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Placebo effects in laser-evoked pain potentials

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9 Abstract

10 Placebo treatment may affect multiple components of pain, including inhibition of nociceptive input, automatic or deliberative
 11 appraisal of pain, or cognitive judgments involved in pain reporting. If placebo analgesia is due in part to an attenuation of early noci-
 12 ceptive processing, then pain-evoked event-related potentials (ERPs) should be reduced with placebo. In this study, we tested for placebo
 13 effects in P2 laser-evoked potentials at midline scalp electrodes. We found that placebo treatment produced significant decreases in P2
 14 amplitude, and that P2 placebo responses were large enough to reflect a meaningful difference in nociceptive processing. However, we
 15 also found evidence that the very robust placebo-induced decreases in reported pain are not solely explained by early reductions in P2.
 16 N2 amplitude was affected by neither placebo nor reduction of laser intensity. These results suggest that placebo treatment affects early
 17 nociceptive processing, but that another component of placebo effects in reported pain occurs later, either in retrospective evaluation of
 18 pain or cognitive judgments about pain reports.
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20 **Keywords:** Placebo; Laser; Pain; EEG; ERP; LEP; P2; Evoked potentials; Placebo effect
 21

22 1. Introduction

23 A major theme in contemporary neuroscientific research
 24 is that subjective experience is not a direct reflection of
 25 events in the world, but rather is constructed within the
 26 brain. According to this view, sensory signals are only
 27 one component of an experience, whether that experience
 28 is the perception of an object or the feeling of pain (Bruner
 29 et al., 1951). These “bottom-up” signals are integrated with
 30 “top-down” information about the context of the experi-
 31 ence (Miller and Cohen, 2001), including memories of rel-
 32 evant past experiences, expectations for the future, and the
 33 significance of the experience for the self. Recent research
 34 suggests that placebo effects emerge from such interactions,
 35 as cognitive expectations interact with ongoing processes in
 36 the brain and body. Placebo effects and other context
 37 effects are particularly powerful in pain, a multifaceted

experience that is closely tied to physical and mental 38
 well-being (Koyama et al., 2005; Lorenz et al., 2005; 39
 Melzack and Casey, 1968; Price, 2000; Sawamoto et al., 40
 2000; Wager et al., 2004). 41

A question with major implications for understanding 42
 the neurobiology of expectation and brain–body interac- 43
 tions is the question of how deep into the body placebo 44
 effects reach. Though a number of studies have reported 45
 reliable placebo effects in reported pain (e.g., Benedetti 46
 et al., 1999; De Pascalis et al., 2002; Pollo et al., 2001; Price 47
 and Barrell, 2000; Vase et al., 2002), reports are cognitively 48
 constructed representations of experience. Judgment of 49
 pain is an active neurobiological process that appears to 50
 engage affective and decision circuits in the brain (Moulton 51
 et al., 2005). Like other forms of judgment, pain reports 52
 may be highly susceptible to expectancy-induced biases in 53
 a variety of settings (e.g., Ericsson, 1980; Manis et al., 54
 1991). 55

Two primary issues that bear on the physiological 56
 ‘depth’ of placebo effects are whether placebo treatments 57

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58 have active psychobiological effects (Wager, 2005a,b), as
59 opposed to resulting from demand characteristics or statisti-
60 cal artifacts (Hrobjartsson and Gotzsche, 2001; Kienle
61 and Kiene, 1997); and if placebo effects are active, whether
62 they affect biological processes related to physical health
63 and mental well-being. A recent fMRI study provided evi-
64 dence that placebo treatment involves active recruitment of
65 cortical regions involved in the regulation of attention and
66 pain (Wager et al., 2004), suggesting that the placebo
67 response is an active psychobiological process. The study
68 also found that placebo treatment suppressed pain-induced
69 activity in the insula, thalamus, and anterior cingulate cor-
70 tex, suggesting that it alters ongoing processing of pain.
71 Zubieta et al. (2005), using PET, found evidence that place-
72 bo treatment both reduces pain and elicits increases in
73 endogenous opioid activity (cf. Benedetti et al., 1999).

74 However, these neuroimaging studies are limited in their
75 ability to address a critical question about the physiological
76 ‘depth’ of placebo: whether placebo treatments can alter
77 nociceptive processing, rather than or in addition to pain
78 affect, evaluation, and judgments about pain. The fMRI
79 study of Wager et al. found decreases in pain regions only
80 late during pain, after the stimulus had been turned off,
81 though strong responders also showed evidence for greater
82 decreases in anterior cingulate activity during the first sev-
83 eral seconds of painful stimulation. Either effect could be
84 related to the evaluation of pain, rather than to the sup-
85 pression of nociceptive processing, particularly since a
86 key area showing decreases—the insula—is also involved
87 in cognitive judgments of pain (Moulton et al., 2005).
88 The Zubieta et al. study provides converging, but also indi-
89 rect, evidence: opioid systems are involved in pain, but also
90 in affect, reward, and motivation, and so the evidence that
91 placebo effects inhibit nociceptive processing remains
92 indirect.

93 In the present study, we recorded brain potentials
94 evoked by painful laser stimuli to test for placebo effects
95 on early nociceptive responses. Laser-evoked potentials
96 (LEPs) are a reliable, objective marker of pain processing
97 (Bromm and Treede, 1984), and they are considered by
98 many to be the best tool for probing the function of noci-
99 ceptive pathways (Cruccu et al., 2004). Laser stimuli selec-
100 tively activate A-delta and C nociceptive fibers, and so
101 activate the nociceptive system without activating touch
102 and vibration pathways (Bromm and Treede, 1984). LEPs
103 are influenced by arousal and attention (Kakigi et al.,
104 2000), as is pain-induced fMRI activity (Petrovic and Ing-
105 var, 2002), consistent with the idea that pain processing is
106 sensitive to behavioral context. However, unlike measures
107 of fMRI or PET activity, which may reflect the process
108 of making subjective cognitive judgments about pain, LEPs
109 arise from nociceptive processes that occur before most
110 evaluation and decision processes begin. Some studies have
111 suggested that strategic response processes do not affect
112 stimulus processing until relatively late (at least 450 ms;
113 Ratcliff and McKoon, 1981), and that strategic control is
114 unlikely to affect responses faster than 700 ms (Seymour

115 et al., 2000). Thus, the cognitive biases known to affect
116 decisions about sensory experience and other types of
117 self-report are unlikely to affect LEPs.

118 A demonstration that placebo treatment affects LEPs
119 would provide converging evidence that placebo treatment
120 can affect early (pre-evaluative) nociceptive processing,
121 with implications for the relationship between cognitive
122 expectations and the function of one of the body’s most
123 basic systems for avoiding harm. There are both theoretical
124 and evidential reasons to expect such effects. The theory is
125 that cognitive expectations maintained in prefrontal cortex
126 may activate the PAG, which has the capability to inhibit
127 pain signals at the level of the spinal cord (e.g., Fields,
128 2004). The evidence comes from two recent studies, in addi-
129 tion to the fMRI and opioid placebo studies discussed
130 above. Matre et al. (2006) induced secondary hyperalgesia
131 by heating the skin to 46 °C for 5 min. Sensitization of the
132 skin area surrounding the stimulation site is known to
133 result from sensitization in the spinal dorsal horn. Expecta-
134 tion of pain relief reduced the size of the secondary hyper-
135 algesic area, compared to a control session where pain
136 relief was not expected, implicating a spinal mechanism
137 in the placebo effect. Converging electrophysiological evi-
138 dence comes from a study by Lorenz et al. (2005), who
139 found that expectations about the intensity of a laser stim-
140 ulus produced systematic changes in laser-evoked magneto-
141 encephalogram (MEG) potentials. They delivered laser
142 stimuli of high and low intensities, and crossed intensity
143 with a manipulation of whether the expected intensity
144 was high or low. They found that MEG potentials localized
145 approximately to SII—a cortical area critical for nocicep-
146 tive processing—were reduced in the low-expectation
147 condition and increased in the high-expectation condition.

