The effect of temporal information on placebo analgesia and nocebo hyperalgesia

(Running Title: Temporal information modulate pain perception)

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Abstract

Objective. Expectations are known to be key determinants of placebo and nocebo phenomena. In previous studies, verbal suggestions to induce such expectations have mainly focused on the direction and magnitude of the effect while little is known about the influence of temporal information.

Methods. Using an experimental placebo and nocebo design, we investigated whether information about the expected onset of a treatment effect modulates the start and time-course of analgesic and hyperalgesic responses. Healthy volunteers (N=166) in three placebo and three nocebo groups were informed that the application of an (inert) cream would reduce (placebo groups) or amplify pain (nocebo groups) after 5, 15 or 30 minutes. Two control groups were also included (Natural History and No Expectations). Participants' pain intensity rating of electrical stimuli administered before and 10, 20 and 35 minutes after cream application were obtained.

Results. Mixed-method analysis of variance showed a signifcanat interaction between group and time $F(12,262)=18.172$, $p<0.001$, $p\eta2=0.454$, suggesting that pain variations differed across time points and between groups. Post hoc comparisons revealed that placebo and nocebo groups began to show a significantly larger change in perceived pain intensity than a no-expectancy control group at the expected time-point ($p<0.05$) but not earlier ($p>0.05$). Once triggered, the analgesic effect remained constant over the course of the experiment whereas the hyperalgesic effect increased over time.

Conclusions. Our results indicate that temporal suggestions can shape expectancy-related treatment effects which – if used systematically - could open up new ways to optimise treatment outcome.

Keywords: Placebo effect, Nocebo effect, Pain, Expectation

Introduction

The outcome of analgesic treatment has been shown to considerably depend on an individual's expectations. The anticipation of pain relief can boost the treatment effect whereas expectations of increased pain can aggravate pain (1–3). Studies using brain imaging techniques have begun to unravel the neural basis of expectancy effects on pain perception. They identified a network of brain regions including the dorsolateral prefrontal cortex, rostral anterior cingulate cortex, periaqueductal gray and amygdala which modify pain-related brain activity in a top-down fashion depending on the expectation of the individual (4–6). In experimental contexts, expectancy effects on the perception of pain have mainly been studied using placebo or nocebo paradigms where participants are led to believe that an inert substance or procedure will have an analgesic effect (placebo) or a hyperalgesic effect (nocebo) (2,3,7,8). However, the modulatory influence of expectations has also been demonstrated in clinical studies on pain (9–11) and other health conditions (12). These observations have recently inspired a new wave of research aiming to harness the potential of positive expectations and avoid the detrimental effect of negative expectations in clinical populations (13).

So far, research in this field has mainly focused on two characteristics of expectation, namely its direction (i.e., whether the treatment is expected to improve or aggravate symptoms) and magnitude (i.e., how strong the expected effect will be). However, any expectation will also be linked to aspects of time – for instance, when the treatment is going to take effect and for how long it is going to last. Although some studies include information about the expected onset and duration of the effect (14–17), the influence of temporal information on treatment outcome has not been studied systematically.

Here, we investigated in healthy volunteers whether the onset of placebo analgesia and nocebo hyperalgesia can be modulated by varying the information participants receive about the onset of the expected treatment effect. We hypothesise that the onset of the treatment effect (i.e., analgesia in placebo groups and hyperalgesia in nocebo groups) depends on the information provided and coincides with the expected onset time.

Methods

Participants

166 healthy volunteers were recruited from the student population of the University of Turin. Sample size calculation has been calculated using G*Power (see Sample size calculation Supplemental Digital Content 1). Students were recruited during classes and were informed about the possibility to participate in a study investigating the onset of action of an analgesic or hyperalgesic cream. Those interested in participating in the study were contacted by phone. Participants provided written informed consent before starting the experiment. Signing the consent, all participants agreed that some details of the experimental procedure would have been omitted during the experiment and that they would be debriefed via email about the details of the study at the end of the experiment. Both in the informed consent and in the debriefing email, participants were told that they could decide to withdraw their consent and data; no one decided to do so. Participants received neither payment nor credits to participate in the study.

