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# An experimental investigation into the mediating role of pain-related fear in boosting nocebo hyperalgesia

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## Abstract

Nocebo hyperalgesia refers to increases in perceived pain that putatively result from negative expectations regarding a nocebo stimulus (eg, an inert treatment, compared with no treatment). The precise cognitive-emotional factors contributing to the origins of nocebo effects are poorly understood. We aimed to test the effects of experimentally induced pain-related fear on the acquisition and extinction of nocebo hyperalgesia in healthy participants (N = 72). Acquisition and extinction of nocebo hyperalgesia were compared between a group receiving standard nocebo conditioning (Control group) and 2 groups receiving distinct fear inductions: high intensity of pain stimulations (High-pain group) or a threat manipulation (High-threat group). During nocebo acquisition, the Control and High-threat groups were administered thermal pain stimulations of moderate intensity paired with sham electrical stimulation (nocebo trials), whereas high pain intensity was administered to the High-pain group. During extinction, equivalent pain intensities were administered across all trials. Pain-related fear was measured by eyeblink startle electromyography and self-report. Nocebo hyperalgesia occurred in all groups. Nocebo effects were significantly larger in the High-pain group than those in the Control group. This effect was mediated by self-reported fear, but not by fear-potentiated startle. Groups did not differ in the extinction rate. However, only the High-pain group maintained significant nocebo responses at the end of extinction. Anticipatory pain-related fear induced through a threat manipulation did not amplify nocebo hyperalgesia. These findings suggest that fear of high pain may be a key contributor to the amplification of nocebo hyperalgesia, only when high pain is experienced and not when it is merely anticipated.

**Keywords:** Pain, Nocebo hyperalgesia, Fear, Conditioning, Negative suggestions, Learning, Expectations

## 1. Introduction

Negative expectations regarding an inert treatment stimulus have been shown to increase perceived pain intensity, as compared to perceived pain intensity in an untreated, control condition.[3,27,59,63,68] This phenomenon has been termed nocebo hyperalgesia.[3,9] In experimental studies, negative suggestions and classical conditioning play key roles in the acquisition of nocebo hyperalgesia.[5,13,21,53] Negative suggestions regarding the effects of a (sham) treatment on pain and the pairing of this treatment with increased pain administrations can produce negative expectations about this treatment.[4,13] As a result of this learned negative expectation, an inert treatment can evoke increased pain sensitivity.[21]

Expectations installed by classical conditioning and aversive (threat/fear) conditioning are closely intertwined procedurally, but nocebo research has not systematically focused on the role of fear. A focus on fear is important because cognitive–affective neural processing has been implicated in nocebo hyperalgesia,[36,37,40,63] with numerous studies showing a specific role of the amygdala, a primary fear processing region, in nocebo, but not placebo effects.[29,62,63] Studies have used varying pain levels to induce nocebo hyperalgesia, ranging from as low as 5 to as high as 10 on 0 (no pain) to 10 (highest pain imaginable) rating scales.[8,31,36,68,69] These pain intensities may differentially induce fear and, as such, influence nocebo responses. Furthermore, the threatening nature of suggestions also varies between experimental nocebo models. For example, Geuter and Buchel[31] used the negative suggestion that a capsaicin cream would momentarily increase perceived pain, whereas Benedetti et al.[8] suggested that participants may experience severe headaches during a mountaineering trip lasting several days. Whether such differences in perceived pain intensities, threatening suggestions, and fear-related experiences can alter induced nocebo responses remains unexplored.

Pain-related fear may arise as a result of experienced pain or from threatening information regarding upcoming pain. Fear

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caused directly by experiencing high pain during nocebo conditioning may augment the acquisition of negative expectations. Research indicates that stimuli paired with pain can elicit fear responses,[37,47] and such pain-related fear can be acquired through associative learning.[38,41,48,52] In a more anticipatory fashion, threatening suggestions about potential pain outcomes may also induce pain-related fear, which can weigh on future pain experiences and augment nocebo hyperalgesia.[2] It is therefore important to determine whether higher reported pain or threatening suggestions amplify nocebo hyperalgesia and whether pain-related fear is a mediator in this putative effect.

The study of pain-related fear in nocebo models is an important step towards a comprehensive understanding of nocebo responses. This study aimed to investigate whether high pain intensity or threatening suggestions augment the acquisition and hinder subsequent extinction of nocebo hyperalgesia. We hypothesized that compared with lower pain, high pain would produce larger nocebo responses and that these would be more resistant to extinction. The same effects were expected for threatening verbal suggestions, compared with the absence of threatening suggestions. We further hypothesized that self-reported and psychophysiological assessments of fear would mediate these effects. Moreover, we explored whether psychological characteristics such as anxiety are related to nocebo magnitudes.

## 2. Materials and methods

### 2.1. Design

This study used a randomized, mixed (between–within subjects), 3-group design (**Fig. 1**). A randomization list was created by an independent researcher to reduce any risk of bias. All participants underwent nocebo acquisition and extinction procedures by use of classical conditioning and negative verbal suggestions. In the acquisition phase, the Control group and the High-threat group were conditioned with moderate pain intensity stimuli during nocebo trials, whereas the High-pain group was conditioned with high pain intensity stimuli during nocebo trials, with the aim to additionally induce and examine increased pain-related fear in this group. The High-threat group received a threat manipulation, with the aim to additionally induce and examine increased pain-related fear in this group.

### 2.2. Participants

The required sample size for the primary analysis was calculated based on our previous nocebo study[68] comparing the magnitude of nocebo responses between 3 groups that received different conditioning manipulations. The analysis was conducted in G*power 3.1[28] for a mixed-model analysis of variance (ANOVA). The effect size was f = 0.26, alpha error probability was set at $\alpha$ = 0.05, and desired power was set at 0.95. The sample size indicated was 21 participants per group. Given that previous studies that included fear manipulations with similar study designs included samples of 20 to 25 participants[38] and because of the novel manipulations used in this study, we aimed to include 24 participants per group. This sample size is similar to previous studies examining between-group differences using conditioning manipulations.[2,19]

Inclusion criteria were being aged between 18 and 35 years, having a good understanding of the English language, and (corrected to) normal vision and hearing. Exclusion criteria were pregnancy, chronic pain, serious medical or psychiatric conditions that interfere with the study of pain, painful health conditions experienced in the past 6 months, and pain or the use of analgesic medication on the day of testing. Participants would also be excluded if their pain tolerance was too high (ie, when the thermode maximum temperature of 49.9°C was not sufficient to induce at least moderate pain). Participants were recruited through posters and the recruitment web site Sona (Sona Systems, Tallinn, Estonia). Study participation involved a 1.5-hour testing session at a research laboratory of the Faculty of Social and Behavioral Sciences of Leiden University, the Netherlands. All participants provided informed consent and were reimbursed by either cash (€15) or study credits. This study was approved by the Leiden University Psychology Research Ethics Committee (CEP19-0614/347) and preregistered on ClinicalTrials.gov (NCT04197154).

### 2.3. Thermal pain stimulation

Thermal pain stimuli were delivered to participants' nondominant volar forearm through 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 on the arm were rated verbally on a numerical rating scale (NRS) ranging from 0 (no pain) to 10 (worst pain imaginable on the arm). Throughout the experiment, each stimulus was initiated from a baseline of 32°C, increased to the target temperature with ramp up and return rates of 8°C per second, and presented at peak temperature for 4 seconds. The interstimulus interval was 10 seconds.

#### 2.3.1. Sensory and pain thresholds

To test warmth and pain threshold levels, heat stimuli were applied on the arm, and participants were asked to indicate the first moment at which they perceived warmth and pain, respectively, from a baseline of 32°C. After a practice trial of each, the average of 3 warmth and 3 pain detection values were calculated as the threshold values for warmth and pain, respectively. This method follows published standardized and protocolled procedures.[61]

#### 2.3.2. Pain calibration protocol and administered stimuli

##### 2.3.2.1. Pain calibrations and selection of pain intensities

Pain calibrations were conducted to select the temperatures that would be used to induce low, moderate, and high pain in the acquisition and extinction phases (similar to previous studies[39,68]). The calibrations were individually tailored, based on participants' NRS ratings of maximum 30 pain stimuli of varying intensities, ranging from 41 to 49.9°C. Median temperatures that were rated as low, moderate, and high pain were calculated to select temperatures that were consistently given a certain rating. Median temperatures were selected because of the presence of outlier ratings during this early stage of participants receiving pain stimulations of varying intensities. Details of the pain calibration procedure can be found in the supplementary material (available at http://links.lww.com/PAIN/B135).

In the Control and High-threat groups, median temperatures consistently rated and experienced as NRS 1 to 3 were selected and used during control trials, whereas median temperatures rated as 4 to 6 were used during nocebo trials. In the High-pain group, median temperatures consistently rated as NRS 4 to 6 were used during control trials, whereas median temperatures

