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COGNITIVE NEUROSCIENCE

Intracortical modulation, and not spinal inhibition, mediates placebo analgesia

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Abstract

Suppression of spinal responses to noxious stimulation has been detected using spinal fMRI during placebo analgesia, which is therefore increasingly considered a phenomenon caused by descending inhibition of spinal activity. However, spinal fMRI is technically challenging and prone to false-positive results. Here we recorded laser-evoked potentials (LEPs) during placebo analgesia in humans. LEPs allow neural activity to be measured directly and with high enough temporal resolution to capture the sequence of cortical areas activated by nociceptive stimuli. If placebo analgesia is mediated by inhibition at spinal level, this would result in a general suppression of LEPs rather than in a selective reduction of their late components. LEPs and subjective pain ratings were obtained in two groups of healthy volunteers – one was conditioned for placebo analgesia while the other served as unconditioned control. Laser stimuli at three suprathreshold energies were delivered to the right hand dorsum. Placebo analgesia was associated with a significant reduction of the amplitude of the late P2 component. In contrast, the early N1 component, reflecting the arrival of the nociceptive input to the primary somatosensory cortex (SI), was only affected by stimulus energy. This selective suppression of late LEPs indicates that placebo analgesia is mediated by direct intracortical modulation rather than inhibition of the nociceptive input at spinal level. The observed 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; pain is mitigated because of conscious expectation of a pain-relieving effect (McMahon *et al.*, 2013; Atlas *et al.*, 2014). 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 nociceptive circuits (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 (Goffaux *et al.*, 2007; Eippert *et al.*, 2009a,b). These results have been used to support the central role of descending spinal inhibition in placebo analgesia, and the idea that the placebo analgesia effect depends on an early spinal inhibition of the nociceptive input is currently accepted. 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; Lui *et al.*, 2010). In addition, spinal fMRI is technically challenging and prone to false-positive results (van Goethem *et al.*, 2007; Brooks *et al.*, 2008; 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 on a scale of milliseconds (Mouraux & Iannetti, 2008). The brain potential elicited by nociceptive-selective laser pulses (laser-evoked potentials; LEPs) consist of an early lateralised potential (the N1 wave), originating from the primary somatosensory cortex contralateral to the stimulated hand (Valentini *et al.*, 2012), followed by a larger vertex biphasic potential (the N2-P2) originating from the operculoinsular and cingulate cortex (Garcia-Larrea *et al.*, 2003; Valentini *et al.*, 2012). Therefore, LEP data can provide critical knowledge about the timing of the modulation of incoming nociceptive input. 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 changes in N1 amplitude. Indeed, direct comparisons were only per-

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formed between treated and untreated body sides, within the same individuals in which the placebo analgesia was induced. In other words, previous data looking at the amplitude of the N1 wave in placebo analgesia did not perform 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 at both behavioural and neurophysiological levels (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 suprathreshold laser stimulus energies were delivered to the right hand dorsum. We sought to replicate the well-known effects of laser stimulus energy and time-dependent habituation on pain and N1, N2 and P2 LEPs (Valeriani *et al.*, 2003; Hu *et al.*, 2014), to demonstrate the sensitivity of the behavioural and neurophysiological assays employed in the experiment. We tested whether placebo analgesia involves either spinal inhibition of ascending nociceptive input, which should be reflected in the attenuation of both early and late LEPs, or intracortical modulation of the responses elicited by the stimulus, which should be reflected in the selective attenuation of late LEPs (i.e. inhibition takes place after the nociceptive input has entered the cortex).