Data from nine participants were excluded from the analysis because they met our outlier criteria (see section on data analysis), leading to a total sample size of 157 participants (for participants' characteristics see Table 1, Supplemental Digital Content 2). Data collection started in February 2019 and ended in June 2019. Participants had no history of neurological, psychiatric, or other medical conditions and were instructed to refrain from consuming alcohol or taking analgesic medication for at least 12 hours prior to the experiment. Experimental procedures were conducted according to the policies and ethical principles of the Declaration of Helsinki. The study was approved by the Ethics Committee of the University of Turin (registration number: *138875*).

Experimental design

Participants were assigned to one of three placebo groups, three nocebo groups, a no expectancy (NE) group or a natural history (NH) group (see below for details; Figure 1). The NH group was filled first, as a form of pilot study. For this reason, this group has been excluded from the primary analysis and only serves as control for pain perception fluctuations over time. The remaining participants were divided into the seven experimental groups (i.e. NE group, Placebo groups, Nocebo groups) using stratified randomization in order to control for demographic variables (e.g. age and sex) (18).

Placebo groups

Participants in the placebo groups were informed that an analgesic cream would be applied to reduce the painful sensation induced by an electrical stimulation (see section on noxious stimulation). They were led to believe that the analgesic would become effective after 5 minutes (Positive Verbal Suggestion 5 Group, P5 group, N=21), 15 minutes (Positive Verbal Suggestion 15 Group, P15 group, N=20) or 30 minutes (Positive Verbal suggestion 30 Group, P30group, N=20). The 5 minutes interval was chosen to mimic the effect of a fast-acting analgesic, whereas the 15 and 30 minute interval were intended to resemble the effect of analgesics with a delayed onset time.

Nocebo groups

Participants in the nocebo groups were informed that a hyperalgesic cream would be applied to increase the painful sensation induced by the electrical stimulation. Participants were informed that the hyperalgesic effect would set in after 5 minutes (Negative Verbal Suggestion 5 Group, N5 group, N=19), after 15 minutes (Negative Verbal Suggestion 15 Group, N15 group, N=19) or after 30 minutes (Negative Verbal Suggestion 30 Group, N30 group, N=18). The time intervals were identical to those used in the placebo groups.

No expectancy group

Participants assigned to the No Expectancy Group (NE group, N=21) were informed that an inert cream would be applied that would have no effect on their pain perception.

Natural history group

A Natural History Group (NH group, N=19) was added to control for the natural course of pain. In this group, no cream was applied and no verbal suggestions concerning treatment effectiveness were given.

Noxious stimulation

Pain was induced using electrical stimuli delivered by a somatosensory stimulator (Neuroscan, Compumedics, Charlotte, NC, USA). See Noxious stimulation in the Supplemental Digital Content 1 for more details.

Pain intensity ratings

All participants were instructed to rate the perceived intensity of the ten noxious electrical stimuli 10 minutes (Test10'), 20 minutes (Test 20') and 35 minutes (Test 35') after the cream had been applied. Ratings were provided verbally using the same NRS as during the calibration phase (i.e., 0 representing no pain, 1 the beginning of a painful sensation, 5 moderate and 10 unbearable pain) and were recorded by the experimenter.

Assessment of expectations and anxiety

Participants completed the State-Trait Anxiety Inventory (I-II) (19) prior to the actual experiment, to assess potential baseline differences in anxiety level between groups. Moreover, after the experiment, they were asked to rate their expectations about the efficacy of the cream. The question asked was: "Before the experiment, what was your expectation about the analgesic action of the cream?". Five possible answers were proposed: 1) the cream will completely reduce my pain; 2) the cream will partially reduce my pain; 3) the cream will slightly reduce my pain; 4) the cream will not alter my pain; 4) the cream will increase my pain. Questions were reversed for the nocebo groups, in which the hyperalgesic action of the nocebo cream was investigated.

