Psychophysiology, 48 (2011), 1119–1128. Wiley Periodicals, Inc. Printed in the USA.

DOI: 10.1111/j.1469-8986.2011.01178.x

A follow-up fMRI study of a transferable placebo anxiolytic effect

WENCAI ZHANG,[a] SHAOZHENG QIN,[c] JIANYOU GUO,[a] AND JING LUO[a,b]

[a]Key Laboratory of Mental Health, Institute of Psychology, Chinese Academy of Sciences, Beijing, PR China
[b]Learning and Cognition Laboratory, Capital Normal University, Beijing, China
[c]Center for Cognitive Neuroimaging, Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen, Nijmegen, The Netherlands

Abstract

Our previous study showed that placebo expectations can develop in a transferable manner; for example, a placebo expectation developed within an analgesic experience may lead to reduced anxiety. Considering that activities in such emotion-responsive areas as the amygdala and insula can be detected through functional magnetic resonance imaging (fMRI), we used fMRI to further study the transferable placebo anxiolytic effect. A main-effect analysis showed that activity in the amygdala and insula was reduced in the placebo condition, whereas an interaction analysis showed activity in the two regions was selectively attenuated in the placebo condition when unpleasant pictures were viewed. We also observed greater activity in the subgenual anterior cingulate cortex under placebo conditions when either emotionally negative or neutral pictures were viewed. These data suggest that the anxiety-relieving placebo effect arose from a reward-related response underpinned by the participants' expectations.

Descriptors: Placebo anxiolytic effect, Expectation, Negative emotion, Amygdala, Subgenual anterior cingulate cortex, Functional magnetic resonance imaging (fMRI)

The neural correlates of placebo effects have been studied using neuroimaging techniques (Kong et al., 2006, 2008; Petrovic et al., 2005; Petrovic, Kalso, Petersson, & Ingvar, 2002; Wager et al., 2004; Zubieta et al., 2005), and almost all studies employed a parallel paradigm in which reinforcement and testing were perfectly intermatched. For example, the analgesic effects of a placebo were tested by administering an actual potent analgesic drug (Amanzio & Benedetti, 1999; Benedetti et al., 2003) or covertly reducing the intensity of painful stimuli when the placebo is administered (Colloca & Benedetti, 2006; Kong et al., 2006; Wager et al., 2004) in the reinforcement-learning phase. Similarly, anxiolytic placebo effects have been investigated by administering a potent tranquilizer in the reinforcement-learning phase (Petrovic et al., 2005). (In the present study, "reinforcement" refers to coupling an analgesic or anxiolytic response with an inert placebo. "Analgesic" or "anxiolytic" experiences reinforce the association between an analgesic or anxiolytic response and the specific placebo.) However, it is possible that placebo expectations transfer from one domain to another. For example, the belief that an incantation or deity can help one escape danger could be extended to a belief that such things will also have other effects, such as bringing good fortune.

Our previous study showed that a placebo expectation can be transferred from alleviating pain to reducing anxiety (Zhang & Luo, 2009). We first developed the subjects' expectations of an analgesic effect from magnetic treatment (the placebo) by covertly reducing or increasing the intensity of a painful stimulus when the (sham) treatment equipment was turned on or off, respectively. We then examined whether the expectation of the placebo's efficacy altered the level of negative emotional arousal in participants while they viewed unpleasant pictures. There are three important findings in our previous study. First, the results verified that the placebo expectation was transferable. The placebo expectation not only significantly induced a direct placebo analgesic effect on sensory pain and affective pain, but also had a significant transferable placebo anxiolytic effect on the negative emotions induced by viewing unpleasant pictures. Second, we found that negative emotional arousal was reduced only by the reinforced expectation that was pretrained in a transferable way in the experimental group; participant expectations of an anxiolytic effect induced by verbal instruction alone in the control group (e.g., control group received no analgesic reinforcement) were not associated with a reduced level of negative emotional arousal. Third, the results of event-related potentials (ERPs) in participants viewing unpleasant pictures showed that N2 and P2 amplitudes in the placebo condition were significantly increased and decreased, respectively, relative to a control condition

This research was supported by the National Basic Research Program of China (Grant 2010CB833904), the National Natural Science Foundation of China (Grants 30970890 and 30770708), the National Hi-Tech Research and Development Program of China (Grants 2008AA022604 and 2009BAI77B06), the Young Scientists Fund of the Institute of Psychology (Grant Y0CX451S01), and the Knowledge Innovation Program of the Chinese Academy of Sciences (Grant KSCX2-EW-J-8).

Address correspondence to: Jing Luo, Key Laboratory of Mental Health, Institute of Psychology, Chinese Academy of Sciences (CAS), Da-tun Road 4#, Chao-yang District, Beijing 100101, PR China. E-mail luoj@psych.ac.cn

PSYCHOPHYSIOLOGY

(Zhang & Luo, 2009). The present study is based on the important findings from our previous study. In the current follow-up study, we examine the transferable placebo anxiolytic effect with event-related functional magnetic resonance imaging (fMRI).

Placebo regulation of negative emotion processing is poorly understood; to date, only one such study has been conducted by Petrovic and colleagues (2005). However, it is valuable to investigate ways to modulate negative emotional reactions because there is a consensus that negative emotions comprise some of the main causes of suffering and dysfunction in everyday life (Beauregard, Levesque, & Bourgouin, 2001). Petrovic and colleagues (2005) found that extrastriate visual areas showed reduced activities, and the right lateral orbital frontal cortex, rostral anterior cingulate cortex, and ventral lateral prefrontal cortex showed increased activities in the placebo condition compared with the control condition; further, activation in the amygdala was negatively correlated with subjective ratings of placebo efficacy. However, no more correlating fMRI evidence of an emotional placebo effect exists. Thus, the present study aimed to use event-related fMRI to provide valuable evidence of the brain mechanisms underlying a placebo anxiety-relief effect.

To clarify and extend our understanding of the neural basis of an anxiety-relieving placebo effect, we used a "transferable" expectation-reinforcing procedure and event-related fMRI to observe the neural network involved in the anxiolytic effect of placebo treatment on negative emotions and the modulatory network of placebo expectations. In particular, we wanted to examine whether the reduced negative emotional arousal induced by a placebo anxiolytic effect was accompanied by attenuated activity in the amygdala and insula; previous studies revealed that the two brain structures are particularly involved in the processing of negative emotion (Costafreda, Brammer, David, & Fu, 2008; Wright, Martis, McMullin, Shin, & Rauch, 2003) and show reduced activation during the placebo administration or reappraising regulation of negative emotions (Ochsner et al., 2004; Petrovic et al., 2005; Wager et al., 2004). We presented participants with both unpleasant and emotionally neutral pictures to investigate whether an anxiolytic placebo effect could "de-emotionalize" highly aversive pictures by making them affectively similar to emotionally neutral pictures. We also wanted to determine whether a transferred anxiolytic placebo effect, like a standard emotion-related placebo effect (Petrovic et al., 2005), is associated with enhanced activity in the reward-related areas of the brain, such as the ventral regions of the anterior cingulate cortex and prefrontal cortex.

