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Brain Mechanisms Supporting Violated Expectations of Pain

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Abstract

The subjective experience of pain is influenced by interactions between prior experiences, future predictions and incoming afferent information. Expectations of high pain can exacerbate pain while expectations of low pain during a consistently noxious stimulus can produce significant reductions in pain. However, the brain mechanisms associated with processing mismatches between expected and experienced pain are poorly understood, but are important for imparting salience to a sensory event in order to override erroneous top-down expectancy-mediated information. The present investigation examined pain-related brain activation when expectations of pain were abruptly violated. After conditioning participants to cues predicting low or high pain, ten incorrectly cued stimuli were administered across 56 stimulus trials to determine if expectations would be less influential on pain when there is a high discordance between pre-stimulus cues and corresponding thermal stimulation. Incorrectly cued stimuli produced pain ratings and pain-related brain activation consistent with placebo analgesia, nocebo hyperalgesia, and violated expectations. Violated expectations of pain were associated with activation in distinct regions of the inferior parietal lobe, including the supramarginal and angular gyrus, and intraparietal sulcus, the superior parietal lobe, cerebellum and occipital lobe. Thus, violated expectations of pain engage mechanisms supporting salience-driven sensory discrimination, working memory, and associative learning processes. By overriding the influence of expectations on pain, these brain mechanisms are likely engaged in clinical situations where patients' unrealistic expectations for pain relief diminish the efficacy of pain treatments. Accordingly, these findings underscore the importance of maintaining realistic expectations to augment the effectiveness of pain management.

1. INTRODUCTION

The moment-to-moment sensory experience is driven by interactions between prior beliefs, future predictions and incoming afferent information. While individual fluctuations in the moment-to-moment experience can shape pain [47], we also learn to continuously anticipate impending noxious sensory events through associative learning processes [4; 5; 45; 46; 54].

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Repeated pairings between contextual cues and subsequent sensory events shape our perceptual framework [4]. Pain, an adaptive subjective experience, is heavily influenced by such predisposed inferences. Expectations of low pain corresponding to a consistently noxious stimulus can produce significant pain relief (placebo analgesia)[31; 63], while expectations of high pain can produce hyperalgesia (nocebo response)[28]. Placebo-analgesia is associated with reductions in pain-related brain activity and is mediated by activation in executive level brain regions such as the prefrontal cortex (PFC) [18; 31; 63]. In contrast, nocebo hyperalgesia is associated with increased pain-related brain activation [28].

Importantly, the efficacy of expectations for shaping afferent nociceptive processing and the experience of pain may likely be limited by the difference between one's expectations for pain and incoming nociceptive input. Substantial deviations between expected and experienced nociceptive information are of significant importance to the organism in that they allow afferent input to break free from the constraints of expectations. Consider a scenario where mild pain was expected during the placement of an intravenous cannula. However, intense pain was unexpectedly elicited when the needle was inadvertently driven into a nerve. The experience of intense pain when only mild pain was expected was potentially associated with tissue damage and would likely require further evaluation to develop a better understanding of such discrepancies.

The brain mechanisms involved in resolving real-time mismatches between expected and experienced pain remain poorly characterized. However, in the case of visual and gustatory information [10; 61], regions in the left posterior parietal lobe and insular cortex appear to be critically involved in processing sensory information and breaches between expected and experienced sensory information. Therefore, the present study tested the hypothesis that a subset of brain regions in the parietal lobe and insular cortex process violated expectations of pain. To test this hypothesis, participants were first conditioned to expect high and low levels of noxious stimulation following visual cues. The noxious stimulus intensities (47°C, 50°C) were designed to elicit markedly and readily recognizable different experiences of pain intensity. During functional MRI scanning, subjects' expectations for pain were violated by presenting incorrectly cued stimuli. The present study identifies brain mechanisms associated with processing the realization that the experienced feeling of pain is incongruent with the expected level of pain.

2. METHODS AND MATERIALS

2.1 Subjects

Fifteen healthy, pain-free participants (8 males, age range: 23–30, mean age: 26 years) completed psychophysical and functional imaging portions of the study. Twelve participants were White, two were Asian, and one was multi-racial. Seven additional participants completed psychophysical training and fMRI portions of the experiment, but their fMRI data were not available for analysis due to equipment malfunction. Subjects gave written, informed consent acknowledging that they would experience painful, heat stimuli and they were free to withdraw from the study without prejudice. The Institutional Review Board of

the Wake Forest School of Medicine approved all procedures. All participants were debriefed at the end of the experiment.

2.2 Thermal Stimulation Procedures

A thermal sensory analyzer (TSA-II, Medoc Ltd., Ramat Yishai, Israel) with a 16 × 16mm contact sensory probe was employed for heat stimulation. The baseline temperature was 35°C. The stimulus temperature changed with rise and fall rates of 4.5°C/s for 47°C stimuli and 5.0°C/s for 50°C stimuli in order to equate total stimulus duration across temperatures. Thermal probes were placed on the posterior portion of the calf with a custom-designed probe holder. We moved the probe to a new location on the calf after each thermal stimulation series to minimize for potential habituation and/or sensitization.

2.3 Psychophysical Training and Conditioning Session

All subjects were initially familiarized to thermal stimulation with 32, 5-s-duration stimuli (35– 49°C). These training stimuli gave participants experience using the visual analog scales (VAS). A 15 cm plastic sliding VAS scale was used to quantify pain intensity and unpleasantness [Paresian Novelty [48]]. Using an audio analogy, subjects were instructed that pain intensity is similar to the loudness of a song on the radio, while the unpleasantness of pain depends not only on the intensity but also other factors that may affect one's subjective experience [49]. The minimum rating ("0") was represented as "no pain sensation" or "not at all unpleasant," whereas the maximum rating ("10") was designated with "most intense imaginable" or "most unpleasant imaginable".

In order to condition participants to pre-stimulus cues and corresponding heat stimulation, we administered four all correctly cued, thermal heat series (7 stimuli/series; temperature plateaus of 47°C and 50°C) during the psychophysical training session. The thermal probe was placed on the posterior aspect of the right calf during the psychophysical training portion of the experiment. Participants were positioned in front of a computer screen and were instructed to pay attention to 2-second visual presentations of cue type (i.e., Low, High) preceding each thermal stimulus. Participants were instructed that a visual cue of the word "Low" would signal low heat intensity stimulation (5 second plateau, 47°C) and a visual cue of the word "High" would indicate high heat intensity stimulation (5 second plateau, 50°C). Both words were presented in white on a black background. After each stimulus, participants provided a pain intensity rating using a trackball to move a slider of an electronically scanned image of the VAS that was presented on the computer screen. Participants' responses were recorded using custom-written programs within the IDL software package (Research Systems, CO). Data from the psychophysical training/ conditioning session of the study will not be presented in the present manuscript.

2.4 fMRI Experimental Task

On a separate day after psychophysical training, subjects participated in the fMRI portion of the experiment. Each participant's behavioral pain ratings and regional brain signals were assessed across fourteen fMRI series (4 thermal stimulus trials/fMRI series). The thermal probe was placed on the back of the left calf and moved to a new location after each fMRI series. Order of thermal stimulus trials was pseudo-randomized within each series and

presentation of thermal series was counterbalanced across participants. Each fMRI series (4 min 24 sec) included two levels of stimulus intensities [i.e., low (47°C) and high (50°C) stimulation]. Identical to the psychophysical training session, low and high stimulus plateaus were five seconds in duration with rise and fall rates of 4.5°C/s for 47°C stimuli and 5.0°C/s for 50°C stimuli.

All thermal stimuli were preceded with a two second visual cue (cue presentation period) presented through MRI compatible goggles (Resonance Technology Inc., CA). Six seconds (post-cue period) elapsed before initiation of the thermal stimulus (stimulus period). Six seconds after the end of each noxious heat stimulus (post-cue period), subjects were provided 14-seconds to rate (rating period) their experienced pain intensity using a VAS presented via MRI-compatible goggles and a MRI-compatible trackball. After the rating was performed, subjects had a 15-second rest period in addition to the remaining time from the allowed rating duration before presentation of the next cue. The timing of events recorded by the program was also used to construct regressors for the fMRI analysis. The timing of events was recorded using a digital chart recorder (Power-Lab: ADInstruments, Colorado Springs, CO).

In order to reinforce the cue-stimulus conditioning paradigm, the first two thermal series (e.g., 8 stimuli) of the fMRI experiment contained only correctly cued stimuli (See Figure 1 for experimental procedures). Two more all correctly cued series were randomly administered throughout the course of the experiment to reduce potential extinction-related effects related to the conditioning paradigm. Ten out of the fourteen series included one incorrectly cued stimulus and 3 correctly cued stimuli. The order of incorrectly and correctly cued stimuli was pseudo-randomly administered.

2.5 Categorization of Trial Types based on Individual Responses

There were a total of 56 thermal stimuli (4 thermal stimuli/series; 14 series) administered to each subject across the fMRI portion of the study. All trial types were calculated on a subject-to-subject basis in a manner dependent on the cue/stimulation type and the subject's VAS ratings. This was done to account for individual differences in the persistence of placebo/nocebo and violated expectation responses.

Correctly cued stimuli were characterized as stimuli where the pre-stimulus visual cue (Low, High) matched the level of thermal stimulation (47°C, 50°C, respectively). A total of 46 correctly cued stimulus trials were administered. However, only a subset of these trials delivered before the first incorrectly cued stimulus was incorporated into the analysis. This was done to ensure that the presentation of an incorrectly cued stimulus did not disrupt cue-stimulus conditioning and thereby confound the experience of a correctly cued stimulus.

Incorrectly cued stimulus trials corresponded to the ten trials where the cue did not match the stimulus level. There were five incorrectly cued stimuli for each stimulation type (47°C, 50°C). These trials were subdivided into violated expectations, nocebo, and placebo categories based on each subject's VAS ratings (Figure 1 and Table 1). It is important to note that the qualitatively distinct stimulus intensities (i.e., 47°C; 50°C) were chosen specifically to minimize nocebo and placebo effects. Since contact heat ratings increase

exponentially with stimulus temperature [49], the subjective difference between 47°C and 50°C is distinct [35; 43]. Thus, we sought to make the mismatch between cue and stimulus as obvious as possible in order identify the brain mechanisms associated with resolving the mismatch between expected and experienced pain intensity (i.e., violated expectations).

Violated expectation response trials were identified as the first incorrectly cued trial in which the cue exerted minimal effect on VAS ratings. Since ratings of correctly cued stimuli exhibited variability from one presentation to the next, we wanted to define points that lay within this natural variability following the presentation of an incorrectly cued thermal stimulus. This was accomplished calculating the SD of VAS ratings of all correctly cued trials (n = 46 stimuli) and identifying the first incorrectly cued trials with a VAS rating that that fell within two SDs of each subject's respective VAS rating (per temperature) for all correctly cued stimuli that preceded the first incorrectly cued stimulus. Since we were concerned about extinction of cue-stimulus conditioning following subjects' recognition of an incorrectly cued trial, we only included one violated expectation stimulus trial in the analyses.

Variation in pain within an individual can occur across stimulation sites and across time [50; 59]. Given that such within subject variation exists, placebo and nocebo responses cannot simply be defined as responses that are below or above those of control stimuli. However, to the best of our knowledge, placebo/nocebo responses to heat pain have not been defined with regards to within subject variability. In order to objectively and consistently define a placebo/nocebo response, we calculated placebo/nocebo responses as VAS ratings that were 2SD below and above respectively, each subject's mean VAS rating corresponding to a correctly cued trial (Table 1).

Nocebo trials were identified as a 47°C incorrectly cued stimulus trial with a VAS rating 2 SDs above the mean rating of all correctly cued 47°C stimuli. In contrast to the violated expectation response trials, all trials meeting the criteria for a nocebo effects were included in analyses.

Placebo trials were identified as 50°C incorrectly cued stimulus trials with a VAS rating 2 SDs below the mean rating of all correctly cued 50°C stimuli. Similar to nocebo trials, all trials meeting the criteria for placebo effects were included in analyses.