Experimental procedure

The experimental procedure is summarized in Figure 2. Participants sat on a chair with the right arm placed on a desk in front of them. A customised wall clock with 5-minute intervals (i.e., 5 to 55) was positioned in front of them. The clock face also showed an icon of a cream tube at the 12 o'clock position to indicate the time-point at which the cream had been applied. Participants were informed that the clock was used to help them keep track of time and to know when the next test session was imminent. Following calibration of the individual stimulation intensity, participants were familiarised with the experimental setup in a practice run. After a 5 minutes break, the actual experiment commenced with the baseline session. Subsequently, participants were informed about the group they had been assigned to and the cream was applied in all groups except the no treatment group along with the verbal instructions (see 'Verbal suggestions' for details). Immediately after the cream had been applied, the experimenter adjusted the clock so that the minute hand pointed at the 12 o'clock position, indicating the time of cream application ('Time 0'). Although no cream was applied in the no

treatment group, the same procedure was followed and participants were told that 'Time 0' referred to the time at which the cream would have been applied if they had been allocated to the active condition. Ten minutes after cream administration, the first test session was run (Test 10') which was followed by a 10 minute rest period. The second test session was completed 20 minutes after cream application (Test 20') and followed by a 15 minute rest period. Participants underwent the third and final test session 35 minutes after cream application (Test 35'). During the rest periods between test sessions participants were instructed to relax and were given the opportunity to read, study or use their phone. Although participants were able to track time by looking at the watch in front of them, the experimenter communicated verbally that the next test session was about to start by saying "Okay, now we will repeat the test again".

Verbal suggestions

Participants in the placebo groups expected to receive a cream that would decrease their pain (analgesic cream), whereas participants in the nocebo groups expected to receive a cream that would increase their pain (hyperalgesic cream). The instructions were provided at the time-point of cream application. Below, an example of the instructions provided to the P5/N5 group is reported.

"Studies have shown that this is an effective analgesic/hyperalgesic cream. Specifically, they have shown that this cream already takes effect after 5 minutes from its application. Therefore, we expect that all the tests we will do after this moment are under the effect of the cream, for this reason you will feel less/more pain (compared to the first test) in all three test sessions after 10, 20 and 35 minutes [experimenter points at time 10, 20 and 35 minute marks on the clock]".

Participants in the NE group were told that an inert cream would be applied: ''The agent you will receive is an inert cream that only has hydrating properties but no effect on pain perception. The pain at the three test sessions after 10, 20 and 35 minutes [experimenter points at time 10, 20 and 35 minute marks on the clock] will therefore be similar to the first baseline". Participants in the NH group were told that they would receive no treatment and the following instructions were given: "Your group serves to assess natural variations of pain over time. Your pain may vary or may remain the same. Simply report your perceived pain intensity when you are prompted to do so."

Cream

A sham cream was administered in the placebo, nocebo and NE groups. It consisted of AquaGel Solution (2g) and water (17ml) and was presented to participants in a clear plastic tube. The experimenter applied the cream within a radius of 2cm around the electrode and massaged it into the skin to ensure that it was fully absorbed.

Debriefing

Participants were debriefed via email, giving details on the real aim of the study, a list of readings on the topic, as well as the possibility of discussing doubts or concerns with their data being used.

Statistical analyses

Analyses were performed using Statistica Software (StatSoft, version 9 for Windows). First, a mean pain intensity rating was calculated for each time-point (i.e., baseline and T10', T20' and T35') by averaging across the 10 ratings per time-point, resulting in four mean pain intensity ratings for each participant. Second, these average measures were tested for normal distribution using the Shapiro-Wilk test. The test did not report significance for any of the variables.

Before running further statistical analyses, the data were screened for outliers. Participants were defined as outliers and discarded from further analysis if any of their average pain intensity ratings were 2.5 times the standard deviation higher or lower than their group mean value. Two outliers were removed from the NH group (N=19), the N15 group (N= 19) and the N30 group (N=19). One outlier was excluded from the NE group (N=21), the P30 group (N=20), and the N5 group (N=19). The final sample size was therefore 157 participants.

