Intracortical modulation, and not spinal inhibition, mediates placebo analgesia.

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Number of pages: 16

Number of figures: 2

Number of tables: 1

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Keywords: placebo analgesia, intra-cortical modulation, descending inhibition, LEPs, pain.

Abstract

Suppression of spinal responses to noxious stimulation has been detected using spinal fMRI (sfMRI) during placebo analgesia, which is increasingly considered a phenomenon caused by descending inhibition. However, the sfMRI is technically challenging and prone to false-positive results. We employed EEG and recorded laser-evoked potentials (LEPs), which allows neural activity to be measured directly and with high enough temporal resolution to capture the ensemble of cortical areas that are activated by nociceptive stimuli. The hypothesis is that inhibition at the spinal level during placebo analgesia should be evidenced by a general suppression of LEPs, rather than by a selective reduction of late LEP components. LEPs and subjective ratings of pain were obtained in two groups of healthy volunteers: one was conditioned for placebo analgesia, while the other served as unconditioned control. Three different supra-threshold laser stimulus energies (3, 3.5 and 4 Joules) were delivered to the right hand dorsum. Placebo analgesia was associated with a selective reduction of late components of LEPs (P2 wave). In contrast, early component (N1 wave) reflecting the arrival of the nociceptive input to the primary somatosensory cortex (SI) were affected by the magnitude of laser stimulus energies only. The selective suppression of late LEPs during placebo analgesia suggests an underlying mechanism of direct intra-cortical modulation, rather than inhibition at the spinal level of afferent nociceptive input. Furthermore, cortical modulation occurs after the responses elicited by the nociceptive stimulus in the SI, suggesting that higher order sensory processes are modulated during placebo analgesia.

Introduction

Placebo analgesia results from the administration of either an inert substance or a sham procedure that mitigates pain because of conscious expectation of a pain-relieving effect (Atlas *et al.*, 2014) (Wager, 2013). Nociceptive stimuli reported as less painful during placebo analgesia elicit increased activity in the dorsal-medial prefrontal cortex and the perigenual anterior cingulate cortex (ACC), as well as in the supraspinal network for the descending inhibition of spinal nociception (e.g. the periaqueductal grey, PAG) (Amanzio *et al.*, 2013). Recently, two studies have revealed suppression of spinal responses to noxious stimulation after successful conditioning for placebo analgesia (Eippert, Finsterbusch, *et al.*, 2009) (Goffaux *et al.*, 2007). These results have been used to support the central role of descending spinal inhibition for placebo analgesia. So, currently the idea that the placebo analgesia effect depends on a very early (spinal) inhibition of the nociceptive input has been accepted within the scientific community. However, when the neural activity *preceding* the incoming nociceptive stimulus is measured, brain areas involved in descending inhibition of nociception are not active, and only prefrontal areas show an increased response (Wager *et al.*, 2004). In addition, spinal fMRI is technically challenging and prone to false-positive results (Brooks *et al.*, 2008) (Goethem *et al.*, 2007) (Summers *et al.*, 2010).

While fMRI measures neural activity indirectly and with a low temporal resolution, because of the delayed neurovascular response, EEG can resolve neural activities within tenths of milliseconds (Wager *et al.*, 2006) . Therefore, data from laser-evoked potentials (LEPs) can provide critical knowledge about how the modulation of incoming nociceptive input is cortically integrated in time. LEPs consist of an early lateralised potential (the N1 wave), originating from the primary somatosensory cortex contralateral to the stimulated hand, followed by a larger vertex biphasic potential (the N2-P2), originating from the operculo-

