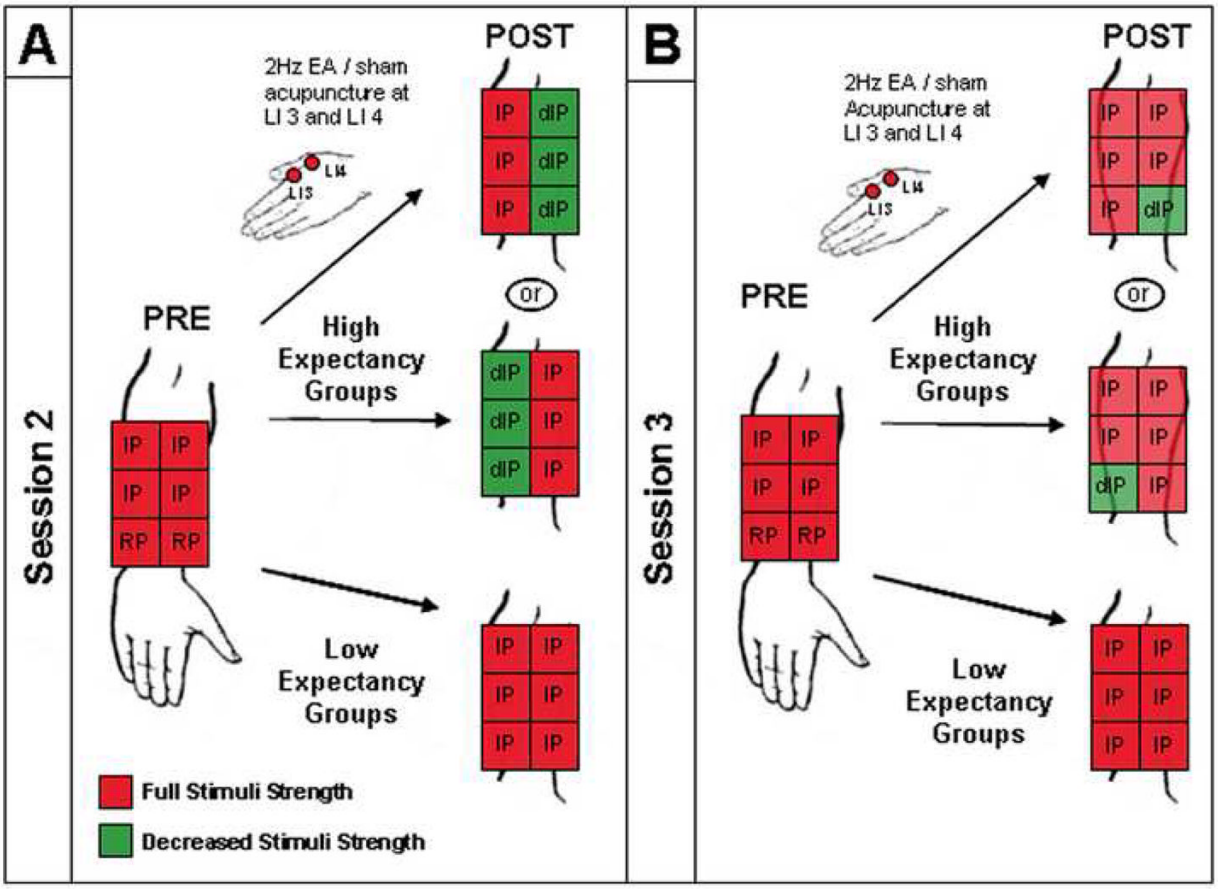

Figure 1.

Details of experimental procedure. In **Session 2**, we used a marker to draw a numbered 2 x 3 grid on the medial aspect of the right forearm and placed the thermal probe in one box of the grid for each of the stimulus sequences (e.g. random pain (RP) and identical pain (IP) sequences). After electroacupuncture treatment in high expectancy groups, decreased stimulus temperatures (dIP) indicated by green color were applied on the meridian side (HE side), but not on the non-meridian side (Control side) to give each subject an unmistakable experience of analgesia. After electroacupuncture treatment in low expectancy groups, the same temperature pre-treatment IP stimuli were applied. In **Session 3**, subjects were told that Session 2 procedures would be repeated during the fMRI scan. However, only one dIP was decreased (green color) on the HE side in high expectancy groups. Original temperatures were administered to the remaining regions of the forearm, thus the pre- minus post- treatment contrast is a subtraction of identical stimuli. The pre- and post- treatment pain ratings and fMRI signal change differences between the meridian side and non-meridian (Control) sides were the primary outcomes of this study.