To test for baseline differences in demographic variables, baseline pain intensity scores and STAI I-II scores, we first compared the eight groups using an analysis of variance (ANOVA) for continuous variables, or Chi-square test for categorial variables.

Turning to pain intensity ratings, to test whether pain perception changed over time irrespective of treatment and treatment onset expectations, changes in mean pain ratings of the NH group for the four different time-points were analyzed separately using repeated measure ANOVA.

Similarly, to investigate whether the application of the cream in itself (e.g. moisture, sensation of freshness) interacts with pain perception, repeated measure ANOVA was used to assess pain rating changes the four different time-points in the NE group.

To test whether placebo and nocebo effects occurred, we conducted 6 repeated measure ANOVAs, one for each experimental group (P5, P15, P30, N5, N15, N30), to evaluate changes in mean pain ratings (raw scores) within each group for the four different time-points.

Subsequentially, we calculated the percentage change in NRS scores from the Baseline Session to each Test Session (delta%, Δ%) in the placebo and nocebo groups. Thus, three Δs were calculated for each group, corresponding to the percentage change in pain perception at T10', T20' and T35' (Δ_{10}, Δ_{20} and $\Delta_{35,}$ respectively). To compare the effect of information regarding the onset of the expected treatment effect between groups, Δ_{10}, Δ_{20} and Δ_{35} of the placebo, nocebo and no expectancy group were entered into a 3 x 7 mixed ANOVA with the within-group factor TIME (3 levels: T10', T20' and T35') and between-group factor GROUP (7 levels: P5, P15, P30, N5, N15, N30 and NE). Significant effects were followed up using pairwise Student-Newman-Keuls (SNK) post hoc tests. To investigate the significant results of the factor GROUP and the interaction between GROUP and TIME (see Results), the placebo and the nocebo groups were compared against the NE group, which served as a reference. To explore the duration of the induced effects in each group, changes in pain intensity relative to baseline (i.e., Δ_{10}, Δ_{20} and $\Delta_{35)}$ were compared separately for P5, P15, N5 and N15.

Furthermore, as done in other studies (20), sex, age and BMI were included as covariates. Since we found no significant effects of these covariates on pain perception nor interactions between these predictors and results were identical to the 3 x 7 mixed ANOVA, we have only included the more parsimonious analysis.

To investigate the possible influence of anxiety (scores of STAI I and STAI II) on pain perception, two correlation analysis were performed: 1) a correlation analysis between anxiety scores and mean pain intensity ratings at Baseline and 2) a correlation analysis between anxiety scores and the percentage (Δ) of pain decrease (in the placebo groups) and increase (in the nocebo groups) after the application of the cream (i.e. Δ10 in P5 and N5 groups, Δ20 in P15 and N15 groups, Δ35 in P30 and N30 groups). To investigate the possible influence of expectations of treatment efficacy on pain perception, a correlation alaysis was performed between expectancy scores and the percentage (Δ) of pain decrease (in the placebo groups) and increase (in the nocebo groups) after the application of the cream.

Note that data from the NH group were not included in further analyses as participants in this group did not show a significant change in pain perception over time (see Results).

Data are presented as mean ± standard error of the mean (SEM), and the level of significance was set at $p < 0.05$.

Results

The groups did not differ with respect to age, Sex, BMI, state (STAI-I) and trait (STAI-II) anxiety scores nor pain intensity at baseline ($p>0.05$ for all comparisons) (see Table 1, Supplemental Digital Content 2).

Effects of no treatment

Repeated measure ANOVA showed no significant changes in mean pain ratings of the NH group for the four different time points [$F(3,54)=0.664$, $p=0.578$, $p\eta2=0.181$]. This indicates that without cream administration and verbal instruction regarding expected changes in pain perception, pain remained stable over the entire course of the experiment. Repeated measure ANOVA showed a significant main of time on mean pain ratings in the NE group [$F(3,60)=2.88$, $p=0.043$, $p\eta2=0.660$]. SNK post hoc tests reported a tendency to significance when comparing baseline with T10 ($p=0.051$) and T20 ($p=0.057$), and significant pain increase between baseline and T35 ($p=0.044$). This suggests that pain perception tends to increase with time when the inert cream is applied with no expectation modulation.