Method

Participants

Participants ($N = 27$; 20.64 ± 1.09 years; 23 women) were recruited from Beijing Forestry University and the University of Science and Technology, Beijing. All participants were healthy and right-handed and had normal or corrected-to-normal vision. None of them reported a history of psychiatric or neurological disease or severe physical or emotional trauma. The procedures of the present study were approved by the local ethics committee, and written informed consent was obtained from the participants before they began the experiment.

There were two separate parts to our experiment: a behavioral portion and an fMRI scanning. All participants first underwent the behavioral portion of the experiment. As per a previous study (Wager, Matre, & Casey, 2006; Zhang & Luo, 2009), the effects associated with interindividual variation were minimized in the fMRI scanning portion of the experiment by testing only the participants who showed a reliable placebo effect (i.e., placebo responders) in the behavioral portion (see the Results section for more details). We consequently performed fMRI on 14 participants, but our analyses were based on only 13 of these (12 women) because 1 was presented with incorrect stimuli.

Pain and Emotional Stimuli

Pain stimuli were delivered with a CO_2 laser stimulator (DIMEI-300, China) with a 2.5-mm spot diameter and a 100-ms pulse duration. Output energy was kept below 300 mJ to avoid skin damage. Stimulation was applied to the dorsum of the right hand, with each stimulus applied to a different spot to avoid habituation. The emotional pictures used for both the behavioral and fMRI portions of the experiment were selected from the International Affective Picture System (IAPS; Lang, Bradley, & Cuthbert, 2001). In the behavioral experiment, only emotionally unpleasant pictures were used ($M \pm SE$: valence and arousal were 2.62 ± 0.87 and 5.74 ± 0.42, respectively). In the fMRI experiment, we used both high-arousal unpleasant (arousal: 5.84 ± 0.10; valence: 2.60 ± 0.10) and low-arousal neutral (arousal: 3.67 ± 0.15; valence: 5.35 ± 0.11) pictures, which differed significantly from each other in terms of both arousal, $t(59) = 10.588$, $p < .001$, and valence, $t(59) = 62.910$, $p < .001$. In general, rating immediately after each individual picture's presentation is ideal for the accurate estimation of emotional arousal, and some evidence indicates an amplified analgesic effect in retrospective ratings of pain (Pierre & Gary, 2006). However, immediate rating also has disadvantages: It makes the scanning session time-consuming and complicated and can introduce cognitive appraisal in emotional processing. The latter side effect can disturb and contaminate the passive perception of pictures and should be avoided, especially in designs using rapid stimulus presentation. In previous studies, some reports on the placebo effect have applied retrospective rating for each block of emotional pictures (e.g., Petrovic et al., 2005), whereas others adopted the immediate rating of each stimulus (e.g., Wager et al., 2004; Kong et al., 2006, 2008). Given that this study aimed primarily to further explore the neural correlates of a transferable placebo effect with an fMRI technique that had already been established in a previous study, we chose to use a retrospective rating design that was optimal for brain imaging. Accordingly, the participants were asked to rate each block of stimuli using an 11-point scale (from 0 = *no pain or no pleasantness* to 10 = *unbearable pain or unbearable unpleasantness*) during the pain and the emotion part of the experiment.

Procedures

In our previous study, we compared a reinforced expectation group (in which the transferable placebo expectation was developed by an analgesic experience in the reinforcement-learning phase) with a verbal expectation group (in which only verbal descriptions of the placebo were provided in the learning phase). The results showed that expectations reinforced by actual analgesia could produce a significant placebo effect on negative emotional arousal. However, expectations that were induced merely

by verbal instruction did not produce these changes (Zhang & Luo, 2009). Based on the results of our previous research, we used fMRI with the transferable reinforced paradigm to investigate the brain functions involved in the anxiety-relieving placebo effect on emotional processing.

Behavioral experiment. All 27 participants were informed that they would be participating in a clinical study examining the ability of magnetic treatment to alleviate pain and negative emotions. In reality, the equipment associated with the magnetic treatment was a sham and simply a pretense for studying the placebo effect. To help develop a belief that magnetic treatment was efficacious, the participants were told that the equipment and treatment procedures were designed in accordance with the acupuncture point theory of traditional Chinese medicine. Specifically, they were told that the equipment would exert an analgesic effect when connected to an electrode on the Hegu acupoint of the hand receiving the painful stimulation. We ensured that the participants knew when the electrode was connected to the equipment and that they thus knew when they were receiving the "magnetic treatment."

After the participants received the introductory explanations mentioned above, they underwent the behavioral experiment. This portion consisted of three phases: familiarization with the painful stimuli, a manipulation designed to induce the expectation that the magnetic treatment would alleviate pain, and a test to determine the presence of a placebo effect on negative emotional arousal. Participants were gradually familiarized with the laser pain stimuli in the first phase via the administration of two sequences of six to seven increasingly intense stimuli. Each sequence had an output energy of 80 mJ, 120 mJ, 160 mJ, 200 mJ, 240 mJ, 280 mJ, and 300 mJ. To relieve any fears and uncertainties about the intensity of painful stimuli to be used in the subsequent phase, the participants were assured that the intensity would not exceed the highest level to which they were exposed in the familiarization phase. In the expectation manipulation phase, the participants received four blocks of painful laser stimulation and were told that the intensity of the stimuli was always the same within and across each of these. In fact, the stimulus intensity varied across the blocks. Six low-intensity stimuli (120 mJ) were delivered in each of the first and third blocks (i.e., the placebo blocks) while the treatment equipment was connected to the electrode (a signal to the participants that the treatment equipment was being used). Conversely, six high-intensity stimuli (220 mJ) were delivered in each of the second and fourth blocks (i.e., the control blocks) while the treatment equipment was disconnected (a signal to the participants that the treatment equipment was not being used). Given that the participants were unaware that the intensity of painful stimuli was less in the placebo blocks, they were expected to believe that the reduced feeling of pain was an effect of the magnetic treatment. This method has been employed in previous studies (Colloca & Benedetti, 2006; Kong et al., 2006). After each block of stimuli, the participants rated the pain using an 11-point scale (from 0 = *no pain* to 10 = *unbearable pain*).

