How negative experiences influence the brain in pain: neuroimaging and biobehavioral insights

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Chapter 4.

Learning mechanisms in nocebo hyperalgesia: The role of conditioning and extinction processes.

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Abstract

Nocebo hyperalgesia is a clinically relevant phenomenon and may be formed as a result of associative learning, implemented by classical conditioning. This study explored distinct nocebo conditioning methods and their consequences for nocebo attenuation methods. Healthy participants (N = 140) were recruited and randomized to the following nocebo hyperalgesia induction groups: conditioning with continuous reinforcement (CRF), conditioning with partial reinforcement (PRF), and a sham-conditioning control group. In the attenuation phase, counterconditioning was compared to extinction. During induction, participants experienced increased thermal pain in 100% of nocebo trials in the CRF groups, while in only 70% of nocebo trials in the PRF groups. During evocation, pain stimulation was equivalent across all trials. During attenuation, pain stimulation was decreased on nocebo trials relative to control trials for the counterconditioning groups, while pain remained equivalent across all trials for the extinction groups. Results showed that both PRF and CRF significantly induced nocebo hyperalgesia, but CRF was a more potent nocebo induction method, as compared to PRF. Counterconditioning was more effective than extinction in attenuating nocebo hyperalgesia. Neither CRF nor PRF resulted in resistance to extinction. However, compared to CRF, conditioning with PRF resulted in more resistance to counterconditioning. These findings demonstrate that the more ambiguous learning method of PRF can induce nocebo hyperalgesia and may potentially explain the treatment resistance and chronification seen in clinical practice. Further research is required to establish whether attenuation with counterconditioning is generalizable to clinical settings.

Introduction

It has been demonstrated that negative expectations regarding treatment outcomes may aggravate pain symptoms [1–4], a phenomenon termed nocebo hyperalgesia [2,5]. In experimental research, nocebo hyperalgesia is defined as a significant increase in pain following a nocebo treatment, relative to no-treatment or a control treatment. Negative expectations may enhance aversive side-effects [6] or produce deleterious effects on pain recovery [7]. Classical conditioning is an important underlying mechanism of nocebo hyperalgesia [8–10]. In conditioning paradigms, the pairing of a conditioned stimulus (CS; e.g., an inert treatment) with an unconditioned stimulus (US; e.g., surreptitiously increased pain) leads to a learned association [11,12]. As a result of this learned association, an inert treatment can evoke increases in perceived pain [10].

The vast majority of nocebo studies induce hyperalgesia by use of conditioning with continuous reinforcement (100% pairing of CS and US). In a more ambiguous type of conditioning with partial reinforcement, stimuli are paired in less than 100% of trials, thus the contingency between pain and an inert nocebo treatment is more variable. Partial reinforcement is of particular clinical interest due to its variable nature, which resembles the more ambiguous and inconsistent learning that may occur in clinical settings [13]. Partial reinforcement conditioning has been successfully used in fear research [14] and was recently also implemented in nocebo research [15]. Colagiuri and colleagues compared continuous and partial reinforcement schedules and found that nocebo hyperalgesia can be induced through partial reinforcement [15]. Additionally, Colagiuri and colleagues [15] investigated the consistent finding from fear studies that partial reinforcement conditioning shows more resistance to extinction than continuous reinforcement [14,16,17]. In contrast to findings in other fields of research [14], extinction was unsuccessful in attenuating nocebo hyperalgesia

irrespective of the conditioning schedule [15]. This indicated that, once established, nocebo hyperalgesia may be especially resistant to extinction; a relevant finding for chronic pain conditions, where learned effects may persist and not become extinct.

If extinction is unsuccessful in attenuating these learned effects, a more active approach may be needed to attenuate nocebo hyperalgesia. A promising novel method is counterconditioning. Unlike in extinction, during counterconditioning the negative stimulus is replaced by a more positive stimulus [18]. Counterconditioning has recently been successful in different fields [19,20]. However, despite its potential as a basis for the treatment of nocebo-augmented pain [21], it remains unclear whether counterconditioning would be an effective intervention for the attenuation of learned nocebo responses.

In this study, we compared two nocebo induction methods, conditioning with partial and continuous reinforcement. Furthermore, we examined the consequences of partial versus continuous conditioning for the attenuation of nocebo hyperalgesia via counterconditioning or extinction. We expected to reproduce earlier findings that partial reinforcement would successfully induce nocebo hyperalgesia and that compared to continuous reinforcement, partial reinforcement conditioning would lead to more resistance to extinction. We furthermore examined counterconditioning as a potential attenuation method for nocebo hyperalgesia. The implementation of novel, clinically relevant learning-based methods for investigating nocebo hyperalgesia is an important step towards eventually diminishing nocebo effects in clinical settings.

Materials and Methods

Participants

One hundred and forty participants were enrolled in this study. The required sample size for the primary analysis was calculated based on a previous similar nocebo study [15]. The analysis was conducted in G*power 3.1 [22] for a mixed model ANOVA. The effect size was f = 0.26, alpha error probability was set at $\alpha = 0.05$, desired power was set at 0.95, and the correlation for repeated measures was set at 0.05 (because of the subjectivity and high variability expected in pain ratings). According to the total sample size indicated, we planned for 140 participants to be enrolled, of which a total of 122 participants were included in the study. This sample size for the primary hypothesis is similar to previous studies examining subtle between-groups differences such as conditioning with partial reinforcement or anxiety correlates [15,23]. The main groups were split in half for the purposes of some of the secondary analyses in this two-by-two design, resulting in subgroups of 24-25 participants, a sample size that has been used in previous nocebo studies that yielded significant results with good effect sizes [21,24,25].

Participants were required to be between 18 and 35 years old, have a good understanding of the Dutch language as well as (corrected to) normal vision and hearing. Exclusion criteria were serious medical or psychiatric conditions, pregnancy, painful health conditions experienced in the past 6 months, and pain or use of analgesic medication at the time of testing. Participants who were determined to have too high of a threshold for pain upon their visit to the department (i.e., when thermode maximum temperatures were not sufficient to induce at least moderate pain) were also excluded from the study. All participants were asked to refrain from alcohol and caffeine consumption, as well as the use of drugs and analgesic medication, in the 12 hours before the testing appointment. Participants were recruited via flyers, social media

advertisements, and the online recruitment website Sona (Sona Systems, Tallinn, Estonia). Study participation involved a 2-hour testing appointment at a research laboratory of the Faculty of Social and Behavioral Sciences of Leiden University, the Netherlands. All participants provided written informed consent prior to the start of the experiment. After completing the experiment, all participants were reimbursed by either cash or study credits for their participation. This study was approved by the Leiden University Psychology Research Ethics Committee (CEP18-0816/318) and pre-registered on ClinicalTrials.gov (NCT03793790).