148 There are several components of LEPs that may be affect-
149 ed by placebo expectancies, with different implications for
150 the cognitive control of nociception. The major components
151 of LEPs are a lateralized mid-latency negativity (N160) likely
152 to be localized in the parietal operculum (SII) and the late
153 N2/P2 complex (Lorenz and Garcia-Larrea, 2003). The
154 N2/P2 complex arises from the activation of A δ fibers and
155 is sometimes followed by an ultralate component thought
156 to arise from C-fiber activation (Bromm and Treede, 1984;
157 Bromm et al., 1984). The P2 increases as a function of both
158 laser intensity and reported pain (Iannetti et al., 2004). It is
159 likely to be separable from the P3, but it may overlap with
160 the P3a and reflect cognitive appraisal or attention to pain
161 (Lorenz and Garcia-Larrea, 2003). This is consistent with a
162 view of P2 LEPs as markers of early brain processing of pain,
163 which may involve attention and appraisal of behavioral
164 context as integral components (Garcia-Larrea et al., 1997,
165 2003; Legrain et al., 2005).

166 A likely source of the P2 is the anterior cingulate gyrus
167 (Garcia-Larrea et al., 2003; Lenz et al., 1998), which plays
168 a central role in both attention and pain, and also appears
169 to be modulated by placebo in studies of pain and emotion
170 (Petrovic et al., 2002, 2005; Wager et al., 2004; Zubieta
171 et al., 2005). Though the cingulate may show both increases

and decreases in different subregions during different phases of pain anticipation and regulation (Porro et al., 2003), the analyses in this study are sensitive to changes occurring within several hundred milliseconds of laser stimulus onset. Slower changes in anterior cingulate activity (i.e., sustained changes beginning in anticipation) will neither influence nor be detected by the measures employed here. The N160 is also of interest because it is a marker for early nociceptive processing, but the midline electrode configuration used in this study was not suitable for examining that component.

One account of placebo effects is that they induce an affective/motivational state that permits reduced attention to pain. The motivational state that regulates the allocation of attention appears to be only partly under voluntary control (e.g., it is very difficult to willfully ignore a rattlesnake next to one's foot), and the effects of placebo serve as a safety signal and permit attention to be directed away from pain. Placebo effects on the mid-frontal P2 would be consistent with this view. Notably, behavioral context (i.e., factors that affect motivated attention) also affects sensory pathways in the dorsal spinal horn (Duncan et al., 1987), which suggests that attentional set can have far-reaching physiological effects. Understanding the psychobiological mechanisms of placebo will likely be an enduring research question. The immediate goal of assessing whether placebo treatment affects early nociceptive processing is a preliminary step towards this understanding. Thus, in this study, we sought to test three specific hypotheses: (a) that placebo treatment would reduce P2 LEP amplitude; (b) that placebo reductions in LEP would correlate with reductions in reported pain; and (c) that the placebo P2 reduction would be comparable in magnitude to an equivalent reduction in the intensity of the laser.

2. Methods

2.1. Subjects

Thirty-nine subjects participated in the study (age: 23.2 ± 5.0 years old; four females). Ten additional subjects participated in a preliminary experiment to measure the relationship between evoked potentials, laser intensity, and reported pain. Of the 39 subjects, four were excluded because LEPs could not be identified reliably, and 11 were additionally excluded because they did not report that the laser stimulus was sufficiently painful (more detail is provided in Section 3). All subjects were free of medication and gave informed consent before testing. The experimental protocol was conducted in accordance with the Declaration of Helsinki and was approved by the Internal Review Boards of the Veterans Affairs Medical Center and the University of Michigan, Ann Arbor, MI, USA.

2.2. Laser stimulation

Laser stimulation was delivered by a Thulium YAG infrared laser (Neurolaser, BAASEL Lasertech, Starnberg Germany), activating heat-sensitive A-delta and C nociceptors. Spot diameter was 5 mm and pulse duration 1 ms. The output energy was kept below 700 mJ to avoid skin damage. The subject and the experimenter wore protective eye goggles.

2.3. Preliminary experiment

N2 and P2 LEP amplitudes were the primary physiological measures collected. Although N2 and P2 amplitudes are sensitive to changes in laser intensity and perceived pain intensity (Kakigi et al., 2000), we conducted a preliminary experiment ($n = 10$) to assess the relationship of these parameters to N2 and P2 amplitudes specifically. By varying the intensity of the laser stimulus within subjects, we hoped to determine roughly what sort of intensity decrease corresponds to a decrease in reported pain of the magnitude observed in placebo studies.

2.4. Experimental protocol

Participants were told that they were taking part in a study that compared brain responses to an analgesic cream (Lidocaine) with a control cream (ineffective). In reality both creams were ineffective (Vaseline skin cream).

The subjects were first tested for warmth-insensitive fields (WIFs) by touching different spots on the volar forearm for 3 s with a thermode heated to 41°C (Green and Cruz, 1998). Any WIFs were marked on the skin. Six 16-by-16 mm patches were then marked on the skin of the volar forearm, avoiding WIFs (Fig. 1). 'Analgesic' (placebo) cream was applied to patches 1 and 2 and control cream to patches 5 and 6 (location counterbalanced across subjects). Control cream was also applied to patches 3 and 4, which served as a calibration area. In the first section of the experiment, contact-heat stimulations were applied to the treated areas. These data were not analyzed in the present study.

Subjects were then prepared for the evoked-potentials section, which went over three phases (Calibration, Manipulation, and Test), as shown in Fig. 1. In the calibration phase, 10–20 laser stimuli of various intensities (300–700 mJ) were delivered to patch 3 and 4 in Fig. 1 to identify stimulus intensities corresponding to low- (level 1), medium- (level 2), and high-intensity (level 3 or above) pain. Many participants reported low or medium pain at the maximum intensity of 700 mJ, in which case this value was used in subsequent testing.

A manipulation phase followed to enhance participants' expectations of pain relief and thereby increase placebo responding. In this phase, pain was surreptitiously reduced in the placebo condition (Price et al., 1999). Five stimuli were applied to the placebo-treated ('analgesic' cream) patches of skin, and subsequently five stimuli were applied to the control-treated patches (order counterbalanced across participants). Participants were told that all stimuli were at level 'high.' However, they were administered at level 'low' in the placebo-treated patch and at level 'high' in the control-treated patch.

Finally, during the critical *test* phase, two runs of stimuli were administered to placebo- and control-treated patches of skin (80 stimulations in total: 2 patches \times 2 runs \times 20 stimuli). Locations of placebo and control patches and testing order were counterbalanced across subjects. A short warning beep (1 kHz tone) alerted the subject at pseudorandom intervals 6200–7900 ms before each laser stimulus. As before, participants were told these were at level 'high,' but all were delivered at level 'medium,' in keeping with the paradigm used in Price et al. (1999). Because the stimuli on placebo and control patches were identical, any differences in reported pain and evoked potential amplitude (control–placebo) during this phase are attributable to placebo effects.

2.5. Psychophysics

After a 1.5 s inter-stimulus interval following each laser stimulation, a beep prompted the subject to rate the perceived intensity. The subjective rating was done verbally on a 13-point numerical ratings scale ranging from -2 to 10 , with anchor points described by the following verbal instructions: -2 was 'not perceived,' -1 was 'non-painful warmth,' 0 was 'non-painful pinprick,' 1 was a painful pinprick, and 10 was 'worst pain imaginable.' The verbal ratings were recorded by the experimenter.