In the test phase of our behavioral experiment, we used unpleasant pictures to determine whether an analgesic placebo expectation of magnetic treatment transferred to negative emotional arousal, thereby reducing it. The participants were informed that if the magnetic equipment was connected to an electrode at the Jiuwei acupoint on the upper abdomen, any negative emotional arousal would be reduced. They were then presented with two blocks of unpleasant pictures. The arousal and valence values of the pictures were equivalent between the two blocks. For one block, the participants passively viewed the unpleasant pictures with the magnetic treatment equipment connected to the Jiuwei acupoint electrode (i.e., the placebo condition). In contrast, for the other block, the participants viewed the pictures with the electrode disconnected from the equipment (i.e., the control condition). The order in which the two blocks were presented was counterbalanced across participants. Each block contained four high-arousal, unpleasant pictures. Each picture was presented for 3 s and followed by a fixation cross ("+") for 3 s. Blocks were separated by a 1-min rest period in order to avoid the potential confounding of feelings. Immediately after each block, the participants provided ratings of unpleasantness using an 11-point scale (from 0 = *no unpleasantness* to 10 = *unbearably unpleasant*). These ratings were used to identify participants in whom a behavioral placebo effect on negative affectivity had been induced. Fourteen participants (seven participants beginning with the placebo condition and seven participants beginning with the control condition) showed a greater reduction in reported unpleasantness in the placebo condition than the mean group reduction (placebo responders; see the Results section for more details) and were invited to return for follow-up fMRI scanning. We did not inform participants of the sham nature of the treatment immediately after the experiment because our participants came from the same campus and may have known each other and disclosed experiment details to one another. Therefore, we reinterviewed and debriefed them about the true purpose and the deceptive nature of the study after the entire experiment had concluded. The delay interval between participation in the experiment and debriefing about the deception of the magnetic apparatus was 2 to 8 days. In the debriefing interview, the experimenter revealed the study's aim and design in detail, in particular, the deception of the magnetic apparatus and its necessity for the study. Participants' questions, if there were any, were answered in detail, and their verbal and nonverbal reactions during the interview were carefully observed. None of the participants were aware of the deception in the study. Most of them expressed surprise upon learning the truth. Some of them remarked that the experiment was interesting and important. None mentioned any undue influence or were upset about the deception.

fMRI experiment. Only responders selected from the behavioral experiment participated in the event-related fMRI experiment (Figure 1B,C). The fMRI experiment consisted of three phases that were similar to those of the behavioral experiment. To reinforce the placebo responders' belief that the magnetic treatment equipment was effective, they again underwent the expectation manipulation phase used in the behavioral experiment to establish the placebo's pain-alleviating effect. Participants were told that, unlike the behavioral experiment a few days before, the present experiment would further observe brain responses produced by the "magnetic acupoint treatment." Specifically, they were told that fMRI activity would be recorded during the anxiolytic treatment, but electroencephalograph (EEG) activity rather than fMRI activity would be recorded during the analgesic treatment because laser pain-stimulating equipment is strictly forbidden during fMRI. In actuality, the ostensible purpose of the pain experiment was to investigate EEG changes during the second round of analgesic treatment, but its real purpose was to reinforce the placebo effect. Because the participants might think there was no need for a second pain treatment if no brain activities were recorded during it, and,

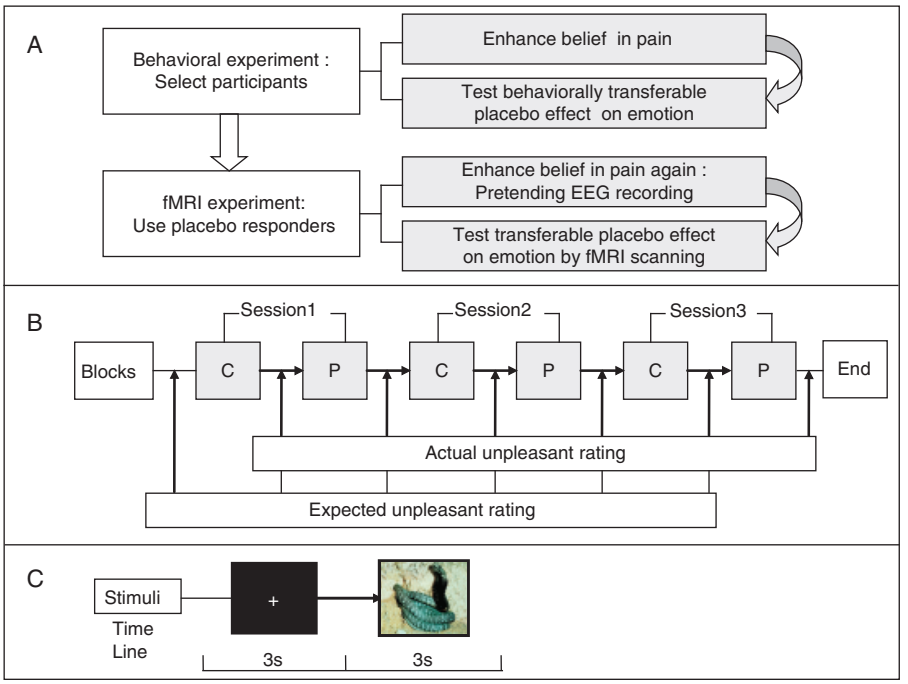

Figure 1. Experimental design. (A) The general procedure consists of a behavioral experiment and an fMRI scanning. The behavioral experiment was conducted to select placebo responders, and then followed by fMRI scanning only for placebo responders. (B) The fMRI scanning consisted of three sessions; each session included one placebo block and one control block. The order of presentation conditions was counterbalanced across all participants. Participants' expected unpleasantness ratings and actual unpleasantness ratings were respectively obtained before and after each block. (C) Timeline for events on each trial. Each picture was presented for 3 s at full-screen size with an interstimulus interval of 3 s, during which a fixation cross was presented.

therefore, develop doubts about the magnetic treatment, the sham EEG recording during analgesic treatment was necessary to justify to the participants why we were repeating the pain experiment. The placebo effect on negative emotional arousal was then investigated using fMRI scanning. This testing used three runs of alternating placebo- and control-condition stimulus blocks. The runs began with a placebo-condition block for half of the participants and a control-condition block for the other half. Each block consisted of 10 unpleasant and 10 neutral pictures. The arousal and valance values of the pictures were equivalent across all blocks. Participants were instructed to carefully view each picture during its 3-s presentation period. Unpleasant and neutral pictures were pseudorandomly intermixed, and pictures from the same category were presented no more than three times in a row to avoid habituation effects. Before and after each block, the participants separately reported their expected unpleasantness ratings (i.e., ratings provided before the block of pictures was presented) and their actual unpleasantness ratings (i.e., ratings provided after the block of pictures was presented) on an 11-point scale.

fMRI Data Acquisition and Analysis

The fMRI imaging was performed with a 3.0 Tesla MRI scanner (Siemens, Magnetom Trio, Germany) using the standard radio frequency head coil. Each participant's head was fixed with foam pads throughout the experiment to minimize head movements. Thirty-two transverse slices of functional images that covered the whole brain were acquired with a T2*-weighted echo-planar imaging sequence based on blood oxygenation level-dependent (BOLD) contrast (repetition time [TR] = 2 s; echo time [TE] = 30 ms; image matrix = 64 × 64; slice thickness = 4 mm; gap = 0.4 mm; FOV = 200 × 200 mm; flip angle [FA] = 90°). For each participant, a high-resolution anatomical scan was acquired at the end of the experiment with a T1-weighted 3D magnetization-prepared rapid gradient-echo pulse sequence (TR = 2530 ms, TE = 3.37 ms, FA = 7°, FOV = 256 × 256 mm, voxel size = 1 × 1 × 1.33 mm, 144 contiguous 1.33-mm-thick sagittal slices, slice matrix size = 256 × 256).

