Placebo analgesia is not due to compliance or habituation: EEG and behavioural evidence

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This study was designed to resolve whether experimental placebo responses are due to either increased compliance or habituation. We stimulated both forearms and recorded laser-evoked potentials from 18 healthy volunteers treated on one arm with a sham analgesic cream and an inactive cream on the other (treatment group), and 13 volunteers with an inactive cream on both arms (controls). The treatment group showed a significant reduction in the pain ratings and laser-evoked potentials with both the sham and inactive creams. The control group showed no evidence of habituation to the laser stimulus. The results indicate that the reduction in pain during experimental placebo response is unlikely to be due to sensory habituation or compliance with the experimental instructions. *NeuroReport* 18:771–775 © 2007 Lippincott Williams & Wilkins.

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Introduction

Placebo analgesia is the reduction in pain sensation following the administration of a pharmacologically inert substance in the guise of an analgesic drug or pain-relieving procedure. Understanding the psychophysiological mechanisms mediating placebo analgesia is important when designing and interpreting clinical trials. Experimental placebo analgesia has been demonstrated using expectation and conditioning cues [1–4]. Watson *et al.* [4] suggested that individuals can respond to placebo conditioning differently depending on their expectation of pain relief; with some participants showing a placebo response in unconditioned sites. It could, however, be argued that the subjective report of reduced pain could be due either to habituation to the painful stimulus or to compliance (conforming to the expected behaviour by overriding the actual pain sensation and reporting a reduced pain rating). In which case, there would be no physiological reduction in nociceptive processing in response to the laser stimulus itself. It is therefore important to determine whether experimental placebo responses reflect a real difference in nociceptive processing.

Laser-evoked potentials (LEPs) are used as an indicator of cortical responses to pain [5], their amplitudes reflecting both stimulus intensity and reported pain [6,7]. The largest LEP peak is maximal at the vertex, and is thought to originate in the anterior cingulate gyrus [8] which plays a central role in pain modulation by placebo [9]. Wager *et al.* [10] have shown a reduction in LEP amplitude after placebo treatment providing an objective measure of placebo effect.

In this study, participants received both a placebo and a control cream in the same session and on the same arm. Although the order was randomized, participants habituated to the laser stimulus. It was therefore not possible to know to what extent the placebo effect was due to habituation. Wager *et al.* suggested that separate groups of control and placebo participants could resolve this ambiguity.

The aim of this study was to investigate whether the apparent experimental placebo responses observed on conditioned and unconditioned sites are due to participant compliance or habituation or a combination of both.

Methods

Participants

The study was approved by the Local Research Ethics Committee. Thirty-one healthy, right-handed participants (age range 19–36 years) gave their informed consent to take part in the study. Participants were randomly divided into two groups: a treatment group (eight women, ten men) and a control group (six women, seven men). Participants who had previously used local anaesthetic creams were excluded from the study.

Laser stimuli

The pain stimuli were delivered by a CO_2 laser (pulse duration 100 ms, beam diameter 15 mm) at 10-s intervals to a 5×3 cm stimulation area marked on the dorsal surface of each forearm. Stimuli were randomly moved around each stimulation area, to minimize sensitization and/or habituation. For each stimulation block, 20 laser stimuli were delivered to each arm in random order.

Participants were trained to rate the pain of each laser stimulus using a 0–10 pain scale, where 0=no sensation, 4=just painful and 10=worse imaginable pain possible. This scale allowed the participants to rate stimuli they perceived as nonpainful. At the start of the study, we determined the laser energies corresponding to each individual's nonpainful level 3 ($5.9 \pm 2\,mj/mm^2$) and moderately painful level 7 ($11.0 \pm 1.7\,mj/mm^2$) using a series of stimuli of ascending intensities, and we checked for reproducibility.

Experimental design

Treatment (placebo) group

The participants' forearms were randomly labelled A and B (counterbalanced across participants). Arm A was subsequently conditioned in the treatment group (but not in the control group). Participants in the treatment group were told that they would receive a local anaesthetic on one arm, but they were not told which arm. They were also told that inactive cream would be applied to the other arm. In fact, participants received an inactive cream on both arms. The experiment was divided into three blocks. The cream was applied in between blocks 1 and 2.

Block 1 (preconditioning)

Before the application of the cream, participants received 20 moderately painful (level 7) laser stimuli to each arm. They rated the level of pain of each stimulus.