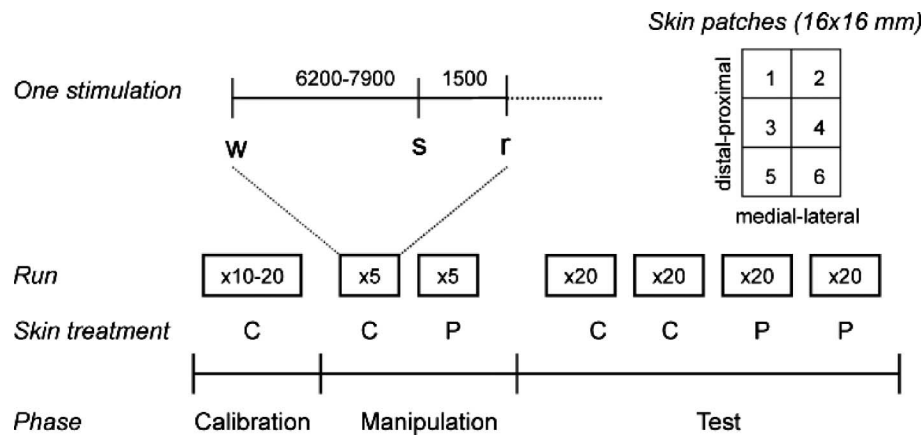


Fig. 1. Experimental protocol. Six 16-by-16 mm patches were marked on the skin of the volar forearm and treated with an analgesic cream or a control cream. The experiment involved three phases: calibration, manipulation, and test. Each laser stimulus was cued by an auditory warning cue (a beep) presented 6200–7900 ms before the stimulus, and followed by another auditory cue 1500 ms post-stimulus that signaled the participant to make a rating of reported pain. After a calibration phase to determine each individual's pain intensity vs. applied laser intensity, a manipulation phase followed in which participants received laser stimuli to the control- and placebo-treated areas at 'high' and 'low' intensities, respectively. During the test phase, subjects received 2 runs of 20 stimuli each to the control (C)- and placebo (P)-treated areas at medium intensity. The figure shows the order of testing for a C-first subject; order was counterbalanced across participants. The manipulation and test phases were performed on different skin patches.

288 2.6. Evoked potential acquisition

289 Electroencephalographic (EEG) registrations were made from four
 290 midline electrodes (FCz, Cz, CPz, and Pz) according to the international
 291 10–20 system, using a standard EEG cap and Neuroscan software (Scan
 292 4.2, Compumedics, El Paso, Texas). The recordings were referenced to
 293 linked bilateral earlobes (A1 + A2). Electrooculogram (EOG) was record-
 294 ed from supra- and infraorbital electrodes for offline artifact rejection. The
 295 impedance was maintained below 5 k Ω . The signals were amplified
 296 100,000 times (Synamp, Compumedics), sampled at 500 Hz, and bandpass
 297 filtered at 0.1–50 Hz. Room temperature was 22–23 $^{\circ}$ C and skin tempera-
 298 ture was always above 30 $^{\circ}$ C. The subjects were instructed to keep their
 299 eyes open, to focus on a fixed point at the wall and to avoid blinking,
 300 particularly in the interval between the warning and rating beeps.

301 2.7. Data analysis

302 Individual pain intensity ratings were averaged for each run of 20 stim-
 303 ulti. Individual behavioral effect scores were then calculated for each run by
 304 subtracting placebo ratings from control ratings (C–P). Furthermore, an
 305 overall pain score was calculated for each subject as the average across
 306 all 80 laser stimuli to determine each participant's pain sensitivity. Because
 307 the effect scores and average pain scores were positively skewed, nonpara-
 308 metric analyses were conducted on ranks of placebo effect scores and
 309 ranks of average pain scores.

310 EEG data were preprocessed using Neuroscan's built-in functions.
 311 First, ocular artifact rejection was done on the continuous recording.
 312 Manually counting the number of eye blinks or saccades in ten randomly
 313 selected runs (each lasting \sim 5 min) showed a variation between 16 and 65
 314 ocular artifacts. Blinking was most pronounced in the intervals between
 315 stimuli, as we instructed subjects to avoid blinking between the warning
 316 and rating beeps. To avoid sampling bias resulting from the manual rejec-
 317 tion of some sweeps (e.g., those with the highest subjective pain, which
 318 may also lead to increases in ocular activity), we employed a computa-
 319 tional correction method that corrects for artifacts rather than deleting
 320 sweeps.

321 The Neuroscan EOG correction procedure involved the following
 322 steps. First, the EOG channel was scanned for the maximum eye move-
 323 ment potential. EOG deviations of more than 10% from the maximum
 324 were used as indicators of blinks, and these were used to estimate an aver-
 325 age blink artifact response for each channel. The procedure discarded arti-
 326 facts starting <400 ms before a previous artifact, to avoid double

detection. If less than 20 blinks were detected, no correction was made. 327
 Otherwise, EEG data were corrected using a regression approach. From 328
 the average EOG ocular artifact, transmission coefficients (b) were com- 329
 puted for each EEG channel by estimating the covariance of the averaged 330
 potentials of the EOG channel with the EEG channels according to this 331
 equation: $b = \text{cov}(\text{EOG}, \text{EEG}) / \text{var}(\text{EOG})$. A fraction of the average 332
 EOG artifact ($b \cdot \text{EOG}$) was subtracted from each EEG channel on a 333
 sweep-by-sweep, point-by-point basis. After ocular artifact correction, 334
 the continuous EEG signal was split into epochs (–500 to 2000 ms relative 335
 to stimulus onset). Finally, each epoch was baseline corrected by subtract- 336
 ing 100-ms pre-stimulus EEG from each point in the epoch. This proce- 337
 dure prevents slow changes that may begin during anticipation of pain 338
 from influencing P2 amplitude estimates. 339

To extract peak LEPs, the 20 sweeps in each run were averaged and the 340
 latency and amplitude of the first major negative (N2) and positive (P2) com- 341
 ponent was extracted for each subject and each electrode using a peak-dete- 342
 ction function. The search interval was limited a priori to 150–350 ms (N2) 343
 and 250–500 ms (P2) relative to stimulus onset. For each run, each detected 344
 peak was confirmed by visual inspection before the estimates were accepted. 345
 In six subjects, no peaks were identified by the automatic procedure. In two 346
 of these subjects the N2/P2 complex was identified by visual inspection of the 347
 averaged sweeps. In the remaining four subjects, no valid N2/P2 complex 348
 could be identified in the search interval, although an ultralate positivity 349
 was identified with peak amplitude between 600 and 1000 ms. Data from 350
 these four subjects are not included in the remaining analysis, leaving 35 sub- 351
 jects. The visual inspection resulted in minor corrections to the automatic 352
 procedure in 4 of the remaining 140 runs (35 subjects \times 4 runs each). These 353
 were typically cases where the EEG signal was distorted by relatively large α - 354
 band (\sim 10 Hz) components. 355

356 2.8. Statistics

357 Statistical analysis was done in the General Linear Model (GLM) 358
 framework using SPSS (SPSS, Chicago, Illinois). Placebo effects in report- 359
 ed pain (RP) were analyzed separately for Run 1 and Run 2 with a 1 with- 360
 in (Placebo, C vs. P), 1 between (Order, C first or P first) GLM. Due to 361
 habituation of pain reports and LEPs, we expected the strongest placebo 362
 effects in Run 1. Follow-up analysis included Run as a factor in a 2 \times 2 363
 within, 1 between ANOVA.

364 LEP amplitudes were analyzed in two ways. First, we analyzed ampli- 365
 tudes in a 4 \times 2 within, 2 between GLM for each run. Within-subjects fac- 366
 tors were Electrode (FCz, Cz, CPz, and Pz) and Placebo (C vs. P).

367 Between-subjects factors were Order (C first or P first) and ranked Reported
368 Placebo.¹ Runs were analyzed separately because reported pain and P2
369 amplitudes both showed strong habituation effects, and we expected the
370 test to be sensitive to placebo effects only when subjects were experiencing
371 pain (i.e., primarily in Run 1). Supplementary analyses added Run as a
372 within-subjects factor. Nonsphericity is not an issue for the effects of Placebo
373 and Order, and Huynh-Feldt correction for nonsphericity was used
374 for effects of Electrode (Kesselman, 1998); thus, fractional degrees of freedom
375 are reported for these effects. MANOVA analyses (Keselman, 1998)
376 revealed a qualitatively identical pattern of results (data not shown).

377 In the second analysis we divided the sample into tertiles based on RP,
378 and looked for differences in P2 amplitudes between RP placebo Responders
379 (those in the highest third, $n = 8$) and Nonresponders (the lowest third,
380 $n = 8$). The GLM model for each run was the same as in the previous analysis,
381 that is a 4×2 within (Electrode and Placebo), 2-between (Order and
382 Responder Status) design.

383 We used one-tailed tests for one-sided hypotheses about which we had
384 a priori expectations, including that placebo would decrease P2 amplitude
385 (main effect of Placebo, particularly in Run 1) and that the placebo effect
386 would be weaker in Run 2 (Placebo \times Run interaction) due to habituation
387 in both pain and P2 amplitude. T-contrasts are presented for these comparisons
388 to test the one-sided alternative (the F statistic gives equivalent
389 results, but for a 2-sided alternative). Two-tailed tests were conducted
390 on other effects. An α level of 0.05 was used throughout.

