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TEMPORAL INFORMATION CAN MODULATE THE ONSET OF NOCEBO HYPERALGESIA: A COLD PRESSOR TEST EXPERIMENT.

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The data and the results of this thesis are fictitious.

Abstract

Nocebo and placebo effects play a crucial role in the persistent pain experience. The former occurs whenof nocebo, the temporal information associated with a given nocebo treatment that influences its onset has yet to be explored. Hence, this study aims at investigating the modulatory effect of temporal information on both the onset and duration of nocebo hyperalgesia at the Cold Pressor Test (CPT), and its influence on heart rate (HR). Forty-eight healthy subjects were randomly allocated into three groups. The first group believed that the nocebo treatment effect took place after 5 minutes, the second after 30 minutes, and the third knew that the treatment was inactive cream. The nocebo treatment consisted of an inert cream that participants believed to have a hyperalgesic effect. We asked the participants to resist at three CPT repetitions till the pain became unbearable. Only in the nocebo groups, we found a statistically significant difference in CPT tolerance either at baseline and in the follow-up tests. Conversely, we did not find any differences in HR between the groups, suggesting that nocebo hyperalgesia is not associated with heart rate changes. We demonstrated that the onset of nocebo hyperalgesia varies accordingly with the temporal information delivered with the nocebo treatment on experimentally induced pain.

1 Introduction

The IASP defines pain as "An unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" [40]. In line with that definition, in the last few decades, the pain concept shifted from a biomedical paradigm, which merely considered pain as an organic response to tissue damage, into a biopsychosocial one, which takes into account pain as a complex interaction of biological, psychological and social factors [40]. Pain is the most disabling symptom that characterises musculoskeletal disorders [42]. When it lasts beyond the average healing time, it becomes persistent. In this chronic condition, different physical, psychological and social factors, that go under the umbrella term "Contextual factors", become crucial in the pain experience [31]. Contextual factors characterise the therapeutic encounter, elicit patients' memory, expectations and emotions that influence their health-related outcome. They modulate neurophysiological mechanism of different intervention such as manual therapy by producing nocebo and placebo effects [3,16,33,37].

In particular, the former effect consists of a worsening of a condition after the administration of an inert treatment [6]. Negative verbal information provided with a nocebo treatment negatively shapes expectations, leading to worsening of clinical symptoms [14]. Specifically, negative verbal information has repeatedly demonstrated to induce nocebo hyperalgesia in the context of pain [15]. Nocebo hyperalgesia modifies individual pain perception by lowering the specific positive effects associated with a particular medical treatment or intervention [12]. Although the scientific literature has established the influential role of verbally-induced expectations in the context of nocebo, the temporal information associated with a given nocebo that influences its onset has yet to be explored. In particular, temporal information refers to which information we provide the patient with in terms of the time of action of a given treatment or nocebo.

The cold pressor test (CPT) represents an excellent experimental paradigm to induce a type of pain that mimics the effects of chronic conditions [28]. Moreover, cold-pressor pain leads to cardiac autonomic function response, which can be measured by employing Heart Rate (HR) [23]. Specifically, HR refers to the variation over time of consecutive heartbeats; this interval is referred to as the R-R interval and represents sympathetic and parasympathetic activity [41]. Experience of pain activates the sympathetic system, and the HR can detect this

activity [41]. It follows that cold pressor pain represents an excellent model to study pain perception in healthy subjects under an ecological paradigm allowing to measure also its physiological correlates [28].

In line with the statements above, we aim at using this type of pain induced by the CPT to investigate whether manipulation of temporal information associated with the given treatment (i.e. a nocebo cream) can modulate nocebo treatment onset of action and its relation to HR. By doing so, we are addressing a completely new area in nocebo research.

2 Methods

2.1 Participants

Healthy volunteers were recruited from the student population of the Vrije Universiteit Brussel (VUB), Belgium and through different social media outlets. Participants between 18 and 45 years old were considered eligible to join the study. In contrast, participants that were in cure with antidepressants or anxiolytics, had a history of cardiovascular disease and suffered from psychiatric, neurological, chronic musculoskeletal and pain-related disorders were not considered eligible to participate in the study. Moreover, we instructed the participants not to consume alcohol or analgesic medications twelve hours before the experiment. We informed the participants that they would have taken part in a study investigating the time of action of a newly developed hyperalgesic cream. We disclosed the actual purpose of the study only once the experiment ended. Participants provided written informed consent stating that they would have been debriefed with all the details of the study at the end of the experiment. All experimental procedures followed the policies and ethical principles of the Declaration of Helsinki. The Ethics Committee of the Vrije Universiteit Brussel approved this study (BUN45147458).

