Nocebo hyperalgesia and pain progression: prediction, acquisition, and recovery

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Prediction, acquisition, and recovery

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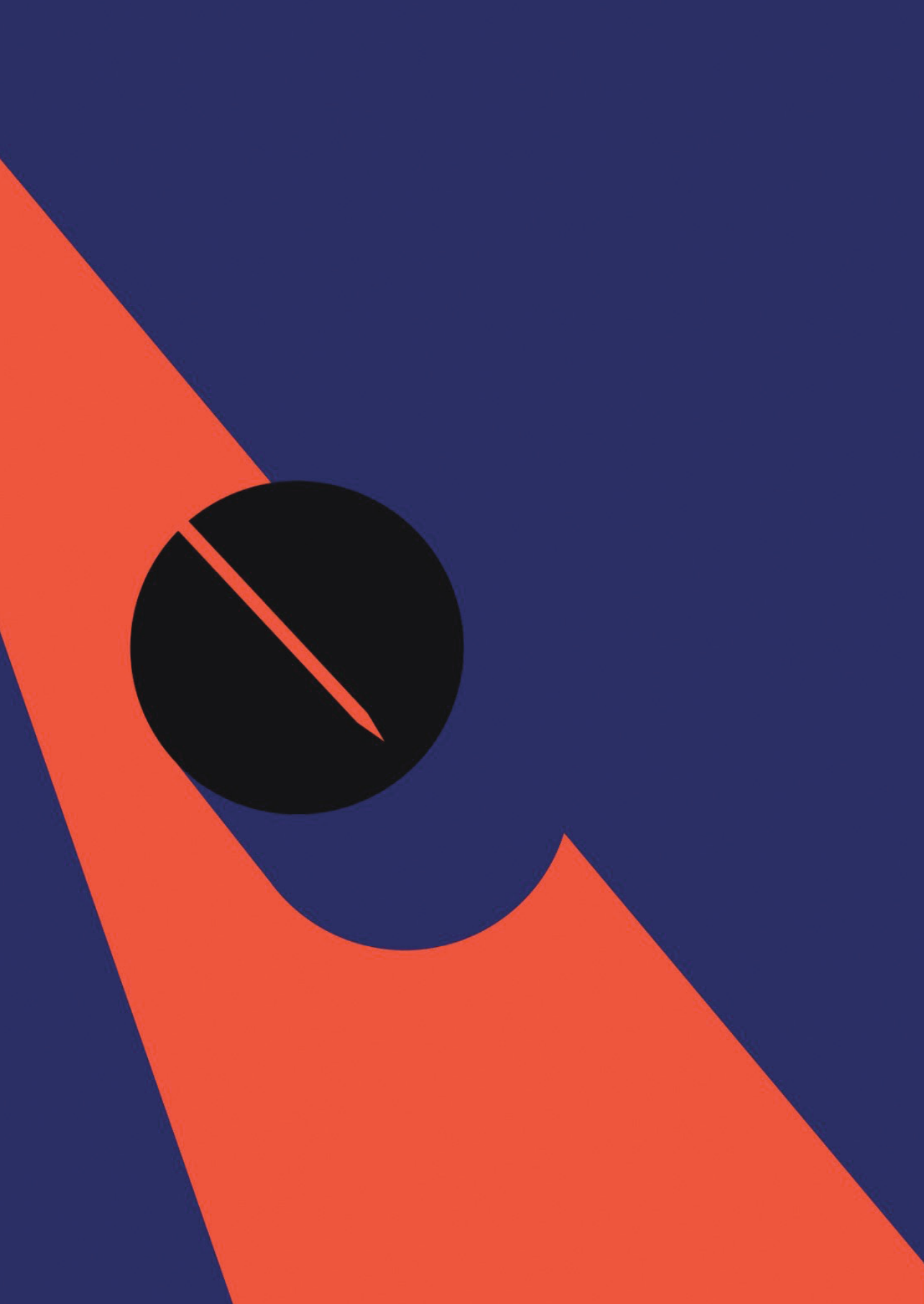

CHAPTER 1

General Introduction

Imagine you are parched and in desperate need of water. After drinking a few drops, you start to feel better again. But how could water quench your thirst in a few seconds when in reality it takes about 20 minutes to reach your bloodstream? The answer lies in prediction and how our expectations interact with the sensory information reaching our bodies[1]. In everyday life, we constantly make predictions about the likelihood of event outcomes, often without conscious awareness[2]. These predictions interact with the sensory signals reaching our brain that form our perceptions, such as knowing that acquiring physical injuries would result in pain perception; therefore, one likely avoids situations with an injury risk[3]. In treatment context, expectations of symptom improvement or worsening related to a treatment can result in perceived pain improvement or worsening, also called placebo or nocebo effects, respectively. The nocebo effect is far less researched than its counterpart the placebo effect[4], and refers to the emergence of adverse treatment outcomes that cannot be attributed to active treatment components[5,6]. A common example of nocebo effects is the experiencing of side effects after disclosing the potential side effects of a medication, regardless of the medication's pharmacological properties[5]. This effect is guided by expectations established through learning experiences regarding the adverse outcomes of taking medication and receiving suggestions on potential side effects[6,7]. Accumulating evidence suggests that nocebo effects can be observed in both healthy and clinical populations[8,9], and across a wide range of symptoms such as pain, itch, fatigue, dyspnea, and nausea[10]. Nocebo effects have a large social and economic impact on society. In clinical practice, nocebo effects can negatively influence the efficacy of therapeutic outcomes and affect patients' health-related quality of life[11].

How nocebo effects are induced: Expectancies and learning

Expectancies are represented as major determinants of behavior and experiences in many psychological learning theories[12]. Expectancies also lie at the core of placebo and nocebo effects. Nocebo effects can be induced by learning processes such as classical conditioning (prior personal experiences) and instructional learning (verbal suggestions).

Classical conditioning occurs when an unconditioned stimulus (US; e.g., high-intensity pressure stimulus) that elicits an unconditioned response (UR; e.g., pain) is repeatedly paired with a neutral stimulus (e.g., an inactive treatment such as a sham Transcutaneous Electrical Nerve Stimulation (TENS) device) until presenting the neutral stimulus alone starts eliciting the conditioned response (CR; i.e., pain) as it becomes the conditioned stimulus (CS). Research findings suggest that a longer conditioning process, i.e., longer repeated pairing of US with neutral stimulus, results in stronger nocebo hyperalgesia that is more resistant to extinction[13]. Moreover, recent studies indicate that explicit expectations (i.e., conscious awareness of CS-US contingency) may not be necessary for mediating conditioned nocebo hyperalgesia[14,15]. Research suggests that nocebo

hyperalgesia can be also established without contingency awareness, demonstrated by designs using implicit conditioning (e.g., pairing abstract figures with high and low heat pain)[16]. Furthermore, findings show that nocebo hyperalgesia can generalize to novel stimuli that fall into the same conceptual category as the original neutral stimulus that was used for conditioning (e.g., conditioning with one set of animal pictures elicits nocebo effects for another set of novel animal pictures)[17]. These findings could have implications for chronic pain, such that contextual stimuli that are repeatedly associated with chronic pain could potentially strengthen nocebo hyperalgesia in patients and make it more resistant to extinction[18]. Meanwhile, exposure to contextual stimuli that are unconsciously registered during a chronic pain episode could also potentially re-activate, intensify, or generalize the chronic pain experiences[18].

Nocebo hyperalgesia can be induced also by instructional learning, i.e., through verbal suggestions about the symptom-worsening properties of an (inactive) treatment, for instance given by healthcare providers. In this form of learning, negative expectancies are formed through explicit verbal information shared about the adverse outcomes of a treatment[19]. Verbal instructions could paradoxically also reverse the efficacy of pharmacological treatments[20,21]. One study found that providing opposing verbal suggestions about the analgesic and also the hyperalgesic properties of a sham treatment could modulate pain in opposing directions[20]. Similarly, providing nocebo information while administering a topical analgesic cream was found to reverse analgesia to hyperalgesia[21]. Interestingly, verbal suggestions of pain increase after pre-conditioning with a pharmacological analgesic (ketorolac) also induced a strong nocebo hyperalgesic effect, emphasizing the role of explicit (i.e., consciously accessible) verbal suggestions in conscious processes such as self-reported pain[20].

Classical conditioning is often investigated together with verbal suggestions[8]. According to a recent meta-analysis, the magnitude of nocebo hyperalgesia induced by combining classical conditioning with verbal suggestions yielded a stronger effect size compared to the magnitude of verbal suggestions alone[22]. In line with this, the current perspectives suggest that nocebo effects can be induced by conditioning and verbal suggestions as complementary implicit and explicit learning processes that together modulate expectancies and strengthen previous learning[19,23]. However, nocebo learning processes have been mainly researched with healthy participants, whereas findings from clinical populations are still limited.

How nocebo effects are maintained and reduced: Stability, extinction, and counterconditioning

In general, 'bad' events occurring in daily life are thought to be more powerful than 'good' ones since negative associations are thought to form quicker and could become more resilient to disconfirmation than positive associations[24,25]. Yet, not much is currently known about the stability and maintenance of nocebo effects over time. To potentially reduce nocebo effects, or in other words to recover from nocebo effects, learning-based intervention strategies are deemed necessary. Research with healthy participants shows that learning processes can be utilized to also reduce nocebo hyperalgesia. Primarily, the role of extinction has been widely researched in nocebo effects on experimental heat[26,27] and electrical[13,28,29] pain. Extinction refers to learning that a CS-US relationship is no longer present by no longer reinforcing CS with US, for instance by no longer pairing a (sham) treatment with high-intensity pain stimuli[30]. Compared to placebo effects, nocebo effects have been found to be more resistant to extinction in the lab[13,29,31]. We do not yet know much about the stability and maintenance of nocebo hyperalgesia over time and extinction of these effects in both healthy and patient populations.

