‘External timing’ of placebo analgesia in an experimental model of sustained pain.

Running title: ‘External timing’ of placebo analgesia

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Conflicts of Interest

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Significance Statement: Placebo research on placebo effects mainly focuses on the influence of information about direction and magnitude of the expected effect but ignores temporal aspects of expectations. In our study in healthy volunteers, the reported onset of placebo analgesia followed the temporal information provided. Such ‘external timing’ effects could not only aid the clinical use of placebo treatment (e.g., in open-label placebos) but also support the efficacy of active drugs.

Introduction

Pain is understood to not only be determined by noxious afferent input but also by the individual's expectations (Tracey, 2010). Although this influence has been demonstrated in various experimental paradigms, placebo analgesia remains the most intriguing of them. The mere information that one has received a potent painkiller can lead to substantial pain reduction – even though the 'painkiller' contains no analgesic properties. Experimental and clinical studies aiming to harness the power of expectations have focused on providing information about the direction (i.e., pain reduction (Barbiani et al., 2018; Kam-Hansen et al., 2014; Piedimonte et al., 2017; Pollo et al., 2001; Vase et al., 2002) and magnitude of the expected effect (Carlino et al, 2019; Price et al, 1999) but have so far ignored that expectations also include a temporal aspect that determines when the desired effect is expected to set in and how long it lasts.

In a recent study we demonstrated that temporal information about the expected onset of the placebo effect determines the reported start of pain reduction suggesting that 'external timing' of placebo effects is possible (Camerone et al., submitted for publication). While participants who were told that the placebo effect would commence after five minutes reported early pain reduction, those who were instructed that the analgesic effect would only set in thirty minutes after cream application showed a delayed onset in their analgesic response. Like many experimental pain studies, we used short-lasting electrical stimuli to probe the influence of temporal information. While this phasic pain model offers several advantages in an experimental setting (e.g., more repetitions for a higher number of trials), it has been criticized for its limited ecological validity (Edens & Gil, 1995; Rainville et al, 1992). Furthermore, the short duration and low stimulus intensity might have made it easy for verbal suggestions to bias perception. According to contemporary models of perception, verbal suggestions shape expectations which serve as a prior in an inferential process that interprets incoming sensory

information (Friston, 2005). Importantly, the relative impact of sensory information in this process critically depends on its precision. Expectations are more likely to impact perception when stimuli are weak, noisy and ambiguous (Pinto et al., 2015; de Lange et al., 2018), such as the short-lasting, low-intensity electrical stimuli we used in the previous study. The temporal shift in the onset of the placebo response we found might therefore at least partly be explained by these stimulus features.

Here, we investigated whether the modulatory effect of temporal information on the onset and duration of placebo analgesia extends to more intense, longer-lasting (tonic) pain in a Cold Pressor Test (CPT). The test assesses pain tolerance operationalised as the time participants are able to keep their hand in ice-cold water before the pain becomes unbearable. This paradigm allowed us not only to investigate whether tonic pain is equally susceptible to temporal information as phasic pain, but also to use a behavioural outcome measure (i.e., time until the hand is withdrawn from the water) instead of verbal pain reports only.

Methods

Participants

Seventy-seven healthy volunteers were recruited from the student population of the Vrije Universiteit Brussel (VUB), Belgium. Participants had no history of neurological, psychiatric, or other chronic medical conditions and were instructed not to consume alcohol or analgesic medication twelve hours prior to the experiment. 29 participants had to be excluded. 28 participants did not withdraw their hand within 10 minutes from immersing their hand into the water which had been defined as the maximum exposure time for safety reasons (Cheung & Daanen, 2012). One participant developed muscle cramps in her arm during the experiment. The final sample therefore comprised 48 participants. Participants were informed they would take part in a study investigating the onset of the effect of a newly developed analgesic cream.

All participants provided written informed consent in which they also agreed to be debriefed about the details of the study at the end of the experiment. All experimental procedures were conducted in accordance with the policies and ethical principles of the Declaration of Helsinki. The study was approved by the Ethics Committee of the Vrije Universiteit Brussel (B.U.N. 143201940102).