Materials and methods

Subjects

Twenty-eight healthy volunteers (14 women) aged 18–35 (23.5 ± 5 years; 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 $n = 14$, eight females; mean age 22.3 ± 4.8; control group $n = 14$, six females; mean age 24.7 ± 5). All participants gave written informed consent, and all experimental procedures were approved by the Ethics Committee of University College London and performed 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 fibre, and its diameter was set at ~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 area of ~5 × 5 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), both 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 related to the activation of Aδ nociceptors. 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

The experimental design is summarised in Fig. 1. 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

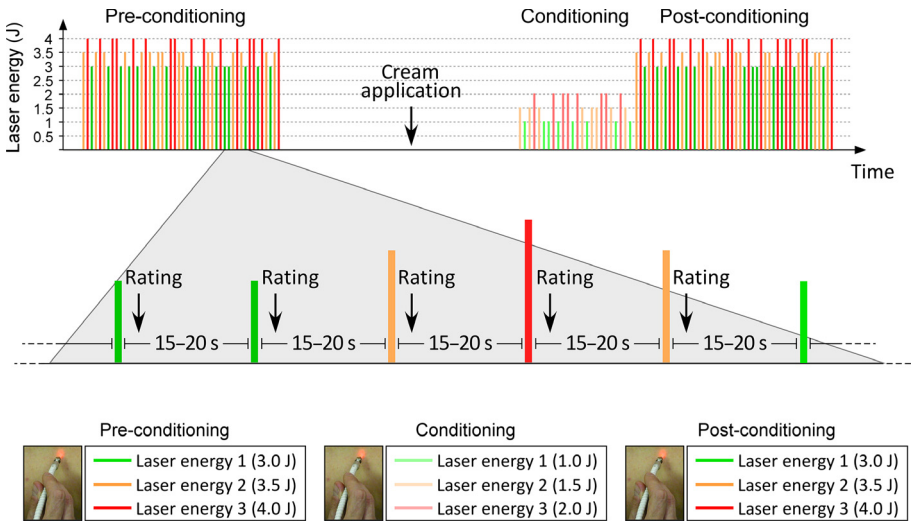

FIG. 1. Experimental design. The experiment was conducted in two groups of healthy participants (placebo and control). In both groups, laser-evoked EEG responses were recorded following the stimulation of the right hand dorsum, in three blocks (pre-conditioning, conditioning and post-conditioning) on 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 ISI varying randomly between 15 and 20 s. In each block, three different energies were used (3, 3.5 and 4 J in the first and third blocks, and 1, 1.5 and 2 J in the second 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 first and second blocks, an inert cream was applied to the dorsum of the right hand. Participants of the placebo group were told that the cream was analgesic, and in the second 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 in the stimulus energy in the second block.

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 familiarise the participants with the stimuli. Participants were told that three different stimulus energies would be employed during the actual experiment.

In the first period (pre-conditioning) 16 laser pulses for each of the three energy levels (3, 3.5 and 4 J) were delivered in pseudo-random sequence (Fig. 1). In the second period (conditioning) the same experimenter applied the cream to the hand dorsum of each subject. The hand dorsum was then covered with gauze. After 10 min the cream was carefully wiped off, and 16 laser pulses of 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

TABLE 1. *P*-values obtained by Tukey *post hoc* comparisons of numerical rating scale (NRS) and peak amplitudes for placebo and control groups