The preprocessing and statistical analysis of images were performed using SPM5 software (http://www.fil.ion.ucl.ac.uk/spm). The first four functional Echo Planar Imaging (EPI) volumes were discarded to allow for T1 equilibration. Preprocessing of the remaining functional EPI images included slice correction, motion correction, and normalization. Functional images were transformed into a standard anatomical space ($3 \times 3 \times 3\ mm^3$ isotropic vexes) based on the Montreal Neurological Institute (MNI) template. Functional images were spatially smoothed using a Gaussian filter with an 8-mm full-width half-maximum. The data were statistically analyzed using general linear models and statistical parametric mapping.

To assess the neural activity corresponding to the processing of the two different types of pictures under each of the experimental conditions, four separate regressors were created (CU, viewing unpleasant pictures in the control condition; CN, viewing neutral pictures in the control condition; PU, viewing unpleasant pictures in the placebo condition; PN, viewing neutral pictures in the placebo condition). These were time-locked to the onset of picture presentation and then convolved with a canonical hemodynamic function. In addition, motion realignment parameters were modeled to account for variability related to

head movements. A high-pass filter with a cutoff frequency of 1/128 Hz was used to correct for low-frequency components, and serial correlations were accounted for with an autoregressive AR (1) model.

The relevant parameter contrasts generated on an individual level were submitted to a group analysis using a random effect model. A 2 × 2 full factorial analysis of variance (ANOVA) was conducted with data from all participants; the factors were experimental condition (placebo vs. control) and emotional picture type (unpleasant vs. neutral). Unless otherwise specified, only whole-brain search results from the random effect analysis with a threshold at $p < .001$ (uncorrected) and a spatial extent of more than 50 continuous voxels and clusters significant at $p < .05$ (corrected for multiple nonindependent comparisons) are reported here (Worsley et al., 1996). Given our a priori hypothesis concerning the amygdala, we used an anatomical mask to perform a small volume correction on activity in the bilateral amygdala. Moreover, the activation patterns associated with the two different types of pictures in each of the experimental conditions were characterized by extracting the signal changes from each region of interest using Marsbar (v. 0.41; http://marsbar.sourceforge.net), as recommended by Brett, Anton, Valabregue, and Poline (2002). The MNI coordinates of the local maximum of each cluster were converted into Talairach coordinates, which are reported in each figure and table.

Results

Unpleasantness Ratings in the Behavioral and fMRI Experiments

In the behavioral experiment, placebo responders were defined as the higher half ($n = 14$), whose rating scores of actual unpleasantness decreased by more than the mean group reduction. A similar approach to identifying placebo responders was previously used (Wager et al., 2006; Zhang & Luo, 2009). A two-way mixed ANOVA, with responders versus nonresponders as a between-subjects factor and placebo versus control conditions as a within-subject factor, identified a significant interaction effect in the behavioral experiment, $F(1,25) = 21.90$, $p < .001$. Further, results of simple effect tests indicated that placebo responders showed significantly decreased unpleasantness ratings in the placebo condition relative to the control condition, $F(1,13) = 43.875$, $p < .001$ (Figure 2A), whereas placebo nonresponders did not have these changes, which can be taken as evidence that a placebo effect was indeed induced in the placebo responders.

In the fMRI experiment, unpleasantness ratings were provided both before and after each block of pictures. As shown in Figure 2B, a significant placebo effect was found for both the expected unpleasantness, $t(12) = 5.13$, $p < .001$, and the unpleasantness associated with actually viewing the pictures, $t(12) = 7.13$, $p < .001$; in each case, unpleasantness ratings were lower in the placebo condition than in the control condition. These results indicate that an anxiety-relieving placebo effect was operating during the scanning experiment.

Imaging Results

Brain regions showing attenuated activity in the placebo condition. First, to identify the neural network associated with a generalized placebo anxiolytic effect, we contrasted the control condition with the placebo condition regardless of viewing unpleasant and neutral pictures. We found that the placebo condition was associated with reduced activity in a widespread set of regions in the limbic network that are mainly involved in regulating emotional processes. These include the right amygdala, the right thalamus, the left and right parahippocampal gyrus, and several subcortical regions, such as the caudate and lateral globus pallidus [(CU+CN) – (PU+PN) in Table 1].

Next, to identify the neural network associated with the placebo anxiolytic effect of the special picture, we contrasted the unpleasant picture in the control condition with the unpleasant picture in the placebo condition (CU – PU; Figure 3, top, and Table 1) and found a similar pattern of activity: decreased activation in the right amygdala, right insula, bilateral cingulate, parahippocampal gyrus, right thalamus, and several subcortical regions. These results indicate an overall reduction in activity within a widespread network related to emotional processing in the placebo condition relative to the control condition.

More importantly, we found several significant clusters (Figure 3, middle, and Table 1) in the right amygdala, right insula, and left precentral gyrus when we contrasted scans taken during unpleasant picture viewing and neutral picture viewing under the control and placebo conditions [(CU – PU) – (CN – PN) in Table 1]. In other words, there was a significant interaction between picture type and experimental condition on the activity within these regions. To characterize the pattern of this interaction effect, we extracted parameter estimates from the peak voxel for the right amygdala (Figure 3, middle). The results indicated that there was attenuated activity in an emotion-related network, especially in the amygdala and insula, during the processing of

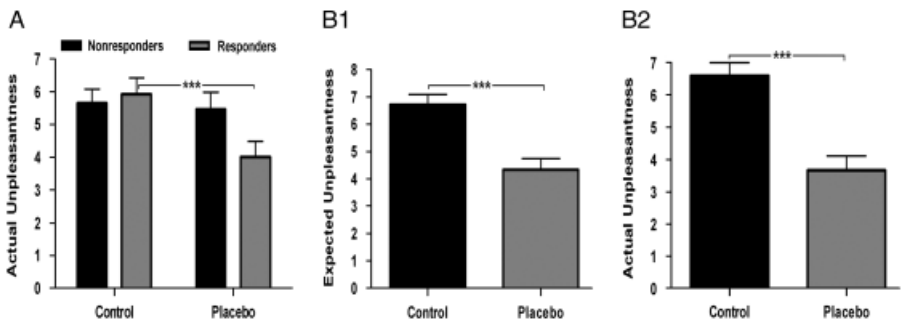

Figure 2. (A) Actual unpleasantness ratings for placebo nonresponders and responders under the placebo and the control conditions in the behavioral experiment. Placebo responders showed significantly decreased unpleasantness ratings in the placebo condition relative to the control condition. ***$p < .001$. (B) Both expected unpleasantness (B1) and actual unpleasantness ratings (B2) in the fMRI experiment were significantly reduced in the placebo condition compared to the control condition.

