Measuring expectation of pain: Contingent negative variation in placebo and nocebo effects

Measuring expectation of pain: contingent negative variation in placebo and nocebo effects

(Short Title: Expectation of pain)

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Abstract

Background: Expectation is an important mechanism underlying placebo response. Here we analyzed expectation of placebo hypoalgesia and nocebo hyperalgesia by using, for the first time, the contingent negative variation (CNV), also known as expectancy wave.

Methods: Subjects were presented a green or red cue followed by a train of either non painful or painful electrical stimuli, and expected hypoalgesia after the green and hyperalgesia after the red cue. In experiment 1, expectation was reinforced using a conditioning procedure whereby the green and red cues were paired with non painful and painful stimuli, respectively (acquisition). In a second session (test) the intensity of the stimuli was kept constant, regardless of cue. In experiment 2 no conditioning was performed and participants expected an altered pain perception indicated by the visual cues. CNV mean amplitude, time necessary to stop the train of stimuli (reaction time) and pain ratings were measured.

Results: A difference in pain perception occurred when electrical stimuli followed the presentation of the green cue compared to the red in the test session, whereas reaction times showed no changes. The same difference occurred in the early CNV component, related to cognitive stimulus anticipation, whereas the late CNV component, related to motor preparation, didn't change. Moreover, these differences in pain perception and CNV amplitude were less robust in the experiment 2.

Conclusion: Placebo hypoalgesia and nocebo hyperalgesia differently affect sensory (pain perception) and motor components (pain avoidance). Furthermore, data show that CNV is an electrophysiological objective measure capable to dissect these components.

Keywords: placebo, nocebo, CNV, expectation, motor responses, pain

Introduction

Placebo effect, and its counterpart nocebo effect, are neurobiological and behavioral changes due to the psychosocial context accompanying a medical treatment (Carlino et al., 2014). An important mechanism underlying these phenomena is expectation (Benedetti, 2008), but different elements are likely to be involved in this process like cognitive, affective, motivational factors and conditioning mechanisms (Price et al., 2008). For example, if a subject has already experienced an effective analgesic treatment, he/she will show a similar analgesic response to a following placebo treatment believed to be the same effective treatment used in the past (Carlino & Benedetti, 2015).

Different studies on placebo hypoalgesia and nocebo hyperalgesia focused on how expectations and previous experiences change pain perception (Colloca et al., 2010, Carlino et al., 2015). However, beside the sensory component of pain, motor preparation to avoid potentially threatening events has a crucial role in pain processing. For instance, the N2 component of laser evoked potentials, related to pain perception, shows higher amplitudes when accompanied by faster defensive motor responses (Moayedi et al., 2015). Furthermore it has been showed that A-δ fibers and the spinothalamic pathway simultaneously activate pain related areas (e.g. SI and SII) as well as different cingulate motor areas, crucial to attention reorienting and motor reactions to painful stimuli (Dum et al., 2009; Frot et al., 2008). Finally, different fMRI studies confirmed the activation of motor related areas, such as the cingulate cortex, during pain perception (Piché et al., 2010; Perini et al., 2013). These evidences indicate that pain perception could be linked with preparation to avoid or stop a painful stimulus.

The aim of the present study was to dissect hypo- and hyperalgesic effects induced by positive and negative expectations, differentiating sensory (pain anticipation) from motor components

(preparation to pain avoidance) using the contingent negative variation (CNV). CNV is an event-related potential related to anticipation (early CNV) and motor preparation (late CNV) for a given event (Brunia and van Boxtel, 2001; Chiu et al., 2004; Nagai et al., 2004). Interestingly, it has been known that CNV has a higher amplitude before high painful conditions (Irwin et al., 1966; Krop & Gerber, 1996; Siniatchkin et al, 2001).

In this study, to elicit a CNV, a warning stimulus (green/red cues) and an imperative stimulus (electrical stimulations) were presented to healthy subjects. Subjects expected the painful stimuli to be modulated by the activation/deactivation of two electrodes at their wrist. In the placebo and nocebo conditions, signaled by green or red cues, subjects expected non painful or painful stimuli respectively. In experiment 1, after a conditioning procedure, the intensity of the stimuli was kept constant regardless of the cues while in experiment 2 no conditioning was performed.

From a behavioral point of view, we expected higher pain and lower reaction times when subjects expected higher painful stimuli. Accordingly, from a neurophysiological point of view, before an expected higher stimulus, we sought to observe an higher early and late CNV amplitude. Finally, we expected these differences to be more robust after a conditioning procedure.