Cream application

Inactive aqueous cream was applied to the entire laser stimulation area on both arms. The cream was applied, covered with an occlusive dressing and left in place for 10 min. Participants were told that the cream would take effect during this time. After this time the dressing was removed and the cream wiped off.

Block 2 (placebo conditioning)

The intensity of the laser stimuli delivered to arm A was reduced to each individuals' nonpainful level (level 3), whereas those delivered to arm B remained at the individuals' moderately painful level (level 7). Participants were not told that the intensity had been decreased for arm A. They received 20 laser stimuli to each arm, and rated the level of pain of each stimulus.

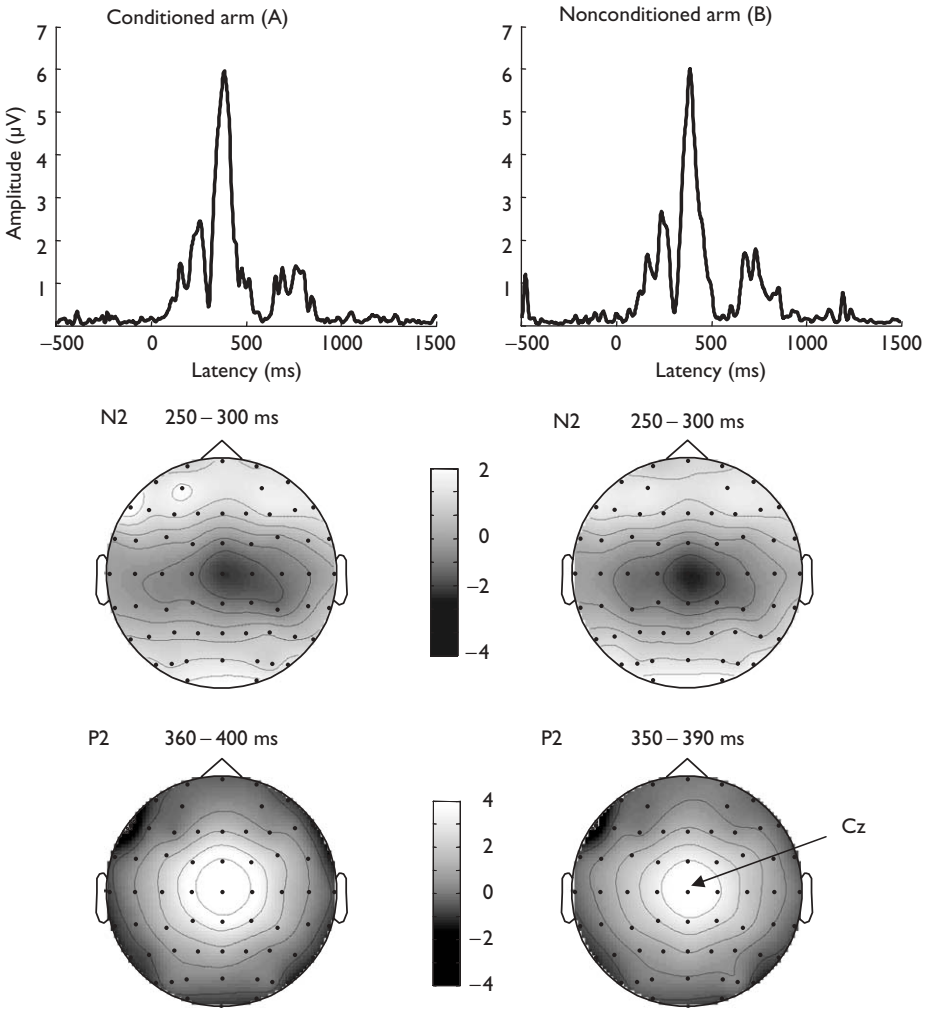

Fig. 1 Global field power (GFP) of the treatment group n=18 and topographic maps for the preconditioning block.

Block 3 (postconditioning)
This block was identical to the preconditioning block; in that 20 moderately painful (level 7) laser stimuli were delivered to each arm, participants rated the level of pain of each stimulus.

Control group
Participants underwent the same procedure as in the treatment group, but with different information. Participants were told that an inactive cream would be applied to both forearms and that, in block 2, the pain stimulus would be reduced to their predetermined nonpainful level on arm A. Participants rated the level of pain of each stimulus.