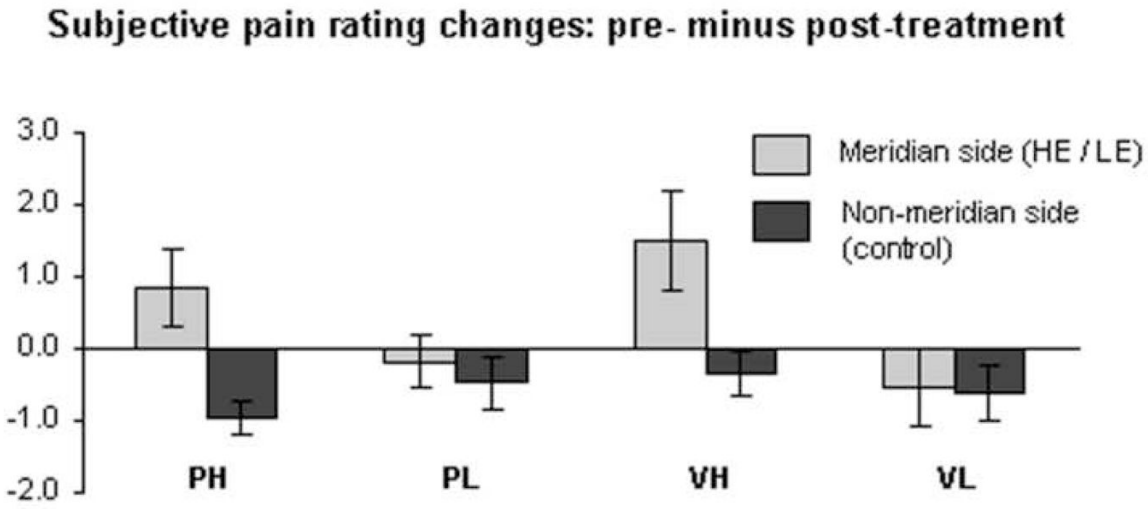

Figure 2.
Subjective sensory pain rating changes (pre- minus post-) on meridian (HE/LE) and Control sides across four groups. VH: verum acupuncture high expectancy group; VL: verum acupuncture low expectancy (VL) group; PH: placebo acupuncture high expectancy group; PL: placebo acupuncture low expectancy group.

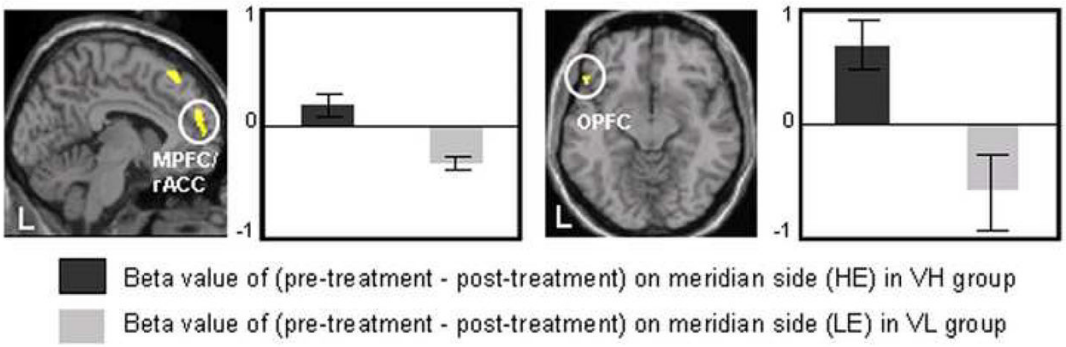

Figure 3.

Representative brain regions showing significantly greater fMRI signal decrease after verum acupuncture with high expectancy (VH) compared with verum acupuncture with low expectancy treatment (VL) on meridian side (VH > VL). The bars indicate averaged beta values (3 mm sphere around the activation peak) of pre- and post-treatment difference in VH group (dark) and VL group (grey) for each of the brain regions indicated on the accompanying image (mean ± SE). L indicates left side of the brain, rACC: rostral anterior cingulate cortex; MPFC: medial prefrontal cortex; OPFC: orbital prefrontal cortex.