Group allocation

Participants were randomised by blind extraction to one of two placebo groups or a control group.

Placebo groups

In the two placebo groups, participants were informed that the inert cream (see details below) that was applied contained an analgesic substance that would reduce the painful sensation induced during the CPT.

The two groups differed in the information they received about the expected onset of the analgesic effect. The first placebo group was led to believe that the analgesic would become effective after 5 minutes (Positive Verbal Suggestion 5 Group, P5 group, N=16), mimicking a fast-acting analgesic (‘‘The agent you will receive is known to have a strong analgesic effect which sets in about 5 minutes after application. You will therefore become less sensitive to pain and be able to keep your hand in the cold water for a longer period of time 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].”).

The second placebo group was informed that the analgesic would become effective after 30 minutes (Positive Verbal suggestion 30 Group, P30 group, N=16), resembling the effect of analgesics with a delayed onset time (‘‘The agent you will receive is known to have a strong

analgesic effect which sets in about 30 minutes after application. You will therefore become less sensitive to pain and be able to keep your hand in the cold water for a longer period of time 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.").

Control group

Participants assigned to the control group were informed that an inert cream would be applied that would have no effect on their pain perception (No Expectancy, NE group, N=16; ''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 analgesic 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".).

Experimental protocol

After providing written informed consent, participants were seated in a comfortable chair positioned next to the CPT device (Figure 1). A stopwatch displayed on a computer screen in front of the participants and a customised wall clock were used 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 cream application. A poster depicting the pain intensity rating scale was positioned on the desk. The experiment started with a 4-minute heart rate measurement at rest during which the participant was asked to breath naturally and relax. Participants were then introduced to the CPT, completed the familiarization run and filled in the psychological questionnaires. Subsequently, all participants underwent the CPT baseline test before they were allocated to one of the three groups, the cream was applied

and participants were provided with information about the nature of the cream and the expected onset of the analgesic effect (placebo groups only). 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'). Afterwards, the CPT was repeated 10 minutes (Test 10') and 35 minutes (Test 35') after cream application (Figure 2).

Cold Pressor Test

During the CPT, participants had to immerse their right hand in seven litres of circulating cold water (7C°, ± .2C°; CPT device: Thermo Scientific™ VersaCool™ Refrigerated Circulating Bath, procedure adapted from Mitchell, Macdonald, & Brodie, 2004). To indicate the level to which participants had to lower their hand, the experimenter drew a red line from the participant's ulnar styloid process to the radial styloid process (wrist level). The CPT was repeated a total of four times (familiarisation, baseline, Test 10', Test 35') with approximately 25 minutes breaks between tests to restore the baseline hand temperature (Figure 2). During the breaks, participants were allowed to read a book. 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, 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 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 ground, but without touching it. The experimenter prompted the participant every 15 seconds to provide a verbal rating of their current pain intensity (see Pain Intensity Ratings below) which the

experimenter recorded manually on a spreadsheet. The participants' task was to keep their hand in the water basin until the pain reached tolerance level. Participants then removed their hand from the water basin and rested it on a towel placed on their knees. The time elapsed between immersion and withdrawal of the hand was recorded as CPT tolerance.

Pain Intensity Ratings

A poster depicting the rating scale including verbal and numerical anchors (0= not painful at all, 25= somewhat painful, 50= moderately painful, 75= very painful, 100= unbearable pain) was positioned in front of the participant (Figure 1). Participants provided numerical pain intensity ratings every 15 seconds during the CPT. Note that pain intensity ratings are not considered primary outcome measures because all participants were instructed to maintain their hand in the water until the pain reached an intensity of 100.

Heart Rate Recording

A decrease in HR has previously been shown to accompany placebo-induced analgesia (Aslaksen & Flaten, 2008; Pollo, A., Vighetti, S., Rainero, I., Benedetti, 2003), thus HR was chosen as potential physiological correlate of this effect.

The ECG signal was measured using a heart rate monitor (Polar V800) which was connected to two standard surface electrodes positioned on the participant's lower end of the sternum. Data was collected at a sampling rate of 700 Hz. The heart rate (HR) was first recorded 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 and avoid hyperventilating when in pain.