	P-values					
	Pre 3 J	Pre 3.5 J	Pre 4 J	Post 3 J	Post 3.5 J	Post 4 J
NRS						
Placebo group						
Pre 3 J		0.0001	0.0001	0.88	0.0002	0.0001
Pre 3.5 J	0.0001		0.0001	0.0001	0.04	0.0001
Pre 4 J	0.0001	0.0001		0.0001	0.0001	0.0001
Post 3 J	0.88	0.0001	0.0001		0.0001	0.0001
Post 3.5 J	0.0002	0.04	0.0001	0.0001		0.0001
Post 4 J	0.0001	0.0001	0.0001	0.0001	0.0001	
Control group						
Pre 3 J		0.0001	0.0001	0.99	0.0001	0.0001
Pre 3.5 J	0.0001		0.0001	0.0001	0.98	0.0001
Pre 4 J	0.0001	0.0001		0.0001	0.0001	1.00
Post 3 J	0.99	0.0001	0.0001		0.0001	0.0001
Post 3.5 J	0.0001	0.98	0.0001	0.0001		0.0001
Post 4 J	0.0001	0.0001	1.00	0.0001	0.0001	
N2 wave						
Placebo group						
Pre 3 J		0.0001	0.0001	1.00	0.35	0.0001
Pre 3.5 J	0.0001		0.0001	0.0001	0.04	0.88
Pre 4 J	0.0001	0.0001		0.0001	0.0001	0.004
Post 3 J	1.00	0.0001	0.0001		0.10	0.0001
Post 3.5 J	0.35	0.04	0.0001	0.10		0.0004
Post 4 J	0.0001	0.88	0.004	0.0001	0.0004	
Control group						
Pre 3 J		0.0001	0.0001	0.99	0.0002	0.0001
Pre 3.5 J	0.0001		0.0005	0.0001	1.00	0.57
Pre 4 J	0.0001	0.0005		0.0001	0.0003	0.20
Post 3 J	0.99	0.0001	0.0001		0.0001	0.0001
Post 3.5 J	0.0002	1.00	0.0003	0.0001		0.40
Post 4 J	0.0001	0.57	0.20	0.0001	0.40	
P2 wave						
Placebo group						
Pre 3 J		0.0001	0.0001	1.00	0.006	0.0001
Pre 3.5 J	0.0001		0.0001	0.0001	0.018	0.90
Pre 4 J	0.0001	0.0001		0.0001	0.0001	0.0072
Post 3 J	1.00	0.0001	0.0001		0.0015	0.0001
Post 3.5 J	0.006	0.018	0.0001	0.0015		0.0002
Post 4 J	0.0001	0.90	0.007	0.0001	0.0002	
Control group						
Pre 3 J		0.0017	0.0001	0.78	0.026	0.0001
Pre 3.5 J	0.0017		0.009	0.0001	0.99	0.015
Pre 4 J	0.0001	0.009		0.0001	0.0006	1.00
Post 3 J	0.78	0.0001	0.0001		0.0002	0.0001
Post 3.5 J	0.026	0.99	0.0006	0.0002		0.001
Post 4 J	0.0001	0.015	1.00	0.0001	0.001	

The *post-hoc* comparisons were performed where ANOVAS showed a significant interaction between at least two of the three factors (Period, Energy, Group). Pre, pre-conditioning; Post, post-conditioning.

first (pre-conditioning) period, and immediately followed the conditioning period. Approximately 2 s after each stimulus, participants were asked to verbally report their pain sensation using a numerical rating scale 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 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 and the other placed lateral to the outer canthus of the right eye. Signals were amplified and digitised at a sampling rate of 1024 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 analysed using Letswave (http://www.nocions.org/letswave/; 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 bandpass-filtered from 1 to 100 Hz using a fast Fourier transform. EOG artifacts were subtracted using 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 lowpass-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 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

All variables were normally distributed (all $P > 0.05$, Kolmogorov–Smirnov test). A mixed-model ANOVA was used to investigate the effect of the two within-subject variables, Energy (three levels: 3, 3.5 and 4 J) and Period (two levels: pre and post), and of the single between-subjects variable, Group (two levels: placebo and control)