Counterconditioning is another potential method for reducing nocebo hyperalgesia. In nocebo research, counterconditioning refers to actively targeting the reversal of painful associations with a (sham) treatment (CS) by replacing the US with a US of opposite valence[32], for instance, by replacing a high-intensity pressure stimulus that is painful with a non-painful pressure stimulus. Counterconditioning has been prevalently researched in the field of fear and evaluative conditioning, where the results are mixed on its efficacy compared to extinction[32]. In nocebo literature, so far two studies have investigated its role in healthy participants and found that counterconditioning was more effective than extinction in reducing nocebo effects on heat pain[26] and itch[33]. Although counterconditioning seems to be a promising learning strategy for reducing nocebo effects in healthy individuals, its efficacy still needs to be tested also in clinical populations.

How individuals are informed about nocebo effects: Closed-label versus open-label learning paradigms

In learning paradigms, such as conditioning and verbal suggestion, the inert nature of the treatment is usually concealed from the participant (i.e., the individual is unaware of taking a placebo). This approach is also used in blinded placebo-controlled randomized trials (RCTs) to demonstrate the therapeutic benefits of the treatment in comparison to the placebo arm of the RCT [5,34]. Experimental research within placebo and nocebo fields are typically conducted using such closed-label paradigms; however, more recent studies

have investigated the extent to which disclosing information about the inert properties of a treatment can influence the induction of placebo and nocebo effects[34–37].

Perhaps one of the most intriguing findings from recent years has been that deception is not required for establishing placebo effects. Open-label placebos (OLPs) have been found to induce placebo effects on a variety of symptoms in both healthy[38–40] and clinical samples[35,37,41], while allowing for an ethical administration of placebo treatments. While the working mechanisms behind OLP effects are still being researched, studies indicate that providing an extensive description of the underlying mechanisms, such as how our brain processes positive expectations, is an effective method for inducing OLP effects[42]. Research on open-label nocebo effects is almost entirely new and relevant for the reduction of previously acquired nocebo effects. For example, investigating the open-label conditioning or instructional learning processes behind nocebo effects could be beneficial for elucidating how these effects can be induced and provide indications on how these could be reduced or reversed by open- or closed-label interventions. Open-label paradigms are more transparent and ethically responsible than closed-label paradigms, and thus have greater potential for application in clinical populations. However, current research comparing open- versus closed-label learning paradigms on the efficacy of nocebo reduction strategies is limited.

How individuals differ in nocebo effects: Predictors of nocebo hyperalgesia

Individuals differ in the degree to which they acquire nocebo effects[23,43]. According to the biopsychosocial approach from Engel[44], biomedical factors such as anatomy and physiology are considered important determinants of symptom and disease progression; however, it is acknowledged that biomedical factors provide only a limited picture of all possible factors that could influence symptom and disease emergence, and progression[43,45]. When examining which characteristics are predictive of nocebo effects, the biopsychosocial approach indicates that not only biomedical traits should be considered, but also psychological characteristics and the social context, whereby the interaction between these three factors continuously shape individual expectancies and disease outcomes[43]. In particular, psychological factors have been more commonly studied as predictors of nocebo hyperalgesia compared to the other factors[46,47]. Previous (systematic) reviews have identified factors such as higher fear, anxiety, pessimism, and physiological suggestibility (i.e., a general tendency to accept verbal suggestions) as possible predictors of nocebo effects[46,48]. However, the current research on the predictors of nocebo hyperalgesia is still limited, and research on the predictors of recovery from nocebo effects is almost entirely lacking, with only a small number of studies that have investigated this during extinction. For example, one study has shown that both heightened autonomic arousal (skin conductance response) and

higher self-reported anticipatory anxiety strongly correlated with resistance to extinction, highlighting the important role of anticipatory anxiety in the persistence of nocebo effects[31]. Another study has shown that only in the high-intensity pain group compared to the control group, nocebo responses were maintained after extinction[27]. This finding suggests that higher pain levels might lead to higher pain-related fear that can augment the nocebo-hyperalgesia response, such that it becomes more resistant to extinction. The same factors predicting the magnification of nocebo hyperalgesia, such as anxiety and pain-related fear, might also play a role in its persistence and potentially in less recovery from nocebo hyperalgesia. However, the predictors of recovering from nocebo effects have not yet been investigated for a broader range of predictors and for nocebo reduction strategies other than extinction, such as counterconditioning.

Identifying individuals at risk of acquiring and maintaining nocebo hyperalgesia, as well as of recovering therefrom, has important clinical implications. Potentially, early implementation of, for example, learning-based nocebo intervention strategies in these individuals can help maximize therapeutic benefits during treatment. Therefore, more prediction studies are needed to better understand the tendencies that lead to acquiring, maintaining, and recovering from nocebo hyperalgesia.

How findings about nocebo effects translate to clinical settings: Fibromyalgia as a framework for studying nocebo hyperalgesia in practice

So far, research has mainly focused on nocebo effects on acute symptoms. Compared to acute symptoms, persistent physical symptoms can be more difficult to treat and are accompanied by substantial costs for society, healthcare, and patients' health-related quality of life[49,50]. Patients with persistent physical symptoms are exposed to treatment (and potentially also treatment failure) more frequently compared to healthy individuals, both due to the persistent nature of their symptoms and due to the scarceness of treatment options that prove effective in the long term[49,50]. This might impact how patients perceive their treatment context and elicit negative expectancies regarding treatment outcomes. Altogether, this could have consequences for treatment efficacy and maintenance of symptoms. Therefore, in patients with persistent physical symptoms, such as fibromyalgia where the underlying etiopathogenesis is unclear[51], the role of nocebo effects in symptom maintenance and worsening warrants further investigation.

Fibromyalgia is characterized by multiple symptoms, such as chronic widespread pain, muscle stiffness, chronic fatigue, sleep problems, cognitive dysfunction (i.e., fibrofog), depression, and anxiety[52,53]. Research shows that fibromyalgia is more frequently diagnosed in women than in men[54,55]. In terms of treatment options, often individualized treatment plans are tried out, such as psycho-education, physical exercise, analgesics,

or antidepressants[56]. Factors such as existing challenges in diagnosing and treating fibromyalgia, as well as patients' reported feelings of lack of understanding by the medical care system/providers, might cause negative expectancies about treatment and pave the way for nocebo effects, which could further exacerbate existing symptoms[56–58]. Although experimentally-induced nocebo hyperalgesia has not yet been investigated in fibromyalgia, research in other chronic pain conditions such as chronic back pain, post-operative pain, and gastrointestinal disorders suggests that nocebo hyperalgesia can be induced by verbal suggestions of pain increase in these populations[8,59,60]. However, it remains to be investigated whether nocebo hyperalgesia can be experimentally induced in fibromyalgia, for example by conditioning and verbal suggestions, and whether this differs from nocebo hyperalgesia in healthy subjects. Fibromyalgia patients' relationship with pain treatment and the surrounding treatment context (e.g., doctor-patient relationship) might be more complex compared to healthy individuals, particularly due to the persistent nature of their physical symptoms. This could potentially result in stronger expectancies of adverse treatment outcomes (nocebo effects) in patients than in healthy individuals. Moreover, not much is still known about the potential role of nocebo hyperalgesia in fibromyalgia pain in daily life and nocebo-related pain progression over time. Through investigating nocebo hyperalgesia in a fibromyalgia population, specific treatment strategies can be targeted for a better prognosis with minimized pain progression.

Better understanding the impact of nocebo hyperalgesia on not just acute, but also on chronic pain can be useful for improving the long-term clinical practices surrounding chronic pain treatment. For instance, treatment-related expectancies might be improved through focusing on the doctor-patient alliance next to the existing treatment options. However, an ecologically-valid translation of experimental nocebo research is still needed to identify the potential role of nocebo hyperalgesia in pain (progression) in fibromyalgia.

Overview and aims of the current dissertation

The field of nocebo research is more recent than the field of placebo research and most experimental findings on nocebo effects have been established based on research with healthy individuals, particularly targeting pain. Findings in healthy individuals are essential for our fundamental knowledge on nocebo effects. Learning mechanisms behind their acquisition are far less understood in clinical populations, such as with chronic pain. Therefore, the current experimental learning procedures for inducing and maintaining (e.g., conditioning, verbal suggestions) as well as reducing (e.g., extinction, counterconditioning) nocebo hyperalgesia need further investigation in both healthy individuals and patients with chronic pain, such as with fibromyalgia. Moreover, identifying individuals susceptible to nocebo hyperalgesia, and the recovery therefrom, is important for implementing strategies for minimizing nocebo effects and for harnessing

the therapeutic benefits of treatment to prevent pain progression. Therefore, more research is needed to identify the predictors of nocebo hyperalgesia acquisition, recovery, and nocebo-related pain progression. Ultimately, this could help us better understand the daily pain of chronic pain patients and implement (preventive) intervention strategies for minimizing nocebo-related pain progression.

The main aims of the current dissertation are to experimentally investigate learning-based nocebo induction and reduction mechanisms in healthy and fibromyalgia populations, identify predictors of nocebo hyperalgesia acquisition and recovery, and to determine the extent of nocebo-related pain progression in fibromyalgia. To address these aims, first the (open-label) induction of nocebo hyperalgesia was tested in healthy participants using pressure pain, an ecologically-valid pain modality for musculoskeletal disorders such as fibromyalgia. Additionally, open-label counterconditioning was studied, next to extinction, as a novel intervention strategy for ethically reducing nocebo hyperalgesia. Here, we also sought to identify the predictors of nocebo hyperalgesia induction and the recovery therefrom in healthy participants. Next, in a separate lab study, group differences between patients with fibromyalgia and matched healthy controls were tested for the (closed-label) induction and reduction by extinction of nocebo hyperalgesia. The selection of the closed-label, instead of open-label, paradigm was intended to mimic the learning events as they occur in real life. Additionally, the stability and maintenance of the overall magnitude of these experimental effects was tested after one-month follow-up. As the final step, we expanded our research outside of the lab, where we studied via electronic diaries whether experimentally-induced nocebo hyperalgesia predicted change in diary-assessed fibromyalgia pain in daily life to better understand nocebo-related pain progression in fibromyalgia. In different chapters of the current dissertation, the following studies were conducted:

In **chapter 2**, we conducted a two-part RCT to investigate whether nocebo effects on pressure pain can be induced and reduced in healthy female participants. Specifically, we utilized open-label nocebo conditioning combined with open-label verbal suggestions about the pain-modulating function of a sham Transcutaneous Electrical Nerve Stimulation (TENS) device to induce nocebo effects on pressure pain. We compared this group with a sham-conditioning group to assess its efficacy. Subsequently, we tested the efficacy of open-label counterconditioning compared to open-label extinction and open-label continued nocebo conditioning (control) in reducing the previously induced nocebo effects.