391 2.8.1. Test of intensity-reduction hypothesis of placebo

392 Another analysis was performed at electrode Cz, where P2 effects are
393 maximal, to test whether P2 placebo effects were significantly smaller than
394 would be expected if placebo worked only by reducing nociceptive input.
395 We refer to this hypothesis as the “intensity-reduction” hypothesis. We
396 assessed this by testing whether placebo effects in P2 amplitude were significantly
397 smaller than P2 reductions in laser intensity that produce equivalent
398 decreases in reported pain. For this analysis, we began with the
399 placebo effect in reported pain ($n = 24$), and used the estimated curves
400 from the preliminary experiment ($n = 10$) to calculate the reduction in
401 both P2 and laser intensity required to produce an equivalent decrease
402 in reported pain. Performing these calculations for each of the 10 participants
403 allowed us to estimate the variance of predicted P2 reductions. We
404 then calculated the standard error of the difference between observed and
405 expected P2 reductions, as given by the equation

$$407 se_{O-E} = \sqrt{\frac{\sigma_O^2}{n_O} + \frac{\sigma_E^2}{n_E}},$$

408 where σ^2 is the variance, n is the sample size, and O and E refer to the
409 observed P2 effects in the placebo experiment and the expected placebo effects
410 in the intensity mapping experiment, respectively. Degrees of freedom
411 are given by the Satterwaite approximation. This allowed us
412 to perform a t test of the difference between observed and expected P2
413 reductions, where the null hypothesis is a placebo effect produced only
414 by intensity reduction.

415 3. Results

416 3.1. Placebo effects in pain ratings

417 There was a strong effect of Placebo on pain intensity
418 ratings. For Run 1, $C-P = 0.64$, $t(38) = 4.26$, $p = .0001$,

¹ Ranked reported placebo was computed by taking the ranks of data (1– n participants, where the lowest score is assigned rank 1, the second lowest rank 2, and so on) and using those as a continuous predictor in the GLM. This is a typical procedure for making a test nonparametric, because using ranks limits the influence of extreme cases on the regression outcome and avoids problems relating to violations of the normality assumption.

419 indicating that pain ratings were decreased with the
420 placebo treatment. For Run 2, effects were also significant,
421 $C-P = 0.65$, $t(38) = 4.75$, $p < .0001$. Adding Run as a
422 factor, the effect of Run was significant, effect = 0.19,
423 $t(37) = 2.07$, $p = .04$, indicating that participants habituated
424 to the laser stimulus. The effect of placebo remained
425 significant, $C-P = 0.64$, $t(37) = 4.30$, $p < .0001$. There was
426 a trend towards a Placebo \times Order interaction,
427 $t(37) = 1.90$, $p = .066$. Mean pain ratings for the P first
428 group were 2.40 and 1.57 for C and P, respectively, and
429 means for the C first group were 1.47 and 1.15. Placebo
430 effects were significant for both order groups: for P first,
431 $C-P = 0.82$, $t(19.0) = 4.00$, $p = .0008$, and for C first,
432 $C-P = 0.46$, $t(18.0) = 2.90$, $p = .009$. No other interactions
433 were significant.

434 Examining bivariate correlations revealed that placebo
435 scores ($C-P$) in reported pain were positively correlated
436 with overall pain ($r = .41$, $p < .05$), but not with other variables
437 (stepwise regression confirmed these results). After
438 controlling for this variable, placebo effects in reported
439 pain remained significant, $t(22) = 2.25$, $p = .035$. The relationship
440 between placebo and reported pain appeared to be
441 due to the fact that a number of participants ($n = 11$) rated
442 the laser stimuli as non-painful, i.e., an average score below
443 1, where 1 was defined as a “painful pinprick” and 0 was a
444 “non-painful pinprick.” These participants still reported a
445 placebo effect [$C-P$] = 0.33, $t(10) = 2.29$, $p = .04$ (the non-
446 parametric Wilcoxon sign-rank test was also significant,
447 $Z = -2.05$, $p = .04$). However, the placebo effect was much
448 reduced compared with that of the remaining 24 participants,
449 [$C-P$] = 0.79, $t(23) = 3.97$, $p = .001$; Wilcoxon
450 $Z = -2.83$, $p = .005$. Because we were interested in placebo
451 effects in pain, participants who did not find the stimulation
452 painful were excluded from further analyses.

453 3.2. Placebo effects in P2 amplitude

454 A planned test on Run 1 showed that Placebo was significant,
455 $t(21) = 2.37$, $p = .013$ one-tailed (equivalent
456 ANOVA $F(1, 21) = 5.63$). The result, shown in Fig. 2, provides
457 evidence that P2 amplitude was reduced in the placebo
458 condition (a 16.7% reduction on average at Electrode Cz).
459 However, placebo effects were negligible in Run 2. Across runs,
460 Placebo showed a trend towards significance, $t(21) = 1.59$
461 ($F = 2.53$), $p = .063$ one-tailed, an 8.8% reduction at
462 electrode Cz. The Placebo \times Run interaction was significant,
463 $t(21) = 1.75$ ($F = 3.06$), $p = .047$ (one-tailed), demonstrating
464 that as expected, placebo effects were reduced in Run 2. Placebo
465 did not interact with Electrode, $F(2.0, 42.0) = 0.13$, $p > .8$.

466 There was a strong effect of Run, $F(1, 21) = 16.37$,
467 $p < .0001$, indicating that P2 amplitudes decreased substantially
468 with repeated testing. There was a Placebo \times Order interaction,
469 $F(1, 21) = 6.37$, $p = .02$, indicating that placebo effects were
470 stronger in the C first group (3.72 μ V for C first vs. -1.16 μ V
471 for P first at Cz). A significant Electrode \times Placebo \times Order
472 effect $F(2.0, 42.0) = 3.91$, $p = .03$
473

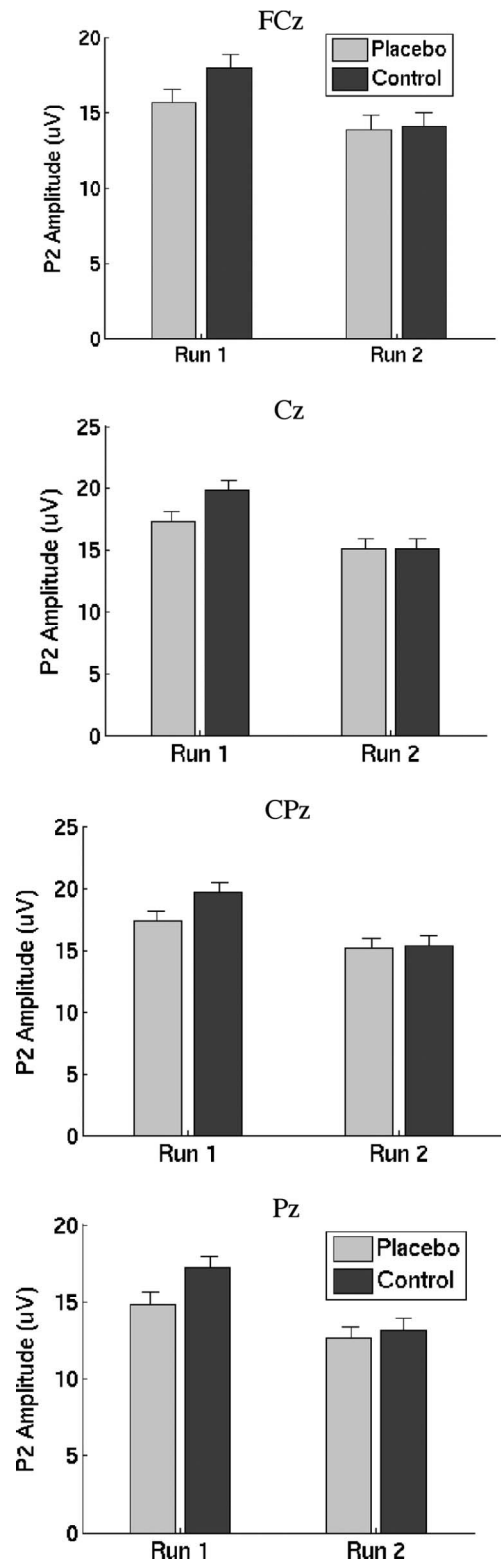


Fig. 2. P2 amplitudes by placebo condition and run for each electrode, controlling for order of testing. Notably, placebo reductions in P2 amplitude are apparent in all electrodes for Run 1, but not Run 2.