insular and cingulate cortex (Garcia-Larrea *et al.*, 2003). The suppression of the N2-P2 complex during placebo analgesia (Wager *et al.*, 2006) (Watson *et al.*, 2007) (Colloca *et al.*, 2008) is well established. In contrast, only a single LEP study has reported that the N1 peak amplitude is unchanged during placebo analgesia (Colloca *et al.*, 2008). However, in that study, there were no positive controls to demonstrate adequate sensitivity for the detection of significant change in N1 amplitude. Indeed, direct comparisons were only performed between treated and untreated body sides, within the same persons where placebo was induced. In other words, previous data pertaining to the N1 wave in placebo analgesia did not entail the key direct comparison with a separate control group, nor demonstrate the variation of N1 peak amplitude with the magnitude of the nociceptive input (Colloca *et al.*, 2008). Indeed, the manipulation of the stimulus energy can be critical for the disclosure of placebo effects both at behavioural and neurophysiological level (Wager *et al.*, 2006).

Here, we randomly allocated healthy volunteers to two groups. One group was conditioned for placebo analgesia (Montgomery & Kirsch, 1997), while the other group served as unconditioned control. Three different supra-threshold laser stimulus energies (Stimulus energy: 3, 3.5 and 4 Joules) were delivered to the right hand dorsum. We sought to replicate the well-known effects of laser stimulus energy and habituation on pain and N1, N2, P2 LEPs (Di Clemente *et al.*, 2013) (Romaniello *et al.*, 2002), to demonstrate the sensitivity for the behavioural and neurophysiological assays employed in the experiment. We tested whether successful placebo analgesia involves either spinal inhibition of ascending nociceptive input, which should manifest as the attenuation of both early and late LEPs, or the selective attenuation of late LEPs (i.e. inhibition takes place after the nociceptive input has entered the cortex).

Method

Subjects

Twenty-eight healthy volunteers (14 women) aged 18-35 (23.5 ± 5; mean ± SD) with no history of neurological or psychiatric disorders participated in the experiment. They were randomly assigned to a placebo or a control group (placebo group n=14, 8 females; mean age 22.3, SD ± 4.8; control group n=14, 6 females; mean age 24.7, SD ± 5).

All participants gave written informed consent, and all experimental procedures were approved by the Ethics Committee of University College London and undertaken in accordance with the Declaration of Helsinki.

Laser stimulation

Noxious radiant heat stimuli were generated by an infrared neodymium yttrium aluminium perovskite (Nd:YAP) laser with a wavelength of 1.34 μm (Electronical Engineering, Florence, Italy). The laser beam was transmitted through an optic fiber, and its diameter was set at approximately 8 mm (50 mm^2) by focusing lenses. The duration of the laser pulses was set at 4 ms. Laser pulses were directed to a square of approximately 5x5 cm on the hand dorsum. The laser beam was slightly shifted after each stimulus to irradiate a different skin spot. Three different and equally-spaced stimulus energies were used (3, 3.5 and 4 J) in the pre-conditioning and post-conditioning periods (Fig. 1). In a preliminary experiment, we found that stimuli with these characteristics always produce painful pinprick sensations. In the conditioning period, the three stimulus energies were reduced to 1, 1.5 and 2 J.

A total of 120 laser stimuli were delivered over the three periods. Within each period, the inter stimulus interval (ISI) varied randomly between 15 and 20 s. The temperature of the hand dorsum was monitored using a KT22 radiation pyrometer (Heitronics, Wiesbaden, Germany).

Mean skin temperature readings did not differ by more than 1 °C between pre- and post-conditioning periods in any individual.

Experimental design and psychophysics

Subjects sat comfortably with their right forearm resting on a table. A wooden frame blocked the view of the right arm. Participants in the placebo group were informed that the aim of the study was to investigate the effects of an analgesic cream on pain-related brain responses. In order to induce positive treatment expectancy, the subjects were deliberately told that the application of the cream would numb their skin and they would feel less pain from the laser stimuli, while in fact the cream was an inert aqueous colloid mixture (E45 cream). A learning phase (conditioning, described below) was included to further enhance placebo effects, according to classical placebo conditioning paradigms (Montgomery & Kirsch, 1997). Participants in the control group were administered the same cream and laser stimulation. However, they were made aware that the cream was inert, and that the laser energies were reduced in the conditioning period.