Adding onto the results from **chapter 2**, in **chapter 3** a series of exploratory analyses were conducted in the same study to investigate individual differences in nocebo

hyperalgesia induction and reduction. We explored whether psychological characteristics (dispositional optimism, state and trait anxiety, pain catastrophizing, fear of pain, and body vigilance) predicted (open-label) nocebo hyperalgesia induction, and whether the same psychological characteristics and susceptibility to experimentally-induced nocebo hyperalgesia predicted nocebo hyperalgesia reduction.

In **chapter 4**, group differences were investigated in inducing and reducing nocebo hyperalgesia on experimental pressure pain in female patients with fibromyalgia and matched healthy controls. Nocebo hyperalgesia was induced via (closed-label) nocebo conditioning with verbal suggestions about the pain-increasing function of a sham TENS device and then reduced via extinction. One month later, the same experimental procedures were repeated in both groups to investigate the temporal stability of nocebo hyperalgesia and of extinction effects.

In **chapter 5**, we aimed to identify whether experimentally-induced nocebo hyperalgesia and diary-assessed expectancy-related factors (i.e., pain expectancy, anxiety, pain catastrophizing, and optimism) are associated with moment-to-moment pain progression in the same fibromyalgia sample as in **chapter 4**. Nocebo hyperalgesia magnitude measured in the lab (in **chapter 4**) was followed by 3 weeks of electronic diary measurements (ecological momentary assessment; EMA) where patients answered questionnaires on expectancy-related factors and pain intensity three times a day.

Finally, **chapter 6** summarizes our main findings and **chapter 7** provides a general discussion on the overarching aims and outcomes of the current dissertation. Also, limitations, implications for research and clinical practice, and considerations for future research directions are discussed.

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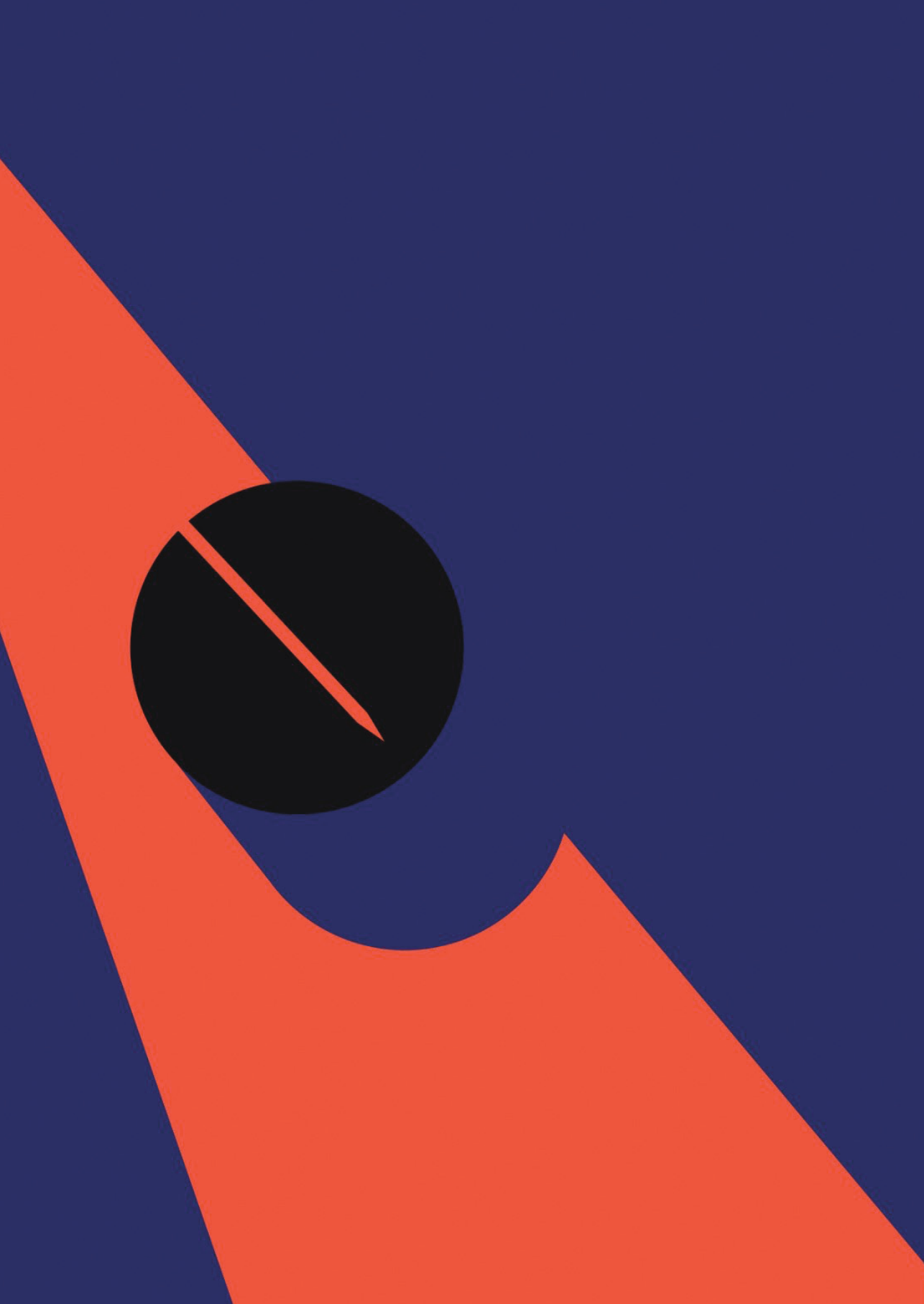

CHAPTER 2

Efficacy of open-label counterconditioning for reducing nocebo effects on pressure pain

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ABSTRACT

Background: Nocebo effects can adversely affect the experience of physical symptoms, such as pain and itch. Nocebo effects on itch and pain have shown to be induced by conditioning with thermal heat stimuli and reduced by counterconditioning. However, open-label counterconditioning, in which participants are informed about the placebo content of the treatment, has not been investigated, while this can be highly relevant for clinical practice. Furthermore, (open-label) conditioning and counterconditioning has not been investigated for pain modalities relevant to musculoskeletal disorders, such as pressure pain.

Methods: In a randomized controlled trial, we investigated in 110 healthy female participants whether nocebo effects on pressure pain combined with open-label verbal suggestions can be 1) induced via conditioning and 2) reduced via counterconditioning. Participants were allocated to either a nocebo or sham conditioning group. Next, the nocebo group was allocated to either counterconditioning, extinction, or continued nocebo conditioning; sham conditioning was followed by placebo conditioning.

Results: Nocebo effects were significantly larger after nocebo conditioning than sham conditioning (d = 1.27). Subsequently, a larger reduction of the nocebo effect was found after counterconditioning than after extinction (d = 1.02) and continued nocebo conditioning (d = 1.66), with effects similar to placebo conditioning (following sham conditioning).

Conclusions: These results show that (counter)conditioning combined with open-label suggestions can modulate nocebo effects on pressure pain, which provides promise in designing learning-based treatments to reduce nocebo effects in patients with chronic pain disorders, particularly for musculoskeletal disorders.

Keywords: nocebo effect, counterconditioning, pressure pain, open-label, counterconditioning, nocebo hyperalgesia

1. INTRODUCTION

It is well known that nocebo effects (i.e., adverse treatment outcomes not attributable to active treatment components) can be induced via learning mechanisms, including classical conditioning and suggestions [1–4]. Much less is known about methods to reduce nocebo effects and their translation to clinical care. First findings indicated counterconditioning to reduce nocebo effects for conditioned thermal pain and itch and may even lead to placebo effects [4,5]. During counterconditioning, the original unconditioned stimulus (US) (e.g., an increase of administered pain) to which a previously neutral stimulus (e.g., activation of a sham electrode) has been paired (conditioned stimulus; CS), is replaced by a US of opposite valence (e.g., decreased pain stimulation). First results have indicated that counterconditioning is more effective than extinction, during which the CS is no longer paired with the US, leading to people gradually learning the US and CS are no longer associated [4,5]. Although not examined yet, pressure pain could be a relevant pain modality in which to examine nocebo-learning strategies, since this elicits a deep tissue pain sensation similar to the pain patients with chronic musculoskeletal pain disorders experience [6,7].

Typically, deceptive (counter)conditioning paradigms have been used in experiments [3,4,8,9], which might lead one to think that deceptive methods are needed to treat nocebo effects. In clinical practice, however, patients need to be informed about their treatment, as deception could harm trust in the healthcare provider and treatment [10,11]. Therefore, it is difficult to translate current findings to clinical practice. A possible solution lies in open-label (counter)conditioning procedures, in which people are informed about using inert treatments, which could provide a non-deceptive opportunity for reducing nocebo effects. Although open-label placebos have been demonstrated to be effective [12–15], open-label nocebo conditioning has only been examined in one study (using itch) and open-label counterconditioning has not been examined. Furthermore, it is unclear whether placebo effects induced after counterconditioning are as strong as placebo effects induced without prior nocebo conditioning. It would therefore be relevant to investigate whether these findings can be replicated in a study using (pressure) pain and to also investigate open-label counterconditioning, as these findings may help develop new treatment opportunities for reducing nocebo effects in clinical care.