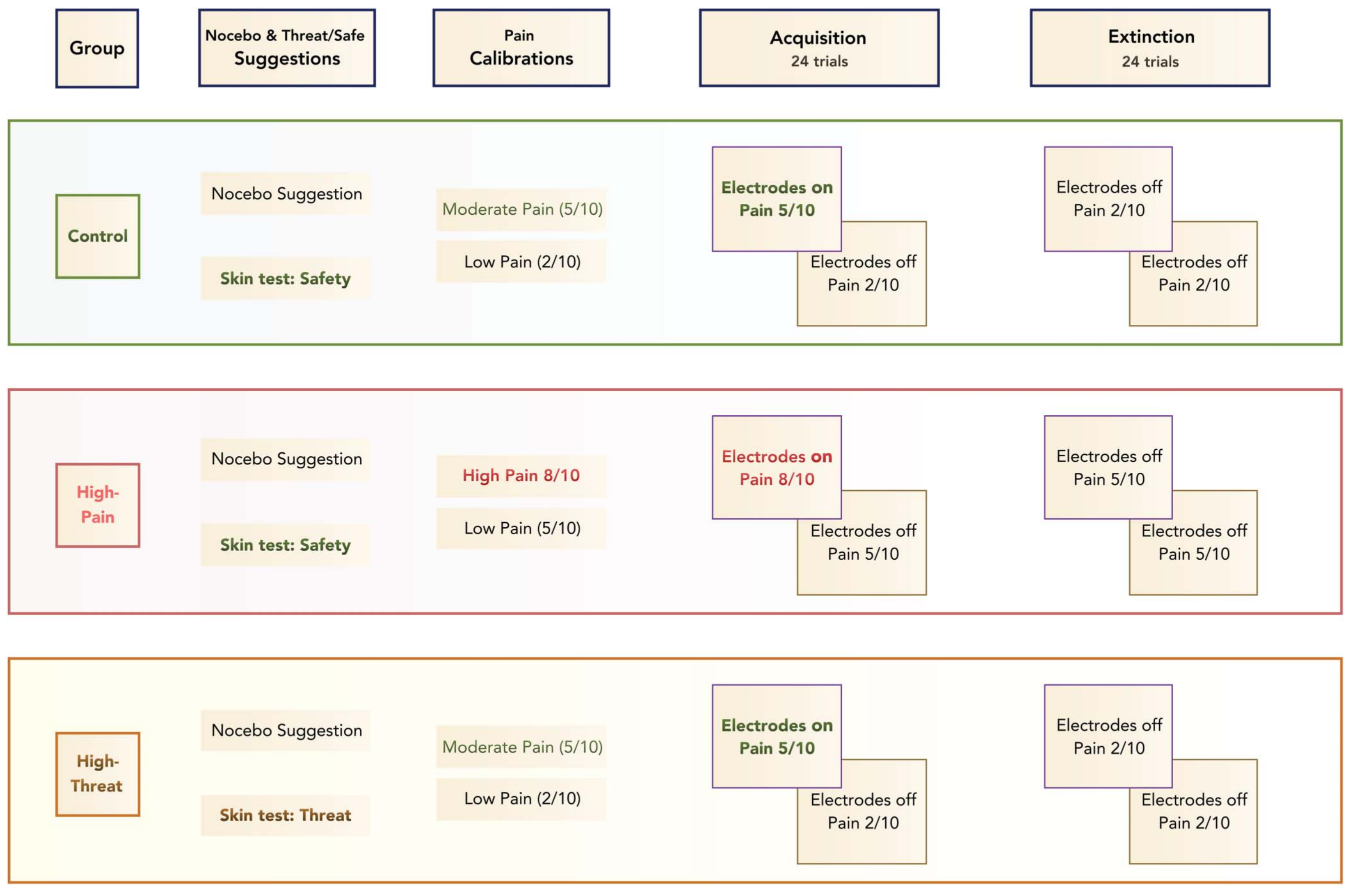


**Figure 1.** Illustration of the experimental design: Participants were randomly allocated to 1 of 3 groups: Control-nocebo, High-pain, or High-threat. Participants in the Control group received lower pain levels during control and nocebo trials and no threat induction. Participants in the High-pain group received higher pain levels during control and nocebo trials and no threat induction. Participants in the High-threat group received lower pain levels during control and nocebo trials and a threat induction. All participants were told that (sham) electrical pulses would increase their pain sensitivity. During nocebo acquisition, higher pain stimulations were delivered during nocebo trials (electrical pulses "on") relative to control trials (electrical pulses "off"). In the extinction phase, all pain stimuli were administered at the same intensity for each participant, to test the acquisition and extinction of nocebo hyperalgesic responses.

rated as 7 to 9 were used during nocebo trials. Consistent with previous nocebo conditioning procedures,[19,31,69] lower pain stimulation was administered during control trials and higher pain was administered during nocebo trials, to condition participants to expect increased pain as a result of the inert nocebo (ie, sham electrical stimulation).

#### *2.3.2.2. Administered pain stimuli during nocebo acquisition and extinction*

During the acquisition phase (described in detail directly below), 12 nocebo and 12 control stimuli were administered in a pseudorandom order, so that no more than 3 trials of the same type were administered in a row. During the extinction phase (also described below), 12 nocebo and 12 control stimuli were administered in a pseudorandom order. To reduce habituation or sensitization to heat pain, the thermode was moved twice to a more proximal site on the same arm (at one-third and two-thirds of the paradigm).

### *2.4. Nocebo manipulation*

A commercial transcutaneous electrical nerve stimulation device (Beurer EM 80) was used to deliver (sham) electrical stimuli, which served as the nocebo manipulation in the nocebo acquisition and extinction procedure, as it represented an inert treatment that was not actually activated in the main experiment. A sham transcutaneous electrical nerve stimulation "treatment" was used to condition nocebo hyperalgesia that may be more ecologically valid, in that negative pain expectations are induced about the effects of a (sham) treatment stimulus. Negative verbal suggestions were used to create expectations regarding the pain-enhancing effects of administering electrical stimuli in combination with thermal pain. Two electrodes (Medi-Trace 200 EKG, 35 mm) were placed in a diagonal line on the base of the thumb and the inner elbow. Before the start of the acquisition phase, participants underwent a short mock calibration procedure during which they felt a light electrical pulse. This pulse was delivered to increase the credibility of the nocebo manipulation. The device was not actually activated during conditioning, but messages displayed on a computer screen through E-Prime 2.0 (Psychology Software Tools, Pittsburgh, PA) signaled the sham activation (conditioned stimulus) of the electrical stimulation during nocebo trials. Negative suggestions indicated to all participants that when the messages "on" (in purple font; nocebo conditioned stimulus) and "off" (in yellow font; control stimulus) were displayed, their pain would be aggravated or not altered, respectively.

In the acquisition phase, the activation of sham electrical stimulation was repeatedly paired with increased pain stimulation during the 12 nocebo trials, whereas the 12 control trials were paired with lower pain stimulation. This is in line with previous nocebo studies[19,68] implementing classical conditioning for the experimental induction of nocebo hyperalgesia. In the extinction phase, both nocebo and control cues were paired with the same lower intensity pain stimulation. The extinction paradigm was also in line with previous nocebo studies and served to attenuate induced nocebo responses.

### 2.5. Fear inductions

Although all groups received nocebo suggestions, the High-pain and High-threat groups were exposed to additional fear-inducing manipulations.

#### 2.5.1. Pain intensity manipulation

The High-pain group received higher pain levels during nocebo acquisition and extinction (2-3 points higher on the NRS), which was intended to increase participants' pain-related fear, especially during nocebo trials.

#### 2.5.2. Threat manipulation

The High-threat group was told that a skin sensitivity test (similar to previous studies,[35] albeit not an identical threat manipulation procedure) indicated that nerves in the skin were hyperresponsive, and therefore, it may potentially be dangerous for them to receive the combination of heat and electrical stimuli. All groups were exposed to the skin sensitivity test, which involved attaching 2 electrodes to the tip of the thumb and index finger that were communicating with a monitor that displayed a scale (**Fig. 2**). The mock scale was an animation that had a bar fluctuating either in the green zone, with the text "recording safe," for the Control and High-pain groups, or in the red zone, with the text "recording unsafe," for the High-threat group. The scale was visible to participants throughout the experiment.

### 2.6. Measures

#### 2.6.1. Pain measures

Participants were provided with an 8-second window to rate their pain on the NRS after each pain stimulation. A message, presented on the computer screen immediately after the pain stimulus returned to baseline, prompted the verbal pain rating.

#### 2.6.2. Fear measures

Pain-related fear was measured through self-report and through electromyography (EMG) of startle eyeblink responses. Participants were prompted to rate their prospective fear levels of the upcoming pain stimulus in one-third of acquisition and extinction trials, after visual cue presentation and before the heat pain application. Pain-related fear was reported on a 0 to 10 NRS from no fear to worst fear imaginable. These measurements were similar to previous studies.[35]

The startle eyeblink reflex was measured as an indicator of conditioned fear, as it is modulated by fear-evoking stimuli and by brain areas responsible for affective processing such as the amygdala and the anterior cingulate cortex.[56] Eyeblink startle response modulation was measured during the presentation of nocebo and control visual cues. Orbicularis oculi EMG activity was recorded with 3-square EL504 BIOPAC electrodes (2.5 × 2.5-cm diameter; BIOPAC Systems, Goleta, CA) filled with electrolyte gel. To reduce interelectrode resistance, participants' skin was scrubbed with an exfoliating gel and cleaned with an alcohol wipe. Subsequently, electrodes were placed on the right side of the face according to the specifications proposed by Blumenthal et al.[12] The raw signal was amplified by an isolated EMG100c amplifier module (BIOPAC Systems). EMG recordings were acquired through AcqKnowledge (AcqKnowledge software; Biopac Systems) at a sampling rate of 2000 Hz, with a low-pass filter of 500 Hz and a high-pass filter of 10 Hz. The eyeblink startle response was elicited by use of a white-noise burst of 100-ms duration (ie, startle probe), with instantaneous rise time, presented binaurally through earphones (Samsung Headset Stereo, model EHS64). The noise was calibrated at approximately 90 dBA, which is safe for hearing.[12] These auditory startle probes were delivered within a random 1-second window, 7 seconds after visual cue presentation and 1 second before heat pain application. The startle probes were presented in two-thirds of the acquisition and extinction trials (trials during which participants were not asked to provide a fear rating), including

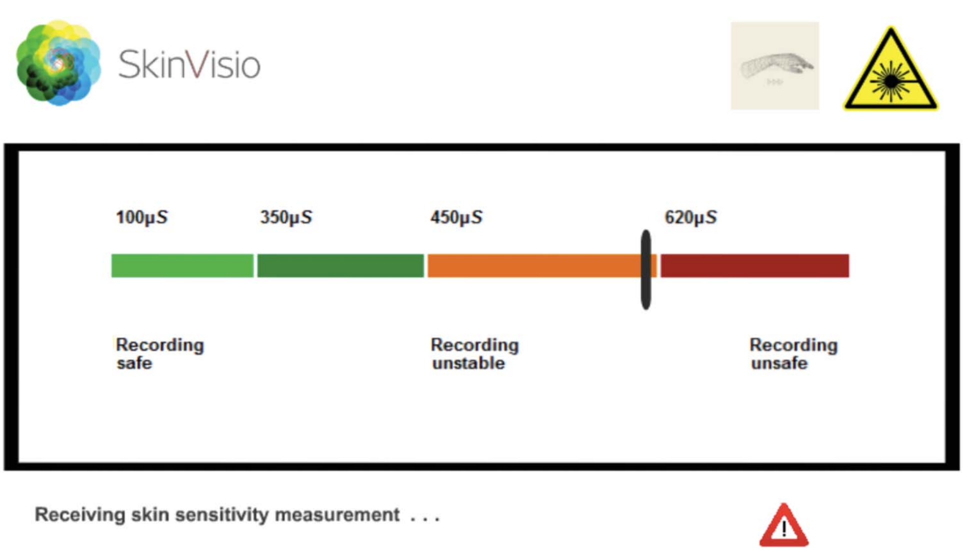


**Figure 2.** The mock skin sensitivity scale that participants viewed as part of the threat manipulation: The scale was displayed on a screen as an animation. For the High-threat group, the scale fluctuated within the orange and red zones. For the Control and High-pain groups, the scale fluctuated within the green zone.

the first and last 2 extinction trials, which were used to calculate the magnitude of nocebo hyperalgesia at the end of acquisition and extinction, respectively.