2.2 Experimental design

We tested the influence of temporal information on the onset and duration of nocebo hyperalgesia using an established nocebo manipulation [27,36] in combination with the Cold Pressure Test. Temporal information provided varied throughout the three groups as described below:

- Control: experimenter explained that the cream is inert, without any effects on pain perception;

- N5: experimenter explained that the cream is a powerful hyperalgesic which took effect after 5 minutes;
- N30: experimenter explained that the cream is a powerful hyperalgesic that took effect after 30 minutes.

2.2.1 Group allocation

We randomly assigned participants to one of the three groups (Control, N5, N30) through a web-based randomization application (random.org). Groups were balanced for age and gender.

2.2.2 Control group

We informed the participants assigned to the control group that we would have applied an inert cream (No Expectancy, control group): "The agent you will receive is an inert cream that only has hydrating properties but no effect on pain perception. Because the cream has no hyperalgesic properties, your test performance after 10 and 35 minutes [experimenter points at time 10 and 35 minute marks on a clock] may be similar to the performance in the first test [CPT baseline], but it can also be longer or shorter than before".

2.2.3 Nocebo groups

Participants in the two nocebo groups received an inert cream (see details below). However, they believed that the cream had a hyperalgesic effect that would have augmented the painful sensation induced during the CPT. We provided both groups with specific details about the onset of action of the hyperalgesic.

The first nocebo group (N5) believed that the hyperalgesic effect would have become active after 5 minutes (negative Verbal Suggestion, N5 group), mimicking a fast-acting hyperalgesic. They received the following instruction: "The agent you will receive is known to have a strong hyperalgesic effect which sets after 5 minutes after application. You will, therefore, become more sensitive to pain and be able to keep your hand in the cold water for a shorter period in the two test sessions after 10 and 35 minutes [experimenter points at time 10 and 35 minute marks on a clock] compared to the first test [CPT baseline]."

Instead, the second nocebo group (N30) believed that the hyperalgesic would have become effective after 30 minutes (negative Verbal suggestion, N30 group), resembling the effect of hyperalgesic with a delayed onset time. They received the following instruction: "The agent you will receive is known to have a strong hyperalgesic effect which sets after 30 minutes after application. You will, therefore, become more sensitive to pain and be able to keep your hand in the cold water for a shorter period in the test session after 35 minutes [experimenter

points at time 35 minute marks on a clock] compared to the first test [CPT baseline] and a second test after 10 minutes."

2.2.4 Experimental protocol

After providing written informed consent, participants seated on a comfortable chair positioned next to the CPT device. The investigator used a stopwatch displayed on a computer screen in front of the participants and a customised wall clock for participants' temporal orientation. The wall clock with 5-minute intervals (i.e., 5 to 55) showed an icon of a cream tube at the 12 o'clock position to indicate the time-point of application of the cream (Figure 1).

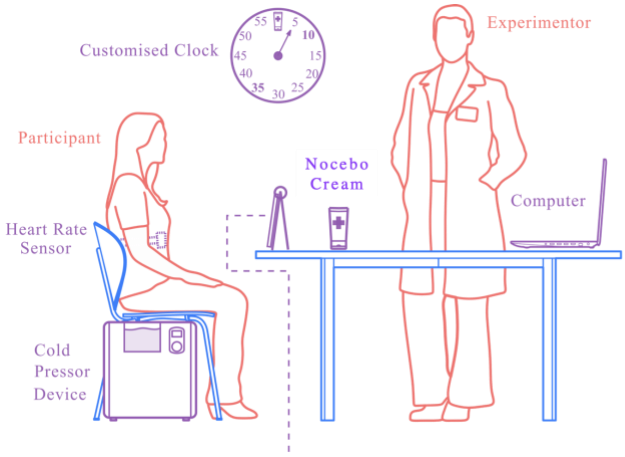

Figure 1. Setting.