In the current study, we aimed to investigate the reduction of nocebo effects on pressure pain through open-label counterconditioning combined with open-label suggestions. We first tested whether a nocebo effect could be induced by open-label conditioning and suggestions by comparing nocebo conditioning with sham conditioning. Secondly, we tested whether counterconditioning works better than extinction, on which commonly-

used treatments (e.g., exposure treatment) are based. Counterconditioning was also compared to continued nocebo conditioning (which mimics a real-life situation in which people repeatedly have negative experiences), and placebo conditioning (to examine the influence of prior nocebo conditioning). We hypothesized that 1) nocebo conditioning induces a stronger nocebo effect than sham conditioning; 2) both counterconditioning and extinction reduce the nocebo effect in comparison to continued nocebo conditioning; and 3) counterconditioning yields a larger reduction than extinction. We further explored 4) whether placebo conditioning and counterconditioning successfully induce a placebo effect, and 5) whether this effect is larger after placebo conditioning than after counterconditioning. Investigating the effects of open-label counterconditioning on pressure pain in healthy participants builds onto prior knowledge on (closed-label) counterconditioning examined in other pain modalities and could provide a first step for new clinically applicable treatment strategies for chronic pain disorders.

2. METHOD

2.1 Ethics statement

This study was approved by the Psychology Research Ethics Committee of Leiden University (reference number CEP18-1114/442) and pre-registered in the International Clinical Trials Registry Platform (number NCT05284383). All participants gave written informed consent and were reimbursed by €15 in cash or study credits. The current paper reports on data from a study entailing different study aims; the current paper focusses on the efficacy of conditioning and counterconditioning for inducing and reducing nocebo effects on pressure pain, whereas in another paper the predictive value of several psychological characteristics, as well as nocebo susceptibility on the strength of the nocebo effect and its reduction will be discussed (M. Karacaoglu, S. Meijer, K.J. Peerdeman et al., unpublished data, October 2021).

2.2 Participants

The sample size required for our primary analysis was calculated using G*Power 3.1 for an independent samples t-test (two-sided, alpha = .05, desired power .80). The expected effect size was d = 0.73, based on a similar study on counterconditioning of nocebo effects[5]. According to the sample size calculation, 31 participants were needed per group. Since the design consisted of 4 groups in the second phase, we aimed for a total of 124 participants.

Participants were recruited through flyers at Leiden University and online via Facebook, as well as via the online recruitment system Sona (Sona systems, Tallin, Estonia).

All participants had to be female, between 18 and 35 years old, and have a good understanding of written and spoken Dutch. The counterconditioning procedure tested in the current study, once found to be effective in healthy participants, is intended to be used in future research with patients with fibromyalgia. As fibromyalgia is more prevalent in women[16], only female participants were tested in the current study, to avoid the possible influence of gender differences.

Exclusion criteria were severe somatic or psychiatric morbidity (e.g., heart/lung diseases, DSM-5 psychiatric disorders), Raynaud's disease, chronic pain complaints at present or in the past (≥ 3 months), current pain complaints (≥ 2/10 on Numeric Rating Scale (NRS)), current use of medication, injuries on the non-dominant hand, refusal/inability to remove nail polish or artificial nails on the thumbnail of the non-dominant hand for the experiment, color blindness, and pregnancy or breastfeeding. Participants were excluded from further participation if their sensory discrimination was poor, i.e., if they were unable to distinguish between three different pressure intensities or if a pain intensity of 4.5/10 on NRS was not reached at maximum pressure levels. Participants were asked not to consume alcohol, recreational drugs, painkillers, and/or sleep medication in the 24 hours prior to testing.

2.3 Design

A randomized controlled trial with a between-within subjects design was employed, consisting of two parts (Figure 1). In part 1 (nocebo induction), participants were randomly assigned (3:1) to the experimental group (open-label nocebo conditioning) or the control group (open-label sham conditioning). A randomization list was made by an independent person and group allocation was noted down on paper and inserted into an opaque envelope, which was opened after the pressure pain calibration procedure, to reduce experimenter bias during calibration. Since all experimental manipulations contained open-label verbal instructions, neither the experimenter nor the participant could be blinded to group allocation. In part 2 (nocebo reduction), participants from the experimental group were randomly assigned (1:1:1) to one of three groups: open-label counterconditioning, open-label extinction, or open-label continued nocebo conditioning. Participants in the control group underwent an open-label placebo conditioning procedure in part 2, to be able to compare the magnitude of placebo effects after counterconditioning (preceded by nocebo conditioning) to the magnitude of placebo effects after placebo conditioning (i.e., an identical procedure, but not preceded by nocebo conditioning).

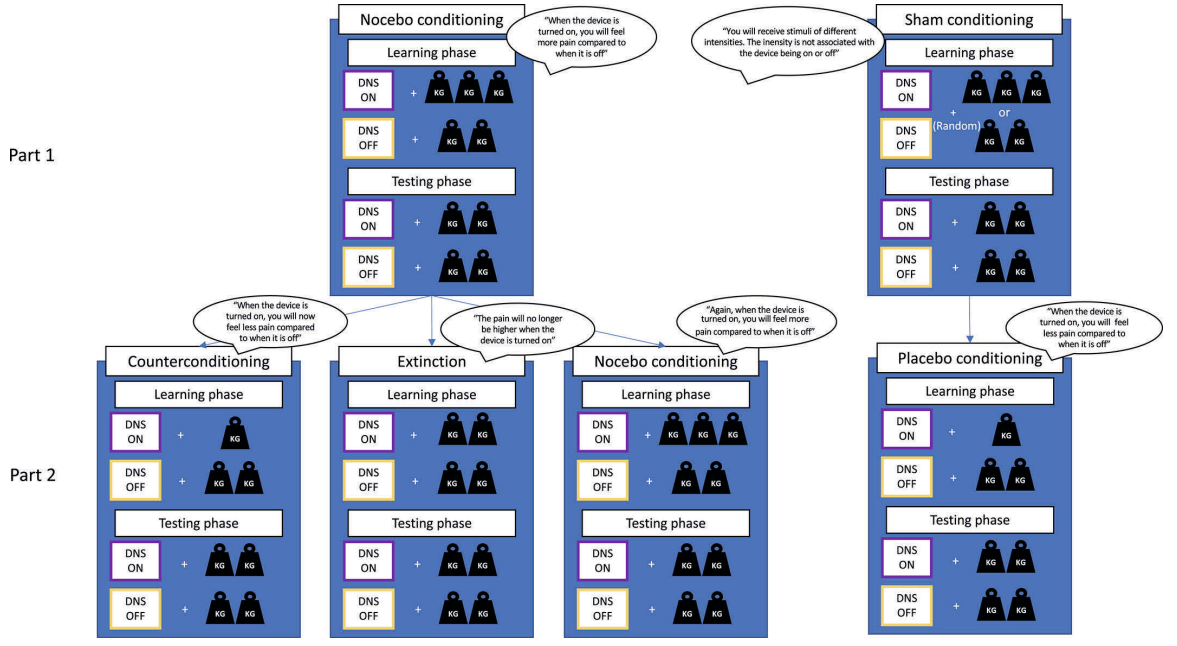

Figure 1. Overview of the study design. In part 1 (open-label nocebo induction), participants were randomly assigned (3:1) to the experimental group (nocebo conditioning) or the control group (sham conditioning). During the learning phase of nocebo conditioning, participants received moderate pain (4.5-5.5 on 0-10 Numeric Rating Scale; NRS) during 'DNS on' trials and slight pain (2-3 on 0-10 NRS) during 'DNS off' trials. The sham group received stimuli of slight and moderate intensity, not specifically associated with 'DNS on' or 'DNS off' (i.e., 10 moderate intensity and 10 slight intensity stimuli were randomly paired to the 20 trials). Both groups received slight pain stimuli for all trials in the test phase. In part 2 (open-label nocebo reduction), participants from the experimental group were randomly assigned (1:1:1) to one of three groups: counterconditioning, extinction, or continued nocebo conditioning. During counterconditioning, 'DNS on' trials were now paired with minimal pain (0-1 on 0-10 NRS) and 'DNS off' trials with slight pain in the learning phase. During extinction, all trials were paired with a slight pain intensity. Continued nocebo conditioning was identical to nocebo conditioning in part 1. Participants in the placebo conditioning group received minimal pain during 'DNS on' trials and slight pain on 'DNS off' trials, which is identical to the procedure of counterconditioning. The test phases in all groups in part 2 were identical to the test phases in part 1.

2.4 Pain induction

To induce pain, pressure pain stimuli were applied to the thumbnail of the non-dominant hand using a custom-made automated pneumatic stimulator, borrowed from the Karolinska Institute in Stockholm, Sweden[17]. The hand-piece of the stimulator (borrowed from Kings College London) has a plastic piston that applies pressure via a 1 cm^2 hard rubber probe. The handpiece has a cylinder opening where participants can insert their thumb, placed such that the probe contacts the middle of the thumbnail. The thumbnail was selected as a neutral location to repeatedly and safely deliver pressure stimuli as has been previously used and reported on for both healthy and clinical samples[17]. Pressure pain was chosen, because this more closely resembles the deep-tissue pain that patients with chronic musculoskeletal pain disorders experience in contrast to the more commonly used method of thermal pain, which relies on applying heat to the skin that leads to a burning sensation. Additionally, patients with fibromyalgia experience a sensitivity to pressure stimuli and applying pressure to certain "tender points" has previously been used in fibromyalgia diagnosis, although not a current criterion [7].

Pressure stimulus duration was set at 2.5 s, with an inter-stimulus interval of 30 s. The minimum intensity of pressure given was set at 50 kPa (≈5 N/cm^2 or 0.5 kgf), while the maximum was set at 850 kPa (≈85 N/cm^2 or 8.7 kgf).