#### *2.6.3. Manipulation check exit questions*

At the end of the experiment, participants completed an exit questionnaire containing manipulation check questions, for instance, regarding pain expectations, trust, and fear. The questions are described in the supplementary material (available at http://links.lww.com/PAIN/B135). All questionnaires were displayed on a computer monitor through the web-based survey software (Qualtrics, Provo, Utah).

#### *2.6.4. Questionnaires*

A screening questionnaire containing demographic and health questions was used to screen participants for inclusion in the study. Four psychological questionnaires were administered. A short State Anxiety version of the State-Trait Anxiety Inventory (STAI-S-s)[43,65] was administered before the start of the experiment, and the STAI-Trait version (STAI-T)[65] was also used. The Pain Catastrophizing Scale[66] was used to assess catastrophizing thoughts related to pain or pain-related worrying.[22] The Fear of Pain Questionnaire-III[44] was used to measure fear of minor, severe, and medical pain. Total scores were used for all questionnaires.

### *2.7. Experimental procedure*

On the day of the laboratory session (lasting approximately 90 minutes), participants received information about the experiment after which they provided written informed consent. Then, participants completed the screening for inclusion, followed by the STAI-S-s. Then, the EMG electrodes were attached,[12] and the mock skin sensitivity test was performed. Warmth and pain threshold levels were then tested, and individual pain stimuli were calibrated. The sham electrodes were then attached to the hand and arm, and a short mock calibration took place. Participants were asked to wear earphones and were exposed to 5 startle probes to achieve startle probe habituation. Then, participants underwent the nocebo acquisition and extinction procedure. After the end of the experiment, participants were asked to answer the exit questions and complete the psychological questionnaires. Then, participants were debriefed and reimbursed for their participation. Reimbursement by cash or study credits was, by chance, equally distributed over groups.

### *2.8. Response definition and statistical analyses*

Behavioral data were analyzed by use of SPSS 23.0 (IBM, Corp, Armonk, NY). For all analyses, the threshold for significance was set at $P < 0.05$, and where multiple comparisons were performed, a Bonferroni correction was used. Partial eta-squared ($\eta_p^2$) was computed as an effect size measure, with $\eta_p^2$ of 0.01 considered a small, 0.06 considered a medium, and 0.14 considered a large effect size.[17,60]

To conduct mixed-model ANOVA, the assumptions of normality and homogeneity of the variances were checked. The assumption of independence was achieved by randomization of participants into groups. For mediation analyses, nonparametric and bias-corrected bootstrapping was used.[26] The independent error assumption was checked with the Durbin–Watson statistic, and multicollinearity was tested through the variance inflation factor.

#### *2.8.1. Pain outcome measures*

Mean pain scores were calculated per trial type for each participant, and nocebo magnitudes were measured within subjects. The magnitude of nocebo responses after acquisition (primary outcome measure) was defined as the difference between the first nocebo and the first control trial of the extinction phase. The first extinction trials were selected because the intensity of administered pain was identical in nocebo and control trials in this phase, and previous studies show the clearest effect of nocebo responses in those trials.[7,19] The magnitude of nocebo responses at the end of extinction was defined as the difference between the last nocebo and the last control trial of the extinction phase. The reduction of nocebo responses was measured as the change in the magnitude of nocebo responses (nocebo minus control) between the start and the end of the extinction phase. One-way ANOVAs were used to assess mean between-group differences in warmth and pain thresholds, temperatures used to induce pain, and NRS pain ratings during the experiment.

#### *2.8.2. Fear outcome measures*

The magnitude of self-reported fear levels was measured within subjects and was defined as the difference in fear ratings for nocebo trials compared with control trials of the acquisition or the extinction phase. Fear-potentiated eyeblink startle responses were analyzed according to typical preprocessing of EMG recordings in the PhysioData Toolbox for Matlab.[64] The EMG signal was digitized at 1000 Hz, Boxcar filtered, and rectified, and each startle trial was segmented. Peak amplitudes were computed, defined as the maximum of the response curve within 21 to 300 ms after startle probe onset. All startle waveforms were also manually inspected, and technical abnormalities or artifacts were eliminated. Each peak amplitude was scored by subtracting it from its baseline score (averaged EMG level between 1 and 20 ms after the probe onset). Finally, raw scores were transformed to T scores, to account for interindividual variation in physiological reactivity. Each 4 consecutive startle probe responses of the same cue (nocebo or control) were averaged for further analyses. Trials during which baseline was higher than the startle response peak (due to no eyeblink response, an occasional blink) were labelled as reject trials.

#### *2.8.3. Hypothesis testing*

##### *2.8.3.1. Acquisition of nocebo hyperalgesia*

First, we examined whether nocebo hyperalgesia was induced and whether it differed between the High-pain and Control groups and the High-threat and Control groups. We expected that the 2 fear inductions (high pain and threat manipulation) would lead to larger nocebo responses, as compared to the control group. To compare each of the fear groups with the control group, two $2 \times 2$ mixed-model ANOVAs were performed, with group as the between-subject factor and trial type as the within-subject factor (first extinction nocebo trial, first extinction control trial).

##### *2.8.3.2. Extinction of nocebo hyperalgesia*

Next, we examined whether the extinction of nocebo hyperalgesia differed significantly between the High-pain and Control groups and between the High-threat and Control groups. We expected that the 2 fear inductions would lead to resistance to extinction, as compared to the control group. To compare each of the fear groups with the control group, two 2 × 2 mixed-model ANOVAs were performed, with group as the between-subject factor and time as the within-subject factor for calculated nocebo magnitudes (start of extinction, end of extinction).

In an exploratory manner, we further analyzed whether the magnitude of nocebo hyperalgesia at the end of extinction differed between groups, for High-pain vs Control and High-threat vs Control. To compare each of the fear groups with the control group, two 2 × 2 mixed-model ANOVAs were conducted, with group as the between-subject factor and trial type as the within-subject factor (last nocebo extinction trial, last control extinction trial).

#### *2.8.3.3. Mediation analyses*

For the High-pain group, we expected that any effects of higher pain stimulation on the magnitude or reduction of nocebo hyperalgesia would be mediated by pain-related fear. Only when ANOVA results were significant, mediation analyses were conducted to assess whether fear mediated the relationship between the pain level and the magnitude of nocebo hyperalgesia. Calculation of indirect effects and bootstrapping tests of mediation were performed, using the PROCESS macro for SPSS,[57,58] with 5000 bootstrap samples. Separate mediation analyses were conducted for the self-report and startle response fear measures (mediator variables). Group (High-pain and Control) was the dichotomous predictor variable. Mediation analyses were not planned for the High-fear group, as an increase in fear is inherent to the threat manipulation.

### *2.8.4. Manipulation checks for fear levels*

We examined whether increased pain levels and the threat manipulation led to higher fear levels. Mixed-model ANOVAs were performed, separately for reported fear and for startle responses, 1 for High-pain group vs Control and 1 for High-threat group vs Control. Group was the between-subject factor, and trial type was the within-subject factor (nocebo, control).

## 3. Results

### *3.1. Participants, temperatures, pain ratings, and startle responses*

A total of 75 participants were enrolled in this study. One participant was excluded for experiencing acute pain due to an injury, 1 participant was excluded because of a severe headache, and 1 participant was excluded because of a chronic pain condition (irritable bowel syndrome). In total, 72 participants were included in the final analyses. Exactly one-fourth of participants reported that they live as a male, stratified for (lived) gender so that each group contained 6 male participants. Randomization resulted in a total of 24 participants in each of the 3 groups.

Calibrated temperature levels and pain ratings during the experiment are reported in **Table 1**. One-way ANOVAs indicated that there were no significant between-group differences in the mean warmth and heat pain threshold levels (**Table 1**). As expected, one-way ANOVAs confirm that there were significant differences in calibrated temperatures and pain ratings during the experiment between the High-pain group and the other 2 groups (**Table 1**).

The EMG recordings of 6 participants were faulty (either the recording was not started because of an error or the sound probe markers were not recorded because of technical difficulties) and were excluded from the analyses. Approximately 20% of trials were marked as nonresponse or reject trials. Although average startle responses range between 100 and 300 μV,[14,50] in this study startle responses overall were smaller than that expected across all groups and trials (**Fig. 3**).

### *3.2. Acquisition of nocebo hyperalgesia*

The mean magnitudes of nocebo responses are presented in **Table 2**. **Figure 4** illustrates differences in pain ratings for the first nocebo and first control extinction trials, across all 3 groups.

#### *3.2.1. High-pain group*

Nocebo responses in the High-pain group were of almost double the magnitude compared with the Control group. The analysis revealed a significant interaction between group (High-pain vs Control) and trial type (nocebo vs control) ($F(1,46) = 4.32$, $P = 0.04$, $\eta_p^2 = 0.09$), indicating significantly larger nocebo responses after higher compared with lower pain administration (**Fig. 4**).