2.6 Image acquisition and processing

MRI data were acquired on a 1.5 T General Electric echo-speed Horizon LX scanner with an 8-channel neurovascular coil. For functional imaging, blood oxygenation level-dependent (BOLD) images were acquired continuously in each contiguous plane by using echo-planar imaging [echo time (TE), 40ms; repetition time (TR), 2s; 28 × 5-mm-thick slices; 3.75 × 3.75 mm in-plane resolution; flip angle, 80°; no slice gap].

High-resolution structural scans were acquired using a BRAVO sequence (inversion time, 600 ms; TR, 11.49ms; flip angle, 12°; TE, 4.74ms; section thickness, 1 mm with no gap between sections; number of sections, 156; in-plane resolution, 0.9375 × 0.9375 mm).

The functional image analysis package FSL [Center for Functional Magnetic Resonance Imaging of the Brain (FMRIB), University of Oxford, Oxford, UK] was used for image processing and statistical analysis. The functional data were movement corrected, temporally filtered with a 100s high pass filter, and spatially smoothed with a 5 mm 3-D isotropic Gaussian kernel. Each subject's functional images were registered to their structural data using a six-parameter linear 3-D transformation and then transformed into standard stereotaxic space [as defined by Montreal Neurologic Institute] using a 12-parameter affine transformation followed by a non-linear transformation [2; 3; 26].

2.7 Psychophysical Data Analyses

Psychophysical data were extracted for trials described in Table 1 and Figure 1. For the analyses of violated expectations (JMP Pro 11.2), a two way repeated measures (RM) ANOVA was performed to examine the main effect of temperature (47°C vs. 50°C) and cue type (correctly cued vs. violated expectation) and the potential interactions between the two main effects. Since two subjects did not report a violated expectation in response to an incorrectly cued 47°C trial, we employed a univariate method in which subjects were modeled as random effects to accomplish the RM ANOVA. Post-hoc analyses were performed to examine significant main effects and interactions.

For the analyses of placebo/nocebo effects, all placebo and nocebo stimulus trials and corresponding correctly cued control stimuli were first averaged within each subject (Table 1 and Figure 1). Then, separate paired-samples t-tests were conducted to compare correctly cued 47°C stimuli with nocebo stimulus trials and correctly cued 50°C stimuli with placebo stimulus trials, respectively.

2.8 Statistical analysis of regional signal changes within the brain

Statistical analysis of regional signal changes was performed on each acquisition series (first level analyses) using a general linear modeling approach with nonparametric local autocorrelation correction [20; 64]. In all analyses, the relationship of the predictive model function to MRI signal intensity was evaluated by calculating a t-statistic on a voxel-by-voxel basis. These t-values were then converted to Z-scores to allow p values to be calculated on the basis of Gaussian random field theory [19; 21; 66]. The predictive model functions for the general linear modeling analysis were derived as follows: we created 20 regressors for analysis of cue presentation, post-cue, stimulus, post-stimulus rest, and rating periods and were described with two regressors based on the cue they were signaled with (low, high). All regressors were orthogonalized to each other to separate a true baseline. Each of the 20 regressors had a period of interest scaled as +1. Only the stimulus period is presented in the present manuscript.

All regressors were convolved with a gamma-variate model of the hemodynamic response (delay 6s, SD 3 s) and its temporal derivative [13; 64]. They were then temporally filtered using the same parameters that were applied to the functional images. We performed inter-series fixed effects (second level) analyses within each subject separately for every regressor and proceeded to inter-subject group analysis (third level) using a random effects model in order to identify clusters significantly activated or deactivated compared to rest condition.

Clusters of voxels exceeding a Z-score>2.3 and p<0.05 (mixed effect, corrected for multiple comparisons) were considered statistically significant [65]. Since we employed the single-epoch design [30], we carefully inspected the output of every first level analysis for residual motion artifacts. Head motion correction parameters were added to the statistical model as covariates of no interest in a total of twelve stimulus trials across six subjects after visual inspection revealed residual motion effects.

Four one sample t-tests were conducted on all 47°C correctly cued, 47°C violated expectation, 50°C correctly cued and 50°C violated expectation response trials, respectively. A two way ANOVA was performed to examine the main effect of temperature (47°C vs. 50°C) and cue trial type (correctly cued vs. violated expectation) and the potential interactions between the two main effects. For each temperature, one violated expectation response trial was compared to 1 correctly cued stimulus trial (see Figure 1 and Table 1 for more details). In order to describe significant interactions, we first created binary masks of individual brain regions exhibiting significant interaction effects. Next, using these masks, we extracted the mean BOLD signal percent change for each condition. Finally, these values were plotted for each brain region exhibiting a significant interaction.

All placebo and nocebo stimulus trials and corresponding correctly cued control stimuli were first averaged within each subject (Table 1 and Figure 1). In order to analyze placebo and nocebo effects, we used all available trials (n =14 nocebo; n= 17 placebo trials) for the subset of individuals (n = 7 nocebo; n = 7 placebo subjects) exhibiting these respective effects. For example, if one individual exhibited a placebo response during three incorrectly cued stimulus trials then these trials would be compared to the three correctly cued stimulus trials preceding the presentation of the first incorrectly cued trial. Separate paired-samples t-tests were conducted to compare correctly cued 47°C trials with nocebo trials and correctly cued 50°C trials with placebo trials, respectively.

Conjunction analyses were conducted to examine if brain regions activated by violated expectations significantly overlap with those activated by nocebo and placebo stimulus trials (z-score threshold = 2.3, *p* value threshold = 0.05) [40]. To address differences in brain activation between the two thermal stimulus levels (47°C; 50°C), two separate conjunction analyses were conducted to identify the potential overlap between 47°C correctly cued and nocebo stimulus trials and 50°C correctly cued and placebo stimulus trials.

2.9.1 Psychophysical Evaluation of Potential Order Effects—In the primary analyses, we compared each subject's violated expectation response trial to the last correctly cued trial preceding the presentation of the first incorrectly cued stimulus. This approach ensured that the presentation of an incorrectly cued stimulus did not disrupt nor confound cue-stimulus conditioning. However, one could postulate that violated expectation of pain trials were simply reflective of the presentation order of our correctly cued comparison trial. Therefore, we examined potential order effect confounds by comparing each subject's violated expectation response trial to the *last* correctly cued stimulus trial of the *entire* experiment [across each temperature (47°C; 50°C)]. Thus, a repeated measure (RM) ANOVA was conducted to identify the main effect of temperature (47°C vs. 50°C) and cue trial type (last correctly cued of the experiment vs. violated expectation). We paralleled this

analysis to our primary analysis. As in the primary analysis, two subjects did not report a violated expectation in response to an incorrectly cued 47°C trial. Therefore, we employed a univariate method in which subjects were modeled as random effects to accomplish the RM ANOVA. Post-hoc analyses were performed to examine significant main effects and interactions.

2.9.2 Functional MRI Evaluation of Potential Order Effects—We examined potential order effect confounds by comparing neural activation corresponding to each subject's violated expectation response trial to the *last* correctly cued stimulus trial of the *entire* experiment [across each temperature (47°C; 50°C)]. Conjunction analyses were also performed to confirm that neural mechanisms supporting violated expectations to pain did not vary as a function of the correctly cued comparison trial. We examined if the A) main effect of violated expectations of pain as compared to the last correctly cued stimulus before presentation of the first incorrectly cued trial significantly overlapped with B) the main effect of violated expectation of pain contrasted with the last correctly cued trial of the experiment.

3. RESULTS

3.1 Violated Expectation vs. Correctly Cued Trials

3.1.1 Psychophysical Pain Ratings—All fifteen subjects reported at least one rating equating to a violated expectation in response to incorrectly cued 47°C stimuli (Figure 2). The majority (n =10) of the subjects reported the first violated expectation in response to the first incorrectly cued 47°C stimulus (Figure 2). The remaining five subjects exhibited a violated expectation after subsequent presentations (i.e., 2nd and 3rd presentation) of an incorrectly cued 47°C stimulus (Figure 2).

In contrast to incorrectly cued 47°C stimulus trials where all subjects exhibited a violated expectation, two subjects unexpectedly did not report a violated expectation in response to incorrectly cued 50°C stimuli (Figure 2). However, twelve out of the 15 participants exhibited a violated expectation during the first presentation of an incorrectly cued 50°C stimulus. One participant exhibited a violated expectation in response to the second presentation of an incorrectly cued 50°C stimulus (Figure 2).

For VAS pain intensity ratings, there was a significant main effect of cue type, $F(1,38.39)=4.73$, $p=.04$ (Figure 3). Despite the significant main effect of cue, post-hoc tests detected no significant differences between a) 47°C correctly cued stimuli and 47°C violated expectation responses, $p= .15$ (Figure 3) or b) 50°C correctly cued and 50°C violated expectation responses, $p= .12$ (Figure 3). The main effect of temperature revealed that 50°C stimulation plateaus were rated significantly higher than temperature plateaus of 47°C, $F(1,39.57)= 81.52$, $p< .001$(Figure 3). There was no significant interaction between temperature and cue type, $F= .03$, $p= .87$, indicating that VE stimulus trials were associated with lower VAS ratings regardless of stimulus temperature (Figure 3).

3.1.2 Neuroimaging Results—Brain coordinates, p-values, and z-scores for brain regions exhibiting task-related activation/deactivation are located in the Supplementary Brain Coordinates Table.

Main Effect of Temperature-Related Brain Activation: Greater activation was found corresponding to 50°C stimulation in the contralateral SI, bilateral thalamus, anterior cingulate cortex (ACC), and cerebellum when compared to 47°C stimulation (Figure 4). Greater activation in the medial frontal gyrus, medial prefrontal cortex, ACC, and superior frontal gyrus was detected for 47°C when compared to 50°C stimulation (Figure 4).

Brain Activation Corresponding to Violated Expectations: Violated expectations of pain, across both temperature types (47°C; 50°C), were associated with greater activation in the left inferior and superior parietal lobe, angular gyrus, intraparietal sulcus, supramarginal gyrus, right cerebellum, and left occipital lobe when compared to correctly cued stimuli (Figure 4). When compared to violated expectations, correctly cued stimuli produced greater activity in the anterior cingulate cortex, premotor cortex, and the contralateral parietal-operculum (Figure 4).

In order to investigate the significant main effect of cue type, separate post-hoc paired samples t-tests were conducted comparing each subject's violated expectation stimulus trial to each respective subject's correctly cued stimuli for each temperature type (47°C, 50°C). During 47°C stimulation, greater activity in the right cerebellum and left occipital lobe was associated with violated expectations when compared to correctly cued stimuli (Figure 5). There was no greater brain activity associated with correctly cued 47°C when compared to violated expectation response trials.

During 50°C stimulation, greater brain activity in the left superior and inferior parietal lobe, including the supramarginal gyrus, angular gyrus, and intraparietal sulcus, was associated with violated expectation stimulus trials when compared to correctly cued stimuli (Figure 5). When compared to violated expectations, correctly cued stimuli produced greater activation extending from the contralateral secondary somatosensory cortex to the parietal operculum, right superior frontal gyrus, contralateral primary somatosensory cortex, right superior parietal lobe, and supplementary motor area.

Interaction Effect: A positive interaction between cue type and stimulus temperature was detected in the left inferior parietal lobe (Figure 6). This positive interaction was characterized by violated expectations evoking different responses across temperatures. During 50°C stimuli, violated expectations produced increased activation in this area relative to correctly cued 50°C stimuli. In contrast, violated expectations during 47°C stimuli produced reduced activity in this region relative to correctly cued 47°C stimuli (Figure 6).

A negative interaction occurred in the right superior frontal gyrus. During 50°C stimuli, violated expectations produced decreases in BOLD signal when compared to correctly cued 50°C whereas during 47°C stimuli, correctly cued stimuli produced decreases relative to

violated expectation stimulus trials (Figure 6). A similar interaction in the right superior parietal lobe was observed (Figure 6).