The energy of laser stimuli during blocks 1 and 3 for the treatment group and control group were identical (the individuals' predetermined level 7 of pain). Therefore, any difference in reported pain and amplitude of the LEP between the two groups (treatment versus control groups) would be attributed to the placebo effect.

Laser-evoked potential recording
Electroencephalographs (EEG) were recorded from electrodes Fz, Cz, Pz, C3, C4, (Quick-Cap system, Neuro Scan, Inc., El Paso, Texas, USA) and the right earlobe as reference, and subsequently transformed offline to common average reference. The sampling rate was 500 Hz, gain of 500, and band-pass filters of 0.15–70 Hz. Vertical and horizontal electrooculograms were also recorded for the purpose of ocular artefact correction. The impedance of each electrode was maintained below 5 kΩ.

Electroencephalograph data analysis
EEG data were analysed using Matlab 7.1 (Mathworks Inc., Natick, Massachusetts, USA). In each experimental block, vertical and horizontal eye movements were removed using independent component analysis [11] carried out on the continuous EEG data. Data were epoched between −500 and +1500 ms from onset of the laser pulse, baseline corrected to the prestimulus period and averaged. Where necessary, data from experimental blocks were smoothed with a 30 Hz low-pass filter (96 dB/Oct slope) before peak analysis, to further reduce muscle artefact. N2 and P2 LEP peak latencies were identified from the global field power plots for each individual for experimental block 1 (preconditioning) for both arms A and B. The different LEP peaks were defined in terms of their latency and topographic distribution.

Statistical analysis
The same statistical analysis was conducted on the behavioural and LEP data. For each individual and each block, the average from the 20 stimuli on each arm was calculated. Comparisons were made using repeated measures analysis of variance with factors blocks (1 and 3) × arms (A and B) × groups (treatment and control). Subsequent differences were further explored using within-group (paired) *t*-tests.

Results
No significant difference exists between the laser energies eliciting moderate (level 7) pain in the treatment and control groups, the same applied to the nonpainful level 3.

Behavioural results
Comparison of the pain ratings in the preconditioning and postconditioning blocks between the control and treatment groups revealed a significant effect of experimental block ($F_{1,29}$=14.71 P=0.001) and a group × block interaction ($F_{1,29}$=21.9 $P<0.001$). This demonstrates that the change in pain rating from the preconditioning to the postconditioning block is different between the two groups.

Paired *t*-tests for the control group showed no significant changes in pain ratings between the preconditioning [arm A 5.5 (SEM 0.24), arm B 5.2 (0.25)] and the postconditioning [arm A 5.7 (0.26), arm B 5.2 (0.30)] blocks.

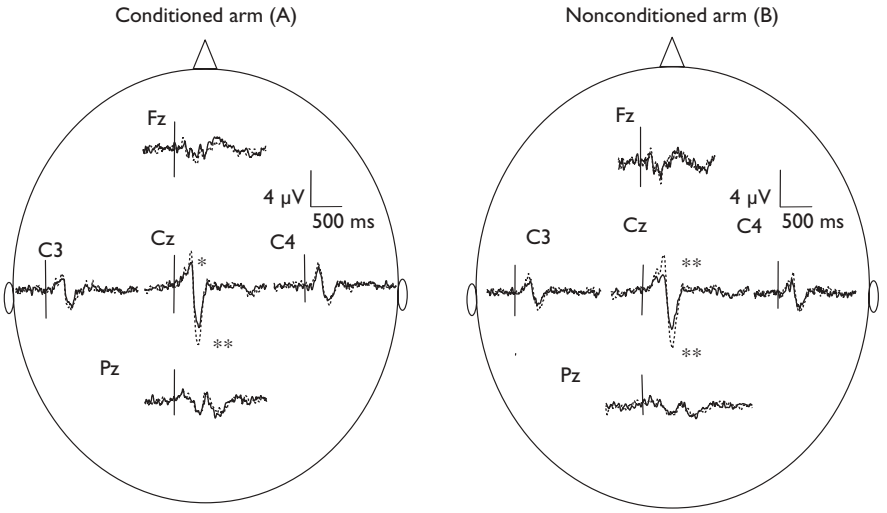

Fig. 2 Treatment group laser-evoked potential (LEP) waveforms (dotted line preconditioning block, solid line postconditioning block, the vertical line on the LEP waveforms indicates when the pain stimulus occurred). The same laser energy was used in the preconditioning and postconditioning blocks, set at each individual's moderately painful level. The placebo response is characterized by a reduction in the N2 and P2 peaks postconditioning at electrode Cz. *$P<0.05$, **$P<0.01$.