Table 1. *Activated Regions in the Contrast of (CU+CN) – (PU+PN), CU – PU, and (CU – PU) – (CN – PN)*

Regions of activation	BA	Talairach coordinate			Voxels	*z*
		X	*Y*	*Z*		
(CU+CN) – (PU+PN)						
Left lateral globus pallidus		– 15	0	– 3	254	4.72
Right parahippocampal gyrus	28	18	– 1	– 10		3.96
Right caudate body		15	– 2	25		3.89
Left parahippocampal gyrus	34	– 24	2	– 10		3.77
Right thalamus		12	– 6	6		3.75
Right amygdala		30	– 6	– 10		3.21
(CU – PU) – (CN – PN)						
Right precentral gyrus	6	45	– 10	36	636	4.44
Right insula	13	33	– 16	20		4.06
Right middle frontal gyrus	6	50	2	41		3.44
Left superior frontal gyrus	6	– 3	0	64		3.92
Right lateral globus pallidus		24	– 9	– 2	100	3.82
Right amygdala		30	– 1	– 10		3.76
CU – PU						
Left lateral globus pallidus		– 15	0	– 3	2816	5.38
Right insula	13	33	– 16	23		4.85
Right hippocampus		30	– 10	– 17		4.73
Left parahippocampal gyrus	34	– 24	2	– 10		4.59
Right thalamus		15	– 5	9		4.44
Right cingulate gyrus/anterior cingulate	32/24/33	18	19	29		4.42
Left thalamus		– 6	– 8	6		4.24
Right precentral gyrus/superior frontal gyrus	6	48	– 5	22		4.22
Left anterior cingulate	32	– 18	27	15		4.11
Left medial frontal gyrus	8	– 9	17	46		4.06
Right amygdala[a]		27	– 7	– 20	20	4.38

Notes: Only clusters (with local maxima coordinates) up to the threshold of $p < .05$ correction with 50 or more contiguous voxels are reported.
[a]Cluster $p < .004$ small volume correction.

unpleasant pictures in the placebo condition compared with the control condition.

Finally, we investigated whether attenuated activity in the placebo condition correlated with the observed behavioral placebo effect. To this end, we conducted two separate simple regression analyses to contrast the control condition and the placebo condition when viewing unpleasant pictures, with the difference of expected and actual unpleasantness ratings between the control and placebo conditions as a covariate. With a less stringent threshold ($p < .001$, uncorrected), we found that the attenuated activations in the right amygdala, right insula, anterior cingulate cortex, parahippocampus, and several subcortical areas positively correlated with decreases in both expected and actual unpleasantness ratings (Table 2). Taken together, our results consistently indicate that an emotion-related network of regions, especially the amygdala and insula, showed significantly attenuated activity under the placebo condition and that these reductions in activity were correlated with a behaviorally demonstrated placebo effect.

Regions common to emotional processing and the placebo effect. To further elucidate whether emotional processing and the placebo-related treatment shared a common neural network, we conducted a conjunction analysis using the contrast between viewings of unpleasant versus neutral pictures in the control condition and the contrast between viewing unpleasant pictures in the control versus the placebo conditions. We found that a widespread network of brain regions, including the bilateral amygdala, right insula, bilateral thalamus, bilateral cingulate, bilateral parahippocampal gyrus, and subcortical regions such as the caudate, putamen, and lateral globus pallidus were affected (Figure 3, bottom, and Table 3). These regions may thus contribute to both emotional processing and a placebo effect.

Modulation network exhibited increased activity in the placebo condition. In contrasting the placebo and control conditions, regardless of whether unpleasant or neutral pictures were viewed, we found significant activity in the subgenual anterior cingulate cortex/left caudate head and the right rostral anterior cingulate cortex (Table 4). Similar results were found when the placebo and control conditions were contrasted separately for the viewing of unpleasant (PU – CU) and neutral (PN – CN) pictures (Figure 4 and Table 4). The pattern of activity in the subgenual anterior cingulate cortex is illustrated in the bar graph in Figure 4. The results indicate that the identified brain regions were significantly activated not only when unpleasant pictures were viewed but also when neutral pictures were viewed.

Discussion

The present study showed that a placebo expectation established by physical pain alleviation also reduced negative emotional arousal. An anxiety-relieving placebo effect was ascertained by both the participants' subjective ratings and the activity of brain regions believed to be critically involved in the arousal and regulation of negative emotions. Both the pain-reducing and unpleasantness-alleviating effects were related to the suppression of anxiety otherwise associated with threatening stimuli. However, there are considerable differences between experiencing painful