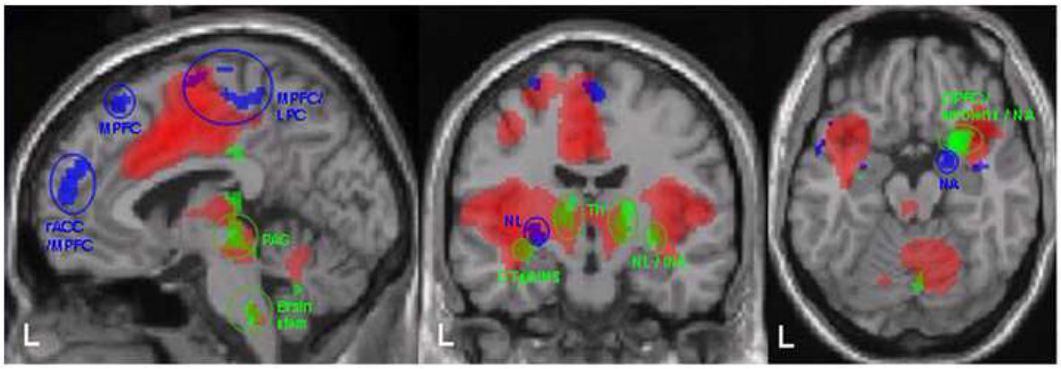

Figure 4.
Representative brain regions involved in expectancy (blue color) and acupuncture treatment (green color) from ANOVA analysis across four groups. The red color indicates the mask of high pain minus low pain across four groups. L indicates left side of the brain, R indicates right side of the brain. rACC: rostral anterior cingulate cortex; MPFC: medial prefrontal cortex; LPC: paracentral lobule; PAG: periaqueduct grey; NL: lentiform nucleus; INS: insula; OPFC: orbital prefrontal cortex; NA: amygdala.

Table 1

Average pain sensory ratings of IP stimuli on HE side and Control side in Session 3 for verum high expectancy (VH), verum low expectancy (VL), placebo high expectancy (PH) and Placebo low expectancy (PL) groups (mean ± SE). MS, meridian side, NMS, non-meridian (control) side.

	VH		PH		VL		PL	
	MS	**NMS**	**MS**	**NMS**	**MS**	**NMS**	**MS**	**NMS**
Pre	13.8±0.7	13.8±0.7	13.4±0.7	13.1±0.8	13.1±0.6	13.5±0.5	12.2±0.7	12.8±0.8
Post	12.3±0.8	14.2±0.5	12.6±0.9	14.0±0.6	13.6±0.5	14.1±0.4	12.3±0.8	13.3±0.8
Difference	1.5±0.7	−0.4±0.3	0.9±0.5	−1.0±0.2	−0.5±0.5	−0.6±0.4	−0.2±0.4	−0.5±0.4

Table 2

Subjects' expected pain reduction in Session 2 and Session 3 in four groups, n=12 in each group (mean ± SE).

Expectancy	VH	PH	VL	PL
S2 pre-treatment	3.3±0.5	5.1±0.7	4.7±0.7	3.0±0.4
S2 post-treatment	9.8±1.4	9.1±1.3	0.7±0.1	1.0±0.1
S3 pre-treatment	10.1±1.5	7.7±1.1	0.8±0.1	1.0±0.1
S3 post-expectancy boost	11.7±1.7	8.7±1.3	1.3±0.2	1.2±0.2
S3 post-final treatment	6.7±1.0	5.5±0.8	1.1±0.2	1.3±0.2

Table 3

Average MASS ratings across different groups (mean ± SE)

	Soreness	aching	deep-pressure	heaviness	fullness	tingling	numbness	dull-pain	sharp-pain	warm	cold	throbbing
VH	2.4±0.5	2.8±0.5	2.6±0.6	1.3±0.4	1.3±0.5	2.0±0.5	2.0±0.5	3.7±0.7	0.8±0.2	0.7±0.2	0.2±0.2	2.4±0.7
PH	0.4±0.2	0.1±0.1	0.5±0.4	1.3±0.7	1.2±0.6	1.3±0.4	1.3±0.6	0.1±0.1	0.1±0.1	0.3±0.3	0.2±0.2	0.0±0.0
VL	2.6±0.7	2.1±0.7	1.6±0.5	1.3±0.6	0.7±0.4	2.5±0.8	2.3±0.6	2.3±0.6	1.2±0.5	0.3±0.2	1.2±0.5	2.5±0.7
PL	0.1±0.1	0.4±0.2	0.2±0.1	0.8±0.4	0.5±0.2	2.2±0.6	1.2±1.2	0.3±0.2	0.1±0.1	0.8±0.3	0.1±0.1	0.3±0.2

Table 4

Between-group comparison in fMRI signal change differences (pre-minus post-treatment) between verum acupuncture high expectancy group (VH) and verum acupuncture low expectancy group (VL) on both meridian side (HE or LE) and non-meridian side (Control).