#### *3.2.2. High-threat group*

The analysis showed that there was no significant interaction between group (High-threat vs Control) and trial type (nocebo vs control) ($F(1,46) = 0.15$, $P = 0.69$, $\eta_p^2 = 0.003$) (**Fig. 4**).

### *3.3. Extinction of nocebo hyperalgesia*

The mean magnitudes of nocebo responses at the end of extinction are presented in **Table 2**. **Figures 5A and 5B** illustrate the reduction of nocebo hyperalgesia and the residual magnitudes of nocebo responses at the end of extinction, respectively. **Figure 6** displays the time course of extinction for all 3 groups.

#### *3.3.1. High-pain group*

The analysis showed that there was no significant interaction between group (High-pain vs Control) and time (nocebo magnitude at the start vs at the end of extinction) ($F(1,46) = 0.58$, $P = 0.45$, $\eta_p^2 = 0.01$).

#### *3.3.2. High-threat group*

The analysis showed that there was no significant interaction between group (High-threat vs Control) and time (nocebo magnitude at the start vs at the end of extinction) ($F(1,46) = 0.04$, $P = 0.84$, $\eta_p^2 = 0.001$) (**Fig. 5A**).

#### *3.3.3. Residual nocebo responses*

We analyzed whether the magnitude of nocebo hyperalgesia at the end of extinction differed between groups. **Figure 5B** illustrates the differences in pain ratings for the last nocebo trial and the last control trial of the extinction phase, across all groups.

##### *3.3.3.1. High-pain group*

The analysis showed a significant interaction between group and trial type, with nocebo responses at the end of extinction (nocebo vs control trials) being significantly different between groups (High-pain vs Control) ($F(1,46) = 4.24$, $P = 0.04$, $\eta_p^2 = 0.09$). We

**Table 1**

**Group means and SDs, as well as between-group *P* values, for sensory thresholds, calibrated temperatures, and reported pain, during the acquisition and extinction phases.**

| Group | Control | | High pain | | High fear | | All groups | | Between-group *P** |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | |
| ˚C warmth threshold | 33.9 | 0.8 | 33.7 | 0.6 | 33.8 | 0.6 | 33.8 | 0.7 | 0.46 |
| ˚C heat pain threshold | 43.8 | 2.4 | 43.3 | 2.7 | 43.3 | 2.2 | 43.5 | 2.4 | 0.65 |
| ˚C moderate heat pain | 45.8 | 1.1 | 47.5 | 0.9 | 45.9 | 1.3 | 46.4 | 1.4 | <0.001 |
| ˚C high heat pain | 47.7 | 0.7 | 49.1 | 0.7 | 47.7 | 0.9 | 48.1 | 1.0 | <0.001 |
| NRS control trials | 2.9 | 1.2 | 4.7 | 1.3 | 2.7 | 1.1 | 3.4 | 1.5 | <0.001 |
| NRS nocebo trials | 5.8 | 1.3 | 7.9 | 0.9 | 6.0 | 1.1 | 6.5 | 1.4 | <0.001 |

Pain scores are reported on a 0 to 10 pain numerical rating scale (NRS). Significant differences were found between the High-pain group and the other 2 groups ($P < 0.001$), driven by the administration of higher pain levels in this group. * One-way ANOVAs were conducted to test for between-groups differences in sensory thresholds, calibrated temperatures, and reported pain levels.
˚C, temperature measured in degree Celsius.

ran repeated-measures ANOVAs separately for the High-pain and Control groups, confirming that nocebo responses (ie, nocebo vs control trials) in the Control group were not significant ($F(1,23) = 1.42$, $P = 0.25$, $\eta_p^2 = 0.08$), whereas nocebo responses in the High-pain group were significant at the end of extinction ($F(1,23) = 18.59$, $P < 0.001$, $\eta_p^2 = 0.45$).

#### *3.3.3.2. High-threat group*

The analysis showed that nocebo responses (ie, nocebo vs control trials) at the end of extinction were not significantly different between the High-threat and Control groups ($F(1,46) = 0.002$, $P = 0.98$, $\eta_p^2 < 0.001$) (**Fig. 5B**).

### *3.4. Nocebo responses mediated by fear*

To test whether the larger nocebo magnitude in the High-pain group compared with the Control group was mediated by fear, a mediation analysis was conducted using the causal steps approach suggested by Baron and Kenny[6] implemented in PROCESS.[57,58] This method uses regression analyses to determine the relationship between the predictor variable and the outcome variable both with and without the mediator in the analysis. The regression was performed in 3 steps (**Fig. 7**). Step 1 (path c) determined that group significantly predicted nocebo magnitude ($F(1,46) = 4.32$, $R^2 = 0.09$, $b = 0.83$, $t(46) = 2.08$, $P = 0.04$). Step 2 (path a) determined that group significantly predicted reported fear ($F(1,46) = 10.99$, $R^2 = 0.19$, $b = 1.37$, $t(46) = 3.32$, $P = 0.002$). Group and reported fear together significantly predicted nocebo magnitude ($F(2,45) = 19.25$, $P < 0.001$, $R^2 = 0.46$), and step 3 (path c′) determined that group did not remain a significant predictor of the nocebo magnitude after controlling for reported fear ($b = -0.02$, $t(45) = -0.05$, $P = 0.96$). The bootstrap analysis confirmed a significant indirect effect of group on the magnitude of nocebo responses through reported fear levels (ab = 0.85, BCa confidence interval [0.34-1.44]). These analyses indicate that full mediation occurred, as the relationship between the group and nocebo magnitude was no longer statistically significant when fear was entered into the model.[58]

The same mediation analysis was performed with EMG fear scores as the mediator variable. EMG startle responses were not a significant mediator of the relationship between the group and the nocebo magnitude, with a nonsignificant indirect effect of group on the magnitude of nocebo responses through EMG fear levels (ab = 0.05, BCa confidence interval [−0.14 to 0.27]).

### *3.5. Manipulation checks for fear levels*

#### *3.5.1. High-pain group*

Differences in reported fear in the High-pain group were more than double compared with the Control group, whereas startle responses were slightly higher for the High-pain group compared with the Control group (**Table 2**). As expected, our analysis confirmed that the High-pain group reported to be more afraid than the Control group during nocebo compared with control trials ($F(1,46) = 11.01$, $P = 0.002$, $\eta_p^2 = 0.19$). No such difference occurred in eyeblink startle responses ($F(1,42) = 0.75$, $P = 0.39$, $\eta_p^2 = 0.018$).

#### *3.5.2. High-threat group*

Differences in reported fear in the High-threat group were more than 50% higher compared with the Control group, and

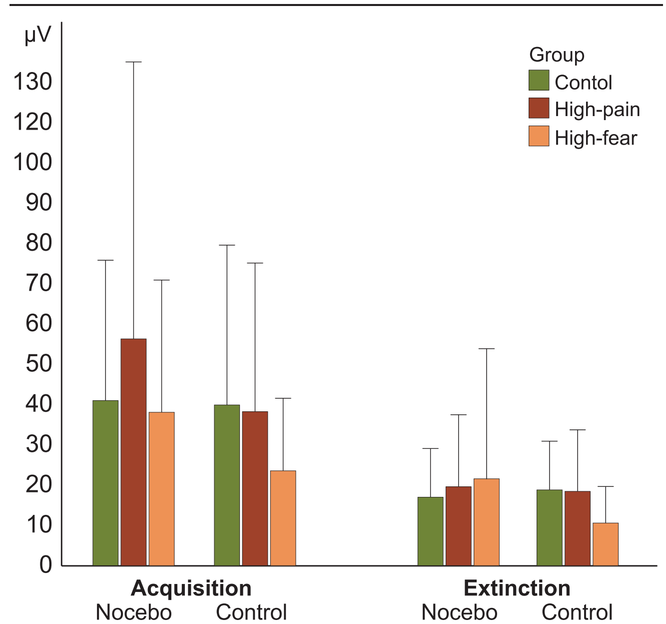


**Figure 3.** Means and SDs of startle responses as measured through electromyography: As compared to the Control group (N = 23), participants in the High-pain (N = 21) and the High-threat group (N = 22) showed larger startle responses during nocebo trials compared with control trials of the acquisition phase. These differences did not reach significance. Overall, mean startle responses were smaller than those expected.

Table 2

**Group means and SDs for fear levels during acquisition and extinction, as well as magnitudes of reported nocebo hyperalgesia after acquisition and at the end of extinction.**

| Group | Control | | High pain | | High fear | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| Acquisition | | | | | | |
| Nocebo magnitude | 0.9 | 1.4 | 1.8 | 1.4 | 1.1 | 1.1 |
| Fear difference (reported) | 1.1 | 1.2 | 2.4 | 1.7 | 1.6 | 1.3 |
| Fear difference (EMG*) | 42.6 | 13.4 | 45.9 | 12.3 | 61.6 | 25.6 |
| Extinction | | | | | | |
| Nocebo magnitude | 0.2 | 1.1 | 0.7 | 0.8 | 0.3 | 0.8 |
| Fear difference (reported) | 0.6 | 0.9 | 1.4 | 1.4 | 0.7 | 1.3 |
| Fear difference (EMG*) | −3.10 | 4.8 | −1.70 | 5.4 | 4.10 | 10.5 |

Pain and fear scores are reported on a 0 to 10 pain numerical rating scale. Magnitudes of nocebo hyperalgesia are shown here as the difference between the control and the nocebo trial, at the start and at the end of extinction (ie, after acquisition and after extinction). * EMG scores are shown here as T scores, to account for interindividual variation in physiological reactivity.
EMG, electromyography.

startle responses were higher for the High-threat group compared with the Control group (**Table 2**). The analysis showed that the High-threat group did not report more pain-related fear than the Control group during nocebo trials compared with control trials (F(1,46) = 3.13, $P = 0.08$, $\eta_p^2 = 0.06$). However, in the High-threat group, startle responses were larger than those in the Control group during nocebo trials compared with control trials (F(1,43) = 9.89, $P = 0.003$, $\eta_p^2 = 0.19$).