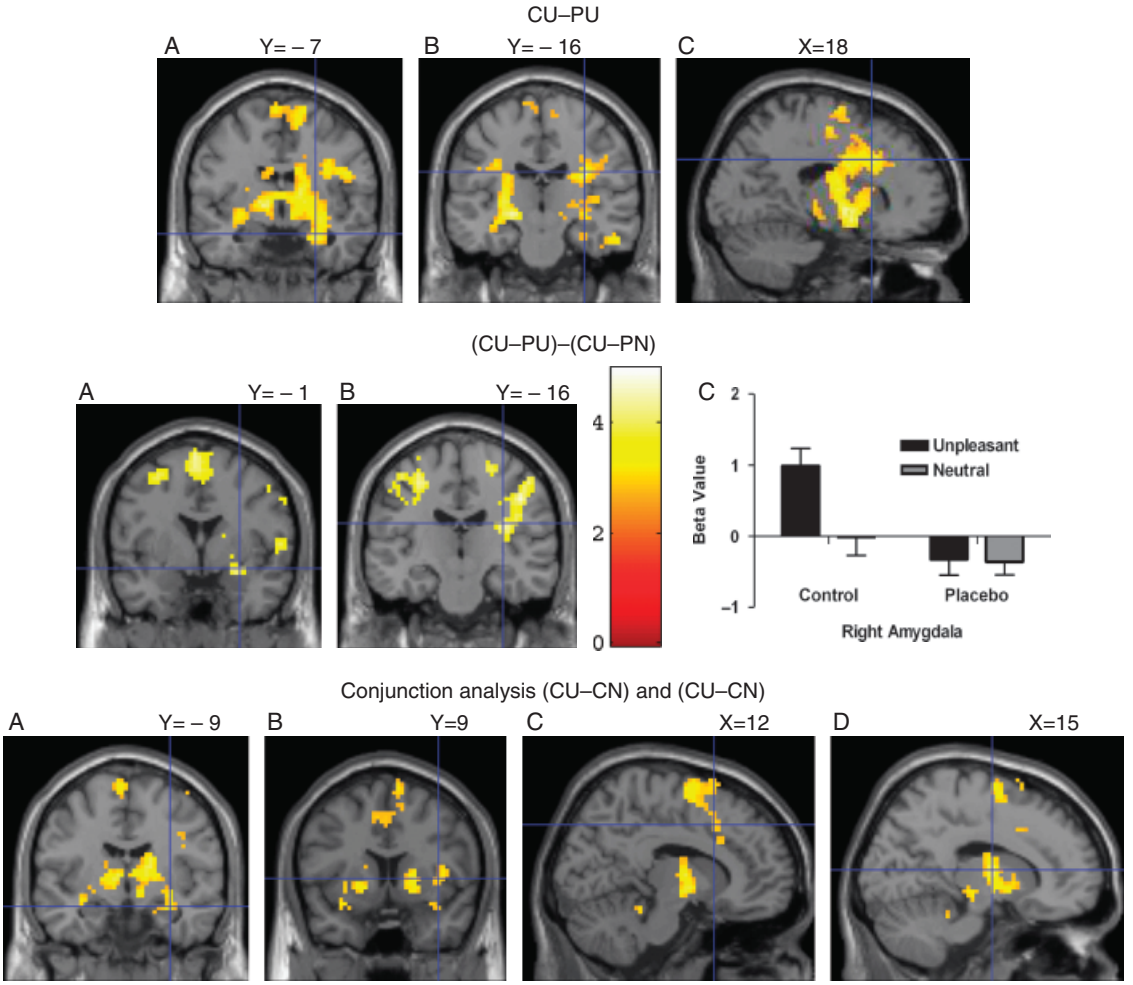

Figure 3. Top: Selected brain regions showing decreased activation in the contrast of CU-PU in the right amygdala, right insula and right cingulate. (A) The right amygdala (27, – 7, – 20); (B) the right insula (33, – 16, 23); (C) the right cingulate (18, 19, 29). Middle: Selected brain regions showing interaction effects (CU – PU) – (CN – PN) and activation patterns in the right amygdala and right insula. (A) The right amygdala (30, – 1, – 10); (B) the right insula (33, – 16, 20); (C) the activiation patterns from region of interest analysis for the right amygdala. Bottom: Conjunction analysis of (CU – CN) and (CU – PU). Selected brain regions showing common activations in both emotional processing and placebo-related treatment. (A) The right amygdala (33, – 9, – 15); (B) the right insula (39, 9, 2); (C) the right cingulate (12, 16, 38); (D) the right thalamus (15, – 5, 9).

laser stimulation and viewing unpleasant pictures. This may exclude, at least in part, the possibility that classical conditioning was responsible for the present placebo effect; instead, psychological expectations could be a more reasonable explanation for this effect. Moreover, unlike a previous emotional placebo study (Petrovic et al., 2005) in which the placebo expectation was de-

Table 2. *Regression (Correlation) Analysis between the CU – PU Contrast and the Behavioral Changes*

Regions of activation	BA	Talairach coordinate			Voxels	*z*
		X	*Y*	*Z*		
Correlated with expectation						
Left lateral globus pallidus/lentiform nucleus/putamen		– 21	– 15	– 4	119	4.82
Left subcallosal gyrus	34	– 24	5	– 13		3.82
Left parahippocampal gyrus	34	– 27	2	– 10		3.77
Right insula	13	33	– 11	20	32	3.89
Right hippocampus		30	– 13	– 20	10	3.73
Right cingulate gyrus	24	21	5	36	8	3.46
Right amygdala[a]		30	– 7	– 20	1	3.23
Correlated with unpleasantness						
Left limbic lobe	28	– 21	– 18	– 7	12	4.28
Right insula	13	33	– 11	20	1	3.12

Notes: The threshold is set at $p < .001$ uncorrected.
[a]Cluster $p < .033$ small volume correction.

Table 3. *Activated Regions by the Junction Analysis of CU − PU and CU − CN*

Regions of activation	BA	Talairach coordinate X	Y	Z	Voxels	z
Left lateral globus pallidus		− 15	0	− 3	416	5.24
Left parahippocampal gyrus	28	− 24	− 21	− 7		4.01
Left putaman		− 30	− 15	− 7		3.87
Left thalamus		− 18	− 23	12		3.43
Left amygdala		− 33	− 1	− 20		3.18
Right lateral globus pallidus		21	− 3	0	417	4.65
Right thalamus		15	− 5	9		4.44
Right amygdala		33	− 9	− 15		3.75
Right insula	13	39	9	2		3.67
Right superior frontal gyrus	6	9	3	66	305	4.13
Left medial frontal gyrus	8	− 9	17	46		4.06
Left cingulate gyrus	32	− 9	16	24		3.59
Right cingulate gyrus	32	12	16	38		3.47

Notes: Only clusters (with local maxima coordinates) up to the threshold of $p < .05$ correction with 50 or more contiguous voxels are reported.

veloped by administering a potent tranquilizer to healthy participants in the reinforcement-learning phase, the present study induced the expectation of an anxiolytic effect by administering an analgesic experience in the reinforcement-learning phase. Therefore, the disadvantages of participants' worries about the potential side effects of drugs were removed.

We found the placebo effect on emotional processing to be associated with significantly decreased activity in the amygdala. This finding is consistent with previous studies on the emotional placebo effect (Petrovic et al., 2005), cognitive reappraisal (Ochsner et al., 2004), and the use of antidepressants and ataractics for major depression and panic disorder (Mayberg et al., 1999, 2002). Our analyses also indicated that (a) there was a significant correlation between decreased activity in the amygdala and reduced ratings for the expected level of negative emotional arousal, and (b) the amygdala was activated during unpleasant picture viewing but not during neutral picture viewing in control/nonplacebo conditions, compared with placebo conditions. This interaction provides further evidence that activity in the amygdala is specifically responsive to negative emotion. Other brain regions showing decreased activity in the placebo condition included, but were not limited to, the insula, dorsal anterior cingulate cortex, thalamus and basal ganglia, superior and medial frontal gyri, hippocampus, and parahippocampus. As discussed in a previous study, decreased activity in the thalamus and basal ganglia might reflect a placebo-related modulation of visual afferent information (Wager et al., 2004) and an attenuated preparedness to warning stimuli for emotionally salient information (Sprengelmeyer, Rausch, Eysel, & Przuntek, 1998). In contrast, altered activity in frontal and hippocampal regions was related to the cognitive aspects of the placebo effect. Attenuated activation of these areas implies that the decrease in emotional arousal engendered by the anxiety-relieving placebo effect was associated with a reduced mobilization of cognitive resources. This process is noticeably unlike that associated with the cognitive reappraisal of emotion (Ochsner, Bunge, Gross, & Gabrieli, 2002; Ochsner et al., 2004), in which there is usually an enhanced mobilization of lateral prefrontal cortex resources to meet the individual's cognitive regulation needs.

Table 4. *Activated Regions in the Contrast of (PU+PN) − (CU+CN), PU − CU, and PN − CN*

Regions of activation	BA	Talairach coordinate X	Y	Z	Voxels	z
(PU+PN) − (CU+CN)						
Left subgenual anterior cingulate cortex	47	− 12	17	− 11	102	4.94
Left caudate head		− 6	14	− 6		4.39
Right medial frontal gyrus	25	12	14	− 13		3.39
PU − CU						
Left subgennual anterior cingulate cortex	11	− 12	20	− 11	67	4.67
Right anterior cingulate cortex	25	3	17	− 8		3.38
PN − CN						
Left subgenual anterior cingulate cortex	47	− 12	17	− 11	83	4.68
Left caudate head		− 6	14	− 6		4.43

Notes: Only clusters (with local maxima coordinates) up to the threshold of $p < .05$ correction with 50 or more contiguous voxels are reported.