Placebo and Nocebo Raw Data Analysis

Three repeated measure ANOVAs for the three placebo groups showed significant changes in mean pain rating for P5 [$F(3,60)=9.77$, $p<0.001$, $p\eta2 =0.997$], P15 [$F(3,57) =22.86$, $p<0.001$, $p\eta2 =1.000$], P30 [$F(3,57)=15.01$, $p<0.001$, $p\eta2=1.000$], for the four different time points. In P5 group, SNK post hoc tests showed a significant reduction in mean pain ratings from baseline to T10, T20 and T35 ($p<0.001$), suggesting that placebo analgesia occurs in all the Test Sessions occurring 10 minutes after cream application. However, no significant difference was shown between T10 and T20 ($p=0.254$), T10 and T35 ($p=0.334$) and T20 and T35 ($p=0.786$), indicating that placebo analgesia remains stable once triggered. In P15 group, SNK post hoc tests indicated a significant decrease in pain ratings from baseline to T20 ($p<0.001$) and to T35 ($p<0.001$). Similarly, a significant decrease was reported from T10 to T20 and from T10 to T35 ($p<0.001$). These comparisons show that placebo analgesia arise in the two test sessions

occurring after 20 minutes from cream application. No significant difference was shown between T20 and T35 (p=0.839) showing, similarly to P5, that once analgesia is triggered it remains stable over time. In P30 group, SNK post hoc tests reported a significant decrease in pain ratings from baseline to T35 (p<0.001), from T10 (p=0.001) to T35 and from T20 to T35 (p<0.001) showing placebo analgesia to arise in the final test session, after 35 minutes from cream application.

Three repeated measure ANOVAs for the three nocebo groups showed significant changes in mean pain rating for N5 [$F(3,54)=26.27$, $p< 0.001$, $p\eta2=1.000$], N15 [$F(3,54) =33.40$, $p< 0.001$, $p\eta2=0.997$], N30 [$F(3,51)=26.44$, $p< 0.001$, $p\eta2=1.000$], for the four different time points. In N5 group, SNK post hoc tests showed a significant increase in mean pain ratings scores from baseline to T10 (p<0.001), T20 (p<0.001), and T35 (p<0.001), suggesting that nocebo hyperalgesia occurs in all the test sessions occurring after 10 minutes from cream application. Additionally, significant increase in pain ratings was reported between T10 and T20 (p=0.045) and between T10 and T35 (p=0.006), suggesting that once triggered, nocebo hyperalgesia increases over time. In N15 group, SNK post hoc tests indicated a significant increase in pain rating from baseline to T20 ($p< 0.001$) and to T35 (p<0.001) as well as from T10 to T20 and to T35 ($p< 0.001$). These comparisons indicate that nocebo hyperalgesia occurs during the two test sessions after 20 minutes from cream application. In this case also, a significant increase was shown between T20 and T35 (p=0.011). Therefore, the N5 and N15 groups agree that once nocebo hyperalgesia is triggered, it increases over time in this study. In N30 group, SNK post hoc tests displayed a significant increase in pain ratings from baseline to T35 (p<0.001), from T10 (p<0.001) to T35 and from T20 to T35 (p<0.001) showing nocebo hyperalgesia to arise in the final test session, after 35 minutes from cream application.