Furthermore, a one-way ANOVA with group (High-threat, Control) as the between-subject group factor confirmed that the High-threat group was significantly more frightened by the mock skin sensitivity test (based on the exit questionnaire) than the Control group, F(1,46) = 10.9, $P = 0.002$, $\eta_p^2 = 0.19$, suggesting that our threat manipulation worked.

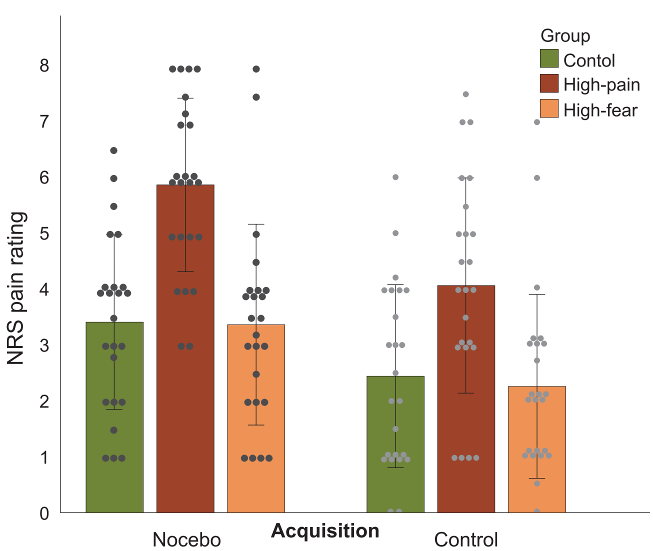


**Figure 4.** Acquisition of nocebo responses: Mean numerical rating scale (NRS) pain ratings and SDs are depicted across all 3 groups (N = 72) for the first nocebo and the first control trial of the extinction phase. In addition, individual scores are presented in gray dots. Although all groups showed significant nocebo responses, the High-pain group showed significantly larger nocebo responses as compared to the Control group. Nocebo responses in the High-threat group were not significantly higher than those in the Control-nocebo group.

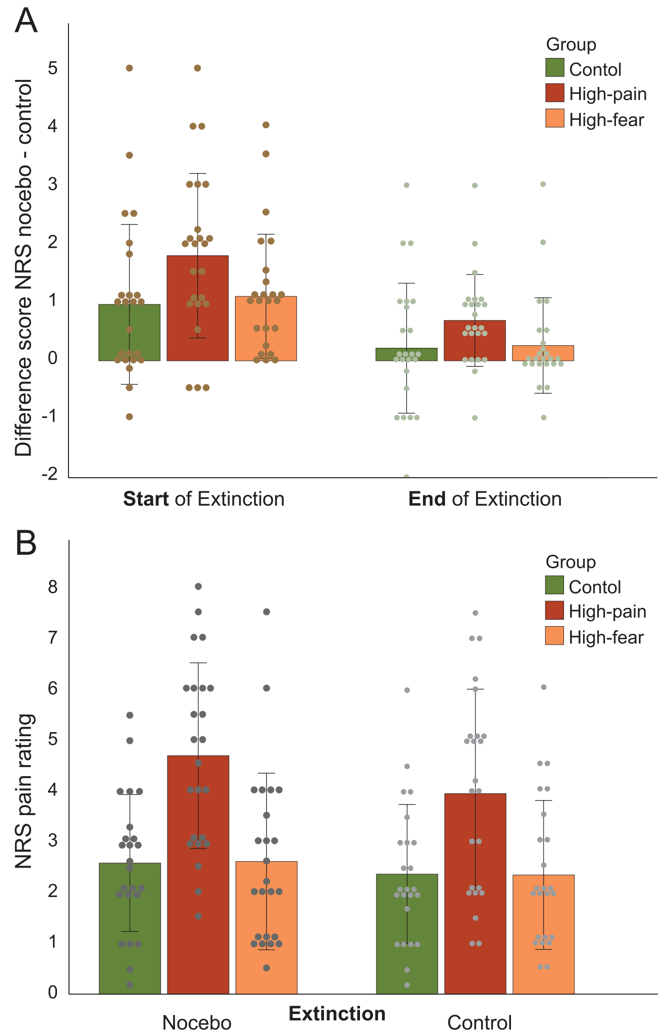


**Figure 5.** Extinction of nocebo hyperalgesia: (A) Extinction of nocebo responses from the start to the end of extinction. Nocebo magnitudes and SDs, based on numerical rating scale (NRS) pain ratings, at the start and at the end of extinction are depicted between all 3 groups. In addition, individual scores are presented in dots. There was no significant difference in the reduction rate of nocebo magnitudes between the High-pain and Control groups, or between the High-threat and Control groups. Negative values signify an effect comparable with a placebo effect (ie, control trials having been rated higher than the nocebo trials). (B) Residual nocebo responses at the end of extinction: Mean pain ratings and SDs for nocebo and control trials at the end of extinction are depicted between all 3 groups. Individual scores are presented in gray dots. At the end of extinction, there was a significant difference in the magnitude of nocebo hyperalgesia between the High-pain and Control groups. At the end of extinction, nocebo responses were still significant for the High-pain group, but not for the Control group.

### *3.6. Exploratory correlations of nocebo responses and fear*

In an exploratory manner, we examined how fear responses influenced the acquisition and extinction of nocebo hyperalgesia. Pearson's correlation analyses across all groups showed significant correlations between reported fear (difference between nocebo and control trials) and the magnitude of nocebo responses ($r = 0.59$, $P < 0.001$), as well as between reported fear and the magnitude of nocebo responses still present after extinction ($r = 0.33$, $P = 0.002$). **Figure 8** illustrates the 2 correlations. **Table 3** lists all correlations between the magnitude of reported fear and the magnitude of nocebo responses for each group and each experimental phase.

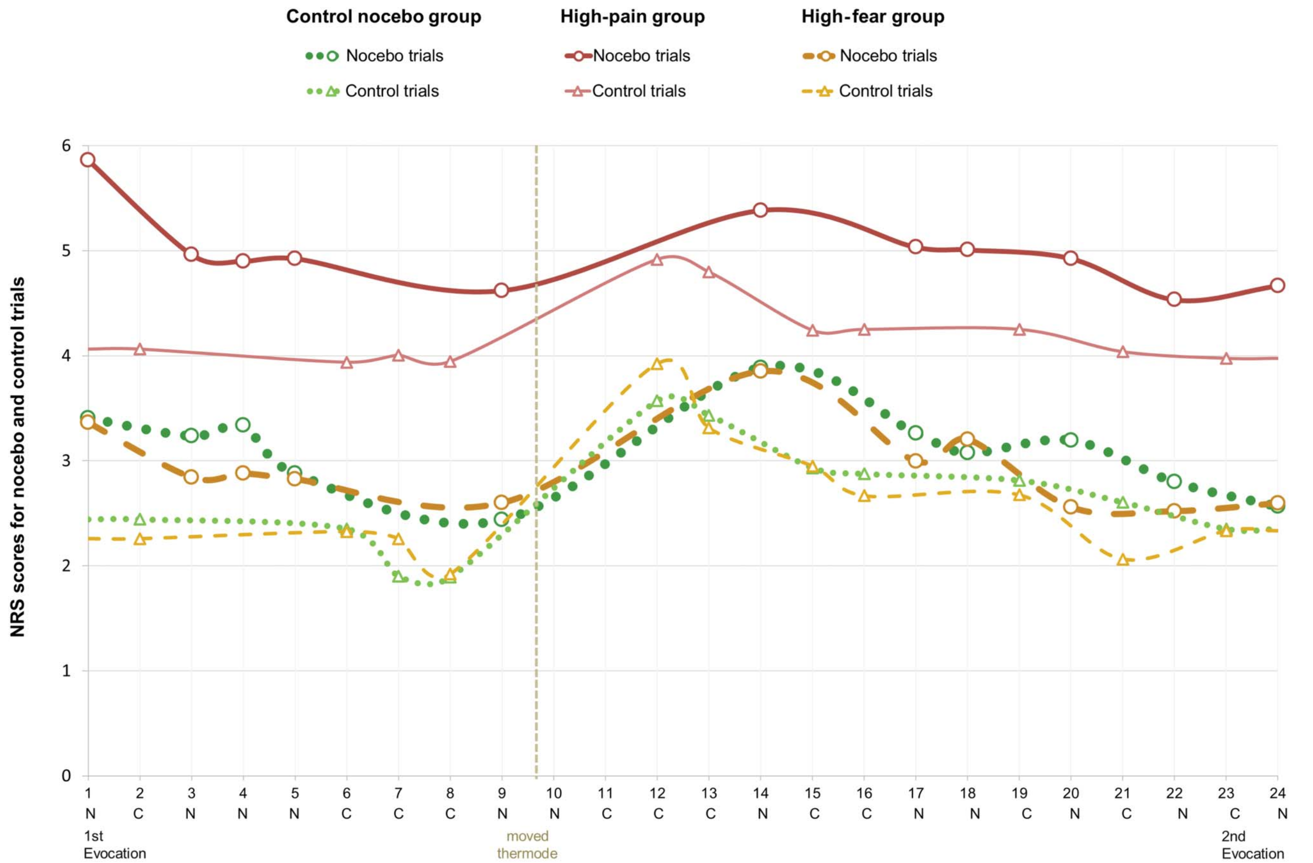


**Figure 6.** Pain ratings for the nocebo and control trials in the extinction phase across all 3 groups: Numerical rating scale (NRS) pain ratings during nocebo and control trials illustrate the evocation of nocebo responses and time course of extinction for the Control-nocebo, High-pain, and High-threat groups. The dotted vertical line indicates the thermode moving point, after which pain ratings suddenly peak due to placing the thermode on a new location on the arm. During the entire extinction phase, all pain stimuli were administered at the same intensity. It is visible that the High-pain group (red lines) consistently rated nocebo trials (thick lines) higher than control trials (thin lines), as compared to the other groups (green and orange lines).

### *3.7. Manipulation checks, questionnaires, nocebo responses, and fear*

Finally, we ran analyses to explore any relationships between nocebo responses, fear responses, and related psychological or cognitive factors.