Design

This study utilized a randomized, two-by-two design, with an additional control group (Figure 1). A randomization list was created by an independent researcher and participants were randomly allocated to one of 5 groups only after the calibration procedure was complete, so to reduce any risk of bias. All participants underwent a two-phase study design of which each phase consisted of two parts. The induction phase (phase 1) comprised an induction part in which associations were learned and an evocation part in which learned associations were tested. The attenuation phase (phase 2) comprised either counterconditioning or extinction to examine the attenuation of the learned responses and a second evocation part to test whether learned associations were still present. Group 1 received conditioning with partial reinforcement and counterconditioning. Group 2 received conditioning with partial reinforcement and extinction. Group 3 received conditioning with continuous reinforcement and counterconditioning. Group 4 received conditioning with continuous reinforcement and extinction. Group 5 (the sham control group) received sham conditioning and also

underwent 'extinction' in order to keep the length and procedures of the experiment identical for all.

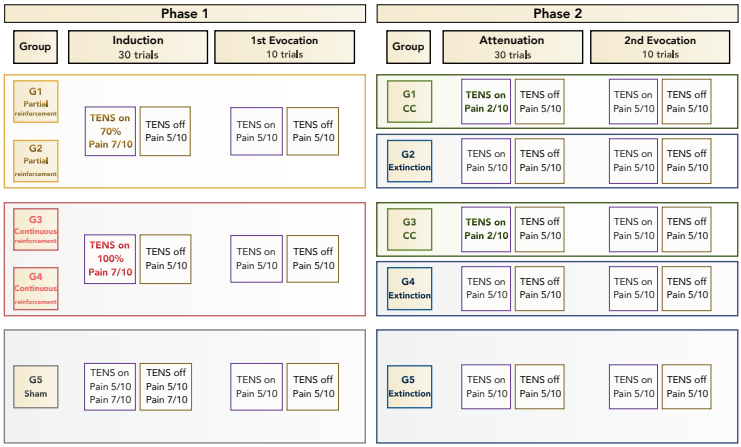

Figure 1. Illustration of the experimental design. During partial reinforcement (G1 and G2) participants received high pain in 70% of nocebo trials and moderate pain in 30% of nocebo trials. The sham group (G5) received high pain in 50% of nocebo trials and in 50% of control trials. In the attenuation phase, during counterconditioning (G1 and G3) participants received low pain in all nocebo trials, while during extinction (G2 and G4) participants received moderate pain for both the nocebo and the control trials. The sham group underwent extinction to keep the procedure equal in length. CC, Counterconditioning.

Thermal pain application

Thermal pain stimuli were delivered to the non-dominant volar forearm using a Thermal Sensory Analyzer with a 3×3 cm thermode probe (TSA-II; Medoc Advanced Medical Systems, Ramat Yishai, Israel). Throughout the experiment, pain intensities were rated on a pain numeric rating scale (NRS) ranging from 0 (no pain) to 10 (worst pain imaginable).

Sensory thresholds

To test warmth and pain threshold levels, heat stimuli were applied and participants were asked to indicate the first moment at which they perceived warmth and the first moment they perceived pain. The average of 3 warmth detection values and 3 heat pain detection values were determined as the threshold values for warmth and pain respectively. This method followed published standardized and protocolled procedures [26].

Pain calibration protocol and administered stimuli

Pain calibrations were conducted in order to select the temperatures that would be used to induce low, moderate, and high pain in phases 1 and 2. The calibrations were individually tailored, based on the NRS ratings of 42 heat stimuli of varying intensities, as well as participants' bodily and facial reactions to pain stimuli. For the calibration procedure as well as throughout the experiment, each stimulus was initiated from a 32°C baseline, increased to a target temperature, and presented for 4 seconds, excluding a ramp up rate of 8°C per second and a return rate of 8°C per second. The inter-stimulus interval was 8 seconds. Median temperatures consistently rated and experienced as NRS 2 to 3 were selected and used to induce low pain, median temperatures rated as NRS 4 to 6 were used to induce moderate pain, and median temperatures rated as NRS 7 to 8 were used to induce high pain. During induction and during attenuation, 15 nocebo and 15 control stimuli were administered in pseudorandom order, so that no three stimuli of the same type were administered in a row. During each of the two evocations, 5 nocebo and 5 control stimuli were administered in pseudorandom order. To reduce habituation to heat-pain, the thermode was moved twice (mid-way through phases 1 and 2) to a more proximal site on the same arm.

Nocebo treatment

A commercial Transcutaneous Electrical Nerve Stimulation (TENS) device (Beurer EM 80) was used to serve as the nocebo treatment in the procedure. Negative suggestions were used to create expectations regarding the pain enhancing effects of the device (Appendix 1). Two TENS electrodes were placed in a diagonal line on the ball of the hand and the inner elbow. Prior to the start of the induction phase, participants underwent a short mock calibration procedure during which they felt a light electrical pulse of the TENS. This pulse was delivered in order to increase the believability of the nocebo verbal suggestion. Participants were told that the device was called "ENS", to avoid that participants would recognize or associate any prior experience with this device. The device was not actually activated during the conditioning procedure, but messages displayed on a computer screen via E-Prime 2.0 (Psychology Software Tools, Pittsburgh, PA, USA) signaled the sham activation and deactivation of the TENS device during nocebo and control trials, respectively.

As part of the nocebo suggestions, participants read an information sheet (see Appendix 1), displayed on a tablet, containing (sham) information regarding the supposed effects of the TENS treatment. During nocebo induction, negative suggestions indicated to all participants that when the messages "ENS on" (in purple font; nocebo cue) and "ENS off" (in yellow font; control cue) were displayed, their pain would be aggravated or not altered, respectively.

Sham TENS activation was paired to surreptitiously increased pain stimulation during nocebo trials, while moderate pain was delivered during control trials during the induction phase. For the partial reinforcement groups, the activation of the TENS device was paired with high pain stimuli in only 70% of nocebo trials (un-paired trials were

pseudorandomized to achieve an approximately even distribution throughout the induction phase). The continuous reinforcement groups received high pain stimuli in 100% of nocebo trials. The control group received sham conditioning, where TENS activation was not consistently paired to the intensity of pain stimuli but rather, this group received high pain in 50% of nocebo trials and in 50% of control trials. In the first evocation phase, all pain stimuli were applied at moderate intensity, preceded by the nocebo and control cues, to evoke conditioned responses. Increased pain reports for the first nocebo trial as compared to the first control trial in this phase indicated nocebo hyperalgesia. During attenuation, the counterconditioning groups received surreptitiously decreased pain stimulations during TENS activation, while TENS deactivation was still paired to moderate pain inductions. During extinction, participants continued being exposed to pain stimuli at only moderate intensity preceded by the nocebo and control cues. During the second evocation phase, all pain stimuli were applied at moderate intensity, preceded by the nocebo and control visual cues, to test whether nocebo responses were diminished after attenuation.