2.5 Pressure pain calibration

A calibration procedure was conducted in order to find the optimal pressure intensity for minimal pain (0-1/10 NRS), slight pain (2-3/10 NRS), and moderate pain (4.5-5.5/10 NRS) for the individual participant, to be used in parts 1 and 2 of the experiment. A minimally painful pressure intensity (0-1 on the NRS) was also accepted for the lowest intensity, as slight sensitization was expected to occur due to the repeated administration of pressure, which could increase the minimally-painful rating above zero. Calibration consisted of three phases. In phase 1, an ascending series of pressure stimuli (50 kPa increments) was applied up to the first pressure intensity participants rated as ≥ 5.5. In phase 2, five different stimuli were applied three times in random order, ranging from the highest pressure intensity rated as 0 in phase 1 up to the highest pressure intensity rated between 4.5 and 5.5. If no pressure intensity during the ascending series was scored between 4.5 and 5.5, a formula was used to calculate the appropriate value (see appendix A). In phase 3, a calibration check was performed. The intensities for phase 3 were determined by taking the median of all intensities in phase 2 rated within the numeric ranges for no, slight, and moderate pain. If participants did not rate any intensity within one or more of the intended ranges, formulas were used again to inter- or extrapolate the intensity corresponding to the intended range of pain scores (see appendix A). The chosen final intensities were administered twice for minimal pain and moderate pain, and thrice for

slight pain. Participants were required to rate at least one out of two (or two out of three for slight pain) stimuli within the intended ranges. If this requirement was not met for any of the three intensities, again formulas were used to calculate the adjusted intensity (see appendix A). If manual adjustments were impossible (due to the requirement of less than the minimum or more than maximum amount of pressure), participants were excluded. In total, participants received up to 38 pressure stimuli during calibration.

2.6 Experimental procedures

2.6.1 Sham TENS device

During the experiment, a sham Transcutaneous Electrical Nerve Stimulation (TENS) device combined with a message indicating its (de)activation on a screen was used as a conditioning stimulus. Depending on the randomized group allocation, participants were taught a contingency between sham (de)activation of this device and the delivery of either a non-painful, slightly painful, or moderately painful pressure intensity by the pneumatic stimulator. To avoid potential interference by participants' possible previous experiences or knowledge on the functions of a TENS, the device was referred to as a Dermal Nerve Stimulation (DNS) device. Two electrodes were attached below each other on the radial side of the participants' non-dominant forearm. As part of the open-label nocebo and placebo induction, it was explained to participants that while the DNS device was sham and therefore inactive, their pain would still be influenced because of the nocebo or placebo effect, respectively. These suggestions were repeated right before the start of each part.

The messages indicating (de)activation of the device were presented to participants on a computer screen, in purple or yellow text (colors associated with either activation or deactivation were counterbalanced across participants). The messages were displayed for 3.5 s, starting 1s before the pressure was administered. Participants were instructed to keep paying attention to the screen. In between stimuli, a fixation cross was shown.

2.6.2 Nocebo-induction part

Nocebo conditioning consisted of a learning and testing phase (see Figure 1). In the learning phase, a button-press on the sham DNS device by the experimenter combined with a computer screen message in either purple or yellow indicating the activation of the DNS device ("DNS ON"), was repeatedly paired with a moderate-intensity pressure pain stimulus (pressure scored as 4.5-5.5 on 0-10 NRS for that participant), whereas the other-colored computer screen message indicating the deactivation of the sham DNS device ("DNS OFF") was repeatedly paired with a slight-intensity pressure pain stimulus (2-3 on 0-10 NRS). In total, the learning phase consisted of 10 experimental trials ("DNS

ON trials") and 10 control trials ("DNS OFF trials"), presented in a standard pseudorandom order (max 2 stimuli of the same trial type (experimental or control) could follow each other). The testing phase consisted of 3 experimental and 3 control trials in random order, all associated with a slight pressure pain intensity. Participants in the nocebo conditioning group were given open-label suggestions about the conditioning procedure and were told conditioning would be used to teach them that the activation of the sham DNS device will increase their pain sensitivity, by manually increasing the intensity of pressure stimuli after experimental trials. The precise verbal suggestions can be found in the supplementary materials.

Sham conditioning deviated from nocebo conditioning only in that pressure intensity was now not associated with sham DNS (de)activation, but randomly paired. For that, a random sequence was created for the 20 pain stimuli (10 slight-intensity stimuli and 10 moderate-intensity stimuli), while the order of the messages ("DNS ON" and "DNS OFF") was identical to nocebo conditioning. Again, max 2 stimuli of the same trial type (experimental or control) could follow each other. Furthermore, participants were explicitly told there was no association between the DNS messages and the pain stimuli.

2.6.3 Nocebo-reduction part

For all groups, the learning phase of part 2 consisted of 20 trials (10 experimental and 10 control trials) and the testing phase was identical to the testing phase for the nocebo-induction part.

The counterconditioning procedure differed from nocebo conditioning in part 1 such that a non-painful pressure stimulus (0-1 on a 0-10 NRS) instead of a moderate-intensity pressure pain stimulus now followed the "DNS ON" message. Again, participants were given open-label suggestions about the counterconditioning procedure and were told counterconditioning would be used now to teach them that the activation of the sham DNS device now decreases their pain sensitivity.

In the extinction procedure, only slightly painful stimuli were given during all trials, in both the learning and the testing phase. Participants were given open-label suggestions about the extinction procedure and were told that the pressure stimuli were no longer manually increased after the CS, to teach them that the activation of the DNS does not influence their pain anymore.

While both counterconditioning and extinction could decrease the nocebo effect, the main difference between the methods is that during counterconditioning the pain intensity paired with experimental trials is actively decreased (to below the level of pain during

control trials), which thus resembles a more active strategy of reducing nocebo effects. During extinction, the pain intensity is identical to the intensity during control trials. This is comparable to either a gradual decrease of a nocebo effect without treatment, or therapies such as exposure, where repeated exposure decreases a certain negative association.

The procedure for the continued nocebo conditioning group in part 2 was identical to nocebo conditioning in part 1 of the experiment and this procedure mimics a real-life situation in which people repeatedly have negative experiences.

The procedure for placebo conditioning was identical to the counterconditioning procedure, apart from following sham conditioning instead of nocebo conditioning in part 1 of the experiment and a slight difference in the verbal suggestions given. Participants were told placebo conditioning would be used to teach them that the activation of the sham DNS device decreases their pain sensitivity. Placebo conditioning mimics a placebo treatment without people negative experiences prior to this treatment (e.g., no existing nocebo effects prior to the placebo treatment).

2.7 Self-report ratings

A questionnaire including demographic and health questions was used to screen participants for inclusion. Furthermore, several validated questionnaires were used to measure baseline psychological characteristics, which is elaborated on further in a separate article as this concerns different study aims (Karacaoglu, Meijer, Peerdeman et al., unpublished data, October 2021). During the experiment, experienced pain intensity was reported after each pressure stimulus on an NRS, ranging from 0 (no pain) to 10 (worst pain imaginable). Participants were allowed to use decimals while scoring their pain. An exit questionnaire consisted of questions on 1) what participants thought the aim of the experiment was (open-ended); 2) level of focused attention during the experiment (0-10 NRS; higher score indicates more focused attention); 3) experienced pain during experimental trials in part 1 (on a scale of 0-10, with 0 indicating less pain compared to control trials, 5 indicating equal pain and 10 indicating more pain compared to control trials, 4) experienced pain during experimental trials in part 2 (on the same scale as question 3), 5) trustworthiness of the experimenter (on a scale of 0-10, with a higher score indicating more trustworthiness), 6) competence of the experimenter (on a scale of 0-10, with a higher score indicating more competence), and 7) whether participants adjusted pain ratings during the experiment to help the experimenter (on a scale of 0-10, with a higher score indicating a higher amount of adjusted answers and thus a response bias). Baseline and exit questionnaires were filled in using Qualtrics software (Qualtrics, Provo, Utah, United States). NRS scores were verbally communicated to the

experimenter, who noted the scores down using an Excel form (Microsoft Corporation, Redmond, United States).

2.8 Experimental procedure

The experiment was conducted in a single session and took approximately two hours, with 5-minute breaks in-between the different parts of the calibration procedure and a 10-minute break between parts 1 and 2 of the experiment. During the experimental procedure, the experimenter always followed a detailed standardized script to ensure procedures for each participant resembled each other closely. After the procedure was explained, participants signed the informed consent form. If participants were eligible based on the screening questions, participants completed all baseline questionnaires. Individual pressure pain levels were then calibrated. Next, part 1 of the experiment commenced (nocebo-induction part), followed by part 2 (nocebo-reduction part). Finally, participants completed the exit questionnaire and were debriefed and compensated for their participation.

2.9 Statistical analyses

All data were analysed using SPSS 25.0 (IBM SPSS Statistics, Chicago, Illinois, USA). Assumptions of all statistical tests were checked through examination of histograms, Shapiro-Wilk tests, Levene's tests, and boxplots. In case of violation, we used a bootstrapping approach or non-parametric tests. The threshold of significance was set at $p < 0.05$, unless stated otherwise. One-way ANOVAs were used to assess between-group differences in calibration values, ability to focus during testing, trust in experimenter, perceived competence of the experimenter, and response bias. An overview of all analyses written below can also be found in the supplementary materials (Appendix C).

2.9.1 Nocebo induction

To examine whether a significant nocebo effect was induced after nocebo conditioning and sham conditioning, two paired samples t-tests were performed separately within the nocebo-conditioning group and within the sham-conditioning group. For this, the average NRS score of experimental trials was compared with the average NRS score after control trials in testing phase 1.