Paired *t*-tests for the treatment group showed that pain ratings were significantly reduced in the postconditioning block compared with preconditioning: Arm A pre 5.5 (SEM 0.22) and post 4.4 (SEM 0.33) (t_{17}=4.18 $P<0.01$); arm B pre 5.4 (0.21) and post 4.3 (0.26) (t_{17}=6.78 $P<0.001$). Therefore, the treatment group demonstrated a significant placebo response on both arms A and B.

Laser-evoked potential data

Global field power and grand average topographic plots for block 1 identified two main peaks: N2 264.8±6.6 ms and P2 374.1±9.8 ms for arm A; and N2 268.2±6.5 ms and P2 366.5±9.6 ms for arm B, maximal at electrode Cz (Figs 1 and 2).

No significant difference exists in LEP latencies for arms A and B between blocks 1 and 3 for either the treatment or the control groups; hence, analysis was confined to changes in LEP amplitudes.

Analysis of variance comparing the preconditioning and postconditioning blocks between the control and treatment groups revealed significant group × block interaction in both peaks (N2: $F_{1,29}$=6.1 $P<0.05$), (P2: $F_{1,29}$=9.2 $P<0.01$).

Paired *t*-tests for the control group showed no significant change in amplitudes between the preconditioning and postconditioning blocks in either arm ($P>0.1$) (Fig. 3a and c). Paired *t*-tests on N2 and P2 amplitudes of the treatment group (Fig. 3b and d) were significantly reduced from preconditioning to postconditioning, in both arms. N2 arm A (t_{17}=−2.19 $P<0.05$); N2 arm B (t_{17}=−2.91 $P<0.01$); P2 arm A (t_{17}=4.24 P=0.001); P2 arm B (t_{17}=3.82 P=0.001). The placebo effect seen in the behavioural results of the treatment group is also seen in the reduction in amplitudes of both N2 and P2 for arms A and B.

Discussion

This study investigated the electrophysiological correlate of placebo-induced reduction in pain ratings, to show that such reduction cannot be the result of compliance