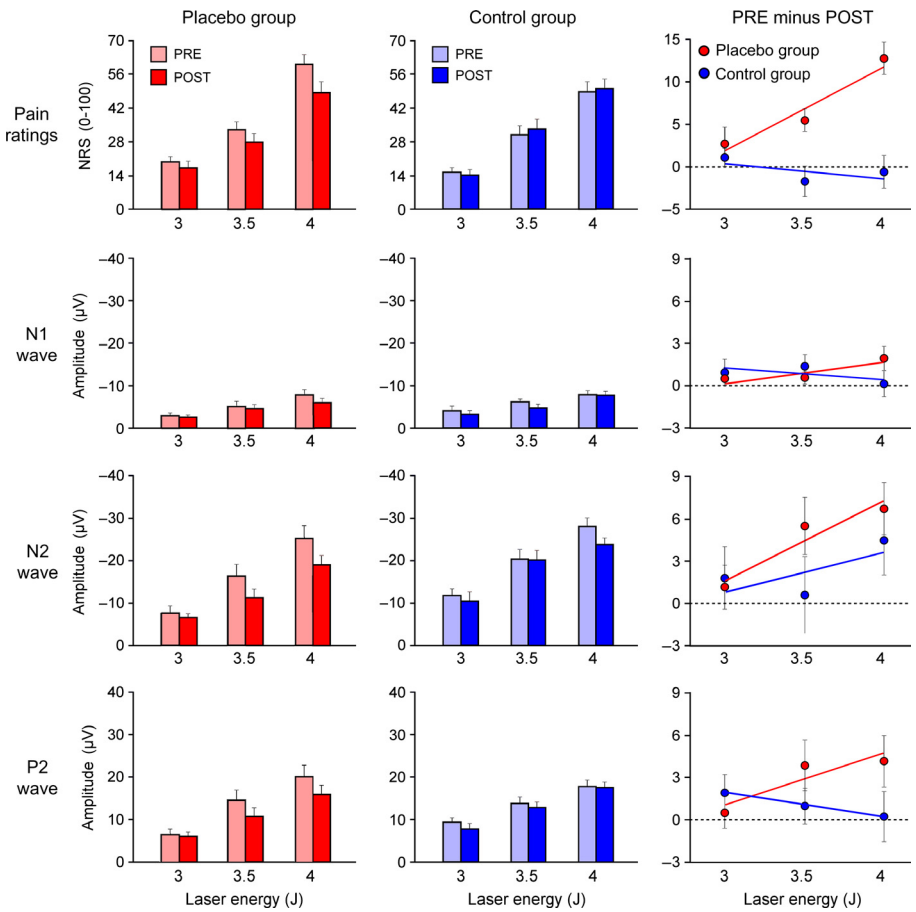

FIG. 2. (Left and middle columns) Pain ratings and amplitudes of nociceptive ERPs in each group (placebo, control), recording period (pre-conditioning, post-conditioning) and level of stimulus energy (3, 3.5, and 4 J). (Right column) Mean differences (pre-conditioning minus post-conditioning) for each dependent variable. Positive values indicate rating and amplitude reductions in the post-conditioning period. Error bars represent variability across participants, expressed as SEM. The analgesic effect increased as the stimulus got 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.

on the subjective pain ratings as well as on the peak latency and amplitude of the laser-evoked N1, N2 and P2 waves. *Post hoc* comparisons were performed using Tukey's test (Table 1). The level of significance was set at $P < 0.05$.

Results

Mean pain ratings and amplitudes of the N1, N2 and P2 waves, as well as their differences between the pre-conditioning and post-conditioning periods, are shown in Fig. 2. Individual differences in subjective pain ratings between the pre-conditioning and the post-conditioning periods are shown in Fig. 3. We observed highly significant main effects of stimulus Energy on both subjective pain ratings and the amplitude of all LEPs (pain: $F_{2,52} = 150.10$, $\eta_p^2 = 0.85$; N1 wave: $F_{2,52} = 29.43$, $\eta_p^2 = 0.53$; N2 wave: $F_{2,52} = 71.32$, $\eta_p^2 = 0.73$; P2 wave: $F_{2,52} = 57.53$, $\eta_p^2 = 0.69$; all $P_s < 0.0001$). Both pain and LEP amplitudes were larger at stronger stimulus energies (Fig. 2). We also found significant main effects of Period on both subjective pain ratings and the amplitude of all LEP waves (pain: $F_{1,26} = 12.2$, $P = 0.002$, $\eta_p^2 = 0.32$; N1 wave: $F_{1,26} = 13.51$, $P = 0.01$, $\eta_p^2 = 0.34$; N2 wave: $F_{1,26} = 6.62$, $P < 0.02$, $\eta_p^2 = 0.20$; P2 wave: $F_{1,26} = 4.33$, $P = 0.047$, $\eta_p^2 = 0.14$). 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 psychophysical and LEP measures.