Then, to test whether the induced nocebo effect was stronger after nocebo conditioning than after sham conditioning, an independent samples t-test was used to compare the induced *nocebo effect* (defined as a difference score between the average NRS score on all 3 experimental trials and the average NRS score on all 3 control trials in testing phase 1) between the nocebo-conditioning group and the sham-conditioning group. A Bonferroni correction was applied to correct for multiple testing and threshold for significance was set at $p < 0.017$.

2.9.2 Manipulation checks

As a manipulation check to see whether the sham conditioning was actually perceived as sham, two paired-samples t-tests were conducted to examine whether experimental trials were on average rated significantly different from control trials during the learning phase of both nocebo and sham conditioning. Additionally, in the nocebo-conditioning group, it was checked whether a difference between experimental and control trials in the test phase were actually due to increased NRS scores during experimental trials, instead of decreased scores during control trials because they think that there should be a difference with the experimental trials. This was done by comparing the average rating of the final control trial from the learning phase with the first control trial in the testing phase, using a paired samples t-test.

2.9.3 Nocebo reduction within groups

In the nocebo-reduction part of the experiment, to determine whether the reduction of the nocebo effect within each group following nocebo induction was significant, three one sample t-tests were performed. *Nocebo reduction* was defined as a difference score between the nocebo effect in testing phase 1 of the nocebo-induction part of the experiment and the nocebo effect in testing phase 2 of the nocebo-reduction part (the nocebo effect in part 2 was subtracted from the nocebo effect in part 1). In each group, the amount of reduction of the nocebo effect was compared to 0, as a significant (positive) deviation from 0 indicates a significant amount of change and thus reduction of the nocebo effect. A Bonferroni correction was applied to correct for multiple testing and threshold for significance was set at $p < 0.013$.

2.9.4 Nocebo reduction: Group differences

Then, we examined whether any differences existed in nocebo reduction between the counterconditioning, extinction and continued nocebo conditioning groups. Since we were only interested in the pairwise comparisons between groups (and specifically the group x time interaction), we conducted three separate 2x2 mixed-model ANOVAs and used Bonferroni to correct for multiple comparisons (i.e. we tested our effects of interest against alpha < .017). These tests compared the interaction of group (1. Counterconditioning vs extinction, 2. Counterconditioning vs continued nocebo conditioning, and 3. Extinction vs continued nocebo conditioning) and time (nocebo effect after part 1 vs nocebo effect after part 2).

Finally, speed of reduction of the nocebo effect by counterconditioning and extinction were compared by examining the interaction between group (counterconditioning and extinction) and time (all 10 experimental trials in the learning phase of part 2) using a mixed ANOVA.

2.9.5 Sensitivity analyses

Sensitivity analyses were conducted to assess the influence of excluding participants for whom no nocebo effect had been induced in phase 1 (i.e., participants for whom the difference between experimental vs control trials in testing phase part 1 was zero or positive) from all analyses on nocebo reduction, as for these participants, there was no nocebo effect to be reduced, which may lead to incorrect inferences on the effects of counterconditioning).

2.9.6 Placebo induction

To test whether a placebo effect could be successfully induced by placebo conditioning (following sham conditioning), a paired samples t-test was performed for the placebo-conditioning group, to test whether the average NRS score on the experimental trials significantly differed from the average NRS score on the control trials during the testing phase of placebo conditioning. Subsequently, a paired samples t-test was performed for the counterconditioning group to test whether a placebo effect was induced after counterconditioning, followed by an independent samples t-test to explore whether placebo effects induced after sham conditioning (placebo-conditioning group) are stronger than placebo effects induced after nocebo conditioning (counterconditioning group). If no difference was found, an equivalency test was run, using the "two one-sided tests" (TOST) approach [18]. The upper and lower equivalence bound were based on the smallest effect size of interest, which was $d = 0.5$ (a medium effect size). Then the 90% confidence interval (CI) for the effect size of the difference between the placebo effect in the counterconditioning group and the placebo conditioning group was calculated, to determine whether the 90% CI fell within the previously established range (which would indicate equivalency).

3. RESULTS

3.1 Participants

Participants were recruited from December 2018 to March 2020. Out of 166 enrolled participants, 56 participants were excluded. Seven were excluded because of fulfilling one of the health-related exclusion criteria, 46 had a too high pain threshold (i.e., they did not reach a moderate pain level during calibration), 2 were excluded because they sensitized during conditioning (1 from nocebo-conditioning group, 1 from sham-conditioning group, part 1) and reported too high pain levels to continue the experiment, and 1 was excluded due to technical difficulties during the experiment. Due to the COVID-19 pandemic, it was decided to end the study prematurely and not to continue to reach the powered 124 participants, as data collected during the pandemic was not considered to be comparable

to previously collected data, due to the additional safety measures (e.g., participant and researcher wearing masks, having a different lab set-up to ensure enough distance between participant and researcher).

In total, 110 participants were included in the final analyses of part 1, whereas 108 participants were included in the analyses of part 2. A flowchart of participant inclusion and exclusion, as well as group allocation is displayed in Figure 2. Descriptive data of calibration values and exit questionnaire scores are displayed in Table 1. During screening, 9 people reported having current pain complaints (of lower than 2 on a 0-10 NRS), which was reported to be either muscle soreness from working out, or mild menstrual pain. No significant differences were found between groups for calibration values, trust in experimenter, perceived competence of the experimenter, and response bias. The nocebo-conditioning group and sham-conditioning group did differ significantly on the self-reported amount of experienced pain (on average) on experimental trials compared to control trials in part 1, as the nocebo-conditioning group reported to have felt more pain after experimental trials than the sham-conditioning group. This indicates that participants perceived the experimental procedures in the expected way. Furthermore, regarding part 2, all groups differed significantly on the self-reported amount of experienced pain (on average) on experimental trials (in comparison to control trials) in part 2, except for the counterconditioning group and placebo-conditioning group, as participants in both groups reported to have felt less pain after experimental trials. In the extinction group, no difference was reported and in in the continued nocebo conditioning group, participants reported to have felt more pain during experimental trials. This indicates all procedures were perceived by participants as intended.

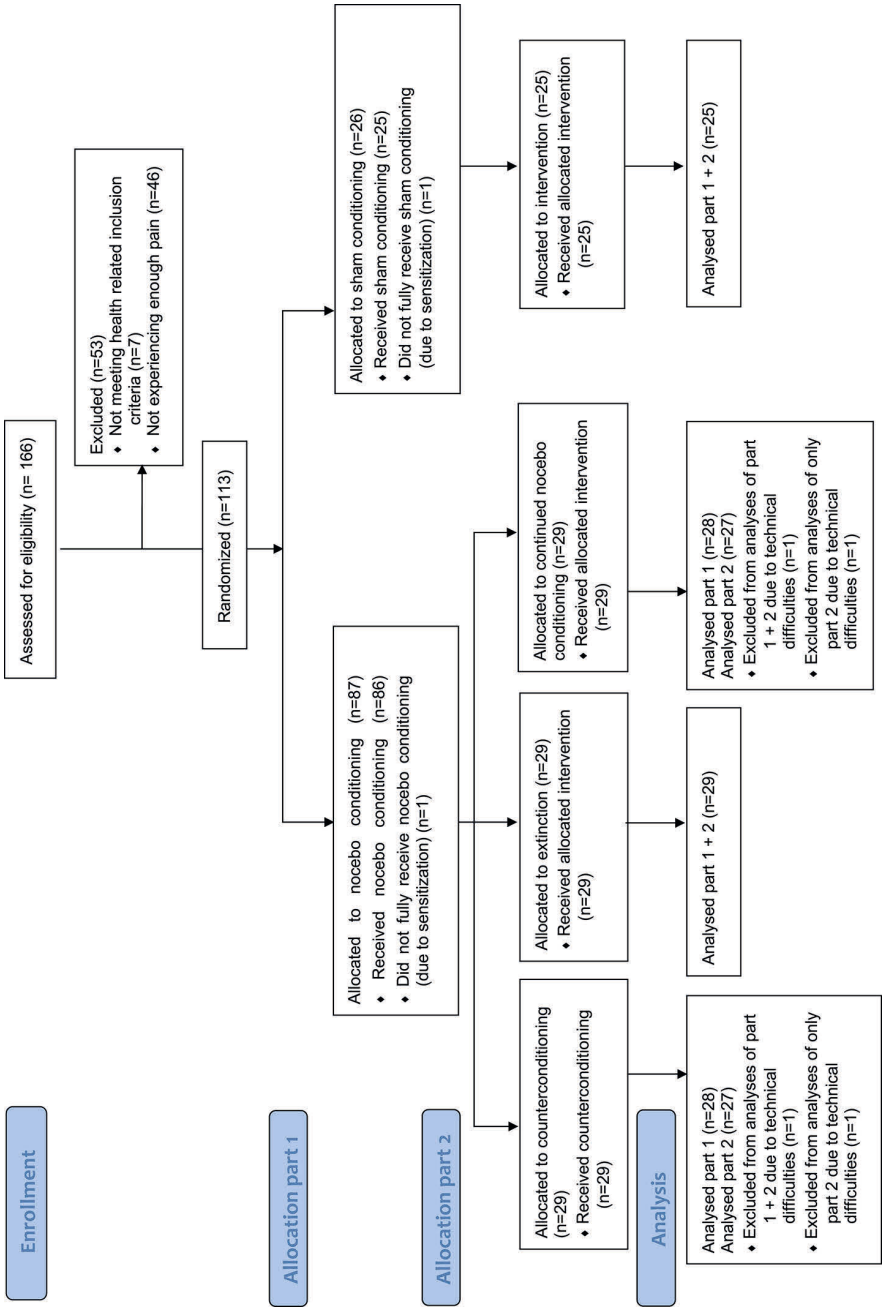

Figure 2. Flow diagram of the RCT.

Table 1
Group means and SDs for calibration values (preceding randomization) and exit questionnaires.