Comparisons			Area (Brodmann Area)	Z score	Number of voxels in cluster	Peak coordinate (x,y,z)
Effect of expectancy (meridian side)	VH>VL		Bilateral MPFC/rACC (32, 10)	3.53	256	−12 54 16
			Left lateral OPFC (47)	3.17	31	−50 28 −14
		ROI	Left MPFC (8)	3.06	65	−6 34 54
			Left DLPFC (44)	2.93	29	−62 6 18
			Right MPFC (8)	2.9	39	12 30 52
		Others	No regions above threshold			
	VL>VH	ROI	No regions above threshold			
		Others	No regions above threshold			
Effect of expectancy (Control side)	VH>VL	ROI	No regions above threshold			
		Others	No regions above threshold			
	VL>VH	ROI	Left subgenual ACC	3.21	29	−8 24 −10
		Others	No regions above threshold			

Note: **Meridian side** indicates where subjects were manipulated to expect good or poor analgesia effect, **Control side** indicates non-meridian side where subjects were told acupuncture can not produce any effect. **ROIs** include the brain regions within the mask of HIGH pain > LOW pain (indicated by italics) and additional *a priori* regions in the DLPFC, OPFC, MPFC, rACC and amygdala. The threshold is set to voxel-wise $p<0.005$ with 20 continuous voxels for predefined ROIs. The threshold for other regions (**Others)**, were set to voxel-wise $p<0.001$uncorrected with 20 contiguous voxels and cluster $p<0.05$ corrected for other regions. Peak coordinates refer to the MNI atlas.

Table 5

Within-group comparison on fMRI signal change differences (pre- minus post-treatment) between HE side and Control side in two groups.

Comparisons			Area (Brodmann Area)	Z score	Number of voxels in cluster	Peak coordinate (x,y,z)
Effect of Expectancy in VH group	HE > Con	ROI	*Left M1/S1 (4)*	3.13	125	−34 −28 62
		Others	No regions above threshold			
	Con > HE	ROI	Right middle frontal gyrus (10)	3.06	53	36 52 6
		Others	No regions above threshold			
Effect of Expectancy in VL group	LE > Con	ROI	No regions above threshold			
		Others	No regions above threshold			
	Con > LE	ROI	No regions above threshold			
		Others	No regions above threshold			

Note: **HE** side in VH group and **LE** side in VL group indicates meridian sides where subjects were manipulated to expect good or no analgesia effect, **Control side (Con)** indicates non-meridian side where subjects were told acupuncture can not produce any effect in both groups. **ROIs** include the brain regions within the mask of HIGH pain > LOW pain (indicated by italics) and additional *a priori* regions in the DLPFC, OPFC, MPFC, rACC and amygdala. The threshold is set to voxel-wise $p<0.005$ with 20 continuous voxels for predefined ROIs. The threshold for other regions (**Others)**, were set to voxel-wise $p<0.001$uncorrected with 20 contiguous voxels and cluster $p<0.05$ corrected for other regions. Peak coordinates refer to the MNI atlas.

Table 6

fMRI signal change differences (pre- minus post-treatment) across meridian (HE/LE) side and non-meridian side (Control) in VH and VL groups

Comparisons				Area (Brodmann Area)	Z score	Number of voxels in cluster	Peak coordinate (x,y,z)
Pre- and post - treatment comparison in VH group	HE side	Pre > Post	ROI	*Left insula/superior temporal gyrus(38)/lentiform nucleus*	3.62	647	−36 0 −16
				Left S1/M1 (3/4)	3.22	107	−26 −32 58
				Left dorsal ACC (24)	3.09	51	−8 −4 38
				Left insula	2.93	38	−42−16 0
				Left paracentral lobule (5)	3.1	24	−12 −32 52
				Left putamen	2.89	26	−24 −6 16
				Right cerebellum	3.49	75	16 −46 −18
			Others	Left lateral OPFC (47)	2.88	33	−48 30 −12
		Post > Pre	ROI	Left superior frontal gyrus (9)	3.23	27	−22 42 30
			Others	No regions above threshold			
	Con side	Pre > Post	ROI	No regions above threshold			
			Others	No regions above threshold			
		Post > Pre	ROI	No regions above threshold			
			Others	No regions above threshold			
Pre- and post - treatment comparison in VL group	LE side	Pre > Post	ROI	No regions above threshold			
			Others	No regions above threshold			
		Post > Pre	ROI	Left MPFC/rACC (9/32)	3.65	80	−12 54 16
				Right MPFC (8)	3.17	33	16 38 42
			Others	No regions above threshold			
	Con side	Pre > Post	ROI	No regions above threshold			
			Others	No regions above threshold			
		Post > Pre	ROI	Right DLPFC/premotor cortex (44/6)	3.61	309	66 6 24
				Left DLPFC/premotor cortex (44/6)	3.16	45	−56 −2 20