provided largely redundant information (max. $F = 2.28$, $p > .11$). Other effects were not significant, including between-subjects effects of RP (all p 's $> .2$). Controlling for Order, partial correlations between C–P P2 effects and RP ranged from $r = 0.01$ to 0.18 across electrodes, and from $r = -.16$ to $.02$ for run 1 only (all $p > .10$). Additional multiple regressions showed no effects of laser intensity or reported pain on placebo responses, suggesting that these were not confounding or masking variables.²

In a second GLM model, we considered only Responder and Nonresponder subgroups. A planned test on Run 1 showed that Placebo was significant, $t(13) = 2.53$, $p = .013$ one-tailed (equivalent two-tailed ANOVA $F(1, 13) = 6.39$, $p = .025$), indicating that P2 amplitudes were reduced with placebo. Again, placebo effects were negligible in Run 2. Across runs, Placebo was significant, $t(13) = 2.11$ ($F = 4.48$), $p = .027$ one-tailed. Placebo \times Responder Status, which would indicate a difference in P2 placebo effects between RP responder groups, was not significant. Neither was Run \times Responder Status. However, the Placebo \times Run \times Responder Status interaction was significant, $F(1, 13) = 6.58$, $p = .02$. Examination of the means suggested that Responders showed placebo reductions in P2 amplitude in both runs, whereas Nonresponders showed it only in Run 1. The Placebo \times Responder Status relationship did not interact with Electrode. Other effects were qualitatively similar to those reported above and do not change the interpretation of results.

Grand averages, illustrated in the top panels of Fig. 3, show the N2 and P2 responses across the 24 subjects included in our analyses. These generally did not show apparent differences between C and P (thin black line and thicker gray line, respectively), which might be expected given the individual variability in the amplitude and latency of reported peaks. The middle and bottom panels of Fig. 3 show grand averages for Responders ($n = 8$) and Nonresponders ($n = 8$), respectively. Responders show an apparent decrease in P2 with placebo, though the effect of Responder Status was not significant in P2 peak amplitude, as described above.

² We tested for effects of laser intensity and overall pain by using R^2 change tests. C–P placebo scores were used as the dependent variable. The basic (reduced) model included only administration Order, Reported Placebo (Placebo), and their interaction. To test effects of intensity, we added intensity and its interactions with Placebo and order. Thus, the full model included Order, laser intensity, Placebo, and all two-way interactions. For Cz, The increase in R^2 compared with the reduced model was 0.20 , $F(3, 17) = 2.29$, $p = .11$, which did not provide convincing evidence for intensity effects. In a second analysis, we added overall pain to the reduced model, so that the full model included Order, Placebo, reported overall pain, and all two-way interactions. This model did not account for significantly more of the variance in P2 placebo effects, R^2 change = 0.11 , $F(3, 17) = 1.09$, $p = .3817$. Analyses showed similar results for other electrodes.

474 suggested that the order effects were strongest at the
475 anterior sites.

476 Electrode did not interact with other effects, consistent
477 with our expectations that the midline electrodes recorded

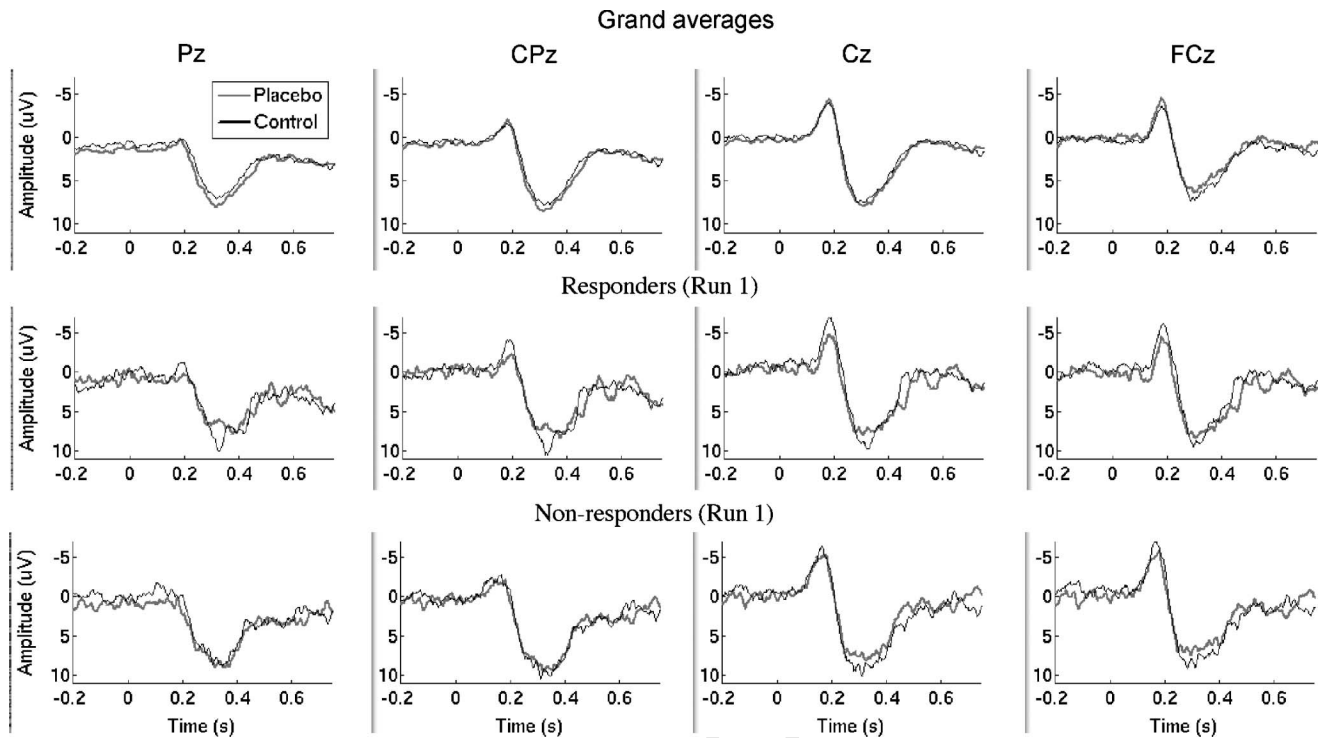


Fig. 3. Top row: grand averages by placebo condition across all runs and participants. For display, data were not adjusted for baseline activity. Responses in the control condition are shown by the thin black lines, and in the placebo condition in thicker gray lines. Middle row: grand averages by placebo condition for the placebo Responder group ($n = 8$). Bottom row: grand averages for Nonresponders ($n = 8$).

518 3.3. Relationship of N2 and P2 amplitude to intensity and 519 reported pain

520 We conducted a preliminary experiment to assure that
521 LEP responses were sensitive to laser intensity variations
522 and test the intensity reduction hypothesis. Results of the
523 preliminary experiment are shown in Fig. 4. The scatterplots
524 show how N2 and P2 vary with reported pain intensity. Dif-
525 ferent subjects are plotted with different symbols. N2 did not
526 vary in a systematic way with laser intensity ($R^2 = 0.0$,
527 $F = 0.0$, $p = .99$) or pain ($R^2 = 0.002$, $F = 0.085$, $p = .77$).
528 These relationships are shown in Figs. 2A and B. Because
529 N2 amplitude was not sensitive to changes in laser intensity
530 or pain, we did not analyze it further.

531 P2 amplitude, however, increased significantly with
532 increasing laser intensity ($R^2 = 0.658$, $F = 57.77$,
533 $p < .0001$) and pain ($R^2 = 0.42$, $F = 24.7$, $p < .0001$). We
534 found that the relationship between P2 amplitude and pain
535 ratings was fit best by a power function (though other func-
536 tions provided similar fits). The best-fitting function was
537 $P2 = 8.1X^{0.78}$ (where $X =$ pain rating), which was roughly
538 linear in the range of reported intensities observed in our
539 experiment (1–5; see Fig. 2C).