Placebo and Nocebo Percentage Change Analysis

The 3x7 mixed ANOVA revealed a significant main effect of TIME ($F(2,262)=7.363$), p=0.001, $p\eta2=0.053$) and a significant main effect of GROUP ($F(6,131)=31.701$, p<0.001, $p\eta2=0.069$). See Placebo and Nocebo Percentage Change Analysis in the Supplemental Digital Content 1 for more details. Most importantly, we found a significant interaction between TIME and GROUP ($F(12,262)=18.172$, p<0.001, $p\eta2=0.454$) (Figure 3). Comparisons between placebo groups (P5, P15, P30) and the NE group showed significantly stronger pain reduction in the P5 group after 10 (p=0.003), 20 (p<0.001) and 35 (p<0.001) minutes. In the P15 group,

the analgesic effect was stronger after 20 ($p<0.001$) and 35 minutes ($p<0.001$) whereas the P30 group only showed a significantly stronger decrease in pain after 35 minutes ($p<0.001$). These results indicate that the analgesic effect strictly followed the verbal information about the expected onset of the analgesic effect. As previously reported in the raw data analysis, P5 and P15 did not show differences in perceived pain after analgesia onset, confirming that once triggered, placebo analgesia is stable over time.

Turning to the nocebo groups (N5, N15, N30), the N5 group showed a significantly stronger increase in pain ratings than the NE group after 10 ($p=0.02$), 20 ($p<0.001$) and 35 ($p<0.001$) minutes. In the N15 group, a significantly stronger pain increase compared to the NE group was found only after 20 ($p=0.006$) and 35 ($p<0.001$) minutes. In the N30 group, a significant pain increase was only detected after 35 minutes ($p=0.007$). Again, these results suggest that the effect on pain perception strictly follows the verbal information provided. In contrast to the placebo effect which remained stable over time, the hyperalgesic effect became stronger over the course of the experiment. In the N5 group, Δ_{35} was significantly larger than Δ_{10} ($p=0.002$). Similarly, an increasing hyperalgesic effect was found in the N15 group with a larger Δ_{35} than Δ_{20} ($p=0.013$). These results replicate what previously shown in the raw scores analysis, and indicate that, once the hyperalgesic effect has set in it continues to increase over time. To further explore this effect, compared to stable placebo analgesic effects, a series of t-test was performed in order to specifically compare Δs between the N5 and P5 groups, as well as between the N15 and P15 groups. Results showed significant differences only between Δ_{35} of N5 compared to P5 ($p=0.028$) and Δ_{35} of N15 compared to P15 ($p=0.011$).

Finally, considering the effect of anxiety scores on pain perception, we found a positive correlation between STAI II and the percentage (Δ) of pain increase after the application of the cream in the nocebo groups ($r(56) = 0.329$, $p = 0.013$), but not in the placebo group. No significant correlation between expectancy scores and the percentage (Δ) of pain decrease (in the placebo groups) and increase (in the nocebo groups) after the application of the cream was found.

Discussion

This study demonstrates for the first time that verbal information regarding the expected onset of a treatment effect can influence the time-course of placebo and nocebo effects. Participants who had been informed that the cream would take effect promptly following application

showed an early analgesic (placebo group) or hyperalgesic effect (nocebo group). Similarly, both effects only set in later in those who expected their treatment effect to unfold after a longer delay.

Previous studies have shown that verbal information can stir placebo and nocebo effects (3,21,22). However, the majority of these studies focused on information about the direction (e.g., increase or decrease of pain) (11,23,24) or the magnitude of the effect (e.g., strong or weak) (25,26). Results of the present study demonstrate that expectations also contain a temporal aspect that determines the onset of placebo analgesia and nocebo hyperalgesia and that can be modulated through information provided to the individual.

Our observation of a delayed analgesic response could therefore be of interest in clinical contexts where - due to the pharmacological properties of the treatment - the effect of an intervention only becomes noticable to the patient after days or even weeks. For instance, the delayed mechanism of action of certain antidepressants requires patients to maintain their positive treatment expectations over an extended period of time until the drug has reached the required blood concentration to take effect. Informing patients about the delayed onset could prevent (premature) abandonment of positive treatment expectations during the period when no treatment effect is detectable (yet) and preserve the supportive effect of these expectations for the time-point when the pharmacological effect sets in. Our findings show that the positive influence of expectations on pain perception could be withheld for at least 30 minutes. Whether these encouraging findings translate to longer delays requires further investigation.