3.2 Distinguishing Violated Expectations of Pain Responses from Order Effects

3.2.1 Psychophysical Results—There was no significant difference in pain ratings between violated expectation and the last correctly cued trial of the entire experiment, $F(1, 51)=.10$, $p=.75$. The main effect of temperature revealed that 50°C stimulation plateaus were rated significantly higher than temperature plateaus of 47°C, $F(1, 51.11)= 45.03$, $p< .001$(Figure 7). There was no significant interaction between temperature and cue type, $F(1,51) = .87$, $p= .36$, indicating that VE stimulus trials were associated with lower VAS ratings regardless of stimulus temperature (Figure 7).

3.2.2 Neuroimaging Results

Brain Activation Supporting Violated Expectations of Pain are Distinct from Order Effects: Analogous to the primary analysis examining violated expectations of pain (Figure 4), we found that when compared to the last correctly cued trial of the experiment, violated expectations were associated with greater activation in the left inferior and superior parietal lobe (Figure 8). When compared to violated expectations, correctly cued stimuli produced greater activation in the bilateral SII and premotor cortex and left primary somatosensory cortex (Figure 8).

Importantly, the conjunction analysis detected significant overlapping violated expectation of pain-related activation in the left inferior and superior parietal lobe between analyses A) corresponding to the last correctly cued stimulus before presentation of the first incorrectly cued stimulus and B) the last correctly cued trial of the experiment (Figure 9). Therefore, we can rule out order effects as a potential explanatory confound.

Additionally, 50°C stimulation produced greater activation in the thalamus, dorsal ACC, and SI corresponding to the stimulation site when compared to 47°C stimulation (Figures 8). In contrast, 47°C produced greater activation in the rostral ACC, superior frontal gyrus, and bilateral superior parietal lobules. There was a significant positive interaction in aspects of the left inferior parietal lobe including the IPS (Figure 8). There was also greater activation in the left superior frontal gyrus. There was no significant negative interaction.

3.3 Placebo analgesia and Nocebo Hyperalgesia

3.3.1 Psychophysical Results—Nocebo trials were associated with significantly higher pain ratings when compared to correctly cued 47°C stimuli, $F(1,6)=88.43$, $p<.001$(Figure 10). Placebo trials were associated with significantly lower pain ratings when compared to correctly cued 50°C stimuli, $F(1,6)=26.67$, $p=.002$ (Figure 10). These findings provide confirmation that the characterization procedures used to define placebo and nocebo effects were valid.

3.3.2 Neuroimaging Results

Nocebo-related Brain Activation: Greater brain activity was detected during nocebo-related stimulus trials in the cerebellum when compared to 47°C stimuli (Figure 11).

Reduced brain activity in the right superior frontal gyrus and inferior temporal gyrus was detected for nocebo stimulus trials when compared to 47°C correctly cued stimuli (Figure 11).

Placebo-Related Brain Activation: Reduced brain activation was detected during placebo stimulus trials when compared to 50°C correctly cued stimuli in SI corresponding to the stimulation site, the contralateral anterior insula, putamen, parietal-operculum, as well as in the cerebellum and supplementary motor area (Figure 11). Placebo-related activation was detected in the medial PFC when compared to correctly cued 50°C stimulation (Figure 11).

Absence in overlapping brain activation between violated expectation, nocebo and placebo stimulus trials: A conjunction analysis of data acquired during 47°C stimulation revealed that brain activation associated with violated expectations did not overlap significantly with brain activation associated with nocebo stimulus trials (z-score 2.3, p value threshold = 0.05). A conjunction analysis of data acquired during 50°C stimulation revealed no significant overlap in brain activation between violated expectation and placebo stimulus trials (z-score 2.3, p value threshold = 0.05).

4. DISCUSSION

Substantial mismatch between expected and experienced pain constitutes an event that is highly salient and significant for the health of the individual. As such, it requires considerable redirection of attentional resources in order to better protect the integrity of the body against unforeseen environmental threats. In the present investigation, we identified the brain mechanisms engaged during subject recognition of a high discordance between expected and experienced pain intensity. Our findings reveal that violated expectations of pain are processed by multiple brain regions supporting salience-driven sensory discrimination, retrieval of incongruent contextual details corresponding to expected pain, and associative learning processes.

4.1 Brain Mechanisms Supporting Violated Expectations of Pain

Violated expectations of pain produced differential activation in functionally distinct regions of the left inferior/superior parietal lobe, as well as the right posterior cerebellum and left occipital lobe (Figure 4). The present findings are remarkably consistent with prior research identifying distinct brain mechanisms within the left parietal lobe that process the divergence between executive level expectations and saliently-driven sensory discrimination [10; 36; 41–43; 61; 67].

The intraparietal sulcus is critically involved in shifting attention to perceptually salient features of a sensory event across multiple modalities [16; 23; 33; 56]. For instance, saliently driven violations of expected taste identity [61] and visuo-spatial target detection [23; 56] are processed within the intraparietal sulcus. However, both the supramarginal gyrus and intraparietal sulcus were engaged during the detection of discrepancies between expected and experienced pain. These two regions may be playing distinct roles in this process. The intraparietal sulcus employs bottom-up attentional processes to “capture” a violated expectation-related sensory event [10; 16; 17; 23]. In contrast, the left

supramarginal gyrus allocates attention to detecting discrepancies between expected and unanticipated sensory/cognitive events [32; 37; 57]. Supramarginal gyrus-mediated detection of unanticipated or infrequent stimuli is largely reflexive and is associated with reorienting attention to appraise present-moment sensations [25; 36; 41]. Moreover, this mechanism is largely independent of memory retrieval processes, suggesting that the role of the supramarginal gyrus is critical to recognizing real-time deviations from expected contextual frameworks [41]. Although existing research is limited, the supramarginal gyrus has been shown to process breaches of expectations across multiple modalities including vision [10] and episodic memory recognition [41]. Therefore, we postulate that this process occurs in a similar fashion across multiple sensory modalities.

In conjunction with the supramarginal gyrus and the intraparietal sulcus, the angular gyrus also processes violations of expected sensory information (Figure 4) [36; 41]. However, in contrast to the supramarginal gyrus and intraparietal sulcus, the angular gyrus is engaged in processing the recognition of violated expectations by retrieving episodic memory details from expected perceptual schemas [11; 12; 25; 41; 53]. In the context of the present study and in light of the existing literature, one could postulate that the supramarginal gyrus and intraparietal sulcus processed real-time deviations of expectations during the experience of pain, while the angular gyrus was involved in the recall of the pre-stimulus cue meaning [7; 8; 24]. We propose that specific regions within the left posterior parietal lobe engage in functionally distinct processes that support the realization of violated expectation.

Visual working memory processes are contingent upon feedback connections between the occipital lobe and the intraparietal sulcus [60; 62] and angular gyrus [24]. The present findings suggest that violated expectations of pain engaged similar feedback connections to process deviations between the recognition of invalid visual cues and real-time thermal stimulation. Violations of expected pain also produced activation in the right cerebellum when compared to correctly cued thermal stimulation. This finding is consistent with previous work implicating the right cerebellum in predictive-learning processes reflecting violations of expected visual [10] and nociceptive [9; 39] input. Furthermore, anatomical connections between cerebellar nuclei and the parietal cortex [1; 55] suggest that the cerebellum may be engaged in processing of deviations from learned pairings between expected and experienced sensory events [10; 24], possibly as part of a parieto-cerebellar loop architecture [51; 58].

4.2 Violated Expectations across Temperature Type

Violated expectations in response to 47°C stimulation produced activation in the occipital lobe and the cerebellum (Figure 5). Cerebellar activation is associated with mediating top-down influences in processing unexpected sensory events [10], while occipital lobe activation has been found to mediate visual working memory processes [62]. Because 47°C stimuli are significantly less painful and salient than 50°C stimulation (Figure 3), subjects were more than likely engaging top-down processes to actively detect discordances between visual cues signaling “high” pain and experienced “low” pain.

Violated expectations in response to 50°C stimulation produced activation across posterior parietal regions hypothesized to capture salience-driven deviations from memory-driven

expectations [41]. Therefore, in contrast to 47°C stimulation, bottom-up attentional processes likely factored more heavily in the processing of violated expectations during 50°C stimulation. Furthermore, activation in the right superior parietal lobe was associated with processing correctly cued 50°C stimulation (Figure 4, 5, and 11), reflecting higher-order evaluation of spatial location of a highly intrusive and noxious signal [34; 35; 42].

4.3 Brain Mechanisms Supporting Placebo and Nocebo Responses

Consistent with prior research [44; 63], placebo analgesia significantly reduced pain-related brain activation and produced greater medial PFC activation when compared to correctly cued thermal stimuli (Figure 11). Similar to the present study, Keltner and colleagues (2006) found that nocebo stimulus trials produced greater activation in the cerebellum when compared correctly cued 47°C stimulation (Figure 11) [27]. Cerebellar activity during nocebo may reflect activation of cortico-cerebellar loops potentially related to conditioned regulation of withdrawal reflexes [27; 39; 46].

4.4 Considerations of Violated Expectations of Pain

The presentation of correctly cued stimuli before an incorrectly cued stimulus was instrumental in reliably executing our conditioning paradigm, but was subject to order effects. To determine if order effects confounded neural mechanisms supporting violated expectations of pain, we conducted a secondary two-way ANOVA comparing each subject's violated expectation response trial to each subject's *last* correctly cued stimulus trials of the entire experiment [across each temperature (47°C; 50°C). Importantly, we found that order effects did not account for our primary findings (Figure 3) as activation in the left inferior and parietal lobe was associated with processing violated expectations of pain when compared to the *last* correctly cued stimulus trial of the entire experiment (Figure 8). Conjunction analyses further confirmed that order effects did not confound our findings. Significant overlapping patterns of neural activity in the left inferior and superior parietal lobe (Figure 9) were detected between violated expectations of pain when compared to A) the last correctly cued stimulus trial preceding the first incorrectly cued trial (Figure 3) and B) violated expectations of pain as compared to the *last* correctly cued stimulus trial of the full experiment (Figure 8). Taken together, these data indicate that activation associated with violated expectations of pain is not related to order effects.

The main findings of this study are derived from violated expectation response trials where only a single violated expectation response trial was included per subject per temperature (47°C; 50°C). Even with this small number of trials, the main effect for stimulus temperature (Figure 3, Figure 8) revealed significant differences in pain-related brain activation between 47°C and 50°C. Thus, there is sufficient power to detect subtle differences in brain activity. Moreover, we employed a conservative whole-brain search that was corrected for multiple comparisons and unbiased by a priori hypotheses.

Given the importance of the anterior insular cortex in processes related to expectation [31] and discrimination [29; 38; 43] of pain, it is surprising that this region exhibited less activation during violated expectations (Figure 3). However, if the anterior insula acts as a neural comparator between expected and experienced sensory information, deviations from

expectations would not necessarily produce differential activation because the comparison process would be relatively constant across both correctly cued and all incorrectly cued conditions.

4.5 Summary

The present study is the first to confirm that multiple regions within the left posterior parietal cortex are differentially engaged when our expectations of an impending painful stimulus are violated. These findings demonstrate that the powerful influence of expectations on the subjective experience of pain can be dramatically attenuated when the discrepancy between expected and experienced pain is large. Violated expectations of pain are characterized by the a) discernible salience of the unanticipated nociceptive sensory event, b) reflexive allocation of attention to the unexpected sensory event, and c) retrieval and comparison of the meaningful details between the expected perceptual framework and experienced pain.

Conjunction analyses revealed that brain mechanisms activated by violations of expected pain did not significantly overlap with nocebo or placebo-related brain activity. These markedly different patterns of brain activity underscore the abrupt termination of expectation-mediated placebo and nocebo responses as a function of violated expectations of pain.