Methods

Subjects

34 healthy right-handed volunteers (16 males, 18 females, age = 23 ± 1.9 years) were recruited from the students of the University of Turin and were engaged in the study after

signing a written informed consent form. Participants were divided in two groups: 17 participants for experiment 1 (exp1) and 17 participants for experiment 2 (exp2). Participants were informed that they were taking part in a study investigating pain perception, in which a train of electrical stimuli would have been delivered on the dorsum of the left hand. All participants were told that they would have received the electrical stimuli with or without the activation of two electrodes placed on the left wrist (actually, two sham electrodes) that would have modulated their pain perception. In particular, participants were told that when a green cue was displayed on a computer screen the electrodes would have been activated leading to a reduction of pain perception (non painful condition), whereas when a red cue was displayed the electrodes would not have been activated (painful condition). Before the experiment, each subject underwent a clinical screening aimed to rule out the consumption of medications (e.g. painkillers) and caffeine beverages in the previous 12 hours. All the experimental procedures were conducted according to the policies and ethical principles of the Declaration of Helsinki. The study was approved by our local ethics committee.

Experimental design

Participants sat on a chair with both hands placed on a desk. The electroencephalogram (EEG) was recorded from 19 scalp locations in accordance with the 10-20 international system (Galileo, EBNeuro, Firenze, Italy) with linked common ears reference. Impedance was less than 5 KΩ in each active lead. Data were collected and digitized at a sampling rate of 512 Hz. After assembling the EEG electrodes, two electrodes were positioned on the dorsum of the left hand and were used to deliver the train of electrical stimuli, whereas the others were sham (placebo)

electrodes, positioned on the left wrist, used only to induce the expectation of pain perception modulation.

The electrical stimuli were square pulses delivered by a somatosensory stimulator (Neuroscan, Compumedics, Charlotte, NC, USA) with a 50 μs duration and a 2 Hz frequency. The stimuli lasted until motor response, and were delivered during a video presented on a 15 inches screen approximately 1 m from the participant. The video contained the following sequence (Fig. 1A): after a 4 s asterisk indicating the fixation point, a warning stimulus consisting in a light (red or green) was presented in the center of the screen for 500 ms. After 3500 ms since the disappearance of the cue (that is 4000 ms after its onset), an imperative stimulus, consisting in the train of electrical shocks to be stopped, was delivered. Finally, a sentence asking participants to rate the stimulus (from 0 to 10) appeared. To do this, we used Presentation software (Neurobehavioral System, Inc).

The intensity of the electrical shocks was based on the individual pain threshold (T) and varied according to the experimental session (see exp1 and exp2). In fact, at the beginning of the experiment, right after the positioning of the skin-electrodes, pain threshold was assessed using the staircase method (Cornsweet, 1962). Electrical shocks were delivered following the ascending-descending staircase method: starting from sub-threshold levels (2 mA of intensity) the intensity was increased with steps of 2 mA at 2Hz frequency until the first no-tactile and low painful sensation was subjectively reported (pain threshold, T_1). Then, pain intensity was increased in order to give two painful shocks and then a descending staircase was delivered with pain intensity reduced, with steps of 2 mA and 2Hz frequency, until the first no-tactile and low painful sensation was reached again (T_2). The final pain threshold (T) was calculated as the mean between T_1 and T_2.

Participants were asked to stop the train of stimuli as soon as possible pressing the left mouse button with the right hand (Fig. 1) and their reaction times were measured (RTs). Participants were also asked to verbally rate the intensity of the electrical stimuli at the end of each stimulation. They were trained to use a numerical rating scale (NRS) from 0 = no painful perception (the lower anchor) to 10=maximal painful perception (the upper anchor). In order to familiarize with the scale, subjects were asked to use the scale also during the pain threshold assessment. During the experiment, after each stimulation, a sentence asking participants to rate the stimulation appeared, with a labelled bar with the two extreme anchors.

This experimental procedure allowed us to investigate: 1) expectation by recording the CNV, 2) motor response using RTs and 3) pain perception using NRS.

Conditioning manipulation and experimental groups

Participants were randomly assigned to two different experiments: 1) conditioning and verbal suggestion (exp1) or 2) verbal suggestion alone (exp2). In exp 1 two different sessions were delivered, namely acquisition session (acq) and test session (test). In exp 2 only one session was delivered, namely verbal suggestion session (ver).