The experiment started with a 4-minute heart rate measurement at rest, during which the participant had to breathe naturally and relaxed. After, participants were explained the CPT, they completed the CPT familiarisation trial and filled in the psychological questionnaires. Subsequently, all participants underwent the CPT baseline test before they were randomly allocated to one of the three groups. Then, the cream was applied, and participants were provided with information about the nature of the cream (hyperalgesic in both nocebo groups and inert cream in the control group) and about the expected onset of the hyperalgesic effect (nocebo groups only). Immediately after the cream application, 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'). Afterwards, the CPT was repeated 10 minutes (Test 10') and 35 minutes (Test 35') after cream application (Figure 2).

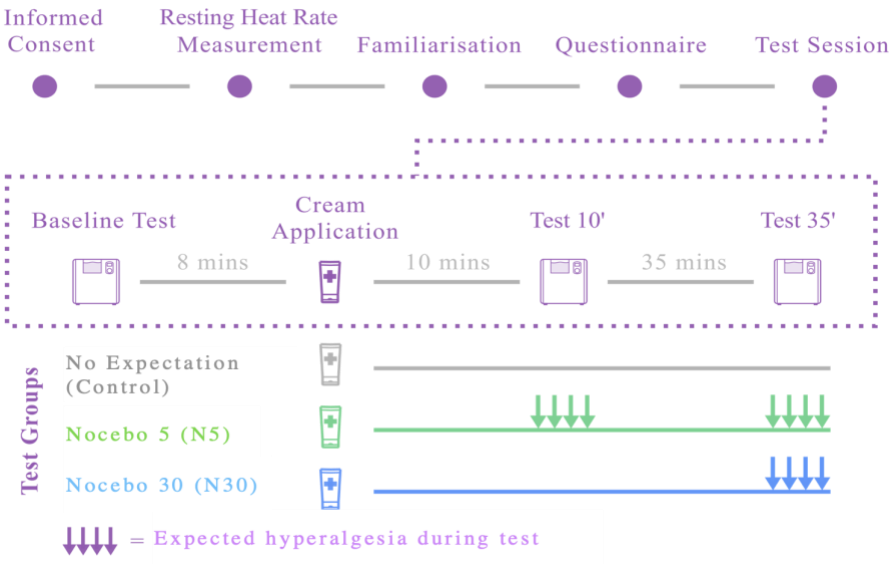

Figure 2. Study Protocol.

2.2.5 Cold Pressor Test

During the Cold Pressor Test (CPT), participants had to immerse their right hand in seven litres of circulating cold water (7 C°, ± .2 C°; CPT device: Thermo Scientific model Haake A 10B, Haake SC 100; Thermo Fisher Scientific, Waltham, MA; procedure adapted from Mitchell et al.) [22]. To indicate the level to which participants had to lower their hand, the experimenter drew a red line from the participant's ulnar to the radial styloid process (wrist level). The CPT was repeated a total of four times (familiarisation, baseline, Test 10', Test 35') with a break of 20 minutes between tests to restore the baseline hand temperature. Each CPT block started with one minute of HR recording at rest, followed by the actual CPT. Ten seconds before participants had to place their hand into the CPT device, and they were alerted by the experimenter to get ready to immerse their hand into the water.

Upon a verbal prompt from the experimenter ("Go"), the participant lowered their hand into the CPT device, and the experimenter started the stopwatch to record the time between the beginning of exposure and hand withdrawal. The stopwatch was displayed on a computer screen located in front of the participant for temporal orientation. Participants were instructed not to move their fingers or hand while they were immersed in the water and to keep their fingers spread with the palm parallel to the bottom of the device, without touching it. The experimenter prompted the participant every 15 seconds to provide a verbal rating of their current pain intensity which the experimenter recorded on a pre-printed grid. The participant's task was to keep their hand in the water basin until the pain in their hand became unbearable. Once this level of pain was reached,

the participant removed their hand from the water basin and rested it on a towel placed on their knees. Between the different measures, the participants filled the psychological questionnaires. The time elapsed between immersion and withdrawal of the hand was recorded as CPT tolerance.

2.2.6 Heart Rate Recording

The ECG signal was measured using a heart rate monitor which was connected to two standard surface electrodes positioned on the participant's sternum with a band. Data was collected at a sampling rate of 700 Hz/sec. The heart rate (HR) had been recording for four minutes during a rest period in which the participant was asked to sit comfortably and breath normally. Subsequently, HR recording started one minute prior to each CPT and continued until two minutes after completion of the test. To limit HR artefacts, participants were instructed to maintain a constant and relaxed breath during each test session, avoiding hyperventilating while feeling pain.