Clinically, the analgesic effects of active pain treatments are significantly augmented when expectations for pain relief are met [22]. In contrast, once expectations of pain relief are violated, the benefits of analgesic treatments are significantly diminished [6; 14; 15; 52]. The present findings indicate the importance of creating realistic expectations for pain relief in order to maintain expectancy-mediated amplification of pain treatments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Amino Y, Kyuhou S, Matsuzaki R, Gemba H. Cerebello-thalamo-cortical projections to the posterior parietal cortex in the macaque monkey. Neurosci Lett. 2001; 309(1):29–32. [PubMed: 11489539]
2. Andersson J, Jenkinson M, Smith SM. Non-linear optimisation. FMRIB Technical Report. 2007
3. Andersson J, Jenkinson M, Smith SM. Non-linear registration, aka Spatial normalisation. FMRIB Technical Report. 2007
4. Atlas LY, Bolger N, Lindquist MA, Wager TD. Brain mediators of predictive cue effects on perceived pain. J Neurosci. 2010; 30(39):12964–12977. [PubMed: 20881115]
5. Atlas LY, Wielgosz J, Whittington RA, Wager TD. Specifying the non-specific factors underlying opioid analgesia: expectancy, attention, and affect. Psychopharmacology (Berl). 2013

6. Benedetti F, Arduino C, Costa S, Vighetti S, Tarenzi L, Rainero I, Asteggiano G. Loss of expectation-related mechanisms in Alzheimer's disease makes analgesic therapies less effective. Pain. 2006; 121(1–2):133–144. [PubMed: 16473462]
7. Binder JR, Frost JA, Hammeke TA, Cox RW, Rao SM, Prieto T. Human brain language areas identified by functional magnetic resonance imaging. J Neurosci. 1997; 17(1):353–362. [PubMed: 8987760]
8. Bonner MF, Peelle JE, Cook PA, Grossman M. Heteromodal conceptual processing in the angular gyrus. Neuroimage. 2013; 71:175–186. [PubMed: 23333416]
9. Borsook D, Moulton EA, Pendse G, Morris S, Cole SH, Aiello-Lammens M, Scrivani S, Becerra LR. Comparison of evoked vs. spontaneous tics in a patient with trigeminal neuralgia (tic douloureux). Mol Pain. 2007; 3:34. [PubMed: 17983481]
10. Bubic A, von Cramon DY, Jacobsen T, Schroger E, Schubotz RI. Violation of expectation: neural correlates reflect bases of prediction. J Cogn Neurosci. 2009; 21(1):155–168. [PubMed: 18476761]
11. Cabeza R, Ciaramelli E, Olson IR, Moscovitch M. The parietal cortex and episodic memory: an attentional account. Nat Rev Neurosci. 2008; 9(8):613–625. [PubMed: 18641668]
12. Cabeza R, Mazuz YS, Stokes J, Kragel JE, Woldorff MG, Ciaramelli E, Olson IR, Moscovitch M. Overlapping parietal activity in memory and perception: evidence for the attention to memory model. J Cogn Neurosci. 2011; 23(11):3209–3217. [PubMed: 21568633]
13. Cohen MS. Parametric analysis of fMRI data using linear systems methods. Neuroimage. 1997; 6(2):93–103. [PubMed: 9299383]
14. Colloca L, Benedetti F. Placebos and painkillers: is mind as real as matter? Nat Rev Neurosci. 2005; 6(7):545–552. [PubMed: 15995725]
15. Colloca L, Lopiano L, Lanotte M, Benedetti F. Overt versus covert treatment for pain, anxiety, and Parkinson's disease. Lancet Neurol. 2004; 3(11):679–684. [PubMed: 15488461]
16. Corbetta M, Patel G, Shulman GL. The reorienting system of the human brain: from environment to theory of mind. Neuron. 2008; 58(3):306–324. [PubMed: 18466742]
17. Corbetta M, Shulman GL. Control of goal-directed and stimulus-driven attention in the brain. Nat Rev Neurosci. 2002; 3(3):201–215. [PubMed: 11994752]
18. Eippert F, Bingel U, Schoell ED, Yacubian J, Klinger R, Lorenz J, Buchel C. Activation of the opioidergic descending pain control system underlies placebo analgesia. Neuron. 2009; 63(4):533–543. [PubMed: 19709634]
19. Forman SD, Cohen JD, Fitzgerald M, Eddy WF, Mintun MA, Noll DC. Improved assessment of significant activation in functional magnetic resonance imaging (fMRI): use of a cluster-size threshold. Magn Reson Med. 1995; 33(5):636–647. [PubMed: 7596267]
20. Friston KJ, Holmes AP, Worsley KJ, Poline JP, Frith CD, Frackowiak RSJ. Statistical Parametric Maps in Functional Imaging: A General Linear Approach Human. Brain Mapping. 1995; 2:189–210.
21. Friston KJ, Worsley KJ, Frackowiak RJ, Mazziotta JC, Evans AC. Assessing the significance of focal activations using their spatial extent. Human Brain Mapping. 1994; 1:214–220.
22. Geers AL, Weiland PE, Kosbab K, Landry SJ, Helfer SG. Goal activation, expectations, and the placebo effect. J Pers Soc Psychol. 2005; 89(2):143–159. [PubMed: 16162050]
23. Geng JJ, Mangun GR. Anterior intraparietal sulcus is sensitive to bottom-up attention driven by stimulus salience. J Cogn Neurosci. 2009; 21(8):1584–1601. [PubMed: 18752405]
24. Horwitz B, Rumsey JM, Donohue BC. Functional connectivity of the angular gyrus in normal reading and dyslexia. Proc Natl Acad Sci U S A. 1998; 95(15):8939–8944. [PubMed: 9671783]
25. Hutchinson JB, Uncapher MR, Wagner AD. Posterior parietal cortex and episodic retrieval: convergent and divergent effects of attention and memory. Learn Mem. 2009; 16(6):343–356. [PubMed: 19470649]
26. Jenkinson M, Bannister P, Brady M, Smith S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage. 2002; 17(2):825–841. [PubMed: 12377157]

27. Keltner JR, Furst A, Fan C, Redfern R, Inglis B, Fields HL. Isolating the modulatory effect of expectation on pain transmission: a functional magnetic resonance imaging study. J Neurosci. 2006; 26(16):4437–4443. [PubMed: 16624963]
28. 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(49):13354–13362. [PubMed: 19052227]
29. 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. 2006; 27(9):715–721. [PubMed: 16342273]
30. Koyama T, McHaffie JG, Laurienti PJ, Coghill RC. The single-epoch fMRI design: validation of a simplified paradigm for the collection of subjective ratings. Neuroimage. 2003; 19(3):976–987. [PubMed: 12880826]
31. Koyama T, McHaffie JG, Laurienti PJ, Coghill RC. The subjective experience of pain: where expectations become reality. Proc Natl Acad Sci U S A. 2005; 102(36):12950–12955. [PubMed: 16150703]
32. Linden DE, Prvulovic D, Formisano E, Vollinger M, Zanella FE, Goebel R, Dierks T. The functional neuroanatomy of target detection: an fMRI study of visual and auditory oddball tasks. Cereb Cortex. 1999; 9(8):815–823. [PubMed: 10601000]
33. Liu T, Slotnick SD, Serences JT, Yantis S. Cortical mechanisms of feature-based attentional control. Cereb Cortex. 2003; 13(12):1334–1343. [PubMed: 14615298]
34. Lobanov OV, Quevedo AS, Hadsel MS, Kraft RA, Coghill RC. Frontoparietal mechanisms supporting attention to location and intensity of painful stimuli. Pain. 2013; 154(9):1758–1768. [PubMed: 23711484]
35. Lobanov OV, Zeidan F, McHaffie JG, Kraft RA, Coghill RC. From cue to meaning: Brain mechanisms supporting the construction of expectations of pain. Pain. 2013
36. Macaluso E, Frith CD, Driver J. Supramodal effects of covert spatial orienting triggered by visual or tactile events. J Cogn Neurosci. 2002; 14(3):389–401. [PubMed: 11970799]
37. McCarthy G, Luby M, Gore J, Goldman-Rakic P. Infrequent events transiently activate human prefrontal and parietal cortex as measured by functional MRI. J Neurophysiol. 1997; 77(3):1630–1634. [PubMed: 9084626]
38. Moayedi M, Davis KD. Theories of pain: from specificity to gate control. J Neurophysiol. 2013; 109(1):5–12. [PubMed: 23034364]
39. Moulton EA, Schmahmann JD, Becerra L, Borsook D. The cerebellum and pain: passive integrator or active participator? Brain Res Rev. 2010; 65(1):14–27. [PubMed: 20553761]
40. Nichols T, Brett M, Andersson J, Wager T, Poline JB. Valid conjunction inference with the minimum statistic. Neuroimage. 2005; 25(3):653–660. [PubMed: 15808966]
41. O'Connor AR, Han S, Dobbins IG. The inferior parietal lobule and recognition memory: expectancy violation or successful retrieval? J Neurosci. 2010; 30(8):2924–2934. [PubMed: 20181590]
42. Oshiro Y, Quevedo AS, McHaffie JG, Kraft RA, Coghill RC. Brain mechanisms supporting spatial discrimination of pain. J Neurosci. 2007; 27(13):3388–3394. [PubMed: 17392455]
43. Oshiro Y, Quevedo AS, McHaffie JG, Kraft RA, Coghill RC. Brain mechanisms supporting discrimination of sensory features of pain: a new model. J Neurosci. 2009; 29(47):14924–14931. [PubMed: 19940188]
44. Petrovic P, Kalso E, Petersson KM, Ingvar M. Placebo and opioid analgesia-- imaging a shared neuronal network. Science. 2002; 295(5560):1737–1740. [PubMed: 11834781]
45. Ploghaus A, Tracey I, Clare S, Gati JS, Rawlins JN, Matthews PM. Learning about pain: the neural substrate of the prediction error for aversive events. Proc Natl Acad Sci U S A. 2000; 97(16): 9281–9286. [PubMed: 10908676]
46. Ploghaus A, Tracey I, Gati JS, Clare S, Menon RS, Matthews PM, Rawlins JN. Dissociating pain from its anticipation in the human brain. Science. 1999; 284(5422):1979–1981. [PubMed: 10373114]

47. Ploner M, Lee MC, Wiech K, Bingel U, Tracey I. Prestimulus functional connectivity determines pain perception in humans. Proc Natl Acad Sci U S A. 2010; 107(1):355–360. [PubMed: 19948949]

48. Price DD, Bush FM, Long S, Harkins SW. A comparison of pain measurement characteristics of mechanical visual analogue and simple numerical rating scales. Pain. 1994; 56(2):217–226. [PubMed: 8008411]

49. Price DD, McHaffie JG, Larson MA. Spatial summation of heat-induced pain: influence of stimulus area and spatial separation of stimuli on perceived pain sensation intensity and unpleasantness. J Neurophysiol. 1989; 62(6):1270–1279. [PubMed: 2600624]

50. Quiton RL, Greenspan JD. Across- and within-session variability of ratings of painful contact heat stimuli. Pain. 2008; 137(2):245–256. [PubMed: 17942227]

51. Ramnani N. Frontal lobe and posterior parietal contributions to the cortico-cerebellar system. Cerebellum. 2012; 11(2):366–383. [PubMed: 21671065]

52. Schenk LA, Sprenger C, Geuter S, Buchel C. Expectation requires treatment to boost pain relief: an fMRI study. Pain. 2014; 155(1):150–157. [PubMed: 24076046]

53. Sestieri C, Corbetta M, Romani GL, Shulman GL. Episodic memory retrieval, parietal cortex, and the default mode network: functional and topographic analyses. J Neurosci. 2011; 31(12):4407–4420. [PubMed: 21430142]

54. Seymour B, O'Doherty JP, Koltzenburg M, Wiech K, Frackowiak R, Friston K, Dolan R. Opponent appetitive-aversive neural processes underlie predictive learning of pain relief. Nat Neurosci. 2005; 8(9):1234–1240. [PubMed: 16116445]