Experiment 1: conditioning

In exp 1 subjects received a conditioning procedure in order to induce a robust modulation of pain perception and two different sessions (acq and test) were delivered. Subjects were informed that the red light would have indicated painful stimuli, whereas the green light would have indicated non painful stimuli through the activation of the skin-electrodes on the wrist. Actually, in the acq session the intensity of the stimuli was decreased and increased

surreptitiously, as already done in previous studies (Colloca et al., 2008; Colloca et al., 2010), as percentages of the subjective pain threshold (T). In particular, green and red cues (conditioned stimuli, CS) were paired respectively with decreased (T-20% mA) and increased (T+100% mA) stimuli (unconditioned stimuli, US). This acquisition session was aimed at reinforcing the verbally induced expectation of pain perception modulation. A total of 40 CS-US pairings were delivered in this session (20 non painful stimuli associated with green and 20 painful stimuli with red light) (Fig. 1B, acq).

In the test session (Fig. 1B, test), pain intensity was always kept constant (T+40% mA), irrespective of the cue, and 20 stimuli were paired to green cues and 20 to red cues. This was done in order to study both the possible reduction of painful perceptions after the red cues (placebo hypoalgesia) and the increase of painful perceptions after the green ones (nocebo hyperalgesia). In order to reinforce the conditioning procedure, 8 additional stimuli (4 preceded by the green and 4 by the red cues) were delivered with the same intensity of the acquisition session but they were discarded in the final analysis. Thus, a total of 48 stimuli were delivered in the test session.

Experiment 2: verbal suggestion

In exp 2, a different group of subjects was verbally informed that the red cue would have indicated painful stimuli, whereas the green cue would have indicated non painful stimuli through the activation of the skin-electrodes on the wrist. However, pain intensity was always kept constant (T+40% mA), irrespective of the cue, from the beginning of the experiment as no conditioning was induced. Basically, in this verbal suggestion session (Fig. 1B, ver) subjects received the same expectations as participants of exp1 without being reinforced by a

conditioning procedure. A total of 40 stimuli were delivered: 20 stimuli were paired to green cues and 20 to red ones.

CNV analysis

EEG continuous data were pre-processed and analyzed using Matlab (Mathworks Inc., Natick, MA, USA), EEGLAB (Delorme and Makeig, 2004) and Letswave 4.0 (Mouraux and Iannetti, 2008). EEG data of each session (acquisition and test) were segmented into 80 epochs of 7 s each (from 1 s before the warning stimulus to 6 s after the warning). Trials were grouped depending on the cue preceding the electrical shocks (green or red lights), and epochs preceded by the same cue were averaged together, time-locked to the onset of the cue. Each epoch was baseline corrected using the pre-warning interval from -1 s to 0 s as reference. EEG epochs were low-pass filtered from 0 to 30 Hz using Fast Fourier Transformation. Electrooculogram artifacts were subtracted using a validated method based on independent component analysis (Jung et al., 2000). A mean of 2 ± 0.66 ICs were removed in both conditions. A second baseline correction was performed using the same -1 s to 0 s reference interval. Finally, epochs with amplitude values exceeding ±75 μV were rejected. These epochs constituted the 4.1% of the total number of epochs.

Thus, for each participant we obtained two averages per condition (acq and test for experiment 1; ver for experiment 2), one corresponding to the responses to electrical stimuli after the red cue, the other to the responses after the green cue. Since the period between the warning and the imperative stimulus was more than 3 s, the CNV could be divided in two components, early and late. We selected these two components based on previous studies on the CNV (Brunia and van Boxtel, 2001; Hart et al., 2012). Indeed, it has been shown that the early component of

this slow related potential lasts usually 1s after the warning stimulus, while the late component starts 1s prior the imperative stimulus (Hamano et al., 1997; Cui et al., 2000). Thus, the early CNV was identified in a time window between 1 and 2 s after the cue onset, whereas the late CNV was identified between 3 and 4 s after the cue onset.

Statistical analysis

In the exp1, acquisition and test sessions were compared. Differences in NRS scores and RTs were tested by means of a 2x2 Repeated Measures ANOVA with Session (acq vs test) and Cue (red vs green) as within factors. Differences in CNV were tested in two time periods (early and late CNV). In each time period differences in mean amplitude were tested by means of a 2x2x3 Repeated Measures ANOVA with Session (acq vs test), Cue (red vs green) and Electrode site (Frontal: average of F3, Fz, F4; Central: average of C3, Cz, C4; Parietal: average of P3, Pz, P4) as within factors. These regions were selected following previous studies on CNV that showed that the activation of fronto-central (Gomez et al., 2004) and centro-parietal (Babiloni et al., 2006) areas are crucial to elicit this slow event related potential.