#### *3.7.1. Exit questions and psychological questionnaires*

On average, participants believed the information they received during the study (M = 8.6, SD = 1.8), they thought the researcher was honest (M = 8.7, SD = 1.5), they were not concerned about what the researcher thought of them (M = 3.3, SD = 1.7), and they were focused on the heat tests (M = 8.7, SD = 1.1). We ran Pearson's correlations between the magnitude of nocebo hyperalgesia and manipulation check exit questions. Participants' expectations about pain during nocebo trials differed per group (Control: M = 5.6, SD = 1.7; High-pain: M = 6.9, SD = 1.7; and High-threat: M = 6.2, SD = 1.9), and pain expectations across all groups were correlated with nocebo magnitudes ($r$ = 0.38, $P < 0.001$). None of the other responses to exit questions were significantly correlated with the magnitude of nocebo responses (for all questions, $P > 0.05$, please see supplementary material, available at http://links.lww.com/PAIN/B135). A one-way ANOVA showed that there were no significant group differences in questionnaire scores (for all questionnaires, $P > 0.05$). Detailed questionnaire results and Cronbach's alpha scores are reported in the supplementary material (available at http://links.lww.com/PAIN/B135).

#### *3.7.2. Manipulation checks for nocebo and fear responses*

Pearson's correlation analyses showed significant correlations between retrospectively assessed fear of the nocebo trials (reported at the end of the experiment) and the magnitude of nocebo responses ($r$ = 0.25, $P$ = 0.02), as well as reported fear differences ($r$ = 0.63, $P < 0.001$). There were no significant correlations between any relevant manipulation check questions or questionnaires and nocebo magnitudes or reported fear (for all questions, $P > 0.05$, please see supplementary material, available at http://links.lww.com/PAIN/B135).

## 4. Discussion

This study investigated the facilitating effects of 2 distinct pain-related fear manipulations on nocebo responses. We expected that higher pain levels would lead to higher pain-related fear, which would augment nocebo responses. We confirmed this by demonstrating that compared with lower pain, conditioning with higher pain administrations produced significantly larger nocebo responses. We also showed that this effect was mediated by reported fear levels, but not by eyeblink startle responses. Contrary to our expectation, nocebo responses extinguished at a

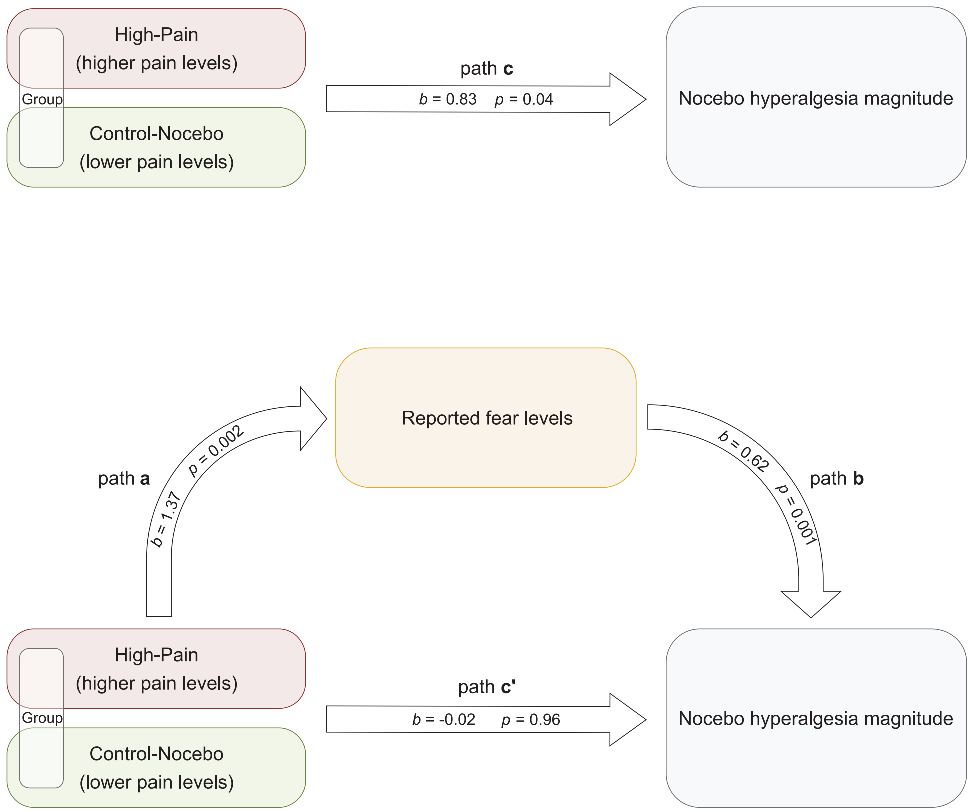


**Figure 7.** Diagram of the hypothesized mediation model and results: Administration of higher pain (High-pain group) compared with lower pain (Control group) significantly predicted the magnitude of nocebo responses, and this effect was mediated by reported fear levels.

similar rate in the High-pain and Control groups. However, we found that nocebo responses at the end of extinction were significantly larger in the High-pain group. A threat manipulation did not amplify nocebo responses. Importantly, nocebo magnitudes across all groups correlated with reported fear during conditioning. These findings bear a number of implications related to both experimental models and clinical practices.

The finding that higher pain levels produced larger nocebo responses and that this was mediated by fear may be linked to previous fear studies.[23,38,47,48] Fear is a response that can be relatively impenetrable to cognitive control[52] and can be learned through classical conditioning.[30,45] Just like nocebo conditioning models, fear-avoidance models consider pain-related fear to be a key factor in certain types of chronic pain.[47] Notably, Crombez et al.[23] studied a sample of patients with chronic back pain and found that pain-related fear may be even more disabling than pain itself. In the current study, we show that during conditioning, fear in response to the experience of high pain may have a direct amplifying effect on the acquisition of nocebo responses. This finding may be a novel link between fear of pain and nocebo hyperalgesia, as both are postulated to play a role in pain conditions.[20,47]

Studying fear in relation to the extinction of nocebo hyperalgesia may also provide insights into pain chronification. Nocebo hyperalgesia is sometimes found to be resistant to extinction.[18,19,42] In this study, nocebo responses were extinguished in the Control group, but in the High-pain group, they remained statistically significant. As the extinction rate of nocebo responses was not hindered by higher pain stimulations, it is apparent that in the High-pain group the substantially larger induced effects led to residual nocebo responses. It is therefore reasonable to expect that after a longer extinction phase, nocebo responses would eventually be extinguished even after higher pain stimulations. Nevertheless, high pain leading to residual nocebo responses bears important implications. In clinical terms, this effect may indicate that compared with lower pain, higher pain not only produces larger nocebo responses, but these responses can also be persistently higher after an initial period of extinction.

Although these findings linking higher pain levels to larger nocebo responses are in line with research into fear and pain chronification, there are some notable differences. Fear-avoidance models[35,38,70] propose that upon the experience of pain symptoms, patients with pain-related fear engage in a negative feedback loop in which fear avoidance and reduced physical activity lead to increased disability and psychological strain.[70] In our study, participants did not engage in avoidance behaviors, yet our results support a separate pathway to pain chronification, in which fear of high pain may be conditioned in parallel with the nocebo response, thereby significantly strengthening the learning process in nocebo hyperalgesia.

In the High-threat group, only startle responses were significantly higher than those in the Control group, and nocebo magnitudes were not affected by the threat manipulation. Previous research also concluded that experimental threat induction is challenging.[35,38] In this study, we informed participants that they may experience sudden, intense pain because of unusual skin sensitivity. Participants were constantly exposed to a mock measurement of this skin test and were reminded to be alert to changes in their sensations. This group generally reported believing the manipulation and being significantly more frightened by it, compared with the Control group that was told that their skin was safe. This may indicate that the threat manipulation did not

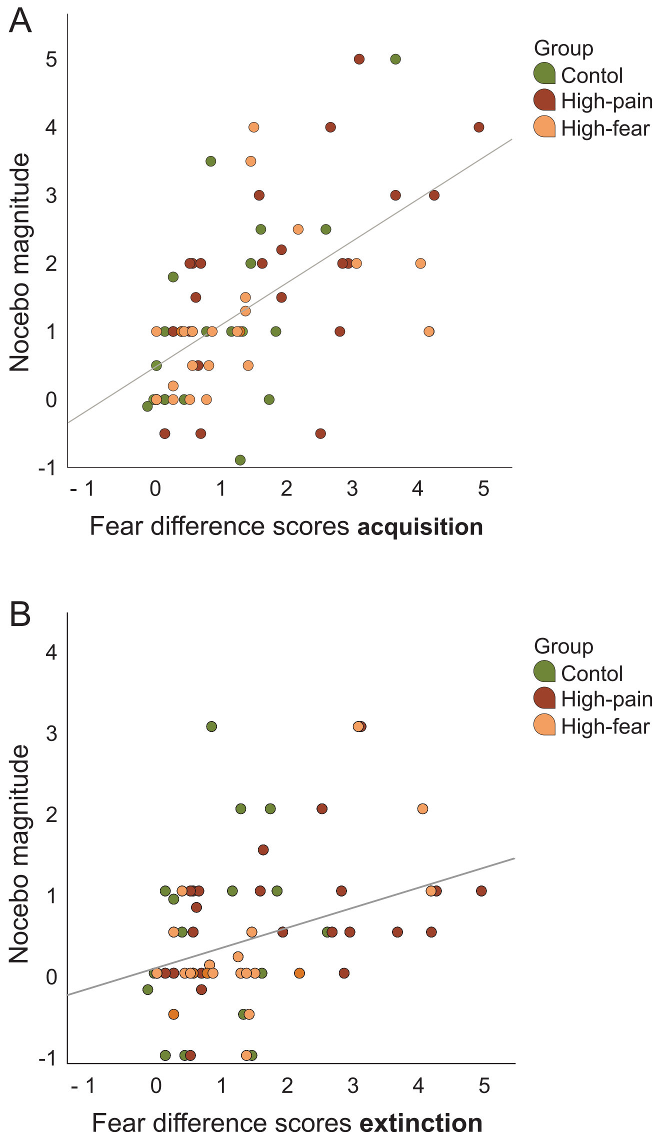


**Figure 8.** Correlations between the magnitude of nocebo responses and reported fear levels: (A) In the acquisition phase, there was a significant high correlation across all groups between nocebo response magnitudes and reported fear levels. (B) In the extinction phase, there was a significant moderate correlation across all groups between nocebo response magnitudes and reported fear. Regardless of the manipulation that participants received, pain-related fear leads to larger magnitudes of nocebo hyperalgesia, both after acquisition and at the end of extinction.

have a direct effect on participants' learning, not because of a lack in credibility but perhaps because of the potential negative effects being only anticipated and never actually experienced, unlike in the High-pain group. It is also possible that participants felt relatively safe and anticipated that no harm would be caused (based on their understanding of ethical standards in research). Differences in learned fear responses resulting from experienced vs anticipated threat have been highlighted in the fear literature[16] and support the differences found in this study between the High-pain and High-threat groups.