55. Shinoda Y, Kakei S, Futami T, Wannier T. Thalamocortical organization in the cerebello-thalamo-cortical system. Cereb Cortex. 1993; 3(5):421–429. [PubMed: 8260810]

56. Shulman GL, Astafiev SV, Franke D, Pope DL, Snyder AZ, McAvoy MP, Corbetta M. Interaction of stimulus-driven reorienting and expectation in ventral and dorsal frontoparietal and basal ganglia-cortical networks. J Neurosci. 2009; 29(14):4392–4407. [PubMed: 19357267]

57. Stevens AA, Skudlarski P, Gatenby JC, Gore JC. Event-related fMRI of auditory and visual oddball tasks. Magn Reson Imaging. 2000; 18(5):495–502. [PubMed: 10913710]

58. Strick PL, Dum RP, Fiez JA. Cerebellum and nonmotor function. Annu Rev Neurosci. 2009; 32:413–434. [PubMed: 19555291]

59. Taylor DJ, McGillis SL, Greenspan JD. Body site variation of heat pain sensitivity. Somatosens Mot Res. 1993; 10(4):455–465. [PubMed: 8310782]

60. Todd JJ, Marois R. Capacity limit of visual short-term memory in human posterior parietal cortex. Nature. 2004; 428(6984):751–754. [PubMed: 15085133]

61. Veldhuizen MG, Douglas D, Aschenbrenner K, Gitelman DR, Small DM. The anterior insular cortex represents breaches of taste identity expectation. J Neurosci. 2011; 31(41):14735–14744. [PubMed: 21994389]

62. Vogel EK, Machizawa MG. Neural activity predicts individual differences in visual working memory capacity. Nature. 2004; 428(6984):748–751. [PubMed: 15085132]

63. 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(5661):1162–1167. [PubMed: 14976306]

64. Woolrich MW, Ripley BD, Brady M, Smith SM. Temporal autocorrelation in univariate linear modeling of FMRI data. Neuroimage. 2001; 14(6):1370–1386. [PubMed: 11707093]

65. Worsley, KJ. Statistical analysis of activation images. In: Jezzard, P.; Matthews, PM.; Smith, SM., editors. Functional MRI: An Introduction to Methods. Oxford University Press Inc; New York, New York: 2001.

66. Worsley KJ, Evans AC, Marrett S, Neelin P. A three-dimensional statistical analysis for CBF activation studies in human brain. J Cereb Blood Flow Metab. 1992; 12(6):900–918. [PubMed: 1400644]

67. Zandbelt BB, Bloemendaal M, Neggers SF, Kahn RS, Vink M. Expectations and violations: Delineating the neural network of proactive inhibitory control. Hum Brain Mapp. 2012

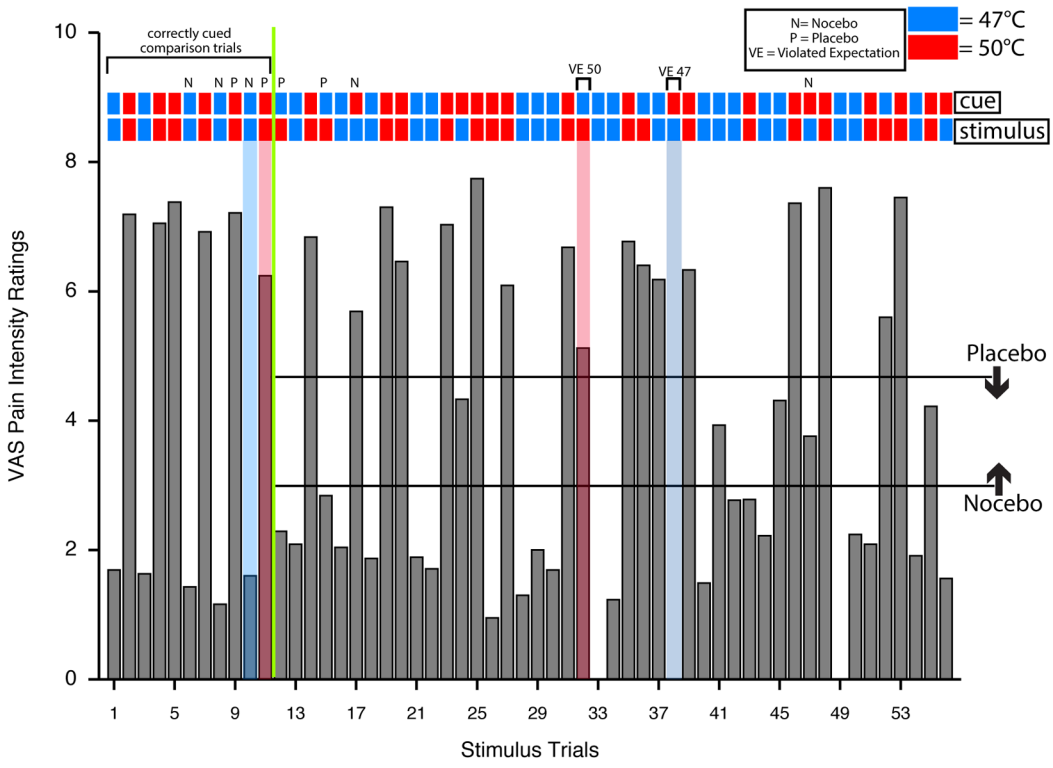

Figure 1.

Definition of Experimental Procedures and Conditions. The following figure depicts data collected from a single study participant during the fMRI portion of the study. Each fMRI series contained four stimuli. The x-axis illustrates all stimulus trials in the order in which they were delivered (i.e., 56) and the y-axis corresponds to VAS pain intensity ratings (0–10). Blue boxes correspond to “Low” cues and 47°C thermal stimuli. Red boxes are associated with “High” cues and 50°C thermal stimuli. Thus, correctly cued stimuli are denoted by cue and stimulus boxes of the same color, while incorrectly cued stimuli are denoted by mismatched colors.

To reinforce the cue-stimulus conditioning paradigm from the psychophysical training and conditioning session, the first two fMRI thermal series (e.g., 8 stimuli; 1–8) contained only correctly cued (CC) stimuli. Due to the randomization of stimulus conditions in the third MRI series of this case, a total of eleven correctly cued stimuli were administered before presentation of the first incorrectly cued stimulus (i.e., all stimuli preceding the green vertical line). Importantly, *correctly cued* stimulus trials to the left of the vertical green line were used as the control comparison trials. *Incorrectly cued* stimulus trials to the right of the vertical green line were used as the experimental conditions [i.e., violated expectation (VE), placebo (P) and nocebo (N) responses]. See Table 1 for full operational definitions of all stimulus response types.

Stimulus trials comparing VE 47°C with CC 47°C are denoted in light blue transparent bars. In order for a 47°C trial to be considered a VE, VAS ratings had to below the nocebo threshold (N) for incorrectly cued 47°C stimuli. The nocebo threshold is two SDs greater than the mean of each subject’s VAS ratings for all CC 47°C stimuli to the left of the vertical green line. In the illustrated case, VAS ratings above 2.87 in response to an

incorrectly cued 47°C were characterized as a nocebo response (n = 3 nocebo responses). Stimulus trials comparing VE 50°C with CC 50°C are denoted in light red transparent bars. In order for a 50°C trial to be considered a VE, VAS ratings had to above the placebo threshold for incorrectly cued 50°C stimuli. The placebo (P) threshold is two SDs lower than the mean of each subject's VAS ratings for all CC 50°C stimuli to the left of the vertical green line. In the illustrated case, VAS ratings below 4.60 in response to an incorrectly cued 50°C were characterized as a placebo response (n = 2 placebo responses). Two more all correctly cued series were randomly administered throughout the course of the experiment to reduce potential extinction-related effects related to the conditioning paradigm (in the present case stimulus trials 25 – 29 and 41 – 44).

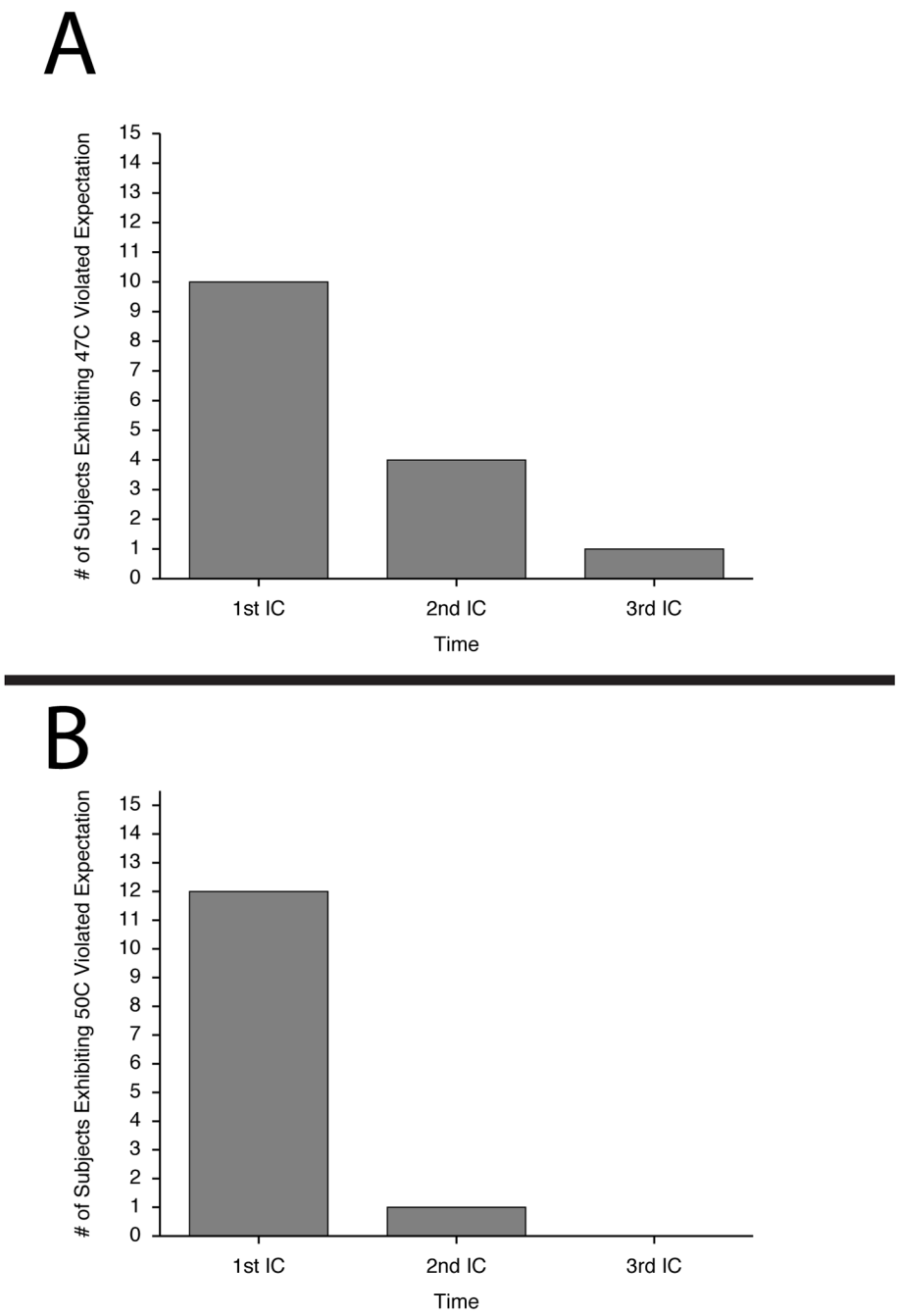

Figure 2.

Subjects Exhibiting Violated Expectations Across Incorrectly Cued Stimuli. A) Number of subjects exhibiting violated Expectation (VE) for 47°C incorrectly cued stimuli. All fifteen subjects reported at least one rating equating to a violated expectation stimulus trial in response to incorrectly cued (IC) 47°C stimulus. Ten participants reported a VE to the first incorrectly cued 47°C stimulus while five exhibited nocebo responses that persisted across one or more incorrectly cued stimulus trials. B) Number of subjects exhibiting VE for incorrectly cued 50 °C stimuli. Two subjects did not report a violated expectation and

exhibited placebo analgesia in response to all incorrectly cued 50°C stimuli. However, twelve participants reported a VE response to the first incorrectly cued 50 C° stimulus and one participant reported a VE response to the second presentation of an incorrectly cued 50°C stimulus.