Questionnaires

Four questionnaires were used to measure baseline differences in psychological characteristics. Total scores were used for all questionnaires. A short State Anxiety version of the State-Trait Anxiety Inventory, (STAI-S) [27,28] was used once before the start (STAI state pre) and once after the end of the experiment (STAI state post). Scores on this questionnaire range from 20 to 80, with higher scores indicating higher state anxiety. Cronbach's alpha in this study were 0.77 (pre) and 0.74 (post). The State-Trait Anxiety Inventory, Trait version (STAI-T) [27] was also used, with scores also ranging from 20 to 80 and higher scores

indicating higher trait anxiety. Cronbach's alpha was 0.83 in this study. The Pain Catastrophizing Scale (PCS) [29] was used to assess catastrophizing thoughts related to pain, with scores ranging from 0 to 52, where higher scores indicate more frequent catastrophizing thoughts. Cronbach's alpha was 0.87 in this study. The revised Life Orientation Test (LOT-R) [30] was used to measure dispositional optimism versus pessimism. Scores on this questionnaire range from 0 to 24, with higher scores indicating higher optimism. Cronbach's alpha was 0.69 in this study. Participants were also asked to rate their tiredness on a 0-10 NRS scale from "not at all" to "very much". Moreover, a screening questionnaire containing demographic and health questions was used to screen participants for inclusion in the study. At the end of the experiment, participants completed an exit questionnaire containing manipulation check questions assessing pain expectations (rated on the pain NRS), how much they trusted the experimenters, and how honest they thought the experimenters were (rated on a 0-10 NRS from "not at all" to "very much"). The exit questionnaire also assessed whether participants believed the cover story or were aware of the real purpose of the experiment (i.e., the manipulation of expectations or use of conditioning). All questionnaires, as well as a debriefing from, were displayed on a tablet via web-based survey software (Qualtrics, Provo, Utah, USA).

Experimental Procedure

On the day of the appointment, participants were first provided with information about the experiment and were asked to provide written informed-consent. Then, participants completed the screening and the psychological questionnaires. Following this, they read the information sheet about the (sham) pain enhancing effects of the TENS device. Warmth and pain threshold levels were then tested and individual pain

stimuli were calibrated. Participants then underwent nocebo induction through conditioning with partial reinforcement, continuous reinforcement, or sham conditioning. The first evocation phase where nocebo responses were tested then followed. Subsequently, participants underwent nocebo attenuation, through either counterconditioning or extinction. A second evocation phase then followed, where the presence of nocebo responses after attenuation was tested. After the end of the experiment participants were asked to complete the exit questionnaire. Then, a debriefing was conducted and participants were reimbursed for their participation.

Statistical Analyses

All data were analyzed by use of SPSS 23.0 (IBM Corp., Armonk, NY, USA). A one-way analysis of variance (ANOVA) was conducted between all groups for mean scores on each of the questionnaires and the tiredness rating, in order to determine whether any personal characteristics could have influenced the results. One-way ANOVAs were also used to assess between-groups differences in state anxiety, trust in the experimenter, and pain expectations, as assessed at the end of the experiment and in temperatures used to induce pain and the NRS pain scores throughout the experiment. As these analyses involved multiple between-groups comparisons, the threshold for significance was set at $P < 0.01$.

Primary and secondary outcome measures

The magnitude of reported nocebo hyperalgesia (primary outcome measure) was measured within-subjects, and was defined as the difference in pain ratings for the first nocebo trial compared to the first

control trial, during first evocation. The reduction of induced nocebo hyperalgesia after attenuation was measured as the change in reported pain for the first nocebo trials between the first and second evocation. The first trials of each testing phase were selected since previous studies indicate the effect to be clearest in those trials [15,31]. Difference scores between nocebo and control trials as mentioned above were only used for manipulation checks and descriptive purposes (Tables 1 and 2). To conduct mixed model analysis of variance (ANOVA), the assumptions of normality, independence and homogeneity of the variances were checked. Unless otherwise stated, the threshold for significance was set at $P < 0.05$. As an effect size measure, partial eta-squared (η_p^2) was calculated for analyses of primary and secondary outcomes, with η_p^2 of 0.01 considered small, 0.06 considered medium, and 0.14 large [32,33].

Nocebo hyperalgesia induction

First, to examine whether a significant nocebo response was present after nocebo induction, a 3x2 mixed model ANOVA was used, treating induction group as the between-subjects factor with 3 levels (partial reinforcement, continuous reinforcement, or sham) and magnitude of the nocebo response as a within-subjects factor with 2 levels (first nocebo trial, first control trial). A conservative Bonferroni correction was applied and the threshold for significance was set at $P < 0.01$. Where a significant interaction is detected, planned contrasts are analysed (2x2 mixed ANOVAs) between each of the pairs of experimental groups.

Nocebo hyperalgesia attenuation

In order to test the hypothesis that counterconditioning would be more effective than extinction in attenuating nocebo hyperalgesia, a 2x2 mixed

model ANOVA was performed with attenuation group as the between-subjects factor with two levels (counterconditioning, extinction) and the nocebo reduction as the within-subjects factor with 2 levels (first nocebo trial of the first evocation phase pre-attenuation, first nocebo trial of the second evocation phase post-attenuation).

Resistance to extinction

To test the hypothesis that conditioning with partial reinforcement would lead to a more durable nocebo effect as compared to conditioning with continuous reinforcement, we explored resistance to extinction. A 2x2 mixed model ANOVA was performed with the induction group as between-subjects factor with two levels (partial reinforcement-extinction, continuous reinforcement-extinction) and the nocebo response as the within-subjects factor with 2 levels (first nocebo trial of the first evocation phase pre-attenuation, first nocebo trial of the second evocation phase post-attenuation). Following this, two repeated measures ANOVAs were conducted with the nocebo response as the within-subjects factor with 2 levels (as described above), to test whether extinction significantly reduced the magnitude of nocebo hyperalgesia within the partial reinforcement group and within the continuous reinforcement group.

Manipulation-check for the time-course of extinction

Because of the unique attenuation paradigm in the experiment, we implemented a design that applied 10 evocation trials, that were essentially extinction trials, before the start of the 30 attenuation trials. In our paradigm, 30 induction trials were followed by 10 evocation trials, which in turn were followed by 30 extinction trials. Evocation trials,

however, are identical to extinction trials. This exposed participants to a longer extinction time (i.e., essentially 40 trials), as compared to the 30 induction trials. In order to verify that any extinction or resistance effects were not present after an equal number of induction and extinction trials, we analyzed the 30th trial after the start of evocation. A 2x2 mixed model ANOVA was performed with the induction group as between-subjects factor with two levels (partial reinforcement-extinction, continuous reinforcement-extinction) and the nocebo response during extinction as the within-subjects factor with 2 levels (20th nocebo extinction trial, 21st control extinction trial).