Before starting the recording, a few laser pulses were delivered to familiarize the participants with the stimuli. Participants were told that three different stimulus energies would have been employed during the actual experiment.

In the first period (pre-conditioning) sixteen laser pulses for each of the three energy levels (3, 3.5 and 4J) were delivered in pseudo-random sequence. In the second period (conditioning) the same experimenter applied the cream on the subject's hand dorsum. The hand dorsum was then covered with gauze. After the cream was left in place for ten minutes, it was carefully wiped off. In the second period (conditioning) sixteen laser pulses for each energy level were delivered in pseudo-random sequence, but the energies were lowered (1, 1.5 and 2 J).

Participants belonging to the control group were told that the laser energies were lowered and that the cream was inert and had no effects on pain sensation. Participants belonging to the placebo group were not told that the laser energies were lowered, and were informed that the cream was an "analgesic" that would reduce their pain sensations.

The third period (post-conditioning) was identical to the first (pre-conditioning) period and followed the conditioning period without pause.

Approximately two seconds after each stimulus, participants were asked to verbally report their pain sensation, using a numerical rating scale (NRS) ranging from 0 ("no pain at all") to 100 ("worst imaginable pain").

Electroencephalographic recordings

The electroencephalogram (EEG) was recorded from 32 Ag-AgCl electrodes placed on the scalp according to the International 10–20 system. The nose was used as a reference. To monitor ocular movements and eye blinks, the electro-oculogram (EOG) was simultaneously recorded from two surface electrodes, one placed over the right lower eyelid, the other placed lateral to the outer canthus of the right eye. Signals were amplified and digitized at a sampling rate of 1,024 Hz and a precision of 12 bits, resulting in an amplitude resolution of 0.195 μV (SD32; Micromed, Treviso, Italy).

EEG data were pre-processed and analyzed using Letswave (http://amouraux.webnode.com; (Mouraux & Iannetti, 2008)) and EEGLAB (Delorme & Makeig, 2004). Continuous EEG data were segmented into epochs of 1.5 s, with 0.5 s pre-stimulus and 1 s post-stimulus. EEG epochs were band-pass filtered from 1 to 100 Hz using a fast Fourier transform. EOG artifacts were subtracted using a validated method based on independent component analysis (ICA (Jung *et al.*, 2000)). In all datasets, ICs related to eye movements had a large EOG channel

contribution and a frontal scalp distribution. After ICA, epochs were baseline corrected using the interval from -0.5 to 0 s as reference, and low-pass filtered with a cutoff of 30 Hz.

Epochs from each participant were averaged according to stimulus energy (3, 3.5 and 4 J) and period (pre-conditioning, post-conditioning). This procedure yielded six average waveforms for each participant. Latency and the baseline-to-peak amplitude of the three main LEP waves were measured in each average waveform, as follows: the N1 wave was measured at the central electrode contralateral to the stimulated side (C3), referenced to Fz, and it was defined as the most negative deflection preceding the N2 wave. The N2 and P2 waves were measured at the vertex (Cz) referenced to the nose. The N2 wave was defined as the most negative deflection after stimulus onset. The P2 wave was defined as the most positive deflection after stimulus onset.

Statistical analyses

A mixed model ANOVA was used to investigate the effect of the two within-subject variables, Energy (three levels: 3J, 3,5J and 4J) and Period (two levels: pre and post), and one between-subject variable, Group (two levels: placebo and control) on the subjective pain reports as well as on the peak latency and amplitude of the laser-evoked N1, N2 and P2 peaks. *Post-hoc* comparisons were performed using Tukey's test (Table 1). The level of significance was set at $p < 0.05$. All variables were normally distributed (all $p_s > 0.05$, Kolmogorov-Smirnov test).