It is worth noting that processing of threatening stimuli is mainly associated with activity in the dorsal anterior cingulate cortex. Only this part of the cingulate gyrus receives high and direct amygdala input and plays a significant role in fear (Vogt, 2005). Both the present study and one by Wager et al. (2004) suggested that the dorsal anterior cingulate cortex was inhibited during the placebo condition only in placebo responders. However, these changes were not observed in other placebo studies with randomly selected participants (Kong et al., 2006; Petrovic et al., 2002, 2005; Zubieta et al., 2005). Another meaningful study (Kong et al., 2008) showed that activity in the dorsal anterior cingulate cortex was significantly enhanced under a pain nocebo condition, indicating that this region played an important role in negative arousal. Given that the anterior cingulate cortex is generally believed to function as an "alarm system" (Eisenberger & Lieberman, 2004) that signals a need for attentive control, the discrepancy between the responders and the randomly assigned participants might be attributed to the different mechanisms underlying their placebo responses. For the placebo responders, the need for attentive control when viewing unpleasant pictures in the placebo condition was so low that they required less anterior cingulate cortex activity than under the control condition.

If the above-mentioned point of view is correct, then the question of how the placebo effects were achieved in the placebo responders is raised. Theoretically, rather than being monolithic, emotional processing covers a broad spectrum that ranges from reactive to effortful (Pessoa, 2008). It is believed that a predominantly ventral system, including the ventral regions of the anterior cingulate and frontal cortices and the ventral striatum, is important for the automatic or reactive regulation of emotional stimuli and that conscious, effortful emotional regulation (e.g., cognitive reappraisal strategies) typically activates widespread regions of the dorsolateral prefrontal cortex (Ochsner et al., 2002, 2004). In the present study, we found that an anxiety-reducing placebo effect was associated with activity in the subgenual anterior cingulate cortex/caudate head (BA 47, BA 11, or BA 25). Activity in these areas is known to be correlated with numerous phenomena, including reward responses (Bjork,

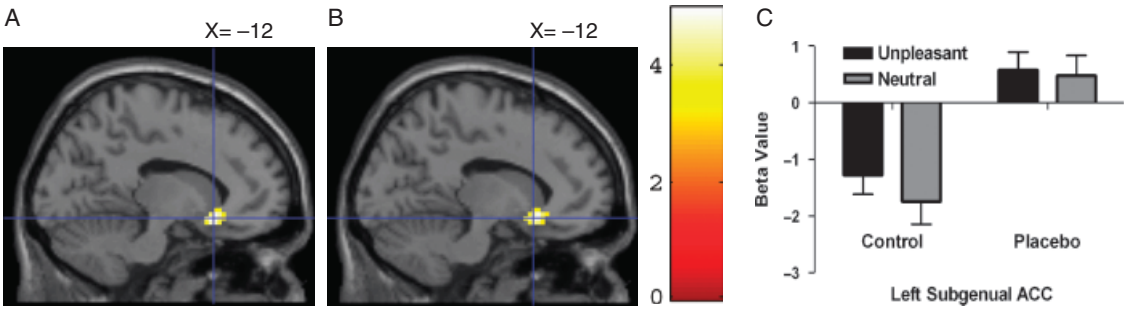

Figure 4. Selected brain regions showing increased activation in the placebo treatment. (A) The subgenual anterior cingulate cortex activated in PU – CU (– 12, 21, – 12); (B) the subgenual anterior cingulate cortex activated in PN – CN (– 12, 18, – 12); (C) activation patterns showing in the bar graph. The pattern of activation in the subgenual anterior cingulate cortex is similar in these two contrasts.

Smith, & Hommer, 2008; Critchley & Rolls, 1996), pleasant stimuli (Lane et al., 1997), inhibition of sympathetic autonomic activity and defensive behavior that is elicited by stimulation of the amygdala (Timms, 1977), antagonization of emotional excitement (Matthews, Paulus, Simmons, Nelesen, & Dimsdale, 2004), and the extinction of fear conditioning (Quirk & Mueller, 2008). A recent report of deep-brain stimulation of the subcallosal cingulate gyrus was used to modulate dysfunctional brain networks that lead to depression (Lozano et al., 2008). These results suggest that the anxiety-relieving placebo effect of the present study was realized through reward-related responses (Bjork et al., 2008) rather than through effortful and attentive cognitive regulation (Ochsner et al., 2002, 2004). In addition, we found that the subgenual anterior cingulate cortex was activated to an almost equal extent when either unpleasant or emotionally neutral pictures were viewed in the placebo condition. In other words, increased activity in the subgenual anterior cingulate cortex was not specifically related to unpleasant pictures, as it was also present when neutral pictures were viewed. This provides further evidence that the placebo regulation might have occurred in an autonomic manner that did not involve the emotional valence of consciously discriminated pictures. Striking incongruities exist between these observations of the anterior cingulate cortex with those of some other placebo studies, in which increased activity was observed in the pregenual anterior cingulate cortex rather than in the subgenual anterior cingulate cortex, and this region was thought to play a critical role in placebo modulation (Kong et al., 2006; Petrovic et al., 2002, 2005; Zubieta et al., 2005). Vogt (2005) proposed that activation patterns in the anterior cingulate cortex vary between different observations and require further explanation.

In conclusion, the present study enhances our current understanding of the neural bases of placebo effects. It revealed that an anxiety-relieving placebo effect was associated with reduced unpleasantness ratings and decreased activity in emotionally responsive brain regions, including the amygdala, insula, and dorsal anterior cingulate cortex. Additionally, the evidence of increased activity in the subgenual anterior cingulate cortex/ventral striatum in the placebo condition implies that the anxiety-relieving placebo effect was realized through a reward-related response underpinned by the participants' beliefs and expectations.

Although this study firmly demonstrated the neural correlates of the transferable placebo effects, it has also some limitations. First, the sample size (12 females and 1 male) of this study was relatively small and gender unbalanced. Although our major experimental observations were proved to be statistically sufficient, further experimentation with a larger and gender-balanced sample size could be more informative. Second, this study adopted a shorter, unjittered interstimulus interval. This may have resulted in a reduced sensitivity to estimate the hemodynamic response function (HRF) properties of a single stimulus, and the problems related to the linearity versus nonlinearity of the BOLD interaction in overlapping HRFs (Amaro & Barker, 2006). In spite of the fact that we found that the correlation between four regressors/conditions was relatively low, indicating the collinearity's confounding was not serious in this study, future research should employ longer and jittered interstimulus intervals to deconvolve the overlapping HRFs as far as possible.

References

Amanzio, M., & Benedetti, F. (1999). Neuropharmacological dissection of placebo analgesia: Expectation-activated opioid systems versus conditioning-activated specific subsystems. *Journal of Neuroscience*, *19*, 484–494.

Amaro, E. Jr., & Barker, G. J. (2006). Study design in fMRI: Basic principles. *Brain and Cognition*, *60*, 220–232.

Beauregard, M., Levesque, J., & Bourgouin, P. (2001). Neural correlates of conscious self-regulation of emotion. *Journal of Neuroscience*, *21*, RC165.

Benedetti, F., Pollo, A., Lopiano, L., Lanotte, M., Vighetti, S., & Rainero, I. (2003). Conscious expectation and unconscious conditioning in analgesic, motor, and hormonal placebo/nocebo responses. *Journal of Neuroscience*, *23*, 4315–4323.