2.2.7 Psychological Questionnaires

Participants completed a set of questionnaires (see supplementary file) to assess psychological traits that seem to correlate with nocebo and placebo responsiveness [8,13,21,43]. In particular:

- Beck Anxiety Inventory (BAI) to test the level of anxiety;
- Behavioural avoidance/inhibition scale (BIS/BAS) to test the motivational systems;
- Fear of Pain Questionnaire (FPQ) to test the fear of pain;
- Revised Life Oriented Test (R-LOT) to test the degree of optimism.

Participants completed the questionnaires between the familiarisation with the CPT and the test sessions.

2.2.8 Cream

An inactive cream was administered in all three groups. It consisted of a water-based gel (KY-gel Johnson&Johnson) which was presented to participants in a transparent plastic tube. The experimenter applied the cream on the volar and dorsal side of the hand up to the red line which had previously been drawn onto the participant's wrist to indicate how deep the hand had to be submerged into the water. The cream was massaged into the skin for approximately one minute to ensure that it was fully absorbed.

2.2.9 Debriefing

To debrief participants they were sent an email that explained in detail the actual purpose of the study and why deception that had been used. Participants were offered to contact the experimenter in case they felt the need to discuss their participation and any concerns related to it. They were also given the opportunity to withdraw their data but none of the participants decided to do so.

2.3 Statistical Analysis

One-Way Analysis of Variance was run to test for baseline differences between the three groups in demographic parameters and psychological constructs assessed via the questionnaires. As data for CPT tolerance at baseline, after 10 (Test 10') and 35 (Test 35') minutes did not follow a normal distribution (Shapiro-Wilk tests $p<.05$), non-parametric tests were used.

Within-group analysis: Friedman Tests were performed to detect differences in CPT tolerance across CPT trials at the three different time points (Baseline, Test 10' and Test 35') within each group. Data are presented as median $\pm$ interquartile range and level of significance was set at $p<.05$. Significant results were followed up using Wilcoxon Signed-Rank Tests, corrected with Bonferroni adjustment to $p<.025$.

Between group analysis: we calculated the percentage change in pain tolerance from baseline to CPT10' (Δ_{10}) and CPT35' (Δ_{35}) for each participant as follow:

Δ_{10} = (CPT Test 10'*100)/Baseline CPT-100;

Δ_{35} = (CPT Test 35'*100)/Baseline CPT-100.

Percentage change (Δ_{10}, Δ_{35}) scores were used instead of raw scores in the between group analysis to rely on more standardised values. Kruskal-Wallis H-Tests was used to compare percentage changes (Δ_{10}, Δ_{35}) in pain tolerance between groups, allowing to directly check nocebo response magnitude differences. Data are presented as median $\pm$ interquartile range and level of significance was set at $p<.05$. Significant results were followed up using pairwise Mann Whitney U Tests. Significance acceptance level for pairwise comparison has been adjusted for the number of comparisons (Adjusted α = α/number of comparisons). Effect sizes were calculated as $\eta_2 = z/\sqrt{N}$ [30].

Lastly, HR recording analysis was run. Mean HR value was calculated for each time-point by averaging HR measurements over the first 15 seconds per time-point, resulting in three mean HR indices for each participant. Data followed a

normal distribution (Shapiro-Wilk tests p>.05), therefore parametric analysis was used. To compare the effect of information regarding the expected onset of treatment upon HR between groups, a three-way mixed ANOVA was run within three groups (group: Control, N5, N30) in three times (Session: HR Baseline, HR Test 10', HR Test 35'). Significant results were followed up using Bonferroni-corrected t-tests.

3 Results

We recruited 77 participants of which 29 participants had to be excluded. In particular, 28 participants did not withdraw their hand from the noxious stimulation within 10 minutes which had been defined as the maximum exposure time for safety reasons. One participant developed cramps in the right arm during the experiment. The final sample size was therefore of 48 participants. One-way ANOVA and Chi-Square tests showed no baseline groups differences (p>.05) with respect to age, BMI, gender and key psychological traits (Table 1-2). Kruskal-Wallis H-Test showed no significant baseline differences between groups in CPT tolerance (p=.998).

Table 1. Participants' descriptive analysis

Groups	**Control**	**Nocebo 5**	**Nocebo 30**
N	16	16	16
Age in months(Mean, SD)	333.7±25.6	297.4±38.0	313±71.9
BMI (Mean, SD)	24.3±2.6	22.2±4.1	22.2±3.1
Gender	9M;7F	6M;10F	9M;7F
Handedness	12R;4L	15R;1L	16R

SD, Standard Deviation; BMI, Body Mass Index; M, Male; F, Female; R, Right; L, Left.