Resistance to counterconditioning

It was also assessed whether a resistance effect to counterconditioning was present. A 2x2 mixed model ANOVA was performed with induction group as the between-subjects factor with 2 levels (partial reinforcement-counterconditioning, continuous reinforcement-counterconditioning) and nocebo response as the within-subject factor with 2 levels (first nocebo trial of the first evocation phase pre-attenuation, first nocebo trial of the second evocation phase post-attenuation). Following this, two repeated measures ANOVAs were conducted with the nocebo response as the within-subjects factor with 2 levels (as described above), to test whether counterconditioning significantly reduced the magnitude of nocebo hyperalgesia within partial reinforcement and within the continuous reinforcement group.

Time-course of attenuation

To explore the time-course and slopes of attenuation, a line graph was plotted. Mean NRS pain ratings were plotted for the nocebo trials after

the end of nocebo induction, in the partial reinforcement-counterconditioning group, the partial reinforcement-extinction group, the continuous reinforcement-counterconditioning group, and the continuous reinforcement-extinction group.

Manipulation-check for control trials

We ran manipulation checks to examine any effect of changes in control trial ratings on the reduction of nocebo responses after attenuation. This was done to assure that the effects of attenuation were not driven by changes in the ratings of control trials (TENS off), which could confound the results, for example if between-groups differences were detected, or in the case that general sensitization or habituation to pain had occurred. First, an analysis of the control trial ratings in all groups was performed. A 5x2 mixed model ANOVA was performed with group as the between-subjects factor with 5 levels (groups 1, 2, 3, 4, and 5), and the first control trial rating of each evocation phase as the within-subjects factor with 2 levels (control pre-attenuation, control post-attenuation). As this analysis involved multiple between groups comparisons, a conservative Bonferroni correction was applied and the threshold for significance was set at $P < 0.01$. A non-significant result would indicate that the control trials did not yield significant changes, confirming that they did not affect the within-subjects results of the analyses. Furthermore, we conducted a 2x2 mixed model ANOVA between the attenuation groups (counterconditioning, extinction) and the pre- to post- attenuation difference score between nocebo and control trials. In this way, we examined the reduction in the magnitude of nocebo hyperalgesia from pre- to post- attenuation, by directly comparing control trials to nocebo trials.

Questionnaires

Finally, we studied the relationship of nocebo hyperalgesia and anxiety, pain catastrophizing, and optimism. Scores obtained through the four psychological questionnaires were analyzed using correlation analyses, to explore whether any of these psychological characteristics were associated with the magnitude of induced nocebo hyperalgesia.

Results

Participants, temperatures, and pain ratings

A total of 140 participants were enrolled in this study (118 females, 22 males). Six participants were excluded due to technical difficulties or noise disturbance in the lab, 4 participants were unable to complete the study due to sleepiness, intense anxiety, or inability to follow instructions, 3 participants were excluded due to exhibiting a too-high threshold for pain (i.e., not reaching a moderate pain rating during calibrations), 2 participants were excluded due to fulfilling one of the health-related exclusion criteria (namely, experiencing moderate head or neck pain at the time of testing), 2 participants were excluded due to knowing the purpose of the experiment as assessed in the post-assessment survey, and 1 participant was excluded due to insufficient understanding of Dutch. A total of 122 participants were included in the final analyses, 102 females and 20 males. Randomization across the five groups resulted in a total of 25 participants in group 1 (partial reinforcement-counterconditioning), 24 participants in group 2 (partial

reinforcement-extinction), 24 participants in group 3 (continuous reinforcement-counterconditioning), 24 participants in group 4 (continuous reinforcement-extinction) and 25 participants in group 5 (sham). Participants were stratified for gender, so that each group contained 5 male participants.

Descriptive data of the questionnaire scores, temperature levels, and pain ratings are listed in Table 1. One-way ANOVAs indicated that there were no significant between-groups differences in the mean scores on any of the psychological questionnaires. The mean warmth detection threshold across all participants was 33.5°C (standard deviation; SD = 0.5) and the mean pain threshold was 42.3°C (SD = 2.9). The results of a one-way ANOVA indicated that there were no significant group differences in the mean temperatures selected to induce low, moderate and high pain. A one-way ANOVA detected a significant group difference in the ratings of the control trials of the induction phase, however when the analysis was performed again with the sham group removed, it was revealed that this difference was merely driven by the sham group, where half of the control trials were purposely paired with high pain stimulation. Despite moving the thermode several times during the experiment in order to avoid habituation to the heat stimuli, an overall decrease in pain ratings over time was observed (see Table 1, Conditioning and Attenuation rows).

Table 1. Group means and standard deviations, as well as between-groups *P* values.

	1 PRF - CC		2 PRF - Extinction		3 CRF - CC		4 CRF - Extinction		5 Sham		All groups		*P* value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
STAI Trait	40.2	8.0	37.2	5.7	37.6	8.1	36.8	7.4	38.8	6.6	38.1	7.2	0.45
STAI State pre	50.8	24.4	52.7	23.1	48.6	25.1	44.3	18.6	44.2	16.6	48.1	21.7	0.56
PCS	13.6	8.1	12.9	6.6	11.9	5.4	13.8	6.8	13.4	8.0	13.1	7.0	0.90
LOT-R optimism	16.9	3.3	14.8	3.5	17.2	2.6	16.1	2.9	15.1	3.4	16.0	3.3	0.33
Tiredness (NRS)	3.4	2.0	2.9	2.1	3.4	2.3	3.9	2.5	3.3	2.3	3.4	2.3	0.60
Low °C	45.0	0.8	45.1	1.1	45.5	1.1	45.0	0.9	45.0	0.5	45.1	0.9	0.31
Moderate °C	46.8	0.7	47.0	0.8	47.0	0.8	46.7	1.1	46.9	0.7	46.9	0.8	0.61
High °C	48.4	0.5	48.6	0.7	48.8	0.6	48.5	0.8	48.5	0.5	48.6	0.6	0.31
Nocebo trials NRS ^	6.6	1.7	6.2	1.7	7.3	1.5	6.6	1.6	5.8	1.6	6.5	1.7	0.03
Control trials NRS	4.6	1.8	4.4	1.8	4.8	1.6	3.7	1.5	5.7	1.7	4.6	1.8	0.002 *
Nocebo trials NRS	3.2	2.0	4.4	2.0	2.6	1.4	3.8	2.0	4.0	2.1	3.6	2.0	0.01**
Control trials NRS ^	4.5	2.0	3.9	2.1	4.1	1.7	3.2	2.0	3.9	1.9	3.9	2.0	0.31
STAI State post	32.3	8.7	29.8	7.5	31.6	10.6	31.3	8.3	31.1	5.8	31.2	8.2	0.87
Trust in researcher (NRS)	8.5	2.1	8.6	1.7	8.7	1.8	8.6	1.7	9.3	0.9	8.7	1.7	0.49
Honesty researcher (NRS)	7.4	2.3	7.6	2.2	7.5	2.2	6.7	2.3	7.2	2.6	7.3	2.3	0.65
Pain expect. Indu.	6.4	1.8	6.0	1.7	6.4	1.7	6.5	1.7	5.2	1.9	6.1	1.8	0.06
Pain expect. Atten.	4.4	1.9	4.9	2.1	3.6	2.2	4.3	1.9	4.1	1.8	4.2	2.0	0.22

Note: Alpha set at 0.01. The NRS was always 0-10.
^ Excluding 1 trial from each phase immediately after thermode was moved (trial 21 of each phase).