Assessment of pain-related psychological traits

Participants completed a set of questionnaires (Table 1) to assess psychological traits that have previously been linked to placebo responsiveness (Broelz et al., 2019; Corsi & Colloca, 2017; Lyby et al., 2010; Zhou et al., 2019). Questionnaires were completed between the familiarization with the CPT and the test sessions. At the end of the experiment, the two placebo groups were asked to retrospectively rate how they had expected the cream to affect (i) their pain during the experiment (*"When the cream was applied on your hand, did you expect it to make you feel less pain during the task?")* and (ii) their ability to keep their hand in the cold water (*"When the cream was applied to your hand, did you expect it to make you keep your hand in the water for longer?"*). Furthermore, participants had to retrospectively rate between 0 (= not at all) to 7 (= very much) to which extent they had believed the information regarding the onset of the analgesic effect *("When the cream was applied on your hand, how much did you agree with the following statement: The cream will start to become effective after 5 minutes"* (P5)/ *The cream will start to become effective after 30 minutes* (P30)").

Cream

A sham 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.

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. None of the participants decided to do so.

Statistical Analysis

Analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 9.6). To test for baseline differences in demographic variables and questionnaire scores, we compared the three groups using an analysis of variance (ANOVA) for continuous variables, or Chi-square test for categorial variables.

Pain ratings (NRS from 0 to 100) were not included as an outcome measure but served to check whether participants had kept their hand in the cold water until tolerance level had been reached. Note that these ratings do not necessarily represent the level of pain at the moment of hand withdrawal, but the last rating participants provided before they removed their hand (e.g. if the hand was removed after 59 second, the last pain intensity rating was obtained after 45 seconds). Median and interquartile range (IQR) are reported in the result section.

As data for CPT tolerance at baseline, after 10 (Test 10') and 30 (Test 35') minutes did not follow a normal distribution (Shapiro-Wilk tests $p>.05$), non-parametric tests were used throughout. First, Kruskal-Wallis H-Test was performed to test for baseline differences in CPT tolerance between groups. Second, Friedman Tests were performed to detect differences in CPT tolerance between the three different time points (Baseline, Test 10 and Test 35) separately for each group. Data are presented as median ± interquartile range (IQR) and level of significance was set at $p<.05$. Significant results were followed up using Wilcoxon Signed Rank Tests, Bonferroni-corrected for multiple comparisons. Effect sizes were calculated as r

= z/√N (Rosenthal, R., Rosnow, R. L., & Rubin, 2000). Third, we compared changes in CPT tolerance between groups using a Kruskal-Wallis H-Test. To this end, we calculated the percentage change in CPT tolerance from baseline to CPT10' (Δ_{10}) and baseline to 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.

Because baseline tolerance level varied considerably across participants (see IQR in Table 3, Results Section), percentage changes were used as a way to scale the results with respect to each participant's baseline tolerance, thus making the increase or decrease more comparable between participants. Significant results were followed up using pairwise Mann Whitney U Tests, Bonferroni-corrected for multiple comparisons. Effect sizes were calculated as $r = z/\sqrt{N}$ (Rosenthal, R., Rosnow, R. L., & Rubin, 2000).

To investigate the possible influence of expectations of treatment efficacy on CPT tolerance, Spearman rank-order correlation analyses were performed in the placebo groups between retrospective expectancy measures and Δ_{10} and Δ_{35}. Expectancy measures assessed how participants expected the cream to affect i) their *pain*, ii) *pain tolerance* as well as their expectations regarding the *onset of the analgesic effect*.

For the analysis of HR data, ECG recordings were first truncated at the shortest tolerance time recorded (i.e., 15 seconds after hand immersion) to ensure comparability across participants. The mean HR for each of the three CPTs (i.e., baseline, Test 10' and Test 35') was calculated for each participant by averaging the number of heartbeats within this time window, resulting in three mean HR indices for each participant. As HR data were normally distributed (Shapiro-Wilk tests $p<.05$), parametric analysis was used. To compare the HR between groups and time-points, an ANOVA with the within-subject factor TIME (Baseline,

Test 10' and Test 35') and between-subject factor GROUP (NE, P5, P30) was used. Significant results were followed up using Bonferroni-corrected t-tests.