Results

We found highly significant main effects of stimulus Energy on reported pain and on all LEP amplitudes (NRS: $F_{2,52} = 150.10$, $\eta^2_p = 0.85$; N1-wave: $F_{2,52} = 29.43$, $\eta^2_p = 0.53$, N2-wave: $F_{2,52} = 71.32$, $\eta^2_p = 0.73$, P2-wave: $F_{2,52} = 57.53$, $\eta^2_p = 0.69$; all $p_s < 0.0001$). Both pain and LEP amplitudes were bigger at stronger stimulus energies (Fig. 2). We also found significant main effects of

Period on reported pain and all LEP peak amplitudes (NRS: $F_{1,26} = 12.2, p = 0.002, \eta^2_p = 0.32$; N1-wave: $F_{1,26} = 13.51, p = 0.01, \eta^2_p = 0.34$; N2-wave: $F_{1,26} = 6.62, p < 0.02, \eta^2_p = 0.20$; P2-wave: $F_{1,26} = 4.33, p = 0.047, \eta^2_p = 0.14$). Both pain and LEP amplitudes were smaller in the post-conditioning period. These findings are consistent with those reported in other LEP studies (Watson et al., 2007), and critically demonstrate the sensitivity of both the psychophysical and LEP measures. We observed a significant triple interaction between Stimulus Energy, Group and Period, for both pain and P2 amplitude (NRS: $F_{2,52} = 7.28, p = 0.002, \eta^2_p = 0.22$; P2: $F_{2,52} = 3.99, p = 0.02, \eta^2_p = 0.13$). Post-hoc Tukey revealed significant reductions of reported pain and P2 amplitudes for the responses elicited by stimuli of highest energies in the post-conditioning period of the placebo group *only* (NRS pre-conditioning vs. post-conditioning at 3.5J: p = 0.044, and at 4J: p = 0.0001; P2 pre-conditioning vs. post-conditioning at 3.5J: p = 0.02, and at 4J: p = 0.007. See table 1 for further details). Critically, there was no significant main or interaction effect of Group on the early N1 wave, which, nevertheless, had exhibited significant modulation related to Period and Stimulus Energy in the same experiment.

These results clearly support the hypothesis that the successful placebo analgesia does not involve early inhibition of ascending nociceptive input at the spinal level, but rather inhibition of the neural activity elicited after the nociceptive input has reached the cortex.

Discussion

Experimental evidence indicates that the N1 reflects more closely the incoming afferent input, while subsequent N2 and P2 waves reflect later processing more related to the perceptual outcome of the stimulus (Lee *et al.*, 2009). Indeed, unlike the N1 wave, the later N2 and P2 waves have been shown to be consistently modulated when laser-induced pain is psychologically manipulated, for example in tasks that vary attentional load or emotional

context(Legrain *et al.*, 2012a) . N2 and P2 waves are also significantly suppressed when subjects fail to detect the second of a pair of laser stimuli in a temporal discrimination task, whereas the N1 wave remains unchanged (Lee *et al.*, 2009). A number of other studies have demonstrated that the amplitude of the N1 wave is better correlated with pain, when pain variability is modulated by changing the energy of the physical stimulus rather than the psychological state of the individual (see (Legrain *et al.*, 2012b) for a review). Hence, the clear dissociation between the strong modulation of the P2 amplitude and the lack of modulation of the N1 amplitude indicates that the observed placebo analgesia was not determined by an inhibition of the nociceptive input at subcortical level, but to a later modulation at cortical level.

A similar finding has been recently observed in the tactile domain, where placebo manipulation of perceived energy of non-noiciceptive stimuli has been shown to modulate only the late cortical components of somatosensory evoked potentials, while the subcortical and early cortical components were not altered (Fiorio *et al.*, 2012). The specific suppression of late but not early cortical potentials during placebo modulation of nociceptive and non-nociceptive input, strongly suggests that placebo manipulation of somatosensation may be an entirely cortically-mediated phenomenon. Nevertheless, our findings do not exclude completely a role of descending spinal inhibition for placebo analgesia. In our case however, the lack of modulation of LEP-N1 suggests that descending spinal inhibition does not occur shortly after the onset of the nociceptive stimulus but may be delayed to at least after the latency period of that early evoked potential. Human FMRI studies have revealed increased PAG activation occurs during noxious stimulation after successful placebo conditioning and suggest that descending inhibition occurs during placebo analgesia (Eippert, Bingel, *et al.*, 2009). However, the temporal resolution of FMRI studies is limited, and it is possible that