Notably, when examining the relationship of pain-related fear with nocebo responses across all 3 groups, we found that fear reports almost always correlated with the magnitude of nocebo responses. This is interesting, given the substantial interindividual variation in fear of pain.[15,51] We further showed that none of the

**Table 3**

**Correlations of nocebo magnitudes and fear magnitudes across all groups and for both the acquisition and the extinction phase.**

| | Nocebo magnitude | | | | | |
|---|---|---|---|---|---|---|
| | Control-nocebo | | High pain | | High fear | |
| | *r* | *P* | *r* | *P* | *r* | *P* |
| Fear magnitude | | | | | | |
| Induction | 0.73 | <0.001 | 0.59 | 0.001 | 0.61 | 0.001 |
| Extinction | 0.17 | 0.21 | 0.32 | 0.04 | 0.69 | <0.001 |

Pain and fear scores are reported on a 0 to 10 pain numerical rating scale. Magnitudes of nocebo hyperalgesia are shown here as the difference between the control and the nocebo trial, at the start and at the end of extinction (ie, after acquisition and after extinction). Reported fear magnitudes are shown here as the mean difference between control and nocebo trials in each phase (acquisition and extinction).

anxiety measures correlated with the magnitude of nocebo responses. This was critical in this study, as we specifically focused on the effects of fear on nocebo hyperalgesia. Fear is a response that is often difficult to disentangle from anxiety, theoretically and physiologically.[54,67] The two may produce similar responses yet involve distinct psychobiological mechanisms, with fear involving more immediate responses to explicit danger and anxiety presenting as a diffuse response to anticipated threat. Based on our findings, fear, as measured both during and after the experiment, produced larger nocebo responses. By contrast, anxiety, as measured after the experiment and the threat manipulation that involved anticipated threat, was not related to larger nocebo responses.

Another method for measuring fear of pain is the measurement of fear-potentiated startle responses. These responses are produced through projections from the central nucleus of the amygdala.[25,49] This role of the amygdala, as well as ample fear research, indicates that startle responses may be more specific to fear states and less specific to states of anxiety.[24,33,34] Average acoustically elicited startle responses range between 100 and 300 $\mu$V.[14,50] Typically, sound probes are delivered through noise-cancelling headphones, which achieve optimal auditory conditions and block sounds in the environment.[1,10] In this study, earphones were used so that participants could verbally communicate with the researcher, which was crucial in our design. Startle responses were observed; however, potentially as a result of using earphones, these were smaller than expected, on average below 100 $\mu$V. Although trends that followed reported fear were observed, on this smaller scale of responses most differences did not reach statistical significance. This is an apparent study limitation that should be addressed in future designs.

Another study limitation may have been the effectivity of the threat manipulation. As mentioned earlier, participants in the High-threat group believed and were more frightened by the mock skin sensitivity test, compared with the Control group. However, this fear did not translate to increased fear during conditioning. It is possible that induced fear levels were not high or specific enough to translate into experienced fear during nocebo trials. However, it was not possible to increase threat levels without risking participants dropping out of the study or it seeming illogical for the researcher to continue the experiment. This is a common obstacle in experimental threat manipulations.[35,38] As noted, however, the threat manipulation may not have increased fear reports because of its anticipatory and obscure nature, rather than a manipulation failure, although it is also plausible that pain may have captured participants' attention and diverted it away from the potentiality of a threat.

Finally, it is important for future studies to address whether clinically relevant extinction effects are affected by fear. For instance, reinstatement of conditioned responses (after experience with unpredictable increased pain) to the conditioned stimulus has been observed in previous studies.[11,32,55,71] Reinstatement translates to clinical practice where patients may be re-exposed to exacerbated pain, even after successful treatment.[71] Similarly, patients may retrieve a previously extinguished effect, upon exposure to an aversive stimulus distinct from pain, such as fear.[46] Based on the results of this study, it is important to further examine whether high pain can also impact the return of learned effects on pain. It is worth noting that controlling for unwanted variability due to age differences in our sample, the generalizability of our findings to the general population is limited. Future studies may consider including broader age ranges.

Overall, this study implemented a novel, clinically relevant learning model that investigated the effects of direct fear inductions on nocebo hyperalgesia. The findings provided evidence that experienced threat in the form of higher pain stimulations led to significantly larger nocebo hyperalgesia, compared with lower pain. Importantly, this effect was mediated by self-reported fear. The anticipation of threat, however, did not impact nocebo magnitudes. This study also indicated that higher pain stimulations induce amplified nocebo responses that persist after a period of extinction. Given the substantial impact of higher pain and pain-related fear on nocebo hyperalgesia, further assessment of these variables in relation to pain aggravation and chronification may be of value.

## Conflict of interest statement

The authors have no conflicts of interest to declare.

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## Appendix A. Supplemental digital content

Supplemental digital content associated with this article can be found online at http://links.lww.com/PAIN/B135.

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## References

[1] Anderson NE, Wan L, Young KA, Stanford MS. Psychopathic traits predict startle habituation but not modulation in an emotional faces task. Pers Individ Dif 2011;50:712–16.
[2] Aslaksen PM, Lyby PS. Fear of pain potentiates nocebo hyperalgesia. J Pain Res 2015;8:703–10.
[3] Atlas LY, Wager TD. How expectations shape pain. Neurosci Lett 2012;520:140–8.
[4] Bąbel P, Bajcar EA, Adamczyk W, Kicman P, Lisińska N, Świder K, Colloca L. Classical conditioning without verbal suggestions elicits placebo analgesia and nocebo hyperalgesia. PLoS One 2017;12:e0181856.
[5] Bajcar EA, Wiercioch-Kuzianik K, Adamczyk WM, Bąbel P. To experience or to Be informed? Classical conditioning induces nocebo hyperalgesia even when placebo analgesia is verbally suggested—results of a preliminary study. Pain Med 2019;21:548–60.
[6] Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. J Pers Soc Psychol 1986;51:1173–82.
[7] Bartels DJP, van Laarhoven AIM, Haverkamp EA, Wilder-Smith OH, Donders ART, van Middendorp H, van de Kerkhof PCM, Evers AWM. Role of conditioning and verbal suggestion in placebo and nocebo effects on itch. PLoS One 2014;9:e91727.
[8] Benedetti F, Durando J, Vighetti S. Nocebo and placebo modulation of hypobaric hypoxia headache involves the cyclooxygenase-prostaglandins pathway. PAIN 2014;155:921–8.
[9] Benedetti F, Lanotte M, Lopiano L, Colloca L. When words are painful: unraveling the mechanisms of the nocebo effect. Neuroscience 2007;147:260–71.
[10] Bennett KP, Dickmann JS, Larson CL. If or when? Uncertainty's role in anxious anticipation. Psychophysiology 2018;55:e13066.
[11] Benson S, Kattoor J, Kullmann JS, Hofmann S, Engler H, Forsting M, Gizewski ER, Elsenbruch S. Towards understanding sex differences in visceral pain: enhanced reactivation of classically-conditioned fear in healthy women. Neurobiol Learn Mem 2014;109:113–21.
[12] Blumenthal TD, Cuthbert BN, Filion DL, Hackley S, Lipp OV, Van Boxtel A. Committee report: guidelines for human startle eyeblink electromyographic studies. Psychophysiology 2005;42:1–15.
[13] Blythe JS, Peerdeman KJ, Veldhuijzen DS, van Laarhoven AIM, Evers AWM. Placebo and nocebo effects on itch. Itch 2019;4:e27.
[14] Bradford DE, Magruder KP, Korhumel RA, Curtin JJ. Using the threat probability task to assess anxiety and fear during uncertain and certain threat. J Vis Exp 2014;9:e88482.
[15] Bradley MM, Silakowski T, Lang PJ. Fear of pain and defensive activation. PAIN 2008;137:156–63.
[16] Britton JC, Lissek S, Grillon C, Norcross MA, Pine DS. Development of anxiety: the role of threat appraisal and fear learning. Depress Anxiety 2011;28:5–17.
[17] Cohen J. A power primer. Psychol Bull 1992;112:155–9.
[18] Colagiuri B, Quinn VF. Autonomic arousal as a mechanism of the persistence of nocebo hyperalgesia. J Pain 2018;19:476–86.
[19] Colagiuri B, Quinn VF, Colloca L. Nocebo hyperalgesia, partial reinforcement, and extinction. J Pain 2015;16:995–1004.
[20] Colloca L, Miller FG. The nocebo effect and its relevance for clinical practice. Psychosom Med 2011;73:598–603.
[21] Colloca L, Sigaudo M, Benedetti F. The role of learning in nocebo and placebo effects. PAIN 2008;136:211–18.
[22] Crombez G, De Paepe AL, Veirman E, Eccleston C, Verleysen G, Van Ryckeghem DML. Let's talk about pain catastrophizing measures: an item content analysis. PeerJ 2020;8:e8643.
[23] Crombez G, Vlaeyen JWS, Heuts PHTG, Lysens R. Pain-related fear is more disabling than pain itself: evidence on the role of pain-related fear in chronic back pain disability. PAIN 1999;80:329–39.
[24] Davis M. Neural systems involved in fear and anxiety measured with fear-potentiated startle. Am Psychol 2006;61:741–56.
[25] Davis M. The role of the amygdala in fear-potentiated startle: implications for animal models of anxiety. Trends Pharmacol Sci 1992;13:35–41.
[26] Efron B, Tibshirani RJ, London NY. An Introduction to the Bootstrap. 1993 p. Available at: http://cindy.informatik.uni-bremen.de/cosy/teaching/CM_2011/Eval3/pe_efron_93.pdf. Accessed April 2, 2020.
[27] Evers AWM, Colloca L, Blease C, Annoni M, Atlas LY, Benedetti F, Bingel U, Büchel C, Carvalho C, Colagiuri B, Crum AJ, Enck P, Gaab J, Geers AL, Howick J, Jensen KB, Kirsch I, Meissner K, Napadow V, Peerdeman KJ, Raz A, Rief W, Vase L, Wager TD, Wampold BE, Weimer K, Wiech K, Kaptchuk TJ, Klinger R, Kelley JM. Implications of placebo and nocebo effects for clinical practice: expert consensus. Psychother Psychosom 2018;87:204–10.
[28] Faul F, Erdfelder E, Lang AG. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 2007;39:175–91.
[29] Freeman S, Yu R, Egorova N, Chen X, Kirsch I, Claggett B, Kaptchuk TJ, Gollub RL, Kong J. Distinct neural representations of placebo and nocebo effects. Neuroimage 2015;112:197–207.
[30] Fullana MA, Harrison BJ, Soriano-Mas C, Vervliet B, Cardoner N, Àvila-Parcet A, Radua J. Neural signatures of human fear conditioning: an