					Subgroups nocebo conditioning					
	Nocebo Conditioning (n=85)		Sham/Placebo Conditioning (n=25)		Counterconditioning (n=27)		Extinction (n=29)		Continued Nocebo Conditioning (n=27)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Calibrations										
No pain (kPa)	182.36	86.72	176.32	84.77	173.63	76.95	176.30	84.61	180.56	78.55
Slight pain (kPa)	356.30	122.12	351.10	108.54	342.45	109.06	336.79	124.05	372.52	118.06
Moderate pain (kPa)	570.79	180.84	572.14	157.76	550.46	172.10	519.05	176.96	622.29	166.74
Exit questionnaire										
Trust in experimenter	8.73	1.30	8.53	1.37	8.60	1.37	8.48	1.45	9.17	0.94
Competence experimenter	8.68	1.43	8.58	1.40	8.15	1.65	8.78	1.31	9.04	1.26
Response bias	0.70	1.26	0.70	0.99	0.68	1.21	0.74	1.33	0.68	1.30
Experienced pain intensity during “DNS on” trials as compared to “DNS off” trials in part 1[a]	2.56	1.23	1.91	1.89	2.81	0.94	2.46	1.28	2.54	1.19
Experienced pain intensity during “DNS on” trials as compared to “DNS off” trials in part 2[a]	-	-	-2.52	1.53	-2.50	1.75	0.06	1.19	2.00	1.49

Abbreviations: DNS, Dermal Nerve Stimulation; SD, standard deviation.
[a] Displayed as amount of deviation from no difference between ON and OFF trials, with a positive score indicating a higher amount of pain during DNS on trials compared to DNS off trials and a negative score indicating a lower amount of pain during DNS on trials compared to DNS off trial

3.2 Induction of the nocebo effect

The mean ratings on experimental and control trials during the testing phase of conditioning and sham conditioning are displayed in Table 2 and Figure 3. We found a significant difference between experimental and control trials in the testing phase of nocebo conditioning; $t(84) = 12.10$, $p < .001$, $d = 1.31$, as well as in the testing phase of sham conditioning; $t(24) = 3.34$, $p = .003$, $d = 0.67$, indicating both procedures led to a nocebo effect;. However, as hypothesized and as displayed in Figure 3, the nocebo effect was significantly larger in the nocebo conditioning group than in the sham conditioning group ($t(84.33) = 6.82$, $p < .001$, $d = 1.27$).

As a manipulation check, we tested whether experimental trials were on average rated significantly differently from control trials during the learning phase of both nocebo and sham conditioning. NRS scores during experimental trials were significantly higher than during control trials ($t(84) = 22.10$, $p < .001$) during nocebo conditioning, consistent with the difference in pressure intensity. As expected, during sham conditioning, experimental trials were not rated significantly higher than control trials ($t(24) = -1.53$, $p = .138$), consistent with the fact that the different pressure intensities were not specifically paired with either experimental or control trials. Additionally, no significant differences were found in the nocebo-conditioning group between the final control trial of the learning phase and the first control trial of the testing phase ($t(84) = -0.13$, $p = 0.942$), indicating that the induced nocebo effect in the testing phase of nocebo conditioning was driven by a higher pain score during the experimental trials, instead of a lower pain score after the control trials.

Table 2

Group means and SDs for reported pain during the learning and testing phase of nocebo induction and reduction, as well as for the magnitude of the nocebo effect and the reduction of the nocebo effect.

	Part 1				**Part 2**							
	Nocebo Conditioning (n=85)		**Sham Conditioning (n=25)**		**Counter-conditioning (n=27)**		**Extinction (n=29)**		**Continued Nocebo Conditioning (n=27)**		**Placebo Conditioning (n=25)**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Nocebo induction/reduction Learning phase												
NRS experimental trials	5.32	1.04	4.20	0.87	0.72	0.76	3.56	1.51	5.74	1.25	1.17	0.99
NRS control trials	2.73	1.09	4.33	0.87	3.79	1.36	3.08	1.43	3.01	1.26	4.01	1.18
Testing phase												
NRS experimental trials	4.04	1.46	3.46	1.43	3.15	1.32	3.49	1.73	4.30	1.54	3.47	1.34
NRS control trials	2.78	1.44	3.16	1.39	3.80	1.37	2.99	1.65	3.03	1.55	4.08	1.55
Nocebo effect	1.26	0.96	0.31	0.46	-0.65	0.74	0.51	0.74	1.27	1.17	-0.62	1.00
Reduction of nocebo effect (part 1 - part 2)	-	-	-	-	1.98	1.47	0.77	0.90	-0.01	0.90	-	-

Abbreviations: NRS, Numeric Rating Scale; SD, standard deviation

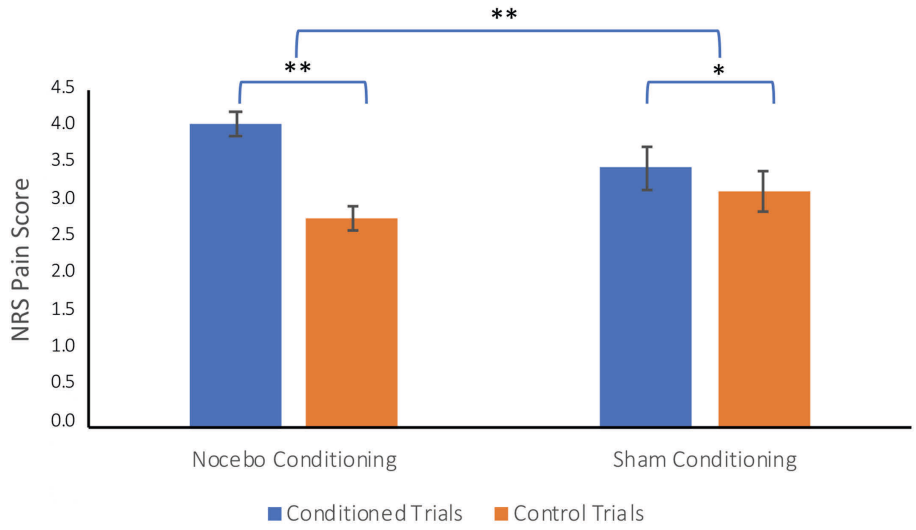

Figure 3. Average NRS ratings and Standard Error of the Mean (SEM) of all 3 experimental trials and control trials during the testing phase of nocebo conditioning and sham conditioning. Both for nocebo conditioning and sham conditioning, experimental trials were rated as significantly more painful than control trials. The magnitude of the nocebo effect was significantly larger in the nocebo conditioning group than in the sham conditioning group. * $p < .01$, ** $p < .001$ (two-tailed)

3.3 Reduction of the nocebo effect

The mean reduction in each group is shown in Figure 4. The nocebo effect was effectively reduced by both counterconditioning ($t(26) = 6.77$, $p < .001$, $d = 1.35$) and extinction ($t(28) = 4.60$, $p < .001$, $d = 0.85$), whereas continued nocebo conditioning showed no significant change in the nocebo effect compared to part 1 ($t(26) = -.047$, $p = .963$, $d = -.01$).

A 2x2 mixed model ANOVA showed a significant interaction between group (counterconditioning vs extinction) and time (nocebo reduction); $F(1,54) = 14.06$, $p < .001$, $d = 1.02$), indicating a significantly larger reduction of the nocebo effect after counterconditioning compared to extinction. Another 2x2 mixed model ANOVA showed a significant interaction between group (counterconditioning vs continued nocebo conditioning) and time ($F(1,52) = 36.01$, $p < .001$, $d = 1.66$), indicating a significantly larger reduction of the nocebo effect in the counterconditioning group compared to continued nocebo conditioning. Finally, the last 2x2 mixed model ANOVA also showed a significant interaction between group (continued nocebo conditioning vs extinction) and time ($F(1,54) = 10.51$, $p = .002$, $d = .86$), which indicated a significantly larger reduction in the extinction group compared to continued nocebo conditioning. For this analysis, our assumption of homogeneity of variances was violated, thus the data in both groups was transformed by taking the 10log(nocebo effect + 10), after which the assumption was

met. Since this led to highly similar results as the original analysis, the results of analysis using non-transformed data was used, to stay closest to the original data.

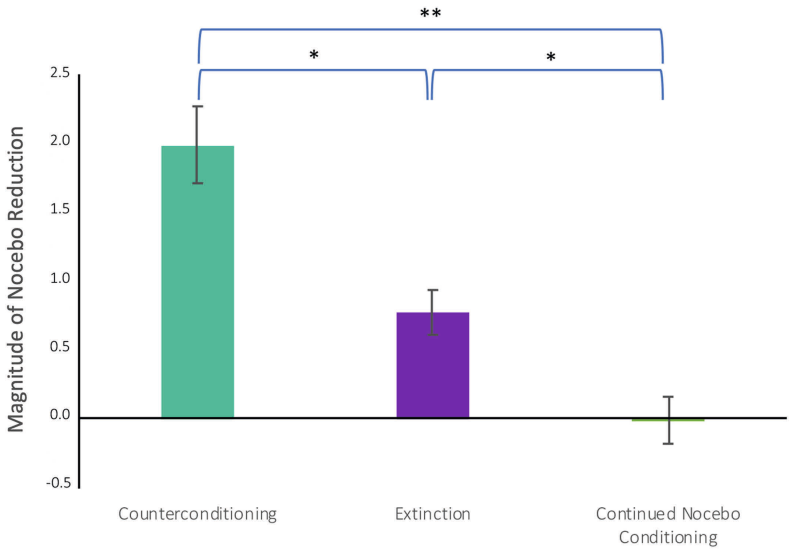

Figure 4. Level of nocebo reduction from the testing phase of nocebo induction to the testing phase of nocebo reduction. Means and SEM's are depicted across the three groups. Both counterconditioning and extinction led to a significant reduction of the nocebo effect, whereas continued nocebo conditioning did not. Counterconditioning led to a significantly larger reduction than extinction and continued nocebo conditioning; extinction also led to a larger reduction than continued nocebo conditioning * $p < .01$, ** $p < .001$ (two tailed)

3.5 Induction of a placebo effect

Reduction of the NRS score during experimental trials is displayed in Figure 5. Speed of reduction did not differ between counterconditioning and extinction, as no significant interaction between group and time (10 experimental trials in learning phase of part 2 of the experiment) was found ($F(5.04) = 0.395$, $p = .853$).