In the exp2, differences in NRS scores and RT were tested by means of t-test for dependent samples. Differences in CNV were tested, as for the exp1, in two time periods (early and late CNV). In each time period differences in mean amplitude were tested by means of a 2x2 Repeated Measures ANOVA with Cue (green vs red) and Electrode site (Frontal: average of F3, Fz, F4; Central: average of C3, Cz, C4; Parietal: average of P3, Pz, P4) as within factors.

Moreover a comparison between exp1 and exp2 was performed. In particular, the test session of exp1 was compared with results collected in the verbal suggestion session of exp2 (test vs ver). Differences in NRS scores and RT were tested by means of a 2x2 Repeated

Measures ANOVA with group (test vs verbal suggestion) as between factor and Cue (red vs green) as within factors. Post-hoc Student-Newman-Keuls was applied for multiple comparisons. Differences in CNV mean amplitude were tested by means of a 2x2x3 Repeated Measures ANOVA with Group (test vs verbal suggestion) and Cue (red vs green) and Electrode site (Frontal: average of F3, Fz, F4; Central: average of C3, Cz, C4; Parietal: average of P3, Pz, P4) as within factors. Post-hoc Student-Newman-Keuls was applied for multiple comparisons.

Finally, in both experiments, a laterality analysis was conducted to check for possible effects due to the motor response of stopping the electrical stimulation. Thus, for each time period (early and late CNV), in experiment 1 differences in mean amplitude between left vs right electrodes were tested by means of a 2x2 Repeated Measures ANOVA with Session (acquisition vs test) and Side (left vs right) as factors. In experiment2 laterality differences for early and late CNV were tested by means of a 2x2 Repeated Measures ANOVA with Group (test vs verbal suggestion) and Side (left vs right) as factors.

For all the analysis, data are presented as mean ± standard error of the mean (SEM), and the level of significance was set at $P<0.05$.

Results

Psychophysics

The mean pain threshold (T) was 22.3 ± 2 mA in exp 1 and 23.3 ± 1.7 mA in exp 2. The groups did not differ in T values.

Experiment 1

In the acquisition session, mean NRS was 7 ± 0.3 after the red cue and 1.7 ± 0.3 after the green cue. In the test session, mean NRS was 5.4 ± 0.4 after the red cue and 3.0 ± 0.3 after the green cue (Fig. 2A). Results of the 2 x 2 ANOVA showed a main effect of Cue ($F(1,16)=215.9$, $P<0.001$) and a significant interaction of Session x Cue ($F(1,16) = 257.3$, $P < 0.001$). *Post-hoc* tests showed a difference in NRS between green and red cues in both sessions ($P<0.001$), thus confirming the presence of a placebo hypoalgesic effect after green cues and nocebo hyperalgesic effect after red cues. As expected, NRS after red cues in the acquisition session was significantly higher than NRS after red cues in the test session ($P<0.001$). Likewise, NRS after green cues in the acquisition session was significantly lower than NRS after green cues in the test session ($P<0.001$) (Fig. 2A).

In the acquisition session, mean RT was 533 ± 49.9 ms after the red cue and 591.6 ± 57.1 ms after the green cue. In the test session, mean RT was 520.9 ± 72.6 ms after the red cue and 477.8 ± 68.6 ms after the green cue (Fig. 2B). Results of the 2 x 2 ANOVA showed a significant interaction of Session x Cue ($F(1,16) = 6.9$, $P=0.018$). *Post-hoc* tests showed that RTs were slower after the green cue compared to red cue in the acquisition session only ($P=0.041$), whereas no significant differences were found in the test session ($P=0.13$). Moreover, RTs after green cues in the acquisition session were significantly slower than RTs after green cues in the test session ($P<0.001$) (Fig. 2B).

Experiment 2

In the verbal suggestion session, mean NRS was 3.7 ± 0.4 after the red cue and 2.9 ± 0.3 after the green cue (Fig. 2A). T-test for dependent sample showed a difference in NRS between green and red cues ($t(16)=3.3$, $p<0.004$), proving that the only expectation of receiving no

painful or painful stimuli actually modifies pain perception inducing a placebo hypoalgesic effect after the green cues and a hyperalgesic effect after the red cues.

Mean RT was 525 ± 129.3 ms after the red cue and 513 ± 138 ms after the green cue (Fig. 2B). T-test for dependent sample showed no differences (P = 0.77) in RTs between red and green cue, confirming that expectations acted only at the sensory level.