Results

The groups did not differ with respect to age, sex, BMI and psychological variables (anxiety, disposition for behavioural inhibition/approach, fear of pain and degree of optimism) (p>.05 for all comparisons). For participants' characteristics see Table 2 (demographics) and Table 3 (psychological traits). Participants' pain intensity ratings served to check whether participants indeed only removed their hand from the ice water when the pain had become unbearable. As shown in Table 4, ratings reached an NRS of 90 or higher in all test sessions.

Placebo Effects: Within group comparison

Friedman Tests for within-group comparisons showed that CPT tolerance changed significantly in both placebo groups [P5, $\chi^2(2) = 18.95$, p <.001; P30, $\chi^2(2) = 21.37$, p <.001] but not in the NE group, $\chi^2(2) = 3.124$, p = .210 (Table 5). Post hoc Wilcoxon Signed Rank tests showed that in the P5 group, CPT tolerance significantly increased from baseline to Test 10' (z=-3.47, p=.002, r=.613) and was still higher than at baseline when assessed at Test 35' (z=-3.34, p=.002, r=.590). No significant difference was found between Test 10' and Test 35' (z=-.710, p=1.434, r=.125). These results suggest that placebo analgesia occurred at the expected time-point and once analgesia had been triggered, it remained stable over time, at least up until 35 minutes after cream application (Figure 3).

In contrast, the P30 group showed no significant difference in CPT tolerance between baseline and Test 10' (z=-.828, p=.224, r=.146). Only at the later test time-point (Test 35'), CPT tolerance was significantly higher than baseline (z=-3.46, p=.002, r=.612) and in Test 10' (z=-

3.52, p=.001, r=.622), indicating that the analgesic effect only set in late in accordance with the instructions provided (Figure 3).

Placebo Effects: Between group comparison

No significant difference in baseline CPT tolerance level between the three groups was reported by Kruskal-Wallis H-Tests (*p*=.988).

Kruskal-Wallis H-Tests showed a significant group difference in Δ_{10}, $\chi^2(2) = 23.05$, p<.001, with a mean rank Δ_{10} of 37.81 in P5, 20.72 in P30 and 14.97 in NE. Post hoc Mann-Whitney U-tests revealed that Δ_{10} was significantly higher in P5 than in both NE (U=16.5, p<.001, r = .743) and P30 (U=26.5, p<.001, r=-.676) but did not differ significantly between the NE group and P30 (U=87, p=.266, r=.274). This indicates that 10 minutes after cream application, pain reduction was stronger in P5 than in NE and P30 (Figure 4). For $\Delta_{35,}$ Kruskal-Wallis H-Test also showed a statistically significant difference between groups, $\chi^2(2)=18.06$, p<.001, with a mean rank Δ_{35} of 29.31 in P5, 31.75 in P30 and 12.44 in NE. Post hoc Mann-Whitney U-tests revealed that Δ_{35} was significantly higher in both P5 (U=38, p=.002, r= .600) and P30 (U=25, p<.001, r= .686) compared to the NE group, indicating that pain reduction after 35 minutes was stronger in the two placebo groups than in the NE (Figure 4).. No significant difference in Δ_{35} was found between P5 and P30 (U=155, p=1.872, r=.179). Median and IQR of percent change in CPT pain tolerance (Δ_{10} ,Δ_{35}) in the three experimental groups are reported in Table 6.

Retrospective expectancy

Median and IQR of retrospective expectancy measures for both P5 and P30 are reported in Table 7. Spearman rank-order correlations between retrospective expectations of i) pain, ii)

CPT resistance and iii) onset of analgesic effect and Δ_{10} and Δ_{35} did not reach significance in either of the two placebo groups.

Heart Rate

HR data showed a significant main effect of TIME ($F(2,90)=19.39$, $p<.001$) but no main effect of GROUP or 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 ($p> 0.05$). Overall means and standard deviations across the three groups as well as for each group separately are reported in Table 8 below.