Regarding the magnitude of the effects found, we reported a 25.6% pain decrease in the placebo groups (that is mean pain reduction from the baseline after the sham analgesic cream took effect) and a 37.5% pain increase in the nocebo groups (mean pain increase from the baseline after the sham hyperalgesic cream took effect). Considering the baseline ratings of the different groups, this result equals an overall mean decrease of 0.6 points in the NRS in the placebo groups and an overall mean increase of 1 point in the NRS in the nocebo groups. While these are small changes from the baseline values, similar ranges of pain ratings have been observed in recent experimental studies using behavioral paradigms (27,28) as well as in classic neuroimaging studies (29). Here we demonstrated the effect of verbal information following the application of an inert substance, but verbal information can also affect the efficacy of active treatments. Information leading to expectations of pain relief doubled the analgesic outcome of an opioid treatment whereas information inducing negative expectations abolished its beneficial effect (6). It follows that the small effects of placebo and nocebos interventions become larger when delivered in association with active treatments, rendering such phenomena

valuable strategies in the clinical context. However, the impact that temporal information can have on the clinical setting is yet to be explored. Future studies are needed to investigate clinical conditions, such as low back pain or neuropathic pain, in order to achieve more ecological results. The effect of temporal information on analgesia should be explored when delivered in association with active treatments.

A second important finding of this study relates to the changes in placebo and nocebo effects once they have been triggered. As shown in Figure 3, the hyperalgesic effect becomes stronger over the course of the experiment while the analgesic effect remained stable. Given that the stimulation was calibrated to the same perceived level in all groups, and neither the placebo nor the no treatment groups showed an increase in pain ratings, it seems unlikely that the hyperalgesic effect was the result of peripheral sensitization. Contemporary models of perception, such as the predictive coding model (30)**,** have offered explanations for such changes in perception which are rooted in the understanding that any type of perception is based on an inferential process. In this framework, incoming sensory information is compared to expectations which the individual holds. If sensory input is as expected, the expectation is confirmed. However, if expectations and incoming information are incongruent, the expectation will be updated following a learning rule that determines the translation of expectancy violation (formalized as prediction error) into expectation updating. Within this framework, two scenarios could explain the difference between the placebo and nocebo group we observed. First, the intensity of the noxious input at T10 confirmed expectations in the placebo group but was stronger than expected in the nocebo group. As a result, expectations would be updated (towards a higher intensity) only in the nocebo group. Alternatively, a similar discrepancy between expectation and incoming information might have been detected in the placebo and nocebo groups but a different learning rule was applied that led to an upwards correction of expectations (and subsequently of the perceived stimulus intensity in subsequent test sessions) in the nocebo group but not the placebo group. A recent study using a cue probability paradigm suggested a similar asymmetry in expectation updating (5) which might be driven by the higher biological relevance of an aggravation of aversive sensory experience (as in the nocebo condition) compared to a turn for the better (as in the placebo condition). Because we did not acquire trial-by-trial expectancy ratings and are therefore unable to verify whether expectancy ratings were adapted between test sessions, further studies are needed to explore the link between the changes in pain perception over time and expectation updating. This study also confirms the crucial role of anxiety on nocebo effects and is in line with previous data that highlight how anxiety affect hyperalgesia (31,32).

Some limitations of the present study need to be considered. The first source of limitation to be discussed here is the number of participants. Even though more than 160 participants were recruited, different shortcomings of the current experiment could be derived from the smaller number of participants included in each experimental group (i.e. NE, P5, P15, P30, N5, N15, N30). Firstly, the use of the SNK post hoc test instead of more strict corrections for multiple comparisons, such as the Bonferroni correction, could be questioned. However, we performed a planned-comparison Bonferroni correction which resulted in the same significant results highlighted by the SNK test, leaving out only the difference between Δ_{10} and Δ_{35} in the N5 group, thus confirming our main results and conclusions. In addition, this study is the first to directly investigate the temporal aspects of placebo/nocebo effects. Therefore, the usage of a less strict post hoc test can be justified because it allows to discover important albeit small differences that are present in new and poorly understood phenomena (33,34). The second limitation stemming from the low number of participants is the lack of a full randomization assignment in favor of a stratified randomization due to the need to balance specific characteristics, such as sex and age between groups. However, adding sex, age and BMI characteristics as covariates to the main analysis did not show any significant impact of these factors on pain perception nor significant differences between groups regarding these variables. Still, future studies, possibly focusing on one single experimental question (e.g. focusing only on placebo analgesia or nocebo hyperalgesia), should reach a higher number of participants per group to avoid these limitations.