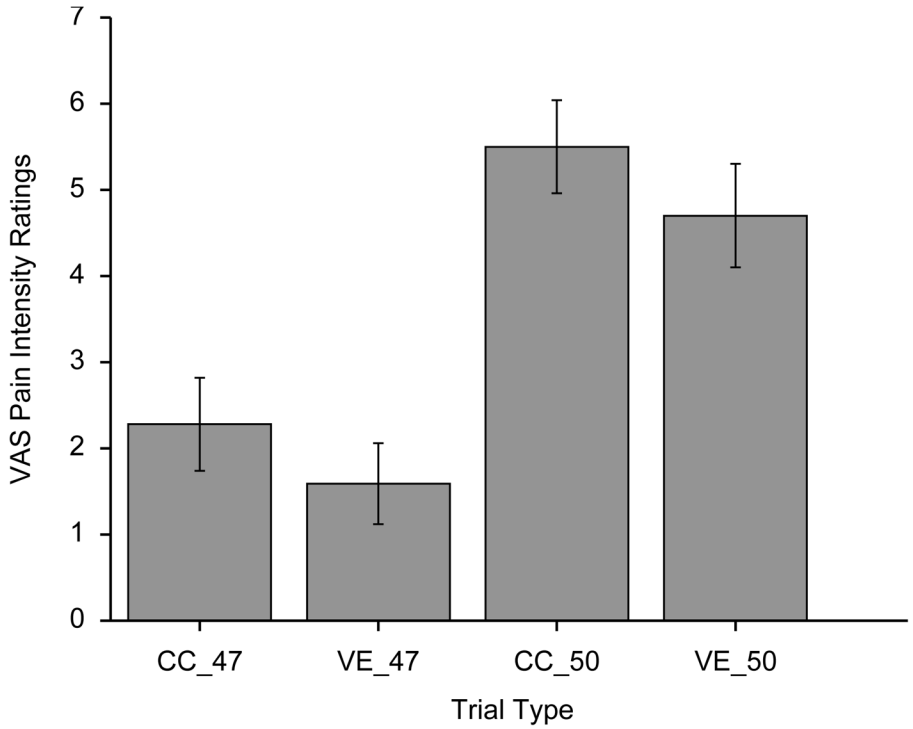

Figure 3.

Visual Analog Scale (VAS) Pain Intensity Ratings for Each Trial Type. Pain intensity ratings (mean ± SEM) did not significantly differ between correctly cued 47°C stimulus (CC_47) and violated expectation 47°C (VE_47) stimulus trials nor between correctly cued 50°C (CC_50) and violated expectation 50°C (VE_50) stimulus trials. Pain intensity ratings were higher in response to 50°C stimuli when compared to 47°C stimuli.

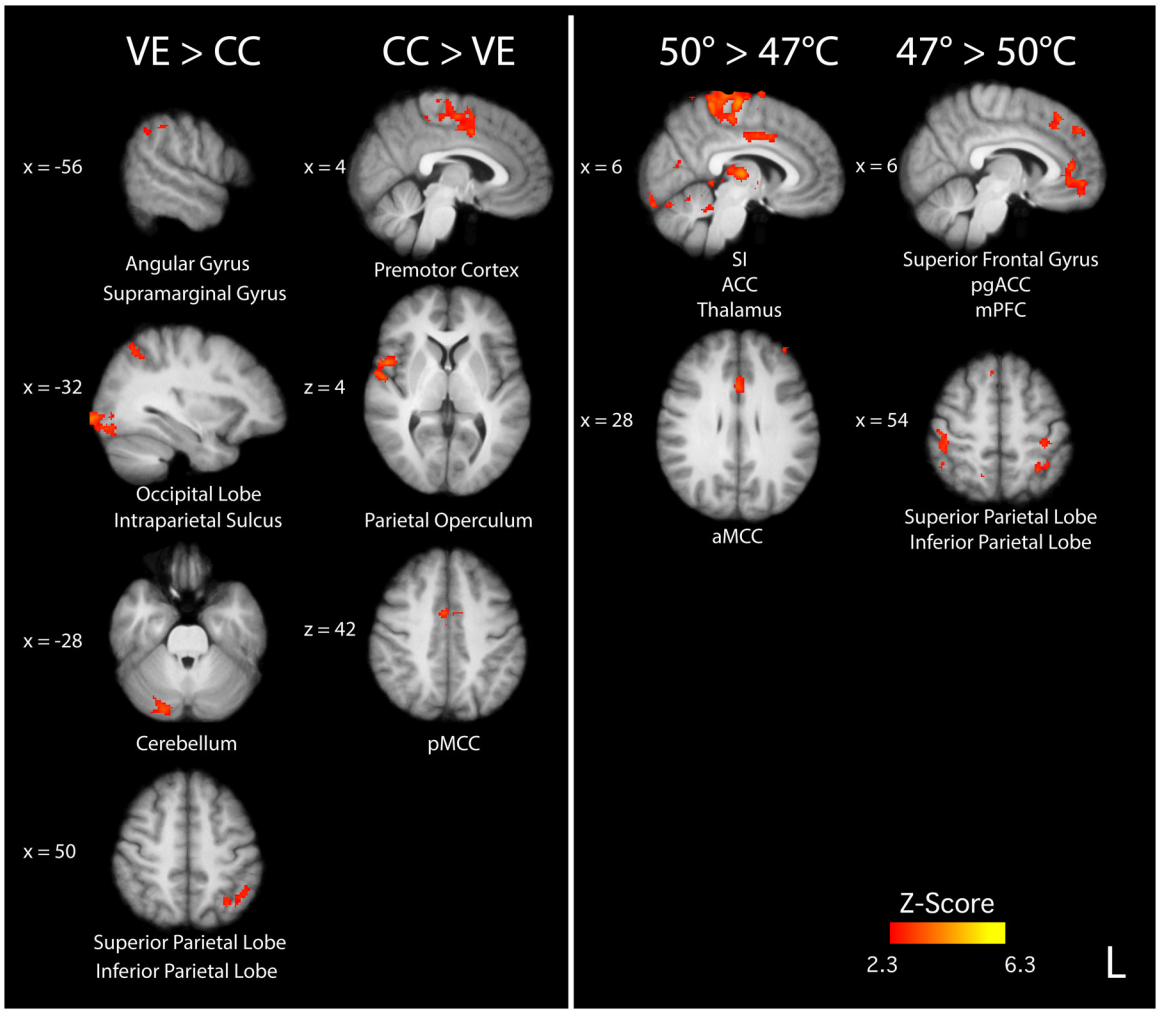

Figure 4.

Brain Activations Related to Violated Expectations and Stimulus Intensity. Brain regions are delineated from left to right. **Left Panel:** Greater violated expectation (VE)-related brain activation was found in the left inferior parietal lobe, including the angular gyrus, supramarginal gyrus, intraparietal sulcus, left superior parietal lobe, occipital lobe, and right cerebellum when compared to correctly cued (CC) stimuli. Greater brain activation during correctly cued stimuli was detected in the premotor cortex, contralateral parietal operculum and the posterior midcingulate cortex (pMCC) when compared to violated expectation-related brain activity. **Right Panel:** Greater brain activation in response to 50°C stimulation was detected in the leg region of the contralateral primary somatosensory cortex (SI), and the anterior midcingulate cortex (aMCC) and thalamus when compared to 47°C stimulation-related brain activation. Greater activation during 47°C stimulation, was detected in the superior frontal gyrus, perigenual ACC (pgACC), medial prefrontal cortex (mPFC), right superior parietal lobe, and left superior/inferior parietal lobe when compared to brain activation associated with 50°C stimulation. R = subject right, slice locations are denoted below images.

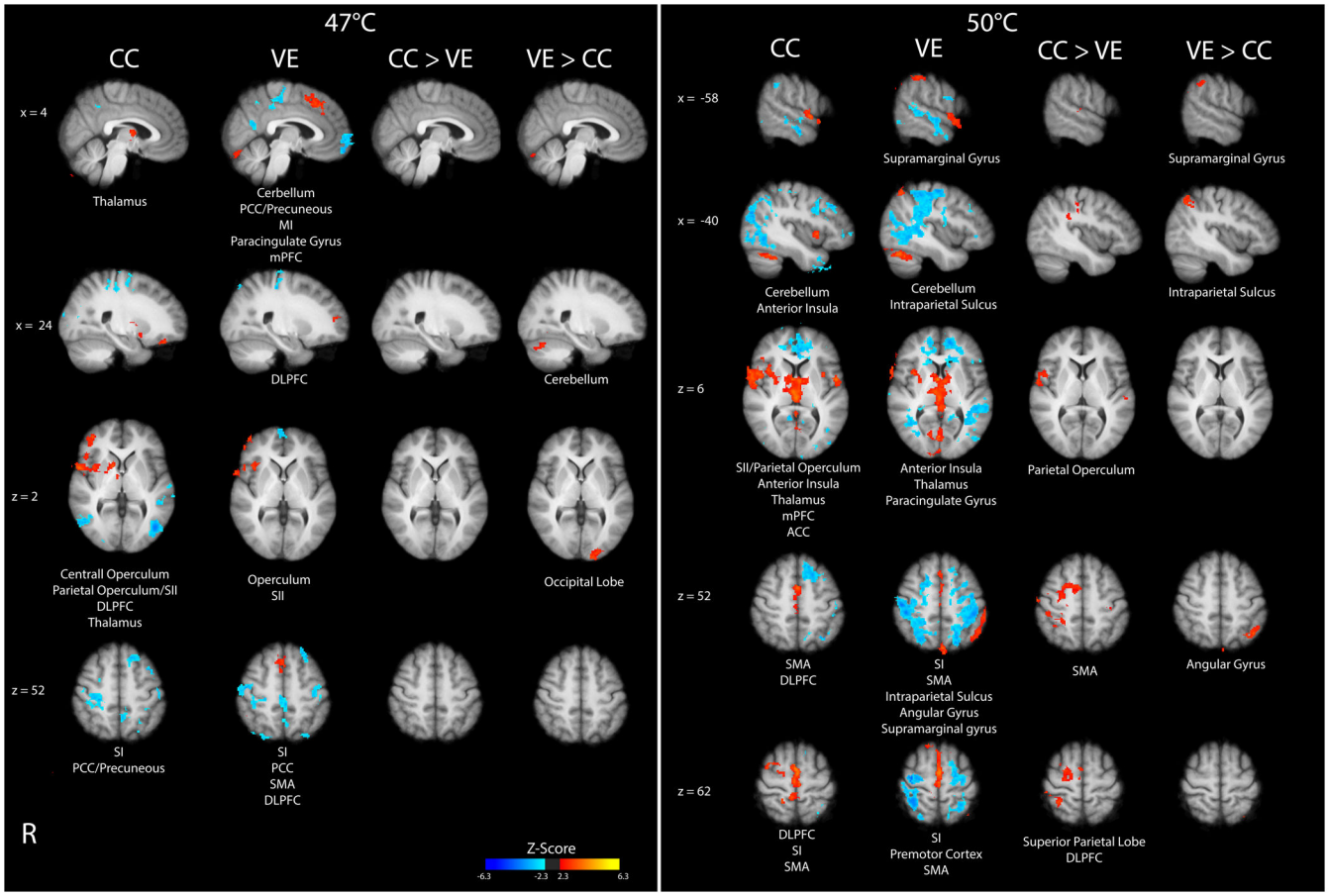

Figure 5.