Discussion

Previous experimental placebo studies have focused on the effect of information about the direction or magnitude of the expected effect on 'treatment' outcome. In a recent study, we demonstrated that the outcome of a placebo manipulation is also influenced by information about the expected time-course of the effect (Camerone et al., submitted for publication). Using low-intensity and short-lasting electrical stimuli, we showed that those who had been informed that the 'analgesic' would become effective shortly after administration displayed immediate (and sustained) pain reduction. In contrast, those who expected analgesia to set in after 30 minutes reported a delayed decrease in pain. Here, we extend these findings by demonstrating a similar effect in an experimental model of sustained pain (CPT) with pain tolerance as an independent behavioural outcome measure.

Our results confirm two key findings of our previous study. First, the onset of analgesia was determined by the temporal information that participants received at the beginning of the

experiment (Figure 4). Only those who had been instructed that the analgesic effect would commence shortly after cream application showed increased pain tolerance at the first test after baseline. The group that was informed that the pain alleviating effect would only set in later showed no analgesic effect at this early test time-point but at the expected time after 30 minutes. Such 'external timing' of placebo effect is noteworthy for several reasons. Neuroimaging studies have shown that placebo effects are mediated by top-down regulatory processes in the brain which alter responses to noxious stimuli at various stages of the neuraxis including the spinal cord (Wager & Atlas, 2015). However, very little is known about factors triggering this cascade. Our findings of an 'external timing' effect suggest that information reaching this top-down modulatory circuit do not necessarily prompt an immediate response but also provide a 'time tag' that determines when the effect is to be set in motion. Where and how temporal aspects of treatment expectations interface with the pain system in the brain needs to be explored using brain imaging technology. The timing effect is also noteworthy from a clinical perspective as it could open up new ways to enhance placebo effects (e.g., open-label placebo treatments; Kaptchuk & Miller, 2018) but even more importantly also the efficacy of active drug treatment (Carlino et al., 2012). Expectancy effects have been shown to contribute substantially to the overall treatment outcome of active drugs (Benedetti et al., 2003; Bingel et al., 2011). Although most drugs develop their maximum effect shortly after administration, some require days or weeks to become effective. For example, the desired effect of some tricyclic antidepressants often only sets in several weeks after start of treatment. Medication discontinuation is therefore a frequent problem in the early weeks of such treatment (Chakraborty et al., 2009; Holvast et al., 2018). Importantly, the lack of noticeable symptoms improvement can cause patients to abandon their initially positive treatment expectations. This means that once the drug has reached its critical concentration and the pharmacological effect unfolds, it may no longer be supported by positive expectations and even be counteracted by

the impression of ‘treatment failure’ which has been demonstrated to squelch also unrelated subsequent treatment attempts (Zunhammer et al., 2017). Our observation of ‘external timing’ of placebo effects suggests that explicitly informing patients about the delayed onset could prevent the abandoning of treatment expectations and instead trigger the supporting placebo effect when the pharmacological drug effect sets in. Because our paradigm only tested whether the onset can be shifted by thirty minutes, further studies are needed to explore more substantial delays.

We also confirmed that once placebo analgesia had been triggered, it was maintained for the duration of the experiment (Figure 3). In the P5 group, which expected and showed an early reduction in pain, analgesia was still present after 30 minutes without a decrease in strength. Note that no specific information regarding the duration of the effect had been provided. Findings from experimental studies indicate that placebo analgesia can at least be maintained for the duration of a single experimental session (Colloca et al., 2008; Geers et al., 2015) and observations from a randomised controlled trial suggest that placebo effects can even increase over time (Quessy & Rowbotham, 2008). However, more systematic investigations are needed to explore the longevity of placebo effects.