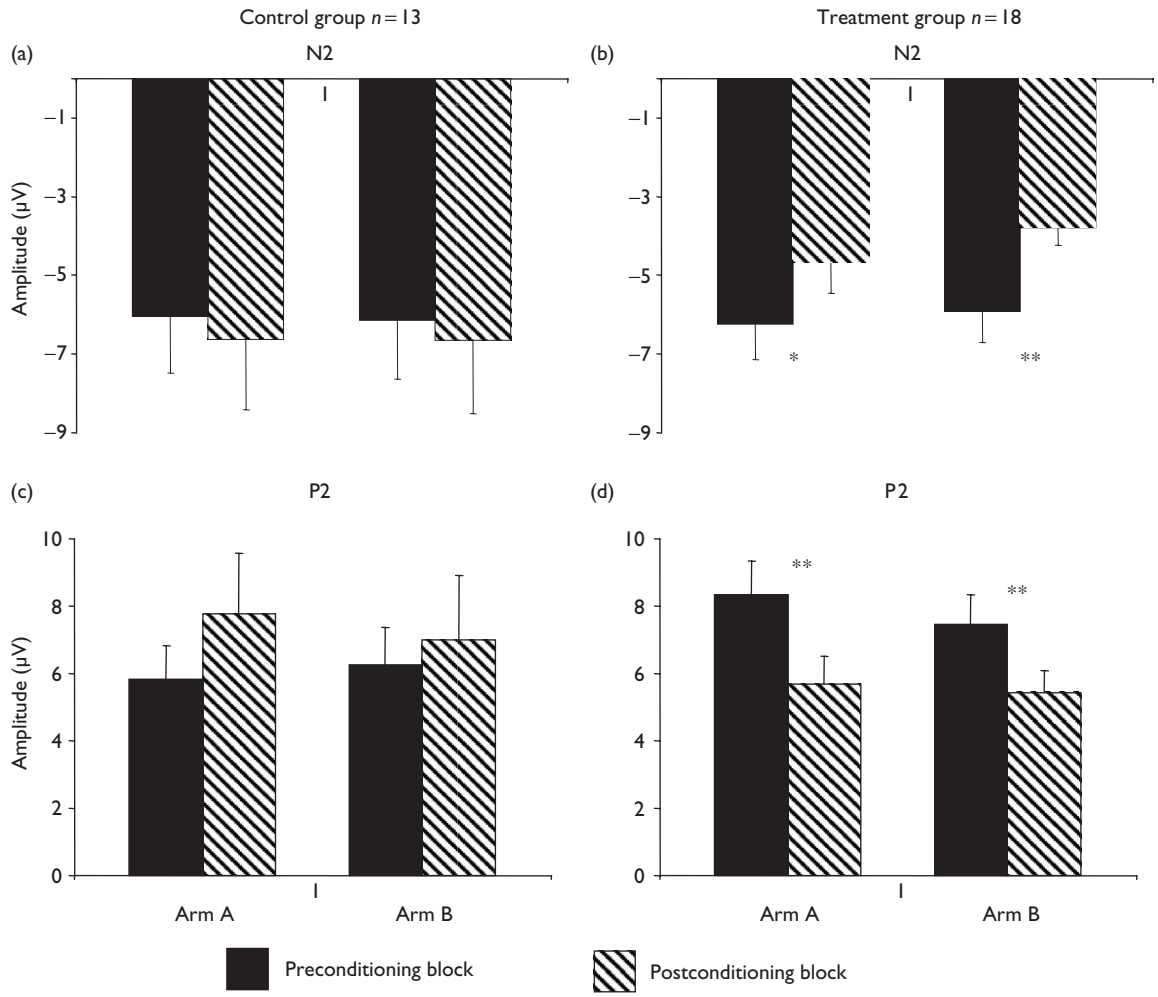

Fig. 3 Mean and SEM of N2 (plots a and b) and P2 (plots c and d) amplitudes at electrode Cz for the pre- and postconditioning blocks. The same laser energy was used in the preconditioning and postconditioning blocks, set at each individual's moderately painful level. (a) Control group preconditioning N2 amplitudes in arms A and B, respectively were −6.0 μV (1.44) and −6.2 μV (1.38); postconditioning −6.65 μV (1.79) and −6.60 μV (1.86). (b) Treatment group, preconditioning −6.30 μV (0.91) and −6.0 μV (0.79); postconditioning −4.70 μV (0.78) and −3.80 μV (0.43). (c) Control group preconditioning P2 amplitude in arms A and B respectively were: 5.82 μV (1) and 6.29 μV (1.1); postconditioning 7.92 μV (1.82) and 6.99 μV (1.95). (d) Treatment group, preconditioning 8.39 μV (1) and 7.46 μV (0.87); postconditioning 5.83 μV (1.82) and 5.48 μV (1.95). *$P<0.05$, **$P<0.01$.

or habituation. We saw a significant placebo-induced reduction in both pain rating and LEP amplitude in the treatment group (Fig. 3), confirming the findings from a previous study by Wager *et al.* [10]. We would not expect to see a reduction in LEP amplitude if the participant was compliant. In other words, participants do not conform to expected behaviour by overriding the actual pain sensation and reporting a reduced pain rating.

Previous placebo analgesia studies [1] have directed the participants to attend to the site of potential analgesia and have measured a site-specific placebo-induced reduction in pain ratings. If no definitive cues or instructions are given regarding the expected site of analgesia, placebo responses may occur at unconditioned sites [4].

In this study, which used nonsite-specific instructions, the treatment group reported a reduction in pain ratings in both the treated (conditioned) and untreated arms, in agreement with previous behavioural findings [4], with corresponding reduction in LEP amplitudes. This suggests that, in the absence of specific instructions, experimental placebo responses may be generalized to include the unconditioned sites. This has important implications for the design of future placebo experiments, as it suggests that it may not be possible to carry out control and placebo experiments in the same individual. It further implies that the expectation of pain reduction is the most likely driver of the placebo response, as previously suggested [12,13].

In conclusion, the reduction in LEP responses on both the conditioned and unconditioned arms is evidence that experimental placebo responses reflect a reduction in pain experience and are not due to increased compliance. The absence of habituation in the control group suggests that the placebo effect is not due to habituation. These insights may in the longer-term aid the design of trials of pharmacological and cognitive interventions that are less susceptible to the variability of placebo response.

Acknowledgement

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