540 We applied the same procedure to the relationship
541 between P2 amplitude and laser intensity to estimate the
542 effective reduction in laser intensity for the observed place-
543 bo effects. The best-fitting function was a sigmoid,
544 $P2 = e^{(5.5 - 1622/X)}$, where $X =$ laser intensity. While the rela-
545 tionship between P2 amplitude and reported pain was

roughly linear, the relationship with laser intensity was 546
nonlinear, as shown in Fig. 2D. In particular, much larger 547
decreases in laser intensity are required to achieve a unit 548
change in P2 amplitude if intensity and reported pain are 549
low. The expected P2 placebo effect, a reduction from 18 550
to 14 μV , is in a sensitive part of the curve in Fig. 2D, as 551
the response is roughly linear between 10 and 20 μV (corre- 552
sponding approximately to 400–700 mJ). The observed P2 553
amplitudes of 18.6 μV for Run 1 and 15.1 μV for Run 2 are 554
also in the sensitive portion of the curve, indicating that P2 555
amplitude was expected to be responsive to placebo 556
suppression of nociceptive input. 557

518 3.4. Testing the intensity reduction hypothesis 558

559 We next compared observed P2 placebo effects with
560 those expected under the intensity-reduction hypothesis,
561 as shown in Fig. 5. The x -axis shows reported pain for both
562 placebo ($n = 24$) and intensity-mapping ($n = 10$) groups.
563 The y -axis shows P2 amplitude in both experiments, for
564 both runs together (left panel) and for Run 1 only (right
565 panel). The solid line is the regression line for P2 amplitude
566 regressed on reported pain ($n = 10$, with 90% confidence
567 bands), and the expected placebo reduction is shown as
568 the lower of two dashed lines on the y -axis. The darker
569 and lighter circles in Fig. 5 show the 90% confidence inter-
570 vals for C and P effects ($n = 24$; though placebo effects were
571 calculated as within-subjects contrasts), and the uppermost
572 dashed line on the y -axis shows the observed placebo effect.

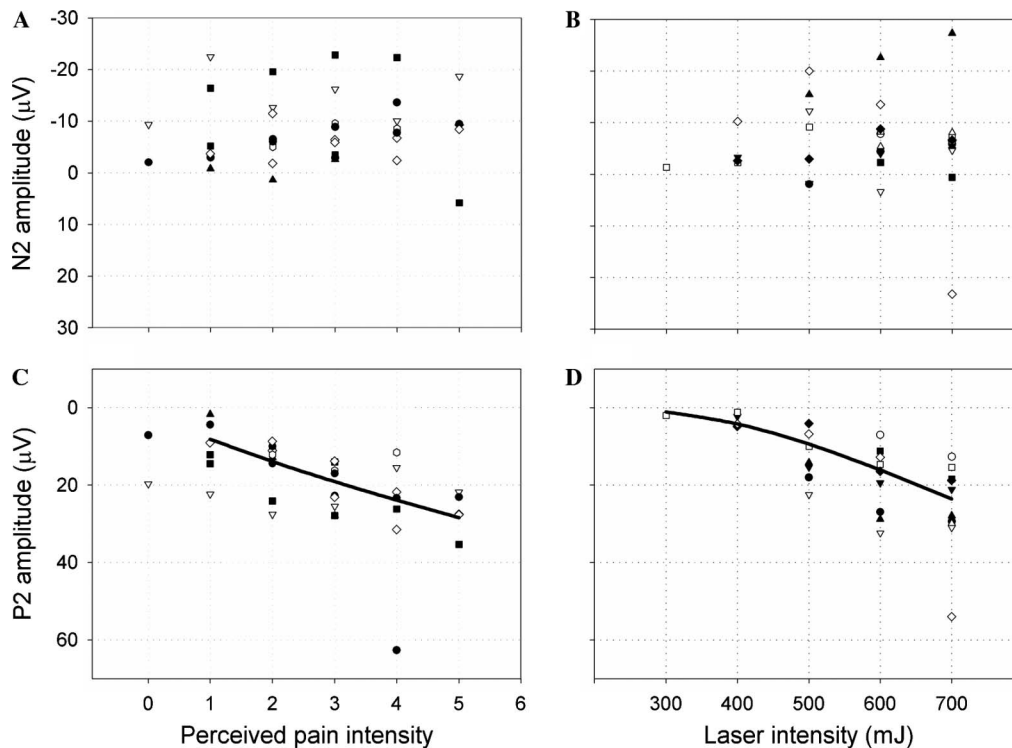


Fig. 4. Scatterplots of N2/P2 amplitude vs. intensity. The relationship between LEP amplitude and perceived and applied intensity was determined in a separate experiment on ten subjects who each received 50 stimuli at five different laser intensities. This enabled us to test whether P2 placebo effects were lower than would be expected if placebo worked only by reducing nociceptive input. N2 amplitude did not change with either perceived intensity (A) or laser intensity (B). P2 amplitude changed with both perceived intensity (C) and laser intensity (D). A power equation best described the overall relationship in (C), though the response was not essentially linear in the range of reported pain placebo effects in the main study, whereas a *S* function best described the relationship in (D). To estimate expected P2 amplitude for a given change in perceived intensity, linear regressions were computed within each participant and the variance in predicted values corresponding to reported pain and placebo effects was assessed across participants.

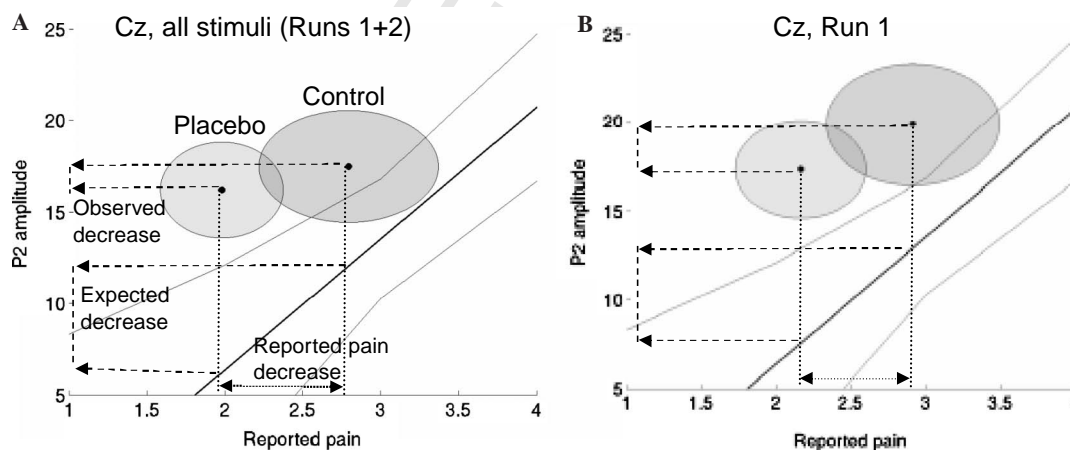


Fig. 5. Test of the intensity reduction hypothesis of placebo. The solid line is the regression slope for the relationship between reported pain and P2 amplitude in the preliminary experiment ($n = 10$). Light gray lines show 90% confidence intervals, corresponding to the one-sided hypothesis of expected decreases in P2 with decreasing pain. The circles show 90% confidence regions in the control and placebo conditions ($n = 24$). Dashed lines on the *y*-axis show the observed placebo decrease and the expected decrease if the same reported pain decrease were produced by an intensity reduction. Confidence regions shown are between subjects, though tests of control–placebo and expected decreases were calculated within subjects. The observed decrease is significantly smaller than the expected decrease across both runs (A), but is not significantly different in the first run alone (B).

573 Notably, P2 amplitudes were lower (and/or reported pain
 574 was higher) for the intensity-mapping group overall than for
 575 the placebo group. This effect may have multiple causes,
 576 including differences between subjects, the placebo context,

and the length of the test. (Reported pain habituates, and
 577 the intensity-mapping test was shorter.) However, what is
 578 critical is the within-subject differences between higher and
 579 lower reported pain. If the relationship between P2 and
 580

581 reported pain is nonlinear, then the difference in offset
582 between groups could make interpretation of the critical
583 reductions in P2 between groups problematic; it would be
584 difficult to tell a whether the difference is due to differences
585 in the magnitude or the scale of the response. However,
586 Fig. 2 shows that the relationship is roughly linear across
587 the range of intensities tested, so the intensity-reduction
588 hypothesis predicts equal reductions in P2 per unit reduction
589 in reported pain for the placebo and intensity-mapping
590 groups.