The second source of limitation is that our study focused on rather low pain intensities (NRS below 5) which may have induced a "floor effect" such that nocebo effects (i.e. changes toward the higher part of the scale) could have been overestimated, while placebo effects (i.e. changes toward the lower part of the scale) could have been underestimated. Nonetheless, the investigation of the magnitude of these effects goes beyond the purpose of the present study. Further research is needed to explore whether our findings persist with the use of more intense pain stimuli as well as in clinical contexts.

The third source of limitation is that verbal instructions used in this study were directive as they not only described the drug effect (i.e., increases/decreases pain) but anticipated what the participant is going to feel. Although it could be argued that these suggestions make it difficult to discriminate between placebo and nocebo responses and a simple 'experimental demand' effect, other studies have used similar instructions (35,36). Future studies should focus on the description of the drug effect and should involve more objective measure of pain perception

(neuroimaging or electrophysiological measure) to confirm that participants are actually reporting their pain changes rather than following experimental demand.

Indeed, our study results are based on participants' pain intensity ratings only. Although these ratings have been shown to correlate with peripheral (e.g., skin conductance and heart rate (18,38) and central measures including activity in brain regions associated with pain processing (4,5), follow-up studies should consider simultaneous recording of these measures to investigate accompanying changes in objective parameters; examples include neuroimaging studies and electrophysiological measures of pain expectations (3,15). Given the multi facet nature of pain perception and the importance of expectation of pain, future studies on the temporal aspects of placebo analgesia or nocebo hyperalgesia should also collect behavioral measures before the experimental sessions such as "a priori" questionnaires on expectations but also subjective reports on the unpleasantness of the pain stimuli during the different time frames.

To conclude, our data suggest that the delivery of temporal information influences the onset of placebo analgesia and of nocebo hyperalgesia. Even if these findings have been collected in an a strictly experimental context, their potential implications in a clinical context are remarkable and additional work is required to explore how our findings relate to the effect of active drugs. Strategic timing of treatment effects through targeted temporal information may have the potential to substantially enhance desired therapeutic effects and delay or abolish unwanted side effects.

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Figure legend

Figure 1. Experimental Groups: Placebo and nocebo groups received different temporal information. P5 and N5 expected the effect of the treatment to set in 5 minutes after cream application, P15 and N15 after 15 minutes and P30 and N30 after 30 minutes. The no expecancy and natural history do not receive temporal instructions.

Figure 2. Experimental protocol: Diagram showing each experimental step (A). Firstly, pain threshold assessment. Secondly, familiarization trial followed, after a 5 minutes break, by the Baseline Session. Thirdly, cream administration along with verbal instructions which varied accordingly with group allocation. Lastly, the three Test Sessions occurring after 10, 20 and 35 minutes from cream application. Stimuli intensity remains stable within and across pain trials. To be noted that the NH group was not included in the randomization and was part of a pilot study. The image next to the diagram (B) shows experiment set up, including the customised wall clock facing the participant.

Figure 3. Experimental results. Results are presented as percentage of change (Δ%) from Baseline in each test (Test 10, Test 20, Test 35). Left side: groups where the expected

onset of action of the treatment was 5 minutes (plus no expectancy group). Middle: groups where the expected onset of action of the treatment was 15 minutes (plus no expectancy group). Right side: groups where the expected onset of action of the treatment was 30 minutes (plus no expectancy group). Gray bars depict the NE group, white bars (plain, striped and dotted) depict placebo groups and black bars (plain, striped and dotted) depict nocebo groups. Asterisks represent significant differences (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$). Error bars represent standard erros of the mean (SEMs).

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