Normality checks

The ANOVA assumptions of normality, independence, and homogeneity of the variances were checked. A non-significant Shapiro-Wilk test and histograms of standardized residuals indicated a normal distribution of the data. Within- and between-groups independence was established by randomization into groups. Homogeneity of variances was tested via a Levene's test, which indicated non-significant results, thereby confirming homogeneity of variance in the data.

Nocebo hyperalgesia induction

The mean magnitudes of reported nocebo hyperalgesia after induction are listed in Table 2. A 3x2 mixed model ANOVA was conducted to establish whether there was a significant difference in the magnitude of induced nocebo hyperalgesia between partial reinforcement, continuous reinforcement, and sham. The analysis revealed a significant group by trial interaction between the 3 induction groups and the magnitude of nocebo responses ($F(2,119) = 20.75$, $P < 0.001$, $\eta_p^2 = 0.26$). Figure 2 illustrates the differences in pain ratings for the first nocebo trial and the first control trial of the first evocation, across all three groups. Three 2x2 mixed model ANOVA planned analyses revealed a significant interaction between the partial reinforcement and sham group and the magnitude of nocebo responses ($F(1,72) = 20.58$, $P < 0.001$, $\eta_p^2 = 0.22$), between the continuous reinforcement and sham group and the magnitude of nocebo responses ($F(1,71) = 45.22$, $P < 0.001$, $\eta_p^2 = 0.39$), and between the partial reinforcement and continuous reinforcement groups and the magnitude of nocebo responses ($F(1,95) = 7.28$, $P = 0.008$, $\eta_p^2 = 0.07$). These results indicated that conditioning with partial reinforcement and with continuous reinforcement were both effective in inducing significant nocebo responses, with continuous reinforcement producing a significantly larger nocebo response as compared to partial reinforcement.

Table 2. Group means and standard deviations for the magnitude of nocebo responses after induction and attenuation, as well as for the reduction of nocebo hyperalgesia after attenuation.

Phase 1								**Phase 2**				
Induction group	Nocebo magnitude		**Attenuation group**	Nocebo magnitude		Nocebo reduction		**Induction - Attenuation group**	Nocebo magnitude		Nocebo reduction	
	Mean	SD		Mean	SD	Mean	SD		Mean	SD	Mean	SD
PRF	0.9	1.0	**CC**	-0.6	1.2	1.8	1.7	**PRF - CC**	-0.2	1.1	1.2	1.8
PRF								**PRF - Ext**	0.3	0.6	0.6	1.0
CRF	1.5	1.1	**Extinction**	0.3	0.6	0.9	1.1	**CRF - CC**	-1.0	1.2	2.3	1.6
CRF								**CRF - Ext**	0.3	0.6	1.3	1.1
Sham	-0.02	0.2										

Note: Scores are reported on a 0-10 pain numeric rating scale.

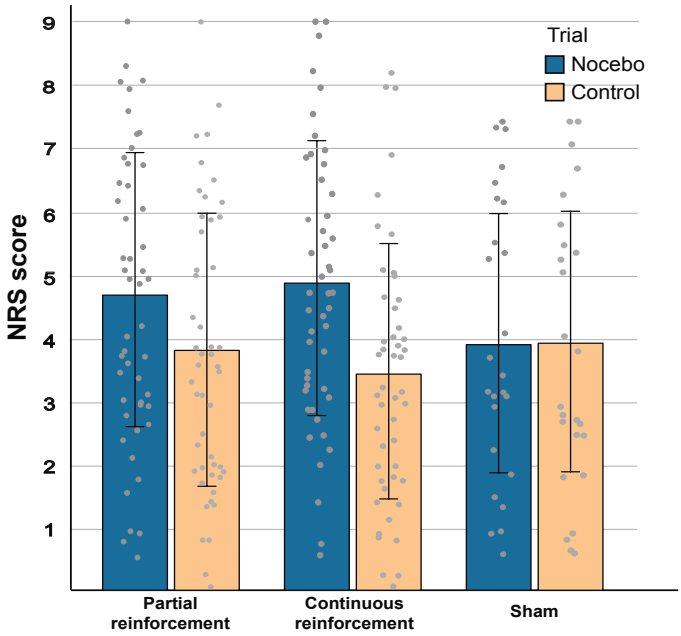

Figure 2. NRS pain ratings for the first nocebo and the first control trial of the first evocation. Mean Numeric Rating Scale (NRS) pain ratings and standard deviations are depicted across all groups (N = 122). Dots represent the (jittered) individual data points. In both the partial reinforcement and the continuous reinforcement groups, evocation pain reports during nocebo trials were significantly higher than pain reports during control trials. Sham conditioning, as expected, did not induce nocebo hyperalgesia. Conditioning with partial reinforcement yielded a significantly smaller nocebo effect than conditioning with continuous reinforcement.

Attenuated nocebo hyperalgesia

Counterconditioning vs extinction

The mean reduction and mean magnitudes of reported nocebo hyperalgesia after attenuation are listed in Table 2. To examine whether counterconditioning was more effective than extinction in attenuating

the induced nocebo responses, a 2x2 mixed model ANOVA was conducted. The analysis revealed a significant interaction between the counterconditioning and extinction groups and the reduction of nocebo responses ($F(1,95) = 6.51$, $P = 0.012$, $\eta_p^2 = 0.06$), indicating significantly higher efficacy of counterconditioning compared to extinction. Figure 3 illustrates the differences in pain ratings for the first nocebo trial of the first evocation and the first nocebo trial of the second evocation, between the counterconditioning and extinction groups.