Brain Activation Associated with One-Sample T-Tests of Correctly Cued and Violated Expectation Stimulus Trials and Brain Activation Associated with Paired T-Tests Comparing Violated Expectation Stimulus Trials to Correctly Cued Stimuli Across Temperature. Brain regions are delineated from left to right. Brain activations are delineated in red and deactivations in blue. **Left Panel:** Correctly cued (CC) 47°C stimulus trials produced activation in the thalamus, contralateral central operculum and secondary somatosensory cortex (SII), right dorso-lateral prefrontal cortex (DLPFC) and deactivation of the primary somatosensory cortex (SI) and precuneous/posterior cingulate cortex (PCC). Violated expectation (VE) 47°C stimulus trials produced activation in the cerebellum, paracingulate gyrus, contralateral central operculum and SII, and the supplementary motor area (SMA). VE 47°C were also associated with deactivation of SI, medial prefrontal cortex (mPFC), and precuneous/PCC. Greater activation in the left occipital lobe and cerebellum was detected for VE stimulus trials when compared to CC stimuli during 47°C stimulation. There was no significant brain activity detected for CC stimuli when compared to VE stimulus trials. **Right Panel:** Correctly cued (CC) 50°C stimulus trials were associated with activation in the cerebellum, contralateral anterior insula, contralateral SII/parietal operculum, bilateral SII, thalamus, SMA, left DLPFC, superior parietal lobe and deactivation of the left DLPFC, ACC, and mPFC. Violated expectation (VE) 50°C stimulus trials were associated with activation in the cerebellum, left intraparietal sulcus, contralateral

anterior insula, thalamus, and deactivation of the paracingulate gyrus. Greater 50°C CC-related brain activation was detected in the contralateral parietal operculum, SMA, and DLPFC when compared to 50°C VE-related brain activity. Greater 50°C VE-related brain activation was detected in the left inferior parietal lobe including the supramarginal gyrus, angular gyrus, and intraparietal sulcus. R = subject right, slice locations are denoted below images.

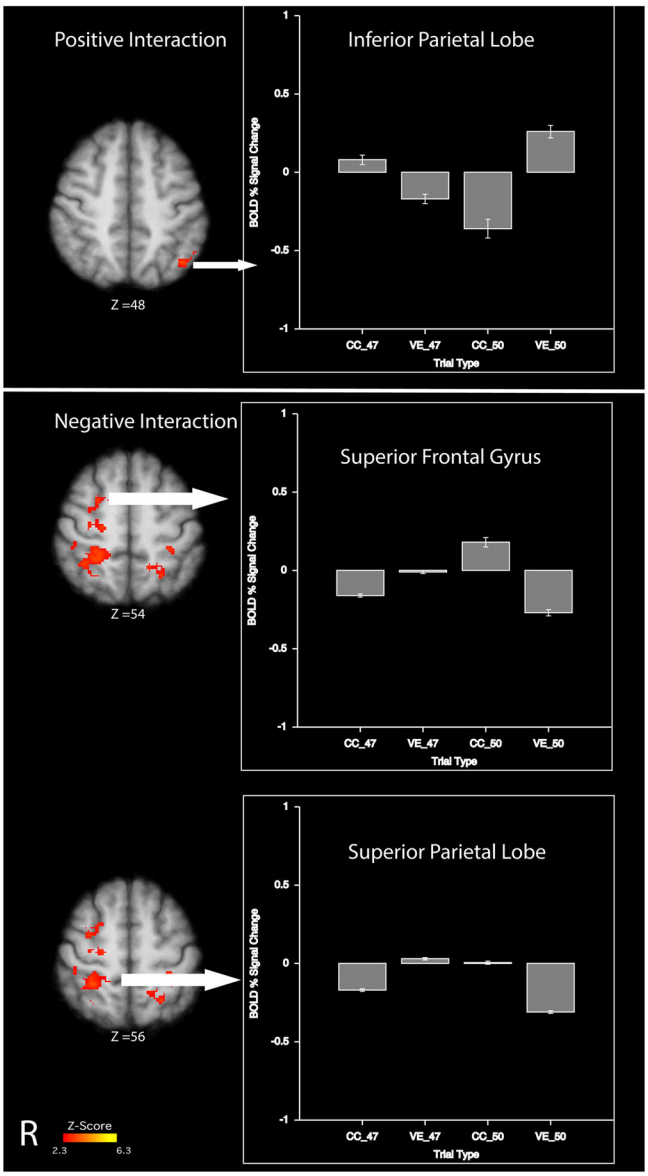

Figure 6.

Brain Activation Exhibiting Differing Responses to Trial Type Across and Temperatures. A positive interaction (top row) between cue type and stimulus temperature was detected in the left inferior parietal lobe. During 50°C stimuli, violated expectations produced increased activation in this area relative to correctly cued 50°C stimuli. In contrast, violated expectations during 47°C stimuli produced reduced activity in this region relative to correctly cued 47°C stimuli. A negative interaction occurred in the right superior frontal gyrus (second row). During 50°C stimuli, violated expectations produced lower BOLD signal relative to correctly cued stimuli whereas during 47°C stimuli, violated expectation produced higher BOLD signal relative to correctly cued stimuli. A similar negative interaction occurred in the right S. parietal lobe (third row).

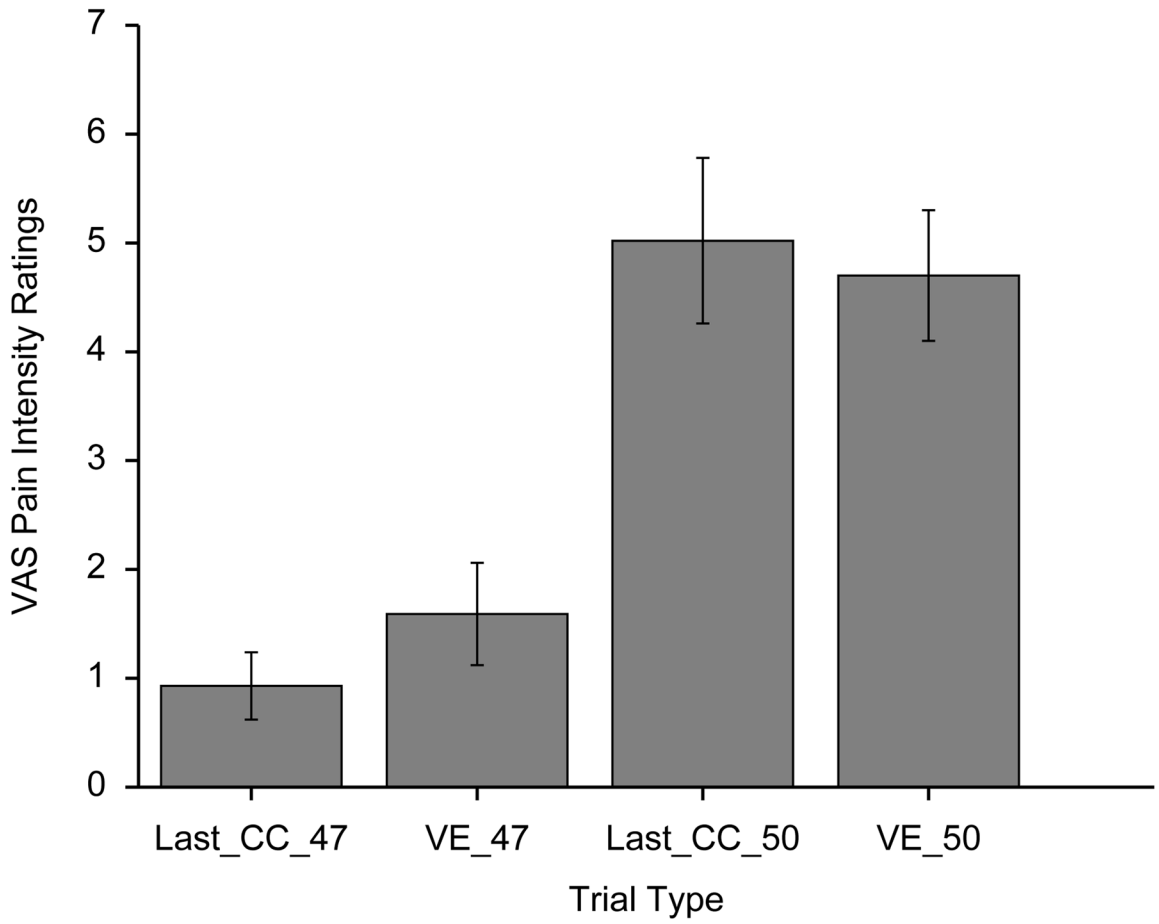

Figure 7.

Visual Analog Scale (VAS) Pain Intensity Ratings comparing the Last Correctly Cued Trial in the Experiment when Compared to Violated Expectation Response Trial Type. Pain intensity ratings (mean ± SEM) did not significantly differ between the last correctly cued 47°C stimulus (CC_47) and violated expectation 47°C (VE_47) stimulus trials nor between the last correctly cued 50°C (CC_50) and violated expectation 50°C (VE_50) stimulus trials. Pain intensity ratings were higher in response to 50°C stimuli when compared to 47°C stimuli.

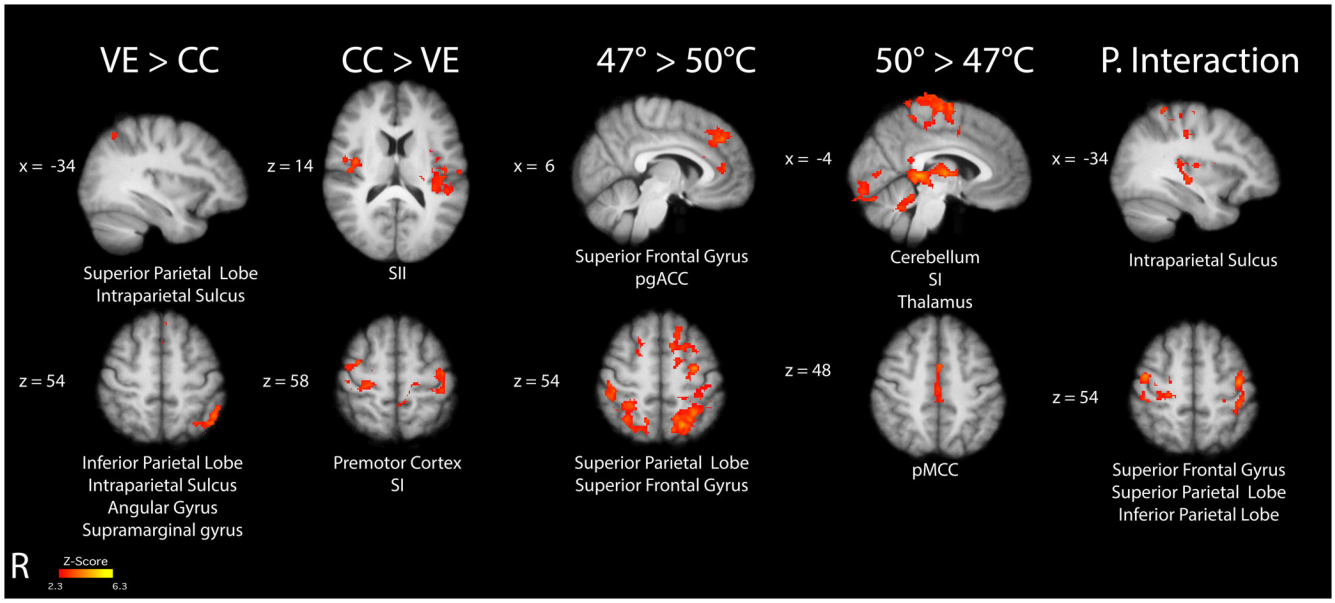

Figure 8.