Comparison between experiment 1 and experiment 2

As far as NRS is concerned, results of the 2 x 2 ANOVA showed a main effect of Cue ($F(1,32) = 45.04$, $P<0.001$) and a significant interaction of Group x Cue ($F(1,16) = 257.3$, $P<0.001$). *Post-hoc* tests showed a difference between NRS after green cue compared to red cue in both sessions ($P<0.001$), thus confirming the occurrence of a placebo hypoalgesia after the green cues and a nocebo hyperalgesia after the red cues in the expectation group. In addition, NRS after red cues in the test session was significantly higher than NRS after red cues in the expectation group ($P<0.003$), suggesting the occurrence of a higher nocebo hyperalgesic effect after the conditioning procedure of the exp1. No differences were observed for the green cues (Fig. 2A).

As far as RTs is concerned, results of the 2 x 2 ANOVA showed no significant differences between groups and sessions (Fig. 2B).

Electrophysiology

Electrophysiological results are presented in Fig. 3. In both experiments, the laterality analysis did not yield significant differences, confirming the bilaterality of the CNV regardless the motor response (Walter et al., 1964).

Experiment 1

In exp1, the acq session was compared with the test session of the conditioning group.

For each electrode site differences in average amplitudes for early and late CNV were tested by means of a repeated measures ANOVA with Session (acq vs test), Cue (red vs green) and Electrode Site (Frontal; Central and Parietal) as within effects. The early CNV component (1-2 s after the warning onset) showed a significant main effect of the Cue in all electrode sites, represented by a decreased early negativity after green cues compared to red cues ($F(1,16)=21,4$ $P<0.001$) in both the acquisition and test session (Fig. 3, Acq and Test). The late CNV component (3-4 s after the warning onset) showed a significant interaction between Session x Cue in all electrodes sites ($F(1,16)=14.14$, $P<0.002$). However, *post-hoc* tests showed a decreased late negativity after green cues compared to red cues only in the acq session ($P<0.001$) but not in the test session. Also, a significant increased late negativity after red cues in the acq session compared to red cues in the test session was found ($P < 0.006$**)** (Fig. 3, Acq and Text).

Experiment 2

In exp2, early and late CNV after red and green cues were compared for each electrode site by means of a repeated measures ANOVA with Cue (red vs green) and Electrode site (Frontal; Central and Parietal) as within effects. The early CNV component (1-2 s after the warning onset) showed a significant main effect of the Cue ($F(1,16)=5.02$, $P=0.04$) in all electrodes sites, represented by a decrease of early CNV mean amplitude after the green cues compared with the red ones. No differences occurred in the late CNV component (3-4 s after the warning onset).

Comparison between experiment 1 and experiment 2

The test session of exp1 (test) was than compared with the verbal suggestion session (ver) of the exp2.

For each electrode site differences in average amplitudes for early and late CNV were tested by means of a repeated measures ANOVA with Cue (red vs green) and Electrodes (Frontal; Central and Parietal) as within effects and Group (test vs expectation) as between factor. The early CNV component (1-2 s after the warning onset) showed a significant main effect of the Cue, represented by a decreased early negativity after green cues compared to red cues ($F(1,32)=12.9$, $P<0.001$) in both groups and all electrodes sites. Moreover a significant main effect of group was found, represented by a reduction of CNV amplitude in the verbal suggestion group ($F(1,32)=11.34$, $P<0.002$) in all electrodes sites (Fig. 3, Test and Ver).

Discussion

The aim of the present study was to investigate how placebo hypoalgesia and nocebo hyperalgesia modulate pain expectancy, pain perception and motor reaction to avoid pain. To accomplish this goal, different painful and non painful electrical stimuli were delivered to healthy subjects. All subjects expected to receive higher painful stimuli after the presentation of a red cue and no painful stimuli after the presentation of a green cue. Subjects were instructed to rate the intensity of pain (sensory component of pain) and to stop the electrical stimulation as soon as possible (motor component of pain). In order to study the neurophysiology of expectancy of pain, differentiating the sensory and motor components, the CNV was used as an objective

measurement of cortical activity related to anticipation of a given event (early CNV) and preparation of the motor response (late CNV).

Two different experiments were conducted. In the first experiment (exp1), after an acquisition session, in which subjects learned to associate a red cue with painful stimuli and a green cue with non painful stimuli, a test session was delivered, in which the intensity of the electrical stimuli was the same. In the second experiment (exp2), a second group of participants received only verbal suggestions of pain modulation, without receiving any reinforcement from a previous conditioning procedure. In this group only one session was delivered, in which the intensity of the electrical stimuli was the same.

Following the recent predictive coding model, placebo hypoalgesia and nocebo hyperalgesia can be interpreted as the combining effect of top-down prior expectations, or predictions of pain decrease/increase, with bottom-up sensory signals (Büchel et al., 2014). Expectations, ex novo or based on previous experiences, play a crucial role when a prediction error or mismatch occurs between the expectation of pain and the actual pain.