591 The observed placebo reduction in P2 was 1.28 μV , with
592 a variance of 20.47 μV^2 ($n = 24$; left panel, top dashed line
593 on y -axis). The expected placebo reduction due to an inten-
594 sity decrease was 5.65 μV , with a variance of 29.36 μV^2 (left
595 panel in Fig. 5, bottom dashed line on y -axis). The differ-
596 ence between observed and expected P2 reductions of
597 4.37 μV was significant, $t(14.5) = -2.25$, $\text{SE} = 1.95$,
598 $p = .02$. The overall difference between P2 amplitudes for
599 the placebo and intensity-mapping groups suggests that
600 P2 amplitudes were higher in the placebo experiment, but
601 the critical effect of interest is the relative drop in P2 ampli-
602 tude with a decrease in reported pain.

603 For Run 1 only (right panel of Fig. 5), the observed
604 decrease was 2.80 μV , with a variance of 41.42 μV^2 . The
605 RP effect of 2.90 μV for C vs. 2.187 μV for P led to an
606 expected P2 decrease of 5.11 μV , with a variance of
607 24.14 μV^2 . The difference between observed and expected
608 P2 placebo effects of 2.31 μV was not significant,
609 $t(22.1) = -1.14$, $\text{SE} = 2.03$, $p = .13$. Adjusting for order
610 did not change the results. Thus, reduction of input (i.e.,
611 early pain inhibition) is a possible cause of reported place-
612 bo effects in Run 1, but is unlikely to be an adequate expla-
613 nation for reported placebo effects overall.

614 4. Discussion

615 In this study, we recorded evoked brain potentials from
616 midline electrodes in response to painful laser stimuli
617 (LEPs) to test for placebo effects on early nociceptive pro-
618 cessing. We compared stimulation of placebo-treated skin
619 (P) with stimulation of control-treated skin (C). The oint-
620 ments applied to each skin area were identical; the only dif-
621 ference was the induction of expectations of pain relief in
622 the placebo condition. We observed robust placebo effects
623 on reported pain, consistent with previous studies (Price
624 et al., 1999; Vase et al., 2005; Voudouris et al., 1989; Wager
625 et al., 2004). In addition, our results show that placebo
626 treatment reduced P2 LEP amplitude, particularly in the
627 first blocks of stimulation (Run 1). These effects were
628 observed to some degree at all electrodes (FCz, Cz, Cpz,
629 and Pz). Adjusting for Run and Order, placebo effects were
630 maximal at Cz (Table 1), although stronger effects of test-
631 ing order, habituation, and overall pain made simple C–P
632 effects most reliable at Pz. The N2 component was insensi-
633 tive to changes in applied pain intensity and to placebo.

634 A reduction in P2 with placebo is consistent with the mod-
635 ulation of pain affect in anterior cingulate suggested by other

636 studies (Rainville et al., 1999; Wager et al., 2004), as cingu-
637 late is a likely generator of the laser-evoked P2 (Garcia-Lar-
638 rea et al., 2003; Iannetti et al., 2004; Lenz et al., 1998).
639 However, we recorded only midline electrodes in the current
640 study and cannot do source localization, which is a limitation
641 of the current study. Because the P2 often has a latency
642 around 300 ms, there is some debate about whether it reflects
643 a P300, but previous work suggests that the P2 and P300 are
644 dissociable (Lorenz et al., 1997a,b; Lorenz and Garcia-Lar-
645 rea, 2003), though the P2 may overlap with or be augmented
646 by the P3a. In our study, the P2 was pain responsive and the
647 stimulus did not vary across trials, making it unlikely that the
648 effect is a classic P3, though a P3a could have produced the
649 responses, particularly as placebo effects were present only
650 in Run 1. The idea that the P2 modulation we observed
651 reflects changes in cognitive appraisal of the pain stimulus
652 is consistent with theories about expectancy-based placebo
653 effects (Wager, 2005a,b).

654 4.1. Factors influencing brain placebo effects

655 The analyses revealed three factors that are important to
656 consider when studying placebo effects in LEPs. The first is
657 overall pain intensity. Consistent with our previous work,
658 if stimulation is less painful (or not painful), then placebo
659 effects are reduced. Thus, care must be taken to ensure that
660 the stimulus is painful enough. One reason for this is that
661 placebo may work by reducing anxiety (Vase et al.,
662 2005), and larger placebo effects may be elicited if the situ-
663 ation is anxiogenic (Staats et al., 2001). A second explana-
664 tion could be that pain and placebo both induce
665 endogenous opioid release, and these two effects are non-
666 additive (see Zubieta et al., 2005, for an example of placebo
667 opioid release specific to pain), implying that both pain and
668 expectation are required for a placebo opioid response
669 (Fields, 2004). Finally, it could simply be placebo effects
670 are lower with reduced pain due to floor effects. In this
671 study, LEP analyses were conducted on participants who
672 reported that the stimulus was painful.

673 A second factor to consider is habituation over time dur-
674 ing testing, which influenced the placebo effect size in P2
675 LEPs, but not in reported pain. As found in other studies
676 of LEPs (Garcia-Larrea et al., 2003), P2 effects habituate
677 more than reported pain does. The habituation of P2 but
678 not reported placebo effects constitutes a striking dissocia-
679 tion between brain and self-report measures of pain process-
680 ing. We anticipated the habituation of placebo effects due to
681 the decrease in reported pain over time, but overall intensity
682 decreases may not be sufficient to explain the effect.

683 What, then, might cause the habituation of placebo
684 effects in LEPs? One possibility is that the effect of placebo
685 on early pain processing is due to anxiety reduction, and
686 thus has a most pronounced effect on the initial pain stimu-
687 li. Participants report that early trials are substantially
688 more painful than later trials, possibly due to anxiety and
689 uncertainty about the stimulus and testing situation. A sec-
690 ond possibility is that placebo treatment can elicit an early,

Table 1
Amplitudes and latencies for evoked potential components N2 and P2 across experimental condition (Placebo or Control), Order of stimulation (P first or C first) and Run

	Placebo				Control			
	P first		C first		P first		C first	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
N2 amplitude (μ V)								
FCz	-10.35 (2.32)	-10.69 (2.71)	-9.59 (2.61)	-6.62 (2.18)	-11.36 (2.59)	-10.67 (2.62)	-11.11 (3.16)	-8.76 (2.44)
Cz	-12.16 (2.24)	-12.43 (2.45)	-8.13 (2.29)	-5.59 (1.93)	-12.69 (2.21)	-11.67 (2.27)	-9.65 (3.06)	-8.28 (2.34)
CPz	-9.19 (1.94)	-10.00 (2.06)	-5.05 (1.56)	-3.11 (1.35)	-8.83 (1.74)	-8.34 (1.95)	-5.91 (2.18)	-5.52 (1.69)
Pz	-5.96 (1.41)	-7.12 (1.48)	-3.14 (0.87)	-2.21 (0.99)	-5.66 (1.17)	-5.90 (1.49)	-3.57 (1.48)	-3.79 (1.22)
P2 amplitude (μ V)								
FCz	16.26 (1.60)	13.72 (1.09)	14.76 (2.49)	14.05 (2.50)	15.45 (1.40)	12.50 (1.40)	20.85 (3.29)	15.84 (3.29)
Cz	18.13 (2.00)	15.43 (1.75)	16.21 (2.60)	14.68 (2.61)	17.76 (2.25)	13.84 (1.49)	22.21 (3.36)	16.58 (3.36)
CPz	17.86 (1.65)	15.49 (1.75)	16.64 (2.66)	14.69 (2.56)	18.04 (2.12)	14.44 (1.39)	21.40 (3.46)	16.29 (3.25)
Pz	15.03 (1.43)	12.89 (1.39)	14.56 (2.38)	12.34 (2.16)	15.69 (1.69)	12.05 (1.04)	18.86 (3.01)	14.41 (2.69)
N2 latency (ms)								
FCz	201.8 (15.3)	187.2 (4.2)	183.7 (7.4)	201.8 (12.5)	190.7 (6.6)	204.3 (14.8)	206.7 (15.4)	209.7 (15.1)
Cz	183.3 (8.2)	180.3 (4.4)	182.8 (7.3)	187.2 (9.9)	186.5 (7.5)	200.3 (7.9)	191.0 (11.5)	202.2 (15.6)
CPz	186.8 (6.8)	191.7 (8.7)	178.3 (8.1)	193.0 (9.8)	196.7 (8.4)	197.5 (7.2)	204.5 (13.5)	184.8 (8.1)
Pz	188.3 (7.4)	193.3 (9.3)	196.3 (12.5)	211.8 (11.4)	203.2 (8.7)	197.7 (8.7)	206.0 (14.2)	196.3 (13.0)
P2 latency (ms)								
FCz	321.5 (18.3)	319.0 (12.8)	327.5 (18.5)	340.3 (15.7)	322.0 (14.1)	333.7 (17.5)	328.3 (13.9)	350.2 (22.2)
Cz	314.5 (16.5)	319.7 (11.8)	338.7 (14.7)	344.2 (14.3)	320.7 (12.3)	321.0 (13.4)	320.8 (11.5)	344.3 (13.1)
CPz	321.0 (16.9)	339.0 (15.5)	334.7 (12.9)	353.7 (10.8)	330.7 (14.6)	331.0 (12.7)	334.5 (12.5)	346.0 (13.0)
Pz	322.3 (16.5)	340.0 (12.9)	343.7 (14.0)	363.2 (13.9)	333.3 (14.5)	339.5 (11.8)	338.5 (14.3)	367.5 (16.6)