descending inhibition is a delayed mechanism that is engaged only when nociceptive stimulation is prolonged, which is the case for the two published studies that demonstrated spinal inhibition during placebo analgesia (Eippert, Finsterbusch, *et al.*, 2009) (Goffaux *et al.*, 2007). A careful analysis of data presented from an early FMRI of placebo analgesia by Bingel and colleagues revealed that activation of the rostral ACC, a region that is functionally connected with the PAG, did not occur prior to or at the onset of laser stimulation. Instead, rostral ACC activity appeared to peak after two to three consecutive noxious laser stimuli that were applied 6-8s apart (Bingel *et al.*, 2006). It remains unclear how quickly the effects from descending inhibition decays after offset of noxious stimulation, and whether the decay rate depends on the duration of noxious stimulation. In this experiment, we employed a range of inter-stimulus intervals (ISIs) that were relatively long (seconds), compared to the duration of nociceptive laser stimulation. We observed suppression of late LEPs only. The finding does not support a tonic or ongoing state of spinal inhibition during placebo analgesia in this study, which would be expected to be associated with suppression of the early LEP as well.

Finally, we note that the observed placebo effect on reported pain was more evident for the more intense stimulus energies. Previous clinical studies on post-operative pain also indicate that placebo analgesia is more effective on severe than on mild painful experiences (Hoffman *et al.*, 2005). In our study, the subjective placebo effect is corroborated by similar findings for the P2-LEP, and hence is unlikely to be an artifact of the close bounded pain rating scale. Both psychophysical and electrophysiological stimulus response functions (SRF) exhibited decreased slopes, and not rightward parallel shifts. In effect, the responses are reduced in proportion to the stimulus energy, rather than by a fixed quantity. This suggest that placebo analgesia may involve a gain control mechanism that is stimulus-dependent (Priebe & Ferster, 2002), rather than a general dampening of the entire nociceptive system. Specifically, the

amplitude reduction of the later P2-LEP, but not N1-LEP, suggest that the gain reduction of nociceptive input occurs after entry into cortex. Indeed, regardless of the functional meaning of N1, this component marks the earliest recordable in-vivo cortical response to afferent spino-thalamic input, and our results show that this is not affected by a successful placebo analgesia induction. A clear modulation instead, takes place at later stages on different cortical areas.

In conclusion, the present findings indicate that placebo analgesia does not only result from a spinal inhibition of the ascending nociceptive input. Instead, they demonstrate that placebo analgesia can occur from cortical modulation of nociceptive input alone, and more precisely, after such input has been processed in the primary somatosensory cortex.

Acknowledgments

We are deeply grateful to Dr. M. Liang and Mr. M. Serafini for their invaluable assistance.

Disclosures

All authors report no competing interests.