In our study, the acquisition session of exp 1 can be conceptualized as a “no mismatch condition” in which no error signal occurs: indeed, during this session, there is no mismatch between the prediction of pain (that is the expectation of perceiving no pain after the green cues and pain after the red cues) and the actual painful signal (lower pain intensity after the green cues and higher intensity after the red cues). According to our results, we observed a decrease of pain perception, as reported by means of the NRS, slow RT after the non painful stimulations (green cues) and an increased pain perception along with faster RT after the painful stimulations (red cues). In line with the behavioural data, a modulation of CNV was observed with a low mean

amplitude when no pain was expected and high mean amplitude when pain was expected, throughout the scalp in both the early and the late phases.

On the contrary, the test session of exp 1 and the verbal suggestion session of exp 2 can be conceptualized as “mismatch conditions”. Indeed, during these sessions a mismatch occurs between the prediction of pain (that is the expectation of perceiving less pain after the green cue and more pain after the red cue) and the painful signal (always kept constant). In the test session of exp1 we observed that RT did not change and only pain perception was modulated with a placebo hypoalgesic effect after the presentation of green cues and a nocebo hyperalgesic effect after the presentation of red cues. Again, CNV data were in line with our behavioral results showing a modulation of early CNV throughout the scalp with low amplitude after the green cues and high amplitude after the red cues. Following these results, expectation of hypoalgesia and hyperalgia seems to affect the sensory component of pain producing a modulation of pain perception while the motor reaction to pain is not affected. Similar results occurred in the exp2, as subjects experienced a placebo hypoalgesic effect after the presentation of green cues and a nocebo hyperalgesic effect after the presentation of red cues at the sensory level (NRS) and not at the motor level (RT). Again we observed only a modulation of the early CNV amplitude. Thus, the predictive coding theory seems to describe the placebo hypoalgesic effect and the nocebo hyperalgesic effect only at the sensory level. It is worth noticing that there is no difference between the RTs after nocebo trials during acq (2T) and RTs after the nocebo trials during in the test and ver conditions (T+40%). A possible explanation is that the motor system, when faced with a physical stimulus intensity that is above the subjective pain threshold (i.e. a painful stimulation), prepares the fastest response possible to avoid/stop pain. This could create a “ceiling” effect, so that differences between participants’ responses after the 2T (noc acq) and

T+40% (plac and noc test and evo) stimulations were not significant due to fact that the motor system programmed the fastest movement possible to stop pain. This effect doesn't occur when the motor system is faced with intensities below the subjective pain threshold (i.e. non painful stimulations) that is the placebo trials during acq (T-20%).

The lack of placebo and nocebo effects at the motor level could be explained by different reasons. First, in our experiments, expectations were only directed to the reduction or increase of pain perception and subjects were not informed about the reduction or increase of RTs. Thus, it is possible that without explicit expectations about reaction times, the mismatch between top (i.e. cognitive information) and bottom (i.e. motor reactions to electrical stimulations) levels still remained. For this reasons, future studies could investigate directly the role of explicit expectations about motor responses to pain perception. Second, due to the low number of stimulations, we did not measure trial by trial changes of RTs, limiting our interpretation of the overall modulation of the RTs and not to the changes of placebo and nocebo magnitude over time. Third, it has to be noted that participants received more pulses during placebo trials, which might have consequently influenced RT´s in placebo and nocebo conditions differently. Further studies should focus on more continuous forms of stimulation to better investigate the relationship between RT and pain intensity.

Finally, as explained below, it is possible that RTs represent a "hard wired" kind of defensive response, i.e. more related to the actual physical intensity of the stimulus and, for this reason, less sensible to expectations.

Interestingly, similar difference between motor and sensory domains has been documented in different visual perception studies. For instance, it has been showed that even though participants visually perceive a specific target whose size or location has been distorted

due to a visual illusion, they are still capable to grasp it or point to it without showing significant mistakes (Aglioti et al., 1995; Bridgeman et al., 1997; Dyde and Milner, 2002). To explain these data, the authors usually mentioned the “two visual systems model” that postulates the existence of two separate visual pathways for perception and action, where the first is influenced by illusion, while the latter remains unaffected (Milner and Goodale, 1995). A similar explanation could be applicable to our data, identifying two different pain sub-pathways, one more related to pain perception and the other more related to pain defensive motor responses. Indeed, different studies on animal models showed that the most important tract for nociception, the spinothalamic pathway, simultaneously activates different areas: the primary and secondary somatosensory cortex, crucial to pain perception, and different motor areas such as the posterior midcingulate cortex, crucial to prepare the motor response to painful stimuli (Dum et al., 2009; Frot et al., 2008). Thus it is possible that, even though motor responses and pain perception are interwoven at a central level, they still can be influenced separately via direct pathways.