Comparisons		Area (Brodmann Area)	Z score	Number of voxels in cluster	Peak coordinate (x,y,z)
	Others	No regions above threshold			

Note: **HE** side in VH group and **LE** side in VL group indicates meridian sides where subjects were manipulated to expect good or no analgesia effect, **Control side (Con)** indicates non-meridian side where subjects were told acupuncture can not produce any effect in both groups. **ROIs** include the brain regions within the mask of HIGH pain > LOW pain (indicated by italics) and additional *a priori* regions in the DLPFC, OPFC, MPFC, rACC and amygdala. The threshold is set to voxel-wise $p<0.005$ with 20 continuous voxels for predefined ROIs. The threshold for other regions (**Others**), were set to voxel-wise $p<0.001$uncorrected with 20 contiguous voxels and cluster $p<0.05$ corrected for other regions. Peak coordinates refer to the MNI atlas.

Table 7

ANOVA results on fMRI signal change differences (post-pain > pre-pain) on the meridian side (where subjects were told that acupuncture can produce effect if they could response to acupuncture treatment) across the four groups.

fMRI signal change difference			Area (Brodmann Area)	Z score	Number of voxels in cluster	Peak coordinate (x,y,z)
Main effect of Acu	Acu > Pla	Within ROI	*Bilateral periaqueduct grey*	*4.02*	*391*	*0 −28 −10*
			Right thalamus	*3.76*		*18 −16 6*
			Left thalamus	*3.11*		*−14 −12 4*
			Left insula/superior temporal gyrus (22)/ parahippocampus (27)	*3.42*	*194*	*−36 −6 −12*
			Right lentiform nucleus/insula	*3.16*	*46*	*32 −14 −4*
			Left pons/medulla oblongata	*3.55*	*27*	*−2 −32 −44*
			Cerebellum	*3.13*	*53*	*0 −54 −26*
			Right OPFC/ambiens/amygada (47, 34)	3.31	297	26 10 −18
			Left superior frontal gyrus (8)	3.41	42	−24 38 46
			Left inferior frontal cortex(45)/insula	3.01	55	−44 26 6
		others	No region above the threshold			
	Pla > Acu		No region above the threshold			
Main effect of Expectancy	HE > LE	Within ROI	*Left posterior insula/operculum*	*3.26*	*45*	*−38 −30 12*
			Left lentiform nucleus	*3.25*	*83*	*−24 −14 −4*
			Left M1/S1 (4, 1)	*2.87*	*33*	*−22 −20 70*
			Bilateral MPFC/rACC	3.91	587	−12 54 18
			Bilateral MPFC/paracentral lobule (6, 4)		695	14 −24 64
			Right amygdala	3.43	58	22 2 −18
			Left superior frontal gyrus (8)	3.39	76	−6 32 58
		others	No region above the threshold			
	LE > HE		No region above the threshold			
Interaction		Within ROI	Left medial frontal gyrus (8)	3.21	34	−14 30 36
			Left inferior frontal gyrus (44)	3.23	32	−62 10 18

fMRI signal change difference		Area (Brodmann Area)	Z score	Number of voxels in cluster	Peak coordinate (x,y,z)
		Right inferior frontal gyrus (44)	2.92	33	52 38 −4
	Others	No region above the threshold			

Note: **Acu** indicates acupuncture, **Pla** indicates placebo, **HE** indicates high expectancy, **LE** indicates low expectancy. **ROIs** include the brain regions within the mask of HIGH pain > LOW pain (indicated by italics) and additional *a priori* regions in the DLPFC, OPFC, MPFC, rACC and amygdala. The threshold is set to voxel-wise $p<0.005$ with 20 continuous voxels for predefined ROIs, and voxel-wise $p<0.001$ uncorrected with 20 contiguous voxels and cluster $p<0.05$ corrected for other regions. Peak coordinates refer to the MNI atlas.