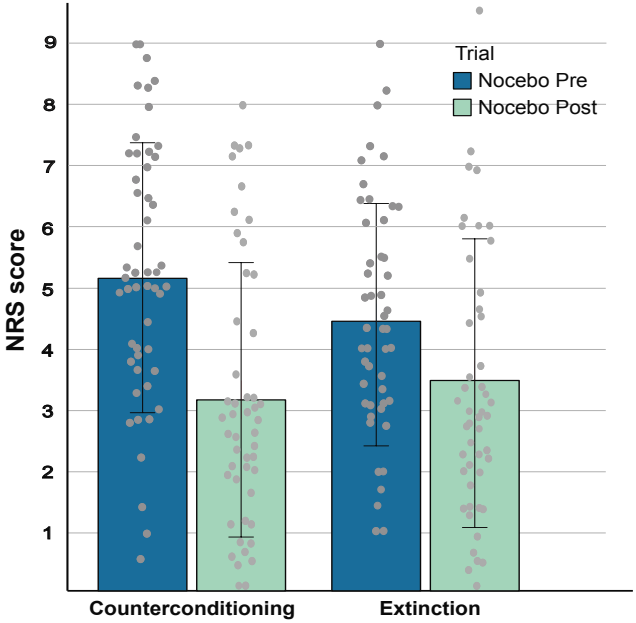

Figure 3. Comparison of nocebo magnitudes after counterconditioning and extinction. Differences in mean Numeric Rating Scale (NRS) pain ratings and standard deviations between the attenuation groups (N = 97) are depicted. Dots represent the (jittered) individual data points. Differences between the first nocebo trial of the first evocation (Nocebo Pre) and the first nocebo trial of the second evocation (Nocebo Post) illustrate the significant reduction of nocebo hyperalgesia achieved by both methods. Attenuation with counterconditioning was more effective in diminishing nocebo responses.

Resistance to extinction

The mean reduction and mean magnitudes of reported nocebo hyperalgesia after extinction are listed in Table 2. We conducted a 2x2 mixed model ANOVA to examine whether conditioning with partial reinforcement resulted in nocebo hyperalgesia that was more resistant to extinction, as compared to conditioning with continuous reinforcement. A non-significant interaction effect showed no significant difference in resistance to extinction between conditioning with partial reinforcement and continuous reinforcement ($F(1,46) = 0.63$, $P = 0.43$, $\eta_p^2 = 0.01$). Figure 4 illustrates differences in pain ratings for the first nocebo trial of the first evocation and the first nocebo trial of the second evocation, between the partial reinforcement-extinction group and the continuous reinforcement-extinction group. Furthermore, two repeated measures ANOVAs showed a significant effect of trial type (first nocebo evocation trial pre attenuation, first nocebo evocation trial post attenuation) in the partial reinforcement group ($F(1,23) = 5.26$, $P = 0.03$, $\eta_p^2 = 0.19$) and the continuous reinforcement group ($F(1,23) = 10.39$, $P = 0.004$, $\eta_p^2 = 0.31$), indicating that extinction significantly reduced nocebo responses in both groups.

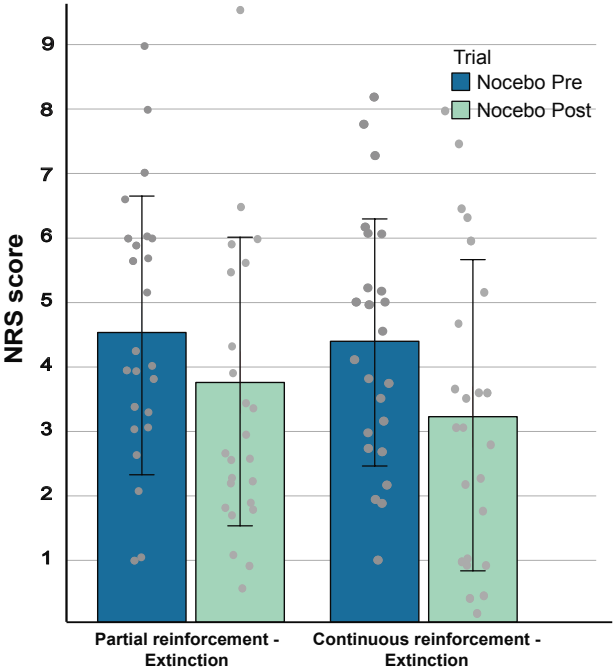

Figure 4. Comparison of nocebo magnitudes after extinction, between the partial reinforcement and continuous reinforcement. Differences in mean Numeric Rating Scale (NRS) pain ratings and standard deviations between the partial reinforcement-extinction group and the continuous reinforcement-extinction group (N = 48) are depicted. Dots represent the (jittered) individual data points. The direction of the difference between the first nocebo trial of the first evocation (Nocebo Pre) and the first nocebo trial of the second evocation (Nocebo Post) pointed towards partial reinforcement resulting in a more durable nocebo response, compared to continuous reinforcement, however this difference did not reach significance.

Manipulation-check for the time-course of extinction

In order to verify that resistance to extinction was not present at an earlier stage during attenuation, we analyzed the 20th attenuation trial, however again, no resistance to extinction was shown. When the 30th

trial after the first trial of evocation was used instead of the 30th trial after the start of attenuation, a 2x2 mixed model ANOVA showed no significant difference in resistance to extinction between conditioning with partial reinforcement versus continuous reinforcement ($F(1,46) = 0.61$, $P = 0.44$, $\eta_p^2 = 0.01$).

Resistance to counterconditioning

The mean reduction and mean magnitude of reported nocebo hyperalgesia after counterconditioning are listed in Table 2. We conducted a 2x2 mixed model ANOVA to examine whether conditioning with partial reinforcement resulted in nocebo hyperalgesia that was more resistant to counterconditioning, as compared to conditioning with continuous reinforcement. The analyses showed a significant difference in the resistance to counterconditioning between conditioning with partial reinforcement versus continuous reinforcement ($F(1,47) = 4.99$, $P = 0.03$, $\eta_p^2 = 0.09$). Figure 5 illustrates the differences in pain ratings for the first nocebo trial of the first evocation and the first nocebo trial of the second evocation, between the partial reinforcement-counterconditioning group and the continuous reinforcement-counterconditioning group. This finding indicated that partial reinforcement led to more resistance to counterconditioning than continuous reinforcement. Furthermore, two repeated measures ANOVAs showed a significant effect of trial type (first nocebo evocation trial pre attenuation, first nocebo evocation trial post attenuation) in the partial reinforcement group ($F(1,24) = 15.96$, $P = 0.001$, $\eta_p^2 = 0.39$) and the continuous reinforcement group ($F(1,23) = 27.65$, $P < 0.001$, $\eta_p^2 = 0.54$), indicating that counterconditioning significantly reduced nocebo responses in both groups.