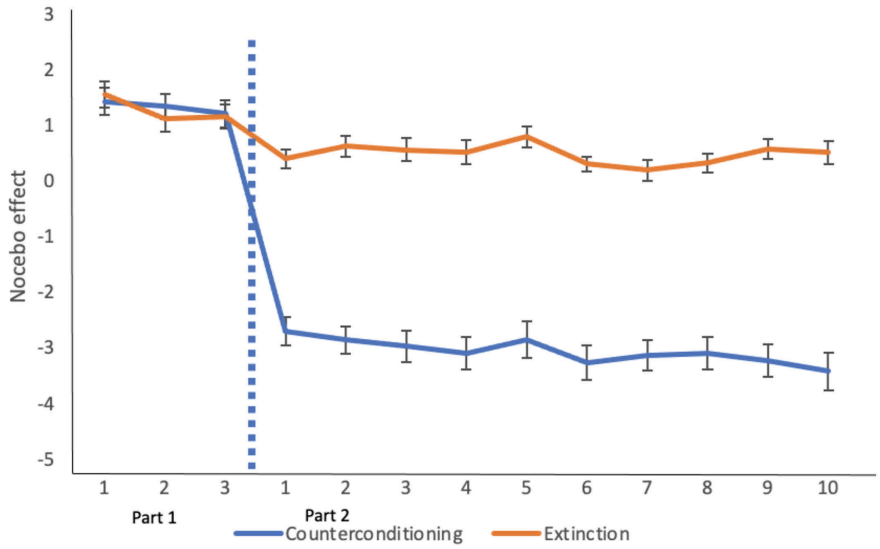

Figure 5. Average nocebo effects and SEM's throughout the testing phase of part 1 and the learning phase of part 2 are displayed for counterconditioning and extinction. The first 3 trials represent the difference in pain between experimental and control trials during the testing phase of part 1, while the next 10 trials represent the difference in pain between experimental and control trials in the learning phase of part 2 (separated by the vertical line). While counterconditioning shows the largest reduction, the speed of reduction does not differ between the groups.

3.4 Sensitivity analyses

After exclusion of 6 participants whom did not show a nocebo effect after part 1 (i.e., 7.2% of the nocebo-conditioning group; 4 showed no change, 2 showed a change in the opposite direction), all analyses on nocebo reduction yielded the same conclusions.

A placebo effect was successfully induced in both the placebo-conditioning group ($t(24) = -3.09$, $p = .005$, $d = -.63$) and the counterconditioning group ($t(26) = -4.42$, $p < .001$, $d = -.84$), since the average NRS rating on experimental trials was significantly lower than on control trials.

The strength of the placebo effect did not differ significantly between the placebo conditioning group ($M = -0.65$ $SD = 0.99$) and the counterconditioning group ($M = -0.62$ $SD = -0.75$; $t(50) = -.14$, $p = .887$, $d = .04$). Equivalency testing showed both groups to be equivalent in terms of the strength of the placebo effect, as the confidence interval (90% CI [-.417, .496] lies within the predetermined range.

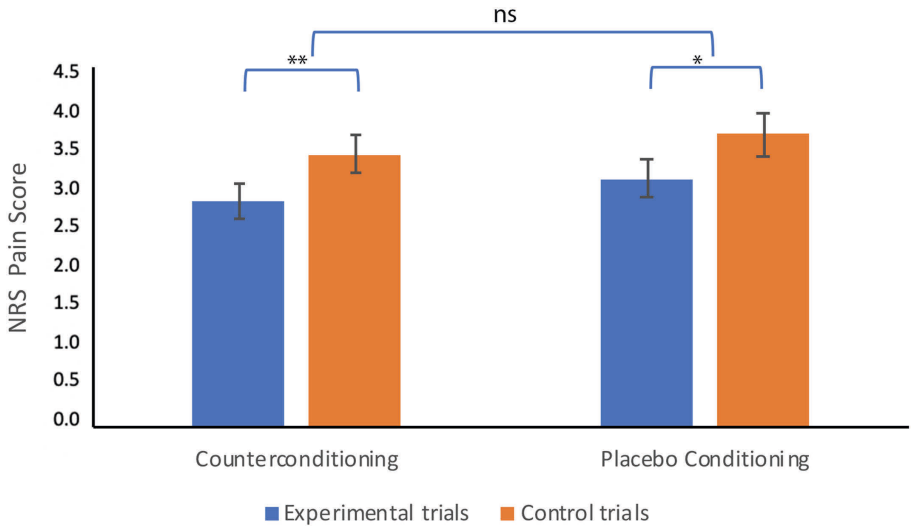

Figure 6. Average NRS ratings and SEMs of all 3 experimental trials and control trials during the testing phase of counterconditioning and placebo conditioning. Both for counterconditioning and placebo conditioning, experimental trials were rated as significantly less painful than control trials. No significant difference in the magnitude of the placebo effect was detected; * $p < .01$, ** $p < .001$, ns = $p \geq 0.05$ (two-tailed).

4. DISCUSSION

The current study investigated the efficacy of open-label nocebo conditioning, and of open-label counterconditioning and open-label extinction on the reduction of induced nocebo effects, using the pain modality of pressure pain. We demonstrated that open-label conditioning can induce a nocebo effect on pressure pain, as participants rated more pain than was actually administered during the test phase of nocebo conditioning. Furthermore, both open-label counterconditioning and extinction combined with suggestions were found to reduce nocebo effects. Both strategies led to an immediate reduction of the nocebo effect from the start of the procedure, instead of a gradual decrease. Counterconditioning yielded a larger reduction than extinction. Counterconditioning not only reduced nocebo effects, but induced a similar level of conditioned placebo analgesia as placebo conditioning (preceded by sham conditioning), as participants rated less pain than was actually administered.

In line with previous research on closed-label nocebo conditioning [1,3,4], open-label conditioning with verbal suggestions effectively induced a nocebo effect. This shows that conditioning is effective even when there is honesty about the procedure, which supports previous findings on the efficacy of open-label nocebo conditioning on itch [19] and of

the use of open-label placebos in clinical trials [12–15]. The current study was the first to show open-label induction of nocebo effects on pressure pain. The use of pressure pain, compared to commonly used methods like thermal and electrical pain, can be beneficial when designing (counter)conditioning-based treatments in patients with musculoskeletal pain conditions, as it more closely taps into the specific sensitivity to pressure and mimics the real-life experience.

As for nocebo reduction, this study was the first to use an open-label counterconditioning procedure to reduce nocebo effects. In line with studies using closed-label procedures [4,5], counterconditioning was found to effectively reduce nocebo effects and to be more effective than extinction. Open-label counterconditioning fully extinguished the nocebo effect and produced a placebo effect. While open-label extinction led to a significant reduction of the nocebo effect, this reduction was smaller than after counterconditioning and the nocebo effect was not fully extinguished (as participants still experienced slightly more pain during experimental trials). This finding slightly contradicts studies showing nocebo effects cannot be reduced by extinction, but it does provide further support for nocebo effects being resistant to complete extinction (i.e., no longer experiencing more pain during experimental trials than during control trials) [3,5,9,20]. It should be kept in mind that the current study used open-label conditioning to induce nocebo effects. This typically is not the case outside of an experimental environment, as pain is not deliberately associated with certain stimuli and people are not aware of being conditioned. Therefore, conditioning in daily life more closely resembles closed-label conditioning, during which people are not informed they are being conditioned. As more resistance to extinction was found in closed-label studies [3,8,9,20] compared to the current study, we should keep in mind that nocebo effects in the real-world context may be more resistant to extinction. This highlights the importance of finding new ways to reduce nocebo effects, such as counterconditioning. A possible explanation for less resistance to nocebo reduction compared to closed-label procedures, could be because possibly no (or little) fear towards the CS was induced during conditioning due to the open-label nature of the study. While trial-by-trial fear (or fear after hearing the suggestions) was not assessed in the current study, the open-label procedure was more predictable than traditional closed-label paradigms, which in turn could lead to participants feeling less anxious about the CS and the pain associated with it. A recent study has shown fear to play an important role in the induction and amplification of nocebo effects, as a larger amount of self-reported fear predicted a larger nocebo effect [21]. Furthermore, several studies using pain conditioning have shown that fear regarding the painful stimuli may arise as a result of conditioning [22–24]. Future studies on open-label (counter)conditioning and extinction should take the possible influence of fear into account, to be able to compare the results of open- and closed-label (counter)conditioning and/or extinction better.

Another explanation for these findings could be the open-label instruction itself, as during closed-label procedures, even when closed-label verbal suggestions are added, it is not mentioned that the amount of administered pain is adjusted by the experimenter. During open-label counterconditioning and extinction, it is specifically told that the experimenter will no longer increase the pain or will lower the pain intensity, meaning there is little to no uncertainty regarding the administered pain. This is supported by our finding that speed of reduction did not differ in the counterconditioning and extinction groups. Typically, conditioned effects extinguish gradually during extinction, but our results showed a decrease right after the open-label instructions that suggested the extinction of pain increase. This illustrates the interaction between conditioning and the role of verbal suggestions in pain regulation [25] and the rapid extinction could thus be due to the explicit suggestion that pain would no longer be increased after presentation of the CS. This indicates that the influence of the provided verbal suggestions could be stronger than the counterconditioning or extinction procedure itself. Bajcar et al., 2021 found that the order of procedures (conditioning vs suggestions) matters: when incongruent verbal suggestions were given after a conditioning procedure, the suggestions and not the conditioning determined the placebo effect. Furthermore, cue validity studies have shown that expectations regarding a certain stimulus, as well as how painful this stimulus