We observed a significant Stimulus Energy × Group × Period interaction, for both pain and P2 amplitude (pain: $F_{2,52} = 7.28$, $P = 0.002$, $\eta_p^2 = 0.22$; P2: $F_{2,52} = 3.99$, $P = 0.02$, $\eta_p^2 = 0.13$). *Post hoc* Tukey's tests revealed significant reductions in reported pain and P2 amplitudes for the responses elicited by stimuli of highest energies in the post-conditioning period of the placebo group only (pain, pre-conditioning vs. post-conditioning: 3.5 J, $P = 0.04$; 4 J, $P = 0.0001$; P2, pre-conditioning vs. post-conditioning: 3.5 J, $P = 0.02$; 4 J, $P = 0.007$. See Table 1 for further details). Critically, there was no significant main or interaction effect of Group on the early N1 wave, which nevertheless exhibited significant modulation related to both Period and Stimulus Energy in the same experiment (Fig. 2).

Discussion

These results clearly support the hypothesis that effective 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. Converging experimental evidence indicates that the N1 wave of LEPs reflects more closely the afferent somatosensory 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.*, 2012). 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.*, 2012, 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 by a later modulation of its processing at cortical level.

A similar finding has been recently observed in the tactile domain, where placebo manipulation of perceived energy of non-