As expected, the behavioural and electrophysiological modulations observed in the verbal suggestion group (exp2) were significantly smaller than those observed in the test session of the conditioning group (exp1). In other words, our results show that, after a conditioning procedure, placebo and nocebo effects occur on pain perception and these effects are higher compared to those obtained only with verbally induced expectations. This is in line with different behavioral and neuroimaging studies demonstrating that reinforcing expectations with previous experiences of hypoalgesia produces more robust placebo analgesic effects (Colloca and Benedetti, 2006; Colloca et al., 2010). Moreover, it has been reported that these subjective analgesic effects are correlated with a reduction of brain responses to nociceptive stimuli assessed by means of laser evoked potentials (Colloca et al., 2008; Colloca et al., 2010; Carlino et al., 2015). Neuroimaging

studies have also demonstrated that during expectation of hypoalgesia, an increased activation occurs in different brain regions, such as the anterior cingulate, precentral and lateral prefrontal cortex and in the periacqueductal gray (PAG) (Amanzio et al., 2013). More specifically, the acquisition session is a crucial step for the development of conditioned hypoalgesia, and it has been shown that anticipation of hypoalgesia is accompanied by signal increases over time in different brain regions, such as medial (MPFC) and lateral (DLPFC) prefrontal foci (Lui et al., 2010).

Traditionally, placebo and nocebo studies have extensively investigated pain perception (Carlino et al., 2014). However, the expectation of pain assessed using the CNV as objective measure of expectation has never been studied in this field. According to our results, CNV appears to be a good candidate to better understand how conditioning and expectations alone shape sensory and motor placebo and nocebo effects. CNV is not a unique wave and is traditionally divided in two different components: namely the early and late CNV (Hamon and Seri, 1987; Forth and Hare, 1989; Cui et al., 2000; Brunia et al., 2001; Chiu et al., 2004; Nagai and Critchley, 2004; Lütcke et al., 2009; Hart et al., 2012). The neural generators for the early CNV wave have been reported to include the prefrontal cortex, anterior cingulate cortex, premotor cortex and supplementary motor area (Gòmez et al., 2004; Lütcke et al., 2009), while basal ganglia, prefrontal and pre-motor cortices, and dorsal anterior cingulate cortex are responsible for the late CNV component (Ikeda et al., 1997; Gòmez et al., 2003; Lütcke et al., 2009). While the late CNV is detected just prior to the onset of the imperative stimulus and is clearly related to motor anticipation and preparation (see also Damen and Brunia, 1994), the early component is more complex. It is thought to be related to initial attention to the warning stimulus, to the cognitive effort to respond to the imperative stimulus, and to motivation to

respond (Hamon and Seri, 1987; Forth and Hare, 1989). It is also worth noting that early CNV is generally thought to index controlled, rather than automatic, psychological processes in response to the warning stimulus in order to anticipate a subsequent imperative target stimulus (Picton and Hillyard, 1988). Thus, from an electrophysiological point of view, these intrinsic differences in CNV can be used to investigate the anticipation phase preceding a placebo or nocebo hypo- or hyperalgesic responses. Indeed the expectation related to placebo hypoalgesia or nocebo hyperalgesia could be dissected in at least two components. A first component, observed in the CNV early phase could represent the somatosensory predictions that in turn elicit the cognitive evaluation of pain perception measured through the NRS. A second component, observed in the CNV late phase, could represent a more "instinctive" motor reaction to the painful stimulus and thus could be more related to pain avoidance measured by means of RTs.

From a behavioural point of view, it is crucial to dissect the expectation of pain, investigating not only pain perception modulation, but also the modulation of motor preparation for pain avoidance. Indeed, expectation of painful stimuli is tightly related to their avoidance, as a safety measure for our organism, and pain avoidance seems to be a crucial mechanism in pathologies such as fibromyalgia (Van Koulil et al., 2011) and back pain (Leeuw et al., 2007). It is worth noticing that the general term pain avoidance could be misleading as the main action to avoid pain is usually represented by withdrawal actions, while in this study participants were asked to stop a train of electrical stimulations to avoid pain. However, even though withdrawal is the most basic defensive action associated with pain, mammalian brains evolved complex actions in order to avoid pain (Morrison et al., 2013), such as using a hand to swat a fly which could potentially hurt the organism. Also from a clinical point view it is more ecological to think about a patient who cannot “outrun” his pain but has to stop it using specific drugs or injections. Still,