The current study extends our previous findings in one very important aspect. While we previously showed an effect of temporal information on placebo analgesia using short-lasting, low-intensity stimuli, we demonstrate here that similar results can be achieved in an experimental model of high-intensity tonic pain. Phasic pain models have been criticised for their lack of ecological validity as their stimuli have little resemblance with chronic pain with respect to duration and aversiveness (Rainville et al, 1992). In contrast, CPT-induced pain increases over time until it reaches tolerance level and participants withdraw their hand. Although this type of pain is still different from clinical pain, it is undoubtedly the better proxy. Stimulus duration and intensity also play a key role for the degree to which expectations can

influence perception. Modern concepts of perception posit that any sensation is determined not only by incoming sensory information but also by the individual's expectations. In this framework, expectations are assumed to have a stronger effect if the afferent input is weak, noisy or ambiguous (de Lange et al., 2018; Pinto et al., 2015), leaving more room for expectations to "fill the gap" and bias the interpretation of sensory information in the expected direction. It could therefore be speculated that temporal expectations induced by verbal suggestions are more likely to impact the onset of placebo analgesia in a model using short-lasting, low-intensity stimuli (as in our previous study; Camerone et al., submitted for publication) than in a high-intensity and long-lasting pain model (CPT). However, a direct comparison of the strength of placebo effects suggests the opposite. While an average placebo effect of r=0.47 was found in our previous study, it was considerably stronger in the current trial (r=.71) (see Supporting Information for details). Of note, a similar result was found in a meta-analysis by Vase et al (2009), who reported larger placebo effects for longer (>20s, d=0.96) than for shorter pain stimuli (<20s, d=0.81). In addition to physical stimulus features, differences in perceived controllability of the stimulation which is known to dampen the perception and neural processing of pain (Salomons et al., 2004, 2007; Wiech et al., 2006) might explain the stronger placebo effects in the current study. In our CPT study, participants had to decide for how long they could keep their hand in cold water. Exposure to noxious input was therefore entirely controllable. In contrast, our previous study used a passive stimulation with no element of control.

Using (self-determined) exposure time as the key outcome also allowed us to quantify the effect of the temporal information in a way that is less prone to report bias than the commonly used pain intensity ratings. Because participants were instructed to reach tolerance level and analgesia was defined as increased exposure time, (deliberate) overreporting, for instance due to social desirability, is unlikely. In pursuit of further changes in objective

parameters, we also tested whether the 'external timing' effect would be reflected in HR variations. However, HR decreased over the course of the experiment in all three groups. So far, studies exploring HR changes related to placebo analgesia have yielded inconsistent results. Studies using CPT-induced pain (Geers et al., 2015; Peerdeman et al., 2017) and electrical stimulation model (Luana Colloca & Benedetti, 2009; Rhudy et al., 2018) found no changes in HR associated with placebo analgesia. However, other studies using ischemic arm pain (Pollo, A., Vighetti, S., Rainero, I., Benedetti, 2003) and thermal pain (Aslaksen & Flaten, 2008) reported a reduction in HR during placebo analgesia.

A limitation of this study is that pain-related expectations were only assessed retrospectively (instead of repeatedly during the experiment) to avoid drawing attention to this variable and potentially disclosing the actual purpose of the experiment. Our data do therefore not allow for any conclusions regarding changes of temporal expectations over the course of the experiment. As expectations not only impact perception but are in turn also continuously updated to reflect past (sensory) experiences, further studies are needed to explore the interplay between both variables.

Taken together, our data confirm previous findings of 'external timing' of a placebo analgesic effect and extend it to an experimental model of sustained pain using pain tolerance as an observable outcome parameter. While these findings hold promise for a systematic use of this effect in therapeutic contexts, further research is required to investigate if and how it translates to clinical pain and different treatment approaches.

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Author contribution statements

Eleonora Maria Camerone conceived and designed the study, collected the data, planned and performed the data analysis and wrote the paper. *Katja Wiech* was involved in developing the data analysis strategy and writing the publication. *Fabrizio Benedetti* and *Elisa Carlino* conceived and designed the study. *Marco Testa* supervised the project throughout, from the design of the experiment to the completion of the final manuscript. *Aldo Scafoglieri* supervised the practical development of the experiment assessing its feasibility from start to end. *Mirko Job* was responsible for the development of data analysis.

All authors discussed the results and contributed to the final version of the manuscript.