Bjork, J. M., Smith, A. R., & Hommer, D. W. (2008). Striatal sensitivity to reward deliveries and omissions in substance dependent patients. *NeuroImage*, *42*, 1609–1621.

Brett, M., Anton, J., Valabregue, R., & Poline, J. (2002). Region of interest analysis using an SPM toolbox [abstract]. *The 8th International Conference on Functional Mapping of the Human Brain (Sendai, Japan), June 2–6*. Available on CD-ROM in *NeuroImage*, Vol 16.

Colloca, L., & Benedetti, F. (2006). How prior experience shapes placebo analgesia. *Pain*, *124*, 126–133.

Costafreda, S. G., Brammer, M. J., David, A. S., & Fu, C. H. (2008). Predictors of amygdala activation during the processing of emotional stimuli: A meta-analysis of 385 PET and fMRI studies. *Brain Research Reviews*, *58*, 57–70.

Critchley, H. D., & Rolls, E. T. (1996). Olfactory neuronal responses in the primate orbitofrontal cortex: Analysis in an olfactory discrimination task. *Journal of Neurophysiology*, *75*, 1659–1672.

Eisenberger, N. I., & Lieberman, M. D. (2004). Why rejection hurts: A common neural alarm system for physical and social pain. *Trends in Cognitive Science*, *8*, 294–300.

Kong, J., Gollub, R. L., Polich, G., Kirsch, I., Laviolette, P., Vangel, M., et al. (2008). A functional magnetic resonance imaging study on the neural mechanisms of hyperalgesic nocebo effect. *Journal of Neuroscience*, *28*, 13354–13362.

Kong, J., Gollub, R. L., Rosman, I. S., Webb, J. M., Vangel, M. G., Kirsch, I., et al. (2006). Brain activity associated with expectancy-enhanced placebo analgesia as measured by functional magnetic resonance imaging. *Journal of Neuroscience*, *26*, 381–388.

Lane, R. D., Reiman, E. M., Bradley, M. M., Lang, P. J., Ahern, G. L., Davidson, R. J., et al. (1997). Neuroanatomical correlates of pleasant and unpleasant emotion. *Neuropsychologia*, *35*, 1437–1444.

Lang, P. J., Bradley, M. M., & Cuthbert, B. (2001). *International Affective Picture System (IAPS): Instruction Manual and Affective Ratings* (Technical Report A-5. Gainsville, FL: The Center for Research in Psychophysiology, University of Florida.

Lozano, A. M., Mayberg, H. S., Giacobbe, P., Hamani, C., Craddock, R. C., & Kennedy, S. H. (2008). Subcallosal cingulate gyrus deep brain stimulation for treatment-resistant depression. *Biological Psychiatry*, *64*, 461–467.

Matthews, S. C., Paulus, M. P., Simmons, A. N., Nelesen, R. A., & Dimsdale, J. E. (2004). Functional subdivisions within anterior cingulate cortex and their relationship to autonomic nervous system function. *NeuroImage*, *22*, 1151–1156.

Mayberg, H. S., Liotti, M., Brannan, S. K., McGinnis, S., Mahurin, R. K., Jerabek, P. A., et al. (1999). Reciprocal limbic-cortical function and negative mood: Converging PET findings in depression and normal sadness. *American Journal of Psychiatry*, *156*, 675–682.

Mayberg, H. S., Silva, J. A., Brannan, S. K., Tekell, J. L., Mahurin, R. K., McGinnis, S., et al. (2002). The functional neuroanatomy of the placebo effect. *American Journal of Psychiatry*, *159*, 728–737.

Ochsner, K. N., Bunge, S. A., Gross, J. J., & Gabrieli, J. D. (2002). Rethinking feelings: An FMRI study of the cognitive regulation of emotion. *Journal of Cognitive Neuroscience*, *14*, 1215–1229.

Ochsner, K. N., Ray, R. D., Cooper, J. C., Robertson, E. R., Chopra, S., Gabrieli, J. D., et al. (2004). For better or for worse: Neural systems supporting the cognitive down- and up-regulation of negative emotion. *NeuroImage*, *23*, 483–499.

Pessoa, L. (2008). On the relationship between emotion and cognition. *Nature Reviews Neuroscience*, *9*, 148–158.

Petrovic, P., Dietrich, T., Fransson, P., Andersson, J., Carlsson, K., & Ingvar, M. (2005). Placebo in emotional processing—Induced expectations of anxiety relief activate a generalized modulatory network. *Neuron*, *46*, 957–969.

Petrovic, P., Kalso, E., Petersson, K. M., & Ingvar, M. (2002). Placebo and opioid analgesia—Imaging a shared neuronal network. *Science*, *295*, 1737–1740.

Pierre, R., & Gary, H. D. (2006). Functional brain imaging of placebo analgesia: Methodological challenges and recommendations. *Pain*, *121*, 177–180.

Quirk, G. J., & Mueller, D. (2008). Neural mechanisms of extinction learning and retrieval. *Neuropsychopharmacology*, *33*, 56–72.

Sprengelmeyer, R., Rausch, M., Eysel, U. T., & Przuntek, H. (1998). Neural structures associated with recognition of facial expressions of basic emotions. *Proceedings of the Royal Society of London, Biological Sciences*, *265*, 1927–1931.

Timms, R. J. (1977). Cortical inhibition and facilitation of the defence reaction [proceedings]. *The Journal of Physiology*, *266*, 98P–99P.

Vogt, B. A. (2005). Pain and emotion interactions in subregions of the cingulate gyrus. *Nature Reviews Neuroscience*, *6*, 533–544.

Wager, T. D., Matre, D., & Casey, K. L. (2006). Placebo effects in laser-evoked pain potentials. *Brain, Behavior, and Immunology*, *20*, 219–230.

Wager, T. D., Rilling, J. K., Smith, E. E., Sokolik, A., Casey, K. L., Davidson, R. J., et al. (2004). Placebo-induced changes in FMRI in the anticipation and experience of pain. *Science*, *303*, 1162–1167.

Worsley, K. J., Marrett, S., Neelin, P., Vandal, A. C., Friston, K. J., & Evans, A. C. (1996). A unified statistical approach for determining significant signals in images of cerebral activation. *Human Brain Mapping*, *4*, 58–73.

Wright, C. I., Martis, B., McMullin, K., Shin, L. M., & Rauch, S. L. (2003). Amygdala and insular responses to emotionally valenced human faces in small animal specific phobia. *Biological Psychiatry*, *54*, 1067–1076.

Zhang, W., & Luo, J. (2009). The transferable placebo effect from pain to emotion: Changes in behavior and EEG activity. *Psychophysiology*, *46*, 626–634.

Zubieta, J. K., Bueller, J. A., Jackson, L. R., Scott, D. J., Xu, Y., Koeppe, R. A., et al. (2005). Placebo effects mediated by endogenous opioid activity on mu-opioid receptors. *Journal of Neuroscience*, *25*, 7754–7762.

(Received May 26, 2010; Accepted December 12, 2010)