Brain Activations Related to Violated Expectations and Stimulus Intensity when compared to the Last Correctly-Cued Stimulus Trial of the Experiment. Brain regions are delineated from left to right. **Violated Expectation (VE) > Correctly Cued (CC) trial types:** VE stimulus trials produced greater activation in regions of left inferior and superior parietal lobe including the intraparietal sulcus, angular gyrus, and supramarginal gyrus when compared to the last correctly cued stimuli administered in the experiment. **CC > VE:** The last correctly cued stimuli administered in the experiment produced greater activation in the bilateral secondary somatosensory cortices (SII), bilateral premotor cortices, and SI when compared to the VE stimulus trials. **47°C > 50°C:** Greater activation in the left superior frontal gyrus, perigenual anterior cingulate cortex (pgACC) and bilateral superior parietal lobe activation was associated with greater 47°C stimulation when compared to 50°C stimulation. **50°C > 47°C:** Greater activation the primary somatosensory cortex (SI) corresponding to the stimulation site, thalamus, and dorsal ACC was exhibited during 50°C stimulation when compared to 47°C stimulation. **Positive Interaction:** The positive interaction was associated with greater activation in aspects of the inferior parietal lobe including the intraparietal sulucs, superior parietal lobe, and left superior frontal gyrus. R = subject right, slice locations are denoted in stereotaxic space.

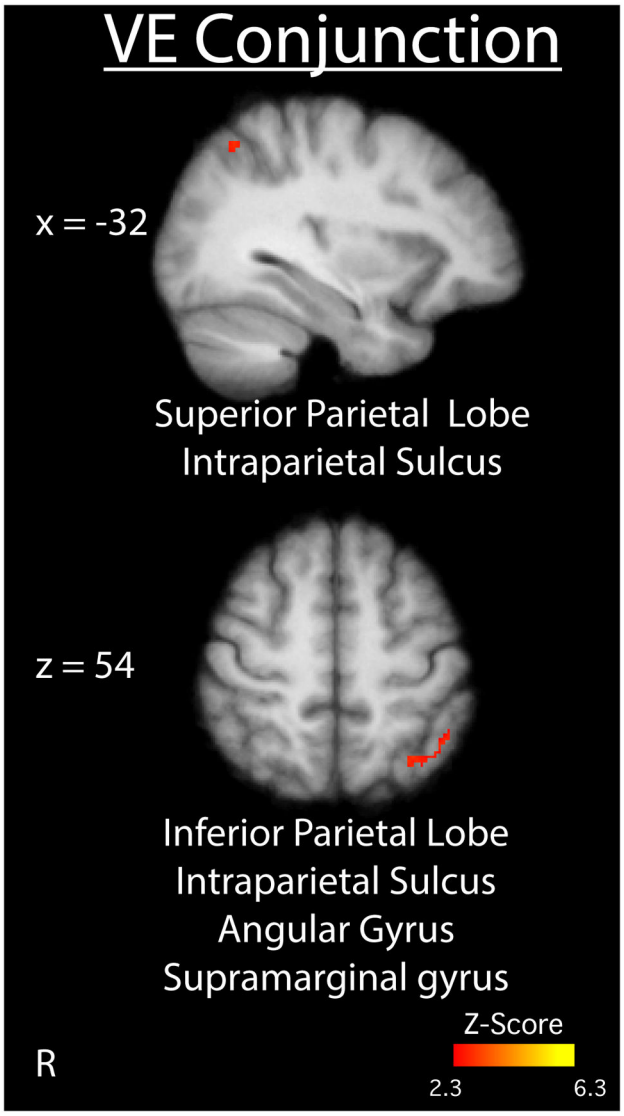

Figure 9.

Significant Overlapping Brain Activation between Violated Expectations (VE) of Pain when Compared to the Last Correctly Cued (CC) Stimulus Trial Preceding the First Incorrectly Cued Trial and Violated Expectations of Pain as Compared to the Last Correctly Cued Stimulus Trial of the Experiment. Brain regions are delineated from left to right. Significant brain overlapping activation was detected in regions of the left inferior and superior parietal lobe including the intraparietal sulcus, angular gyrus, and supramarginal gyrus demonstrating that confounding order effects were not detected. R = subject right, slice locations are denoted in stereotaxic space.

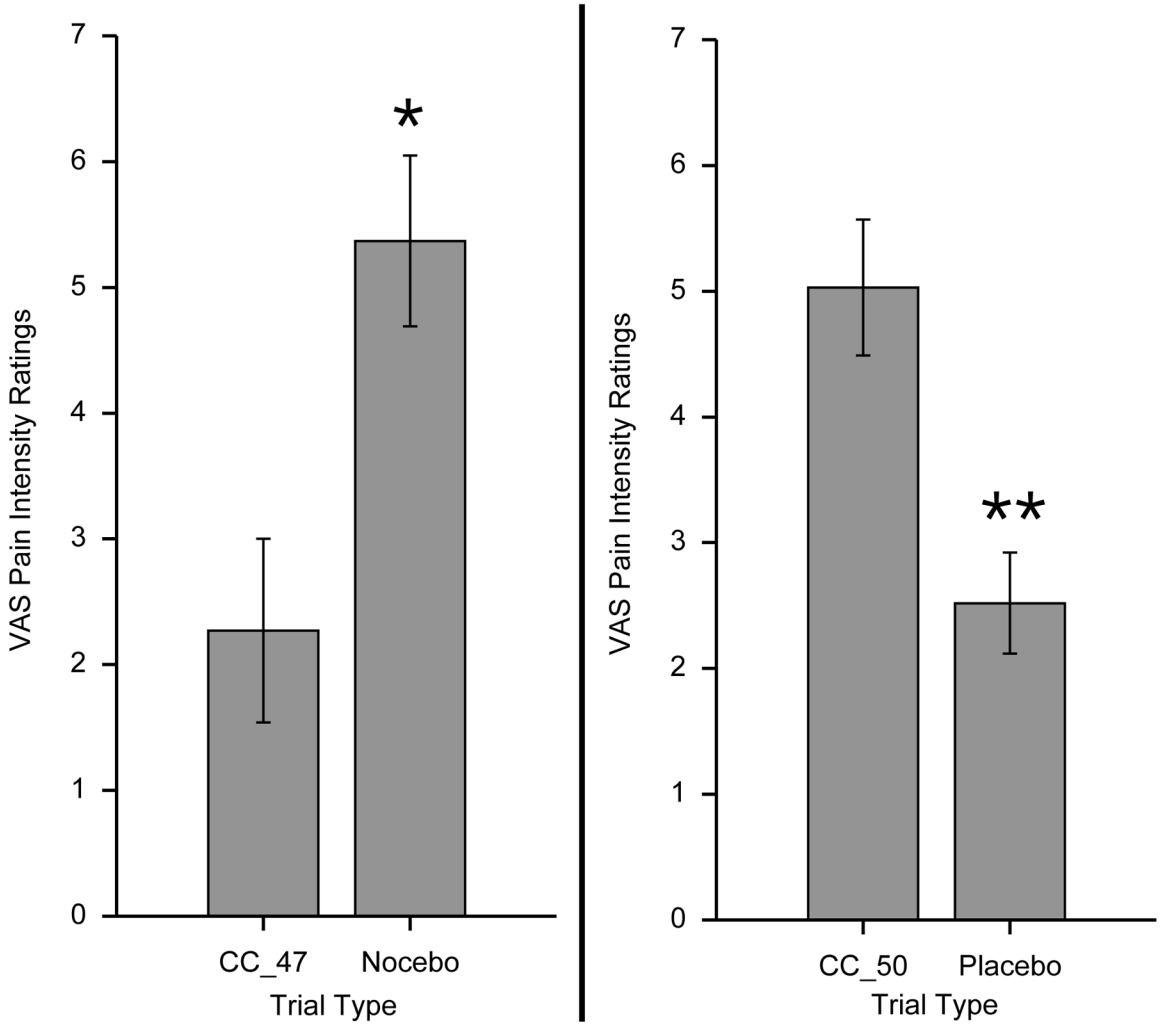

Figure 10.

Psychophysical Ratings of Nocebo and Placebo Effects. **Left Panel**: Visual analog scale (VAS) pain intensity ratings (mean ± SEM) for correctly cued 47°C stimuli and nocebo trials. *Nocebo trials were rated significantly higher than correctly cued 47°C (p<.001). **Right Panel:** VAS pain intensity ratings for correctly cued 50°C and placebo trials. **Placebo trials were rated significantly lower than correctly cued 50°C (p=.002).

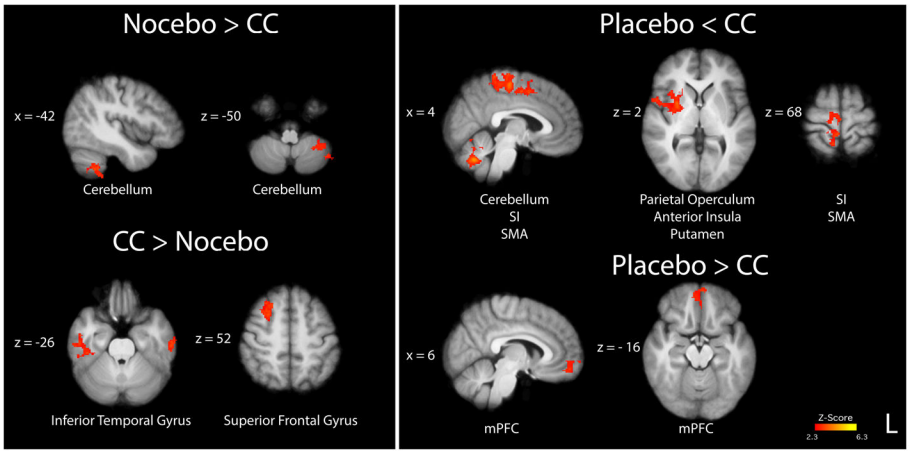

Figure 11.

Brain Activation Associated with Comparing Correctly Cued Stimuli to Nocebo and Placebo Stimulus Trials. **Left Panel:** Greater nocebo-related brain activity was detected in the cerebellum when compared to correctly cued (CC) 47°C stimuli. Greater CC-related activation was found in the right superior (S.) frontal gyrus and bilateral inferior temporal gyrus when compared to nocebo stimulus trials. **Right Panel:** During placebo trials, lower brain activity was detected in the contralateral primary somatosensory cortex (SI), and supplementary motor area (SMA), contralateral secondary somatosensory cortices (SII), anterior insula, parietal operculum, and cerebellum when compared to correctly cued stimuli. Placebo stimulus trials produced greater activation in the medial prefrontal cortex (mPFC) when compared to CC-related 50°C stimulation. R = subject right, slice locations are denoted in stereotaxic space.

Table 1

Definition of violated expectation, placebo, and nocebo stimulus trial types by psychophysical ratings. For each violated expectation response trial type, each subject had only one experimental trial and one control trial. For trials meeting placebo and nocebo criteria, all placebo/nocebo trials per subject were included in the analysis and were compared against an equal number of control trials.

Stimulus Response Type	Experimental Condition	Control Condition
Violated Expectation (47°C)	The 1st incorrectly cued 47°C stimulus trial with a VAS rating within two SDs of each subject's VAS rating for all correctly cued 47°C stimuli that preceded the first incorrectly cued stimulus.	The last correctly cued 47°C stimulus trial before presentation of the first incorrectly cued stimulus trial of either temperature.
Violated Expectation (50°C)	The 1st incorrectly cued 50°C stimulus trial with a VAS rating within two SDs of each subject's VAS rating for all correctly cued 50°C stimuli that preceded the first incorrectly cued stimulus.	The last correctly cued 50°C stimulus trial before presentation of the first incorrectly cued stimulus trial of either temperature.
Placebo	Incorrectly cued stimulus trials corresponding to a VAS rating below two SDs of each subject's VAS rating for all correctly cued 50°C stimuli preceding the first incorrectly cued stimulus.	Correctly cued 50°C stimulus trial (s) before presentation of the first incorrectly cued stimulus trial of either temperature.
Nocebo	Incorrectly cued stimulus trials corresponding to a VAS rating above two SDs of each subject's VAS rating for all correctly cued 47°C stimuli preceding the first incorrectly cued stimulus.	Correctly cued 47°C stimulus trial (s) before presentation of the first incorrectly cued stimulus trial of either temperature.