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Figure Captions

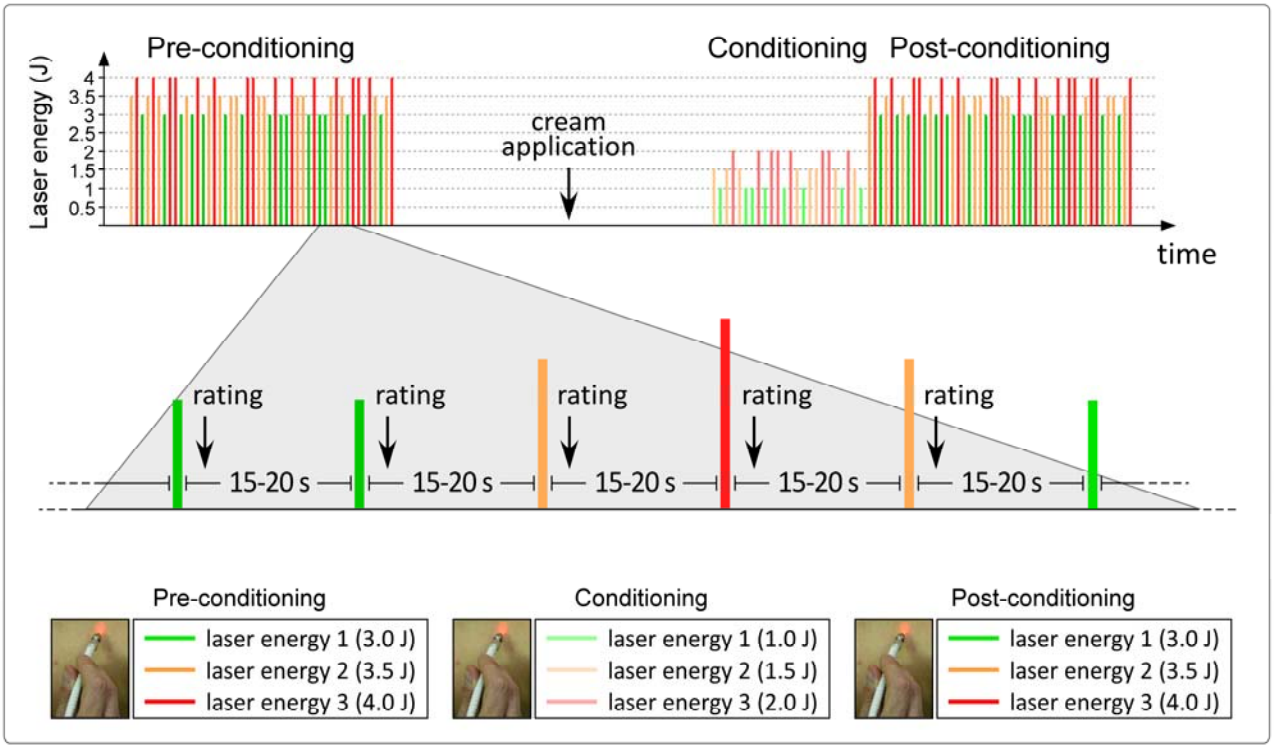

Figure 1. Experimental design. The experiment was conducted in two groups of healthy participants (placebo and control). In both groups, laser-evoked EEG responses were recorded following the stimulation of the right hand dorsum, in three blocks (pre-conditioning, conditioning and post-conditioning) on the same day. Forty-eight nociceptive laser stimuli were provided in the pre-conditioning and post-conditioning blocks while 24 stimuli were given during the conditioning block. Laser stimuli were delivered at an inter-stimulus interval varying randomly between 15 and 20 s. In each block, three different energies were used (3, 3.5 and 4 J in the 1st and 3rd block; 1, 1.5, and 2 J in the 2nd block). After each stimulus participants were asked to rate the intensity of perceived pain using a numerical rating scale ranging from 0 to 100. Between the 1st and 2nd blocks, an inert cream was applied on the

dorsum of the right hand. Participants of the placebo group were told that the cream was analgesic, and in the 2nd block stimulus energies were surreptitiously lowered (conditioning). Participants of the control group were informed of the inert nature of the cream, as well as of the reduction of the stimulus energy in the 2nd block.