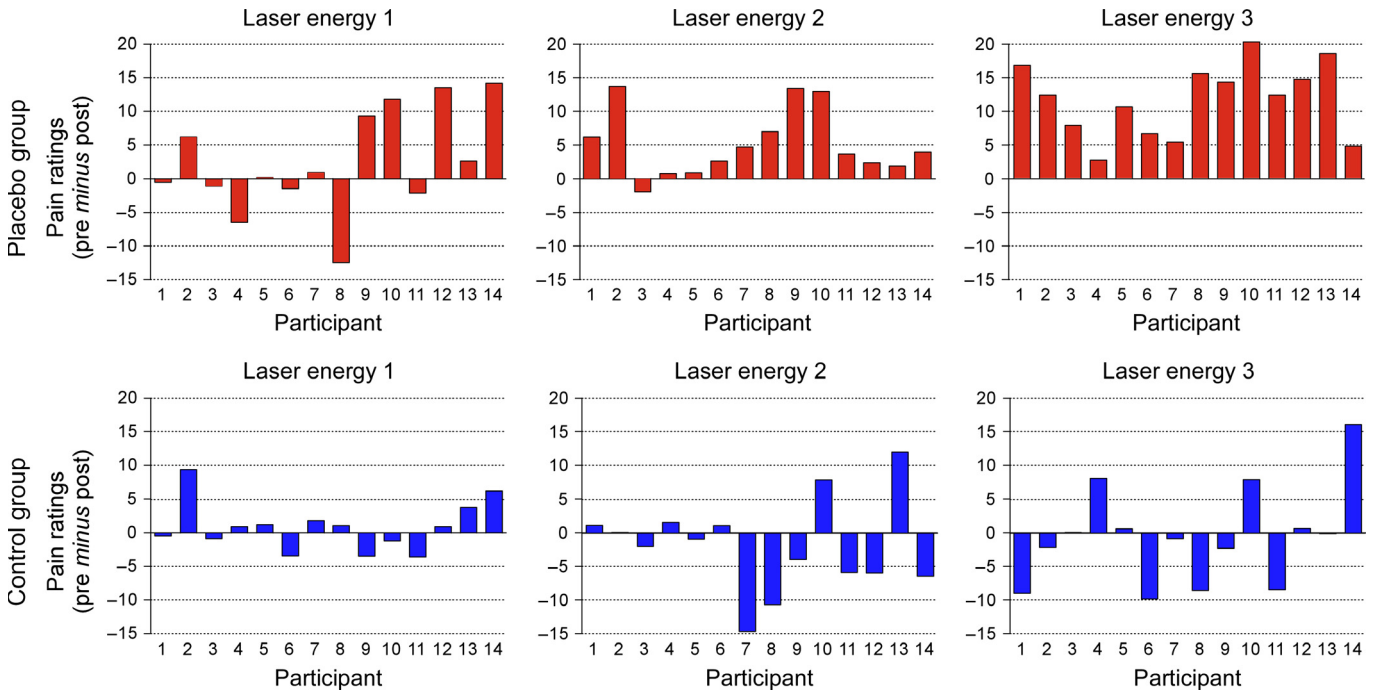

FIG. 3. Individual differences in subjective pain ratings between the pre-conditioning and the post-conditioning periods, at each stimulus energy (columns), for each group (rows). Positive values indicate analgesia in the post-conditioning period. The clear difference in differences in subjective pain ratings between the two groups indicates that the placebo manipulation was, overall, clearly effective.

nociceptive stimuli modulated 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 cannot exclude completely a role of descending spinal inhibition for placebo analgesia. In our case, however, the lack of modulation of LEP-N1 suggests that if descending spinal inhibition occurs it does not start 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 during noxious stimulation after successful placebo conditioning, and suggest that descending inhibition occurs during placebo analgesia (Eippert *et al.*, 2009a). However, the temporal resolution of fMRI is limited, and it is possible that descending inhibition is a delayed mechanism that is engaged only when nociceptive stimulation is prolonged, which was the case in the two studies that demonstrated spinal inhibition during placebo analgesia (Goffaux *et al.*, 2007; Eippert *et al.*, 2009a,b). A careful analysis of data presented from an early fMRI of placebo analgesia by Bingel *et al.* (2006) 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 6–8 s apart (Bingel *et al.*, 2006). It remains unclear how quickly the effect of descending inhibition decays after offset of noxious stimulation, and whether the decay rate depends on the duration of noxious stimulation. In the current experiment, we employed a range of ISIs that were relatively long (seconds) compared to the duration of nociceptive laser stimulation. We observed suppression of late LEPs only. Therefore, this finding does not support a tonic or ongoing state of spinal inhibition during placebo analgesia, 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 percepts (Hoffman *et al.*, 2005). In our study, the placebo analgesia is corroborated by similar findings for the P2-LEP wave, and hence is unlikely to be an artifact of the close-bounded pain rating scale. Both psychophysical and electrophysiological stimulus response functions exhibited decreased slopes rather than rightward parallel shifts. In effect, the responses were reduced in proportion to the stimulus energy rather than by a fixed quantity. This suggests that placebo analgesia may involve a gain control mechanism that is input-dependent (Priebe & Ferster, 2002) rather than a general damping of the entire nociceptive system. Specifically, the amplitude reduction of the late P2 wave, but not of the early N1 wave, suggest that the gain reduction of nociceptive input occurs after its entry into cortex. Indeed, regardless of its functional meaning, the N1 wave represents the earliest recordable *in vivo* cortical response to afferent spinothalamic input, and our results show that it is not affected by a successful placebo analgesia induction. Instead, a clear modulation takes place at later stages on different cortical areas.

A limitation of the present study is the lack of measurement of psychological and cognitive variables (e.g. anxiety, vigilance, empathic trait), as well as of previous exposure to nociceptive stimulation. Indeed, all these factors have been shown to modulate the magnitude of placebo analgesia (Valentini *et al.*, 2013; Geers *et al.*, 2014; Hunter *et al.*, 2014), and they could have been modeled out to improve the significance of its relationship with the electrophysiological measures.

In conclusion, the present findings indicate that placebo analgesia does not 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.

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Disclosure

All authors report no competing interests.

Abbreviations

ACC, anterior cingulate cortex; EEG, electroencephalogram; EOG, electro-oculogram; ICA, independent component analysis; ISI, inter-stimulus interval; LEP, laser-evoked potential; PAG, periaqueductal grey.

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