updated and extended meta-analysis of fMRI studies. Mol Psychiatry 2016;21:500–8.
[31] Geuter S, Buchel C. Facilitation of pain in the human spinal cord by nocebo treatment. J Neurosci 2013;33:13784–90.
[32] Gramsch C, Kattoor J, Icenhour A, Forsting M, Schedlowski M, Gizewski ER, Elsenbruch S. Learning pain-related fear: neural mechanisms mediating rapid differential conditioning, extinction and reinstatement processes in human visceral pain. Neurobiol Learn Mem 2014;116: 36–45.
[33] Grillon C. Models and mechanisms of anxiety: evidence from startle studies. Psychopharmacology (Berl) 2008;199:421–37.
[34] Hamm AO. Fear, anxiety, and their disorders from the perspective of psychophysiology. Psychophysiology 2020;57:e13474.
[35] Den Hollander M, Meulders A, Jakobs M, Vlaeyen JWS. The effect of threat information on acquisition, extinction, and reinstatement of experimentally conditioned fear of movement-related pain. Pain Med 2015;57:e13474.
[36] Icenhour A, Labrenz F, Ritter C, Theysohn N, Forsting M, Bingel U, Elsenbruch S. Learning by experience? Visceral pain-related neural and behavioral responses in a classical conditioning paradigm. Neurogastroenterol Motil 2017;29:e13026.
[37] Jensen KB, Kaptchuk TJ, Chen X, Kirsch I, Ingvar M, Gollub RL, Kong J. A neural mechanism for nonconscious activation of conditioned placebo and nocebo responses. Cereb Cortex 2015;25:3903–10.
[38] Karos K, Meulders A, Vlaeyen JWS. Threatening social context facilitates pain-related fear learning. J Pain 2015;16:214–25.
[39] Keltner JR. Isolating the modulatory effect of expectation on pain transmission: a functional magnetic resonance imaging study. J Neurosci 2006;26:4437–43.
[40] Kong J, Gollub RL, Polich G, Kirsch I, LaViolette P, Vangel M, Rosen B, Kaptchuk TJ. A functional magnetic resonance imaging study on the neural mechanisms of hyperalgesic nocebo effect. J Neurosci 2008;28: 13354–62.
[41] Linnman C, Rougemont-Bücking A, Beucke JC, Zeffiro TA, Milad MR. Unconditioned responses and functional fear networks in human classical conditioning. Behav Brain Res 2011;221:237–45.
[42] Manaï M, van Middendorp H, Veldhuijzen DS, Huizinga TWJ, Evers AWM. How to prevent, minimize, or extinguish nocebo effects in pain: a narrative review on mechanisms, predictors, and interventions. Pain Rep 2019:4; e699.
[43] Marteau TM, Bekker H. The development of a six-item short-form of the state scale of the Spielberger State-Trait Anxiety Inventory (STAI). Br J Clin Psychol 1992;31:301–6.
[44] McNeil DW, Rainwater AJ. Development of the Fear of Pain Questionnaire-III. J Behav Med 1998;21:389–410.
[45] Mechias ML, Etkin A, Kalisch R. A meta-analysis of instructed fear studies: implications for conscious appraisal of threat. Neuroimage 2010; 49:1760–8.
[46] Meulders A, Rousseau A, Vlaeyen JWS. Motor intention as a trigger for fear of movement-related pain: an experimental cross-US reinstatement study. J Exp Psychopathol JEP 2015;6:206–28.
[47] Meulders A, Vansteenwegen D, Vlaeyen JWS. The acquisition of fear of movement-related pain and associative learning: a novel pain-relevant human fear conditioning paradigm. PAIN 2011;152:2460–9.
[48] Meulders A, Vlaeyen JWS. The acquisition and generalization of cued and contextual pain-related fear: an experimental study using a voluntary movement paradigm. PAIN 2013;154:272–82.
[49] Moberg C, Curtin J. Alcohol selectively reduces anxiety but not fear: startle response during unpredictable versus predictable threat. Artic J Abnorm Psychol 2009;118:335–47.
[50] Nesbitt K, Blackmore K, Hookham G, Kay-Lambkin F, Walla P. Using the Startle Eye-Blink to Measure Affect in Players. In: Serious Games Analytics. Springer International Publishing, 2015. pp. 401–434. Available at: https://www.researchgate.net/publication/278783929. Accessed March 19, 2020.
[51] Ochsner KN, Ludlow DH, Knierim K, Hanelin J, Ramachandran T, Glover GC, Mackey SC. Neural correlates of individual differences in pain-related fear and anxiety. PAIN 2006;120:69–77.
[52] Ohman A, Mineka S. Fears, phobias, and preparedness: toward an evolved module of fear and fear learning. Psychol Rev 2001;108: 483–522.
[53] Papadopoulos D, Mitsikostas DD. A meta-analytic approach to estimating nocebo effects in neuropathic pain trials. J Neurol 2012;259: 436–47.
[54] Perusini JN, Fanselow MS. Neurobehavioral perspectives on the distinction between fear and anxiety. Learn Mem 2015;22:417–25.
[55] Piedimonte A, Guerra G, Vighetti S, Carlino E. Measuring expectation of pain: contingent negative variation in placebo and nocebo effects. Eur J Pain 2017;21:874–85.
[56] Pissiota A, Frans O, Michelgard A, Appel L, Langstrom B, Flaten MA, Fredrikson M. Amygdala and anterior cingulate cortex activation during affective startle modulation: a PET study of fear. Eur J Neurosci 2003;18: 1325–31.
[57] Preacher KJ, Hayes AF. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. Behav Res Methods 2008;40:879–91.
[58] Preacher KJ, Hayes AF. SPSS and SAS procedures for estimating indirect effects in simple mediation models. Behav Res Methods Instrum Comput 2004;36:717–31.
[59] Reicherts P, Gerdes ABM, Pauli P, Wieser MJ. Psychological placebo and nocebo effects on pain rely on expectation and previous experience. J Pain 2016;17:203–14.
[60] Richardson JTE. Eta squared and partial eta squared as measures of effect size in educational research. Educ Res Rev 2011;6:135–47.
[61] Rolke R, Magerl W, Campbell KA, Schalber C, Caspari S, Birklein F, Treede RD. Quantitative sensory testing: a comprehensive protocol for clinical trials. Eur J Pain 2006;10:77–88.
[62] Schmid J, Bingel U, Ritter C, Benson S, Schedlowski M, Gramsch C, Forsting M, Elsenbruch S. Neural underpinnings of nocebo hyperalgesia in visceral pain: a fMRI study in healthy volunteers. Neuroimage 2015; 120:114–22.
[63] Scott DJ, Stohler CS, Egnatuk CM, Wang H, Koeppe RA, Zubieta JKK. Placebo and nocebo effects are defined by opposite opioid and dopaminergic responses. Arch Gen Psychiatry 2008;65:220–31.
[64] Sjak-Shie EE. PhysioData Toolbox Version 5. 2019. Available at: https://physiodatatoolbox.leidenuniv.nl. Accessed March 11, 2020.
[65] Spielberger C, Gorsuch R, Lushene P, Vagg P, Jacobs A. Manual for the State-Trait Anxiety Inventory STAI (Form Y) ("Self-Evaluation Questionnaire"). Man State-Trait Anxiety Invent STAI. Palo Alto: Consulting Psychologists Press, Inc, 1983. pp. 4–6.
[66] Sullivan MJL, Bishop SR, Pivik J. The Pain Catastrophizing Scale: development and validation. Psychol Assess 1995;7:524–32.
[67] Sylvers P, Lilienfeld SO, LaPrairie JL. Differences between trait fear and trait anxiety: implications for psychopathology. Clin Psychol Rev 2011;31: 122–37.
[68] Thomaidou MA, Veldhuijzen DS, Peerdeman KJ, Sifra Wiebing NZ, Blythe JS, Evers AWM. Learning mechanisms in nocebo hyperalgesia: the role of conditioning and extinction processes. PAIN 2020;161:1597–1608.
[69] Tu Y, Park J, Ahlfors SP, Khan S, Egorova N, Lang C, Cao J, Kong J. A neural mechanism of direct and observational conditioning for placebo and nocebo responses. Neuroimage 2019;184:954–63.
[70] Turk DC, Wilson HD. Fear of pain as a prognostic factor in chronic pain: conceptual models, assessment, and treatment implications. Curr Pain Headache Rep 2019;14:88–95.
[71] Vlaeyen JWS. Learning to predict and control harmful events. PAIN 2015; 156:S86–93.