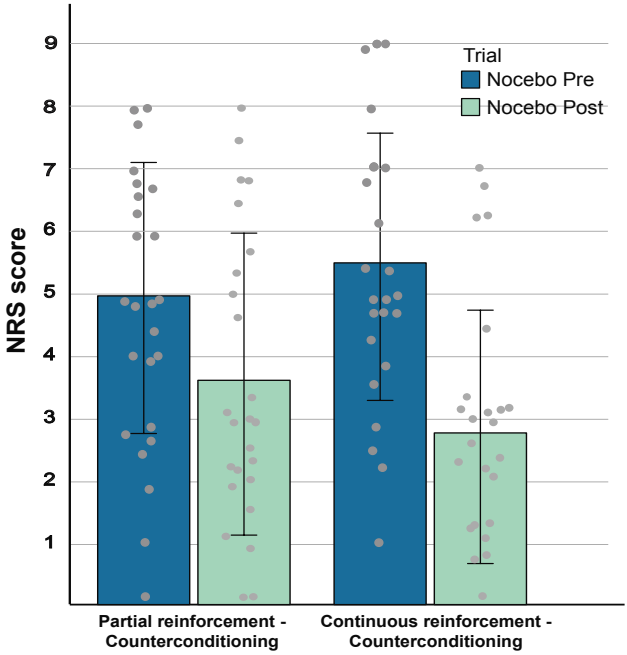

Figure 5. Comparison of nocebo magnitudes after counterconditioning, between the partial reinforcement and continuous reinforcement. Differences in mean Numeric Rating Scale (NRS) pain ratings and standard deviations between the partial reinforcement-counterconditioning group and the continuous reinforcement-counterconditioning group (N = 49) are depicted. Dots represent the (jittered) individual data points.

Time-course of attenuation

To explore the time-course and slopes of attenuation, a line graph was plotted. Figure 6 displays the time-course of attenuation between all four active groups, from the start of the first evocation and throughout the attenuation phase. During attenuation, due to the counterconditioning groups receiving lower pain stimulations than the extinction groups, the nocebo trial NRS scores of the counterconditioning groups were visibly

lower. It is also visible that this persisted as a learned effect, at the start of the second evocation, when all groups received moderate pain. This reduced nocebo response illustrates the higher effectiveness of counterconditioning in diminishing the previously induced nocebo hyperalgesia. Additionally, the attenuation slopes illustrate that participants in the partial reinforcement-extinction and partial reinforcement-counterconditioning groups consistently provided higher pain ratings than participants in the corresponding continuous reinforcement groups, despite the fact that they were receiving pain stimulations of the same intensity (low pain in the counterconditioning and moderate pain in the extinction groups). This points to a tendency for resistance to attenuation after partial reinforcement as compared to after continuous reinforcement, already during learning. However, during the second evocation, the difference between partial reinforcement and continuous reinforcement did not reach significance in the extinction groups.

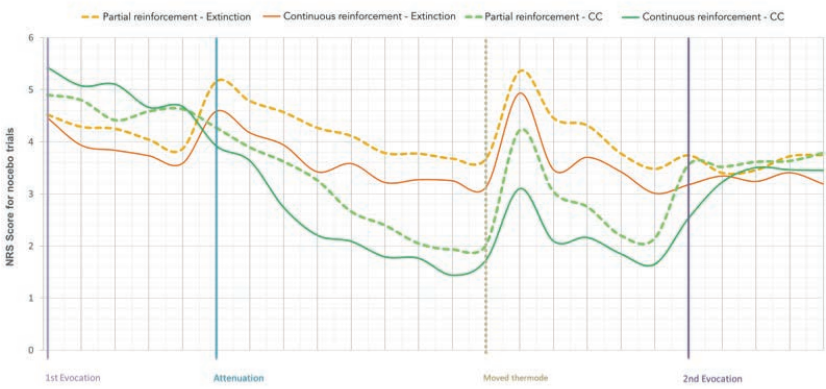

Figure 6. Pain ratings for only the nocebo trials after the end of induction, across all active conditioning groups. Numeric Rating Scale (NRS) pain ratings during nocebo trials illustrate the time-course of attenuation. The dotted vertical line indicates the thermode moving point. In evocations, all stimuli were administered at the same intensity.

Manipulation check for the control trials

Lastly, as a first manipulation check, it was assessed whether changes in the ratings of the control trials influenced the results of the attenuation phase. A 5x2 mixed model ANOVA revealed no significant differences in the NRS pain ratings for control trials before and after attenuation ($F(4,117) = 0.62$, $P = 0.64$, $\eta_p^2 = 0.02$). This result indicates that the control trials did not yield significant changes from pre- to post- attenuation and that the reduction in nocebo hyperalgesia was in fact driven by changes in nocebo responses before and after attenuation. To further examine whether control trials could have affected the attenuation results, a 2x2 mixed model ANOVA was conducted with the attenuation group as the between-subjects factor and the magnitude of nocebo hyperalgesia as the within-subjects factor with two levels (nocebo-control difference score pre-attenuation, nocebo-control difference score post-attenuation). The analysis revealed a significant interaction between the counterconditioning and extinction groups and the reduction of nocebo responses ($F(1,95) = 6.87$, $P = 0.009$, $\eta_p^2 = 0.07$), indicating significantly higher efficacy of counterconditioning compared to extinction (Figure 6), also when the control trials where included in the analysis. Figure 6 depicts time-series data for the evocation and attenuation phases and illustrates that control trials did not yield changes from pre- to post-attenuation that would impact nocebo trials.

Questionnaires

Spearman's Rank-Order Correlation analyses indicated that there was no significant relationship between the magnitude of nocebo hyperalgesia in the active groups and trait or state anxiety, pain catastrophizing, or optimism scores (STAI trait: $r = -0.07$, $P = 0.49$; STAI state pre: $r = -0.06$, $P = 0.55$; STAI state post: $r = -0.13$, $P = 0.19$; PCS: $r = -0.06$, $P = 0.54$; LOT-R: $r = -0.05$, $P = 0.59$).

Discussion

The current study compared distinct and novel methods for the induction and attenuation of nocebo hyperalgesia. We demonstrated that partial reinforcement conditioning was sufficient to induce nocebo hyperalgesia, as was continuous reinforcement conditioning. Furthermore, we showed that counterconditioning is a more potent method than extinction for the attenuation of nocebo hyperalgesia. Interestingly, our results also showed that, despite pain ratings remaining consistently higher in the partial reinforcement group compared to the continuous reinforcement group during extinction, this difference did not reach significance and resistance to extinction after conditioning with partial reinforcement was not observed. Importantly, we found that while counterconditioning was sufficient to attenuate nocebo responses irrespective of induction method, nocebo hyperalgesia was significantly more resistant to counterconditioning when induced via partial, as compared to continuous reinforcement. These findings have a number of implications related to experimental models and clinical practice.