Table 2. Participants' psychological traits

Groups	**Control** (Mean, SD)	**Nocebo 5** (Mean, SD)	**Nocebo 30** (Mean, SD)
BAI	10.3±5.1	9.2±6.8	10.3±5.6
BAS-Drive	8.8±1.8	9.7±2.0	8.7±2.3
BAS-Fun-Seeking	8.3±1.8	8.3±2.2	7.9±1.7
BAS-Reward	8.5±2.1	7.6±1.8	8.0±1.7
BIS	14.8±2.1	15.1±2.6	15.4±2.7
FPQ	72.8±13.2	78.4±14.3	77.0±13.9
RLoT	13.9±3.9	14.4±6.3	14.8±5.0

SD, standard deviation; BAI, Beck Anxiety Inventory; BAS, Behavioural Activation Scale; BIS, Behavioural Inhibition Scale; FPQ, Fear of Pain Questionnaire ; RLoT, Life-Orientation Test-Revisited.

3.1 Nocebo Effects

Within-group analyses using Friedman Tests revealed, in both nocebo groups, a statistically significant difference in CPT tolerance depending on the temporal execution of the CPT test, either at baseline, after 10 (Test 10') or after 35 (Test 35') minutes [Nocebo 5, $\chi2(2) = 18.95$, $p < .001$; Nocebo 30, $\chi2(2) = 21.37$, $p < .001$]. Differently, no significant difference in CPT tolerance across time-points was shown in the Control group, $\chi2(2) = 3.124$, $p = .210$. N5 group showed a significant decrease in CPT tolerance at Test 10' (p=.001) and Test 35' (p=.001) compared to baseline. No significant difference was shown in CPT tolerance between Test 10' and Test 35' (p=.478). N30 group showed no significant difference in CPT tolerance between Test 10' and baseline (p=.408). CPT tolerance significantly increased at Test 35' compared to both Test 10' (p<.001) and baseline (p=.001) (Table 3-4).

Table 3. Median and interquartile range of CPT pain tolerance of all groups at the three test

	Tolerance Baseline		**Tolerance Test10'**		**Tolerance Test35'**	
	Median	IQR	Median	IQR	Median	IQR
Control	69.5	226	62.5	223	62.5	263
N5	69.5	202	51.0	180	53.5	180
N30	55.0	266	51.6	230	37.5	227

IQR, Interquartile Range.

Table 4. Within-group comparisons of CPT tolerance.

Groups	**Comparisons**	**Wilcoxon Signed rank test**	**Effect Size**
Control	No Post Hoc Tests	/	/
N5	T_{10} vs Baseline	Z = -3.47, p=.001	η_2=.87
	T_{35} vs Baseline	Z = -3.34, p=.001	η_2=.83
	T_{10} vs T_{35}	Z =-.710, p=.478	η_2=.18
N30	T_{10} vs Baseline	Z = -.828, p=.408	η_2=.21
	T_{35} vs Baseline	Z = -3.46, p=.001	η_2=.86
	T_{10} vs T_{35}	Z = -3.52, p<.001	η_2=.88

Between-group analysis using Kruskal-Wallis H-Tests showed a statistically significant difference in Δ_{10} between the different groups, $\chi_2(2) = 23.05$, p<.001, Post-hoc Mann-Whitney U-tests showed that Δ_{10} did not differ significantly between the Control group and N30 (p=.122). However, Δ_{10} was significantly higher in N5 than in both Control (p<.001) and N30 (p<.001). For Δ_{35}, Kruskal-

Wallis H-Test showed a statistically significant difference between groups, $\chi_2(2)=18.06$, $p<.001$ (Table 5). Post hoc Mann-Whitney U-tests revealed that Δ_{35} was significantly higher in both N5 ($p=.001$) and N30 ($p<.001$) compared to the Control group. No significant difference in Δ_{35} was found between N5 and N30 ($p=.624$) (Table 6).

Table 5. Median and interquartile range of percent change in CPT pain tolerance (Δ_{10} ,Δ_{35}) in the three experimental groups.

	Δ_{10}		**Δ_{35}**	
	Median	IQR	Median	IQR
Control	-7.5	24	-5.2	26
N5	-29	20	-27.6	18
N30	3.5	21	-37.5	19

IQR, Interquartile Range.