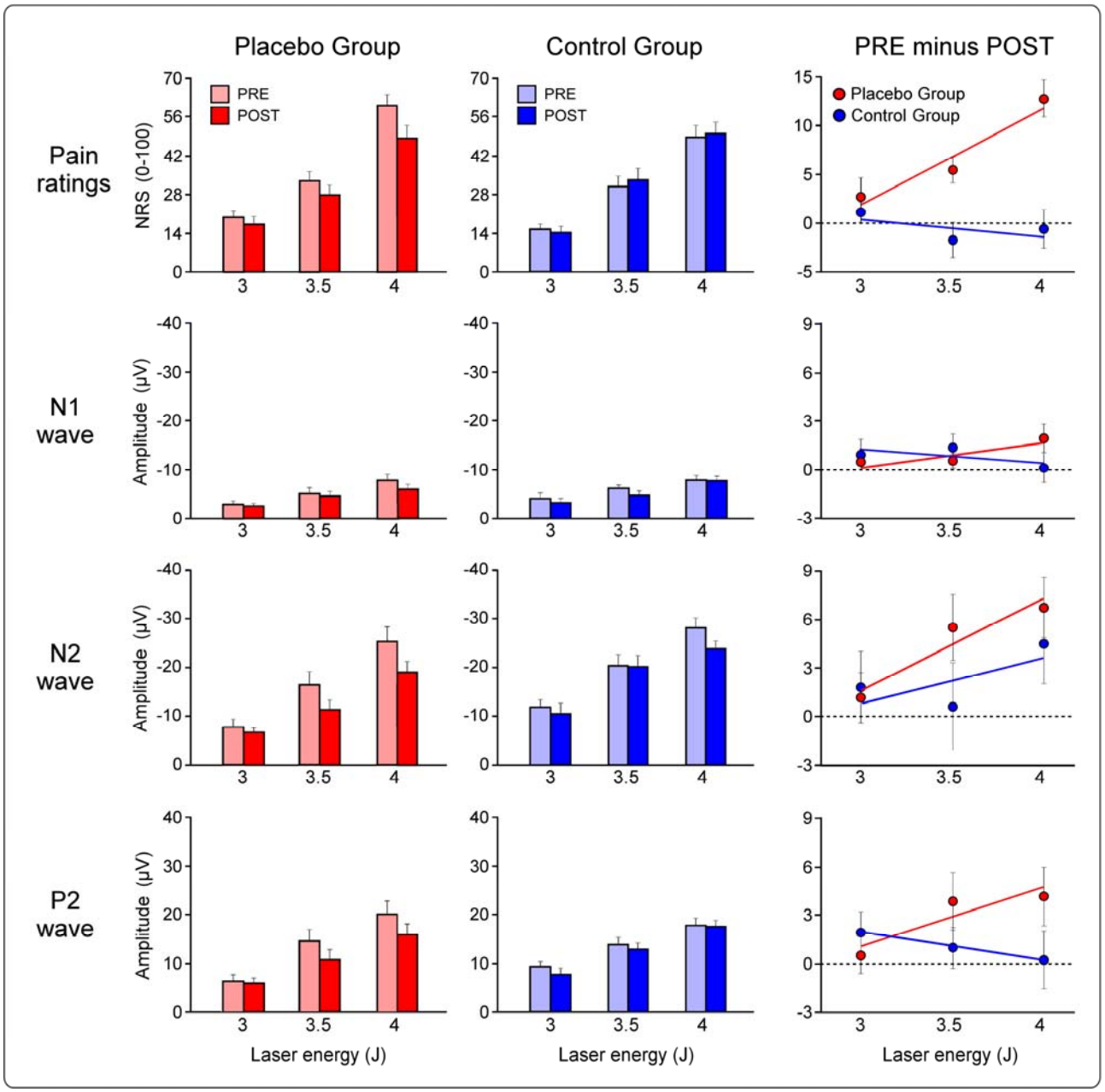

Figure 2. Left and middle columns. Pain ratings and amplitude of nociceptive ERPs in each group (placebo, control), recording block (pre-conditioning, post-conditioning), and level of stimulus energy (3, 3.5, and 4 J). Right column. Mean differences (delta: pre-conditioning minus post-conditioning) for each dependent variable. Positive values indicate rating and amplitude reductions in the post-conditioning block. Error bars represent variability across participants, expressed as standard error. The analgesic effect increases as the stimulus gets stronger only in the placebo group. Note also the dissociation between the lack of modulation of the early-latency N1 component, and the amplitude reduction of the subsequent P2 component.