future studies on CNV and pain expectation could focus on the specific types of motor responses to pain, looking for potential differences between withdrawal and stopping actions. More in general, motor preparation in response to pain and pain perception are strictly linked to each other so that pain could not just signal the presence of a harmful stimulus but instead could signal a harmful state in order to program specific actions (Wall, 1999). Indeed, nociceptive withdrawal reflex neurons localized in the dorsal horn of the spinal cord do not have a somatosensory organization but are instead organized reflecting their different target muscles, thus pain perception is already action-oriented from its early stages (Levinsson et al., 2002; Schouenborg, 2003). Furthermore, consistent activation of motor-related areas like the mid-cingulate cortex during pain perception suggests that these areas are crucial in the control and execution of context-sensitive behavioral responses to pain (Perini et al., 2013).

Some limitations of the present study need to be acknowledged. First, even though we have differentiated CNV effects between areas on the scalp, we have to consider that EEG is not a technique with a high spatial resolution, thus it would be ideal to couple this technique with another, more spatially dedicated, technique like fMRI. Another possible solution to this spatial problem could be represented by the use of a high spatial resolution EEG (i.e. with 64 or 128 channels) and the use of a source analysis program. Second, this is a typical experimental situation with pain induced by electrical stimuli, thus it does not necessarily reflect a clinical situation, in which pain is usually long lasting and not confined to a single cutaneous spot. Third, the anticipatory time lag analysed in the present study is quite limited. In a more natural clinical situation, motivation and motor responses are usually long-lasting, thus the balance between these two factors can be different in clinical pain. However, it should be noted that motivation has been found to play a role in a number of situations (Price and Barrell, 2000; Price et al.,

2001; Price et al., 2008). Fourth, in the present study we did not used a neutral stimulus, mimicking the same experimental procedure used in previous studies in order to reduce the length of experiment (Carlino et al., 2015). Future studies should add a neutral control stimulus with a medium intensity level to better differentiate between placebo hypoalgesia and nocebo hyperalgesia. Finally, other studies should focus on a bigger sample size to consolidate the present data.

This study is an attempt to use CNV as on objective measure of expectations leading to hypo- and hyperalgesic effects, investigating not only the sensory component of pain perception but also the motor preparation to stop an expected painful or non painful stimulation. Our data show that, regardless of the actual intensity of the stimulation, positive expectations lead to lower pain perceptions while negative expectations lead to higher pain perceptions. These results are stronger when verbal suggestions are accompanied by a conditioning procedure. However, interestingly, reaction times (i.e. response to pain) did not change, after positive or negative expectations. In line with these behavioural data, CNV amplitudes completely mimicked these results showing an higher early amplitude when high painful stimulations were expected and a lower early amplitude when no painful stimulations were expected, while late CNV amplitudes were not affected by the expected intensity of the stimulation. Thus, our data show a difference between sensory and motor aspects in the placebo/nocebo responses that can be measured during the expectation of an incoming painful stimulation, through the classic expectancy wave, CNV. Although the experimental approach we used is far from representing a real clinical situation, we believe that it can provide important information on sensorimotor integration in placebo hypoalgesia and nocebo hyperalgesia.

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Conflict of interest

None declared.

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Legends

Fig.1. Experimental setting and paradigm. A) The sequence of visual stimuli on the computer screen is shown. Either the red (R) or green light (G) was paired with painful or non painful stimuli, respectively. B) Sequence of electrical stimuli in both the acquisition and test session. T= pain threshold.

Fig. 2. Psychophysical and behavioral results. A) Pain rating (NRS) for red (right columns) and green stimuli (left columns) in the acquisition (acq) and test (test) session of Exp1 and the verbal suggestion session (ver) of Exp 2. B) Reaction times (RTs) results for Exp 1 (acq and test) and Exp 2 (ver). Columns represent means and error bars represent SEM. *P<0.05; **P<0.01

Fig. 3. Electrophysiological results. A) Early and late CNV topographical distributions across the scalp in acquisition (acq), test (test) and verbal suggestion (ver) sessions after green (upper squares) and red (lower squares) cues. B) Early and late CNV distribution, grandaverage across the scalp, after green (green line) and red (red line) cues in acquisition (acq), test (test) and verbal suggestion (ver) sessions. C) Early and late CNV mean amplitude after green (green bar) and red (red bar) cues in acquisition (acq), test (test) and verbal suggestion (ver) session. Columns represent means and error bars represent SEM. *P<0.05; **P<0.01; ***P<0.001.