Table 6. Between-group comparisons of CPT percental tolerance change.

Group Comparisons	**Dependent variable**	**Mann-Whitney U-Test**	**Effect Size**
	Δ_{10}		
Control vs N5		U=16.5, p<.001	η_2=1.05
Control vs N30		U=87, p=.122	η_2=.38
N5 vs N30		U=26.5, p<.001	η_2=.96
	Δ_{35}		
Control vs N5		U=38, p=.001	η_2=.85
Control vs N30		U=25, p<.001	η_2=.97
N5 vs N30		U=115, p=.624	η_2=.12

3.2 Heart Rate

HR data showed a significant main effect of TIME ($F(2,90)=19.39$, $p<.001$) but no main effect of GROUP nor interaction between both factors (both $p> 0.05$). Bonferroni-corrected post hoc comparisons between the different time-points revealed that the HR decreased significantly between baseline and Test 10' ($p< 0.001$) and between baseline and Test 35' ($p<0.001$). Changes in HR from Test 10' to Test 35' did not reach significance ($p> 0.05$).

4 Discussion

The patient-clinician encounter entails physical, psychological and social contextual factors that can modify patients' outcome, in particular in persistent condition. Their effect is mainly due to the elicitation of patients' memory and

expectations that can modulate placebo and nocebo response to treatment. Verbal suggestion can easily evoke the nocebo effect [5,14]. Following an ethical approach towards patients, clinicians might create unintentionally negative effects while describing the treatment and its adverse effects during the clinical encounter [10]. However, the available literature on nocebo mostly focus on the magnitude effect of this phenomenon. Instead, the temporal onset of it, through a tonic pain model, has yet to be deepen explored. In line with that, in the present study, we showed that nocebo onset follows not only the verbal suggestion *per se* but even the temporal indication.

Specifically, those participants induced to believe that the cream had a fast action time (N5) reported early increased intolerance (Test10'), and this effect lasted over time (Test35'). Moreover, no tolerance difference was found between Test10' and Test35', suggesting that once triggered, nocebo analgesia remains stable over time. Moreover, hyperalgesia onset followed temporal instruction also in those participants believed that the cream had a delayed action time (P30). Increase intolerance was only present at the delayed test session (Test35'), suggesting that expectations can last over time. This data is in line with the one reported by Rodriguez-Raecke *et al.* showing that a single nocebo cue induces effects on patients that had been lasting for eight days [29]. Instead, our study highlighted this effect in a shorter time framework that can represent the duration of a single session with a health professional in which the nocebo effect can occur. Hence, if nocebo response arose, it would modify and disguise the real effect of the delivered treatment.

We also demonstrated that the magnitude of the nocebo effect - percental tolerance change from baseline to each test session - was higher in N5 at Test10 compared to the other two groups. This result showed that early hyperalgesia onset is unique to the early temporal expectation group. Both nocebo groups showed a greater magnitude of the nocebo effect at Test35 compared to the control group, showing hyperalgesia onset delay for N30 and withheld analgesia for N5.

To better understand our data, another focus can be done by considering the underlying differences between phasic and tonic pain. The former is underlined by the engagement of phasic receptors programmed to respond quickly with a burst activity to the incoming stimulus and to adapt (reduce firing rate) if the stimulus persists over time. Differently, the latter depends on the engagement of tonic receptors which adapt slowly to the stimulus and fire at a constant rate over time of the painful stimulation [9,34]. These physiological differences are

mirrored in diverse pain experiences as well as in different experimentally induced pain modalities.

Most of the phasic stimuli used in experimental pain research are very short, leaving limited time to explore the response to the stimulus. Consequently, report ratings such as VAS might have quite a large error margin [18]. Additionally, the quick and sharp nature of phasic pain renders it more difficult to induce high-intensity pain because it does not allow the time for the pain to grow and to reach the level of unpleasantness that characterises tonic pain [28]. For example, high electrically induced pain can be reached by delivering a stimulus that is twice, three or four times the individuals' pain threshold. However, since pain is not a linear phenomenon, three times the pain threshold can be perceived both moderate and excruciating according to the sample's characteristics. In line with ethical allowance, stimulus intensity is often kept at twice the pain threshold, leading to a low-to-medium pain experience [11,26]. Accordingly, participants often describe phasic pain induced with electrical stimulation as a sensation of discomfort rather than pain [18]. Lastly, to quantify the perceptual experience of phasic pain, reports measurements (e.g. NRS, VAS) are most commonly used. However, the subjectivity of these self-reporting assessment methods represents a core bias [17,18].