Values are mean and standard error ($n = 24$).

691 pre-stimulation opioid release—but once the opioid system
692 is activated by painful stimulation, the placebo treatment
693 offers little additional benefit. Either of these scenarios
694 involves the additional assumption that reported pain is
695 influenced by judgments about the experimental context
696 (e.g., the stimulus history) in ways that LEPs are not.
697 For instance, pain reports may be subject to hysteresis or
698 self-consistency biases. That is, if pain is initially high, then
699 participants may form an overall impression of the stimulus
700 as painful and continue to report high levels of pain
701 even when the stimulation is reduced.

702 A third factor is the order of testing (control first or
703 placebo first), which were strongest at Cz. We observed
704 strong placebo effects in the C first group, but no reliable
705 differences in the P first group. This could be because of
706 a real psychological difference between receiving C first
707 or P first, but it could also simply reflect habituation
708 to the stimulus across the first (C or P) and second (C
709 or P) testing blocks, for the following reason: If P2
710 effects habituate, as we observed, then even if there were
711 no true effect of placebo, we would expect to observe
712 C–P differences in the C first group and P–C differences
713 of equal magnitude in the P first group, creating an
714 apparent Placebo \times Order interaction. Another way of
715 saying this is that our design, which was intended to
716 study placebo effects overall but not differences in placebo
717 due to administration order, cannot separate effects of
718 habituation from psychologically caused Placebo \times Order
719 interactions. Thus, we cannot say whether psychobiological
720 placebo effects are larger in the C first group. Future

721 studies with additional subject groups that receive only
722 placebo or only control stimulation across the entire
723 experiment may resolve this ambiguity.

724 Critically for our main hypotheses, however, all our
725 analyses controlled for testing order in the GLM. A significant
726 placebo effect, controlling for order, is statistically
727 equivalent to testing whether C–P effects in the C first
728 group are larger than P–C effects in the P first group,
729 and implies that the placebo treatment had an impact independent
730 of the effects of habituation. We observed strong
731 placebo effects in the C first group, but no reliable differences
732 in the P first group, consistent with the idea that both
733 placebo effects and habituation influence P2 responses.
734 Thus, we can reject the null hypothesis that placebo treatment
735 had no effect, but we cannot assess whether the testing
736 order has a real psychological impact on placebo
737 responses.

738 4.2. The intensity-reduction hypothesis

739 The strongest hypothesis for why brain placebo effects
740 may be observed is that placebo treatment blocks nociceptive
741 input to the brain, thus reducing the impact of those
742 signals on brain activity. Recent evidence from a secondary
743 hyperalgesia paradigm suggests that there may be a spinal
744 inhibition component to placebo (Matre et al., 2006). Thus,
745 goal of this research was to test whether the magnitude of
746 placebo effects in LEPs is consistent with spinal pain inhibition.
747 We equated the reported pain decrease in the placebo
748 experiment ($n = 24$) and a separate laser intensity-

749 mapping experiment ($n = 10$), and asked whether there
750 were also equivalent reductions in P2 amplitude. We found
751 that P2 placebo effects in the first run were strong enough
752 to be consistent with an intensity reduction, but that P2
753 placebo effects collapsing across runs were significantly
754 smaller than would be expected under the intensity reduc-
755 tion hypothesis.

756 This finding is consistent with the finding that placebo
757 effects in P2 were only found in the first run, but equivalent
758 reported placebo effects were found in both runs. Together,
759 the selective habituation of placebo effects in LEPs and the
760 intensity-reduction results suggest that placebo effects on
761 early nociceptive processing (including spinal inhibition
762 and attention- or affect-related effects) are not the only
763 component of placebo analgesia in reported pain. Thus,
764 the model that placebo effects are either completely medi-
765 ated by spinal inhibition or they are not is probably too
766 simplistic. An alternative is that there are multiple compo-
767 nents of a placebo response, including effects on central
768 processing of pain affect (Wager, 2005a,b) and on cognitive
769 judgments about pain (Clark, 1969).

770 An alternative account is that different regions of the
771 cingulate (the presumed source of the P2) have different
772 phasic responses to the laser stimulus, and that placebo
773 effects in different directions (increases vs. decreases in
774 activity) in these regions offset one another, producing
775 smaller than expected placebo decrements in P2. LEP
776 analysis cannot discriminate the activity of multiple cingu-
777 late subregions based on spatial location, but independent
778 components analysis may reveal whether there are multi-
779 ple superimposed effects hidden within the overall P2
780 response. Supplementary independent components analy-
781 sis (ICA) did not show evidence for multiple components
782 that are affected differentially by placebo (data not shown
783 for space reasons). In addition, for this hypothesis to
784 explain the pattern of results, placebo-induced increases
785 in a subcomponent of P2 would have to increase over
786 the course of the session (between Run 1 and Run 2);
787 we are not aware of evidence that might support the exist-
788 tence of such an effect.

789 4.3. Correspondence between P2 and reported placebo effects

790 A related question is whether those participants who
791 reported the largest placebo reductions in reported pain
792 also showed the largest P2 placebo reductions. We looked
793 for these effects in two ways: by using ranked reported
794 effects in the GLM analysis and by comparing P2 effects
795 for the highest and lowest thirds of the group on reported
796 effects. None of these tests were significant, although the
797 grand averages (Fig. 4) show effects of Responders and
798 Nonresponders in the appropriate direction. The low
799 brain-behavior correlations are consistent with the notion
800 that placebo effects on reported pain involve multiple
801 components, only one of which is an effect on early noci-
802 ceptive processing. The existence of both early (LEP-influ-
803 encing) and late (affect or cognitive judgment)

804 components of reported placebo effects would make rela-
805 tionships between P2 effects and reported effects difficult
806 to detect.

807 5. Conclusions

808 In this study, we report that a placebo treatment pro-
809 duced detectable amplitude decreases in the P2 compo-
810 nent of laser-evoked pain potentials. Brain placebo
811 responses were large enough to reflect a meaningful dif-
812 ference in nociceptive processing, but the effects were
813 smaller than the very robust decreases in reported pain.
814 Placebo responses in P2 potentials were smaller than
815 those expected if the entire reported response were pro-
816 duced by a decrease in nociceptive input, suggesting that
817 there are both early and late phases of the placebo
818 response.

819 6. Uncited references

820 Hrobjartsson and Gotzsche (2004), Kirsch (1985), Kirsch
821 and Scoboria (2001), Lazarus (1991), Moerman (2000).

822 Acknowledgments

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