The finding that conditioning with partial reinforcement is, albeit less potent than continuous reinforcement, sufficient to induce nocebo hyperalgesia, is in line with previous research by Colagiuri and colleagues [15]. Reproducing these results and reaffirming the potency of the more ambiguous partial learning method has important theoretical and clinical implications. Conditioning with learning schedules that provide more variable contingencies bears a closer resemblance to what nocebo theories postulate regarding the ambiguity of learning and negative suggestions in clinical contexts [4,15]. Employing a more ecologically valid paradigm can have a crucial impact on our understanding of how and why nocebo hyperalgesia may present in pain patients.

Studying the attenuation of nocebo hyperalgesia provides insights into the mechanisms that may contribute to the chronification of pain. While in the present study extinction was sufficient to attenuate nocebo hyperalgesia, counterconditioning was a more powerful intervention, reversing nocebo responses into an effect resembling placebo responses. Counterconditioning being more powerful than extinction can be explained by counterconditioning involving a paradigm that bears closer resemblance to successful exposure therapy techniques. For example, for the treatment of phobias [34,35] and anxiety [36], the initial association between the aversive stimulus and fear becomes attenuated through a procedure involving the removal of fear or threat [37]. However, in the current study, extinction entailed a reduction of pain to the levels of control (moderate) pain stimulations, rather than the entire removal of these aversive stimuli. In pain paradigms it is often impossible, both experimentally and clinically, to achieve the entire removal of the aversive stimulus during extinction. In counterconditioning however, the painful stimuli were reduced to a level that was perceived as less unpleasant in comparison even to control pain stimulations, leading to a significantly larger reduction of nocebo responses. This is in line with findings by Meulders and colleagues [20] who showed that changing the valence of aversive stimuli might improve fear reduction and potentially prevent relapse. In contrast to the frequently observed lack in effectivity and durability of extinction [34,38,39], this counterconditioning finding indicates that there may be a path to attempt more active ways of minimizing learned responses.

Nocebo hyperalgesia has consistently been found to be resistant to extinction [15,24,40], which may indicate an important mechanism of pain chronification. Moreover, research exploring the learning correlates and effectivity of conditioning with partial reinforcement has previously shown that ambiguous learning schedules produce durable conditioned effects [13,14,16,17], including previous partial reinforcement research on nocebo [15] and placebo effects [13]. In this study, we did not find a

statistically significantly larger resistance to extinction after partial, as compared to continuous reinforcement. Extinction trends pointed towards partial reinforcement resulting in more durable nocebo responses compared to continuous reinforcement, as illustrated in Figures 4 and 6, however this difference was not significant. Moreover, it was observed that during attenuation, pain reports in the partial reinforcement group did remain consistently higher than those in the continuous reinforcement group (Figure 6), despite the fact that after induction, partial reinforcement produced a significantly weaker nocebo response than continuous reinforcement. The effectivity of extinction even after partial reinforcement could be explained by the fact that exposure to extinction was longer than exposure to nocebo induction, when considering the first evocation phase. It is worth pointing out, however, that in real-word contexts, patients may be exposed to shorter periods during which nocebo hyperalgesia is acquired and longer periods of extinction. As such, the current model provides novel evidence that nocebo hyperalgesia can be extinguished over prolonged exposure to extinction, even after partial reinforcement learning.

Interestingly, a partial reinforcement resistance effect was found when attenuation involved counterconditioning. Counterconditioning was still successful in attenuating nocebo effects after conditioning with partial reinforcement, however, counterconditioning was observed to be substantially more effective after conditioning with continuous reinforcement (Table 2, Fig. 5). Importantly, this effect was observed despite the fact that partial reinforcement had resulted in a significantly weaker nocebo response. This counterconditioning-specific resistance effect could be attributed to negativity bias (i.e., the tendency to attend to or remember negative experiences over neutral or positive experiences [41–43]). According to this theory, when provided with inconsistent positive and negative information about the same stimulus, individuals are more likely to retain the negative information [44]. A negativity bias may have taken place following the ambiguous

information provided to participants in the partial reinforcement group and the formation of mixed expectations regarding the activation of the nocebo treatment. The partial reinforcement-counterconditioning group was exposed to a wider range of negative and positive suggestions and associations. It is possible that during the final evocation the negative treatment associations were retained over the positive ones. A resistance to the attenuation with counterconditioning may thus be in line with previous literature about this type of negativity bias [44]. This effect may be of important clinical relevance, as it could shed light on the etiology of pain chronification following exposure to inconsistent, mixed information and experiences. In turn, however, this means that the potency of counterconditioning following ambiguous and variable learning remains uncertain. Gaining a better understanding of the learning mechanisms underlying the process of re-writing negative associations can create great future value for counterconditioning.

One limitation of this study, as mentioned earlier, was the discrepancy in the length of induction and attenuation, which may explain why nocebo responses were not resistant to extinction. Colagiuri and colleagues [15] only applied extinction, allowed the paradigm to comprise an equal number of induction and attenuation trials. In the current study, due to our aim of comparing counterconditioning and extinction, a longer evocation phase was preferred before the start of attenuation. Participants were thus exposed to longer extinction, as compared to induction. Nevertheless, in clinical contexts and chronic pain, unequal lengths of exposure to suggestions, learning, and extinction may also exist. Future research should address the role that the time-course of induction and attenuation may play. Another limitation of this study and a common obstacle in nocebo studies, was related to the nocebo suggestions. In this novel counterconditioning approach, the suggestions had to indicate that the same treatment could increase but also decrease pain sensitivity. The suggestion that pain would be decreased by the same device that previously increased pain sensitivity

could have been confusing to participants. In future research, nocebo suggestions can be optimized by comparing different cover stories and instructions, as these are crucial for influencing expectations. A common limitation in the learning process of extinction is the return of the conditioned response, such as fear, following the passing of time [45]. The current study did not examine this effect, which is thought to result from competing learned effects and deficits in inhibitory learning and more specifically deficits in the neural regulation needed during extinction [45]. Future counterconditioning experiments could shed light on whether – and under which conditions– such a reinstatement could take place following counterconditioning. Further research into the effectivity and durability of counterconditioning is necessary to establish whether this method can provide a basis for clinical interventions targeting nocebo effects. Lastly, controlling for variables in our sample such as caffeine intake and age range, or limiting our sample to higher education students may have created potential confounding variables. In future studies, it would be important to allow for more variance in the participant sample and to collect and check data related to variables such as caffeine intake, age, and education. Overall, future studies should collect and analyze manipulation check data to see whether and how different variables can influence study outcomes.

The present study implemented a complete, clinically relevant model of nocebo hyperalgesia, from acquisition to attenuation. The findings reproduced prior evidence of ambiguous and variable learning being sufficient to induce nocebo hyperalgesia, and that this type of induction method may be more resistant to treatments. This study also provided evidence that counterconditioning is a powerful method for the attenuation of nocebo hyperalgesia. Counterconditioning, however, may be less potent in attenuating effects that have been induced by more ambiguous learning and should therefore undergo further assessment within ecologically valid experimental models.

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