Differently from phasic pain, tonic pain is characterised by an enduring sensation which persists over time; this expanded timeframe leads to several consequences. For instance, tonic pain allows investigating higher intensity pain compared to the phasic model for at least two reasons. First, a tonic pain model can be used to gradually reach maximum tolerance while giving control to participants by asking to remove their hand when the pain becomes unbearable. The perceived sense of control reduced experienced pain intensity and maximised pain resistance [35]. Additionally, the freedom given to the participant allows reaching high pain within ethical permission. A further benefit of using tonic-induced-pain is that measuring the maximum tolerance gives a behavioural and more objective outcome compared to self-report methods. Second, the persistence of pain over time leads to an increase in pain perception that goes beyond nociception. This results in the perception of a highly unpleasant experience, which, arguably, mimics the perception of clinical pain [22].

Unpleasantness, linked to multiple psychological components spanning from anxiety, responses to defence mechanisms and different coping strategies. The longer the pain, the more worried the individual may become of damaging the body area while experiencing the pain (e.g. CPT, damaging hand capillary).

Lastly, tonic pain requires a considerably higher cognitive load compared to phasic pain [32]. The latter is more likely to be an arousal signal that quickly responds to an incoming brief situation which ends before the engagement of other psychological constructs. Conversely, tonic pain, lasting more, is the result of pain signals and psychological resources to face the ongoing 'danger' [32].

Albeit tonic and phasic pain are very different experiences, our results suggest that they respond similarly to temporal expectation modulation both resulting in a shift in nocebo hyperalgesia onset. The similar role played by temporal negative expectations inducing nocebo response across the two different pain modalities is in line with previous evidence on placebo that showed similar mechanisms both on phasic and tonic pain [1,2,38,39,43,44,4,7,19–21,25–27].

Furthermore, the present study aimed at investigating whether the changes in nocebo hyperalgesia onset related to changes in physiological correlates, precisely heart rate. We did not find any differences in heat rate between the groups, suggesting that nocebo hyperalgesia is not associated with heart rate changes. These findings are in line with Peerdeman *et al.* on placebo analgesia on cold pressor pain, finding no heart rate variations in response to analgesia [24]. However, a different study showed heart rate reduction in response to placebo analgesia on ischemic arm pain, suggesting that heart rate may be good physiological outcome measure when inducing pain via other techniques that are not the cold pressor test [26]. Future research shall aim to study alternative physiological correlates that may explain hyperalgesia onset at different time points.

Our data showed that expectations regarding the onset of action of a given treatment play a crucial role upon treatment outcome. We demonstrated that the onset of nocebo hyperalgesia varies accordingly with the temporal information delivered with the nocebo treatment on experimentally induced tonic pain. This data suggests that temporal expectations in nocebo hyperalgesia play a similar role in both phasic and tonic pain. Future research could further deepen our understanding of nocebo hyperalgesia onset variations by looking at functional activation of pain-related brain regions at the different points in time, giving additional objectivity and scientific valence to the phenomenon of time modulated hyperalgesia.

Some limitations of this study need to be discussed. First of all, we had a limited sample size as some participants (N=28) did not withdraw their hand from the noxious stimulation within 10 minutes so that we had to stop the test for safety

reason. Future study should consider this habituation phenomenon in the sample size calculation. Secondly, as mentioned before, expectations mainly modulate placebo and nocebo response. Since we did not take into account participants' expectations during the assessment., we did not know to what extent their expectations of pain onset could have modulated the pain resistance at the CPT test.

Overall, modulation of hyperalgesic nocebo onset appears to be a strong phenomenon worth of further attention. Although tonic compared to phasic induced pain represents a better model to mimic clinical pain, it has yet to be directly investigated whether these findings can be translated to the clinical population. Parallelly, further research is required to investigate if and how the present data relates to active treatment interventions, from pharmaceutical treatments to rehabilitation procedures.

Finally, our results foster not only the magnitude effect of the verbal information alone but also the temporal framework onto which it is delivered. In line with that, clinicians that work in the musculoskeletal field could integrate our findings into their clinical reasoning to enhance the effectiveness of interventions (e.g. manual therapy), improve the efficacy of the decision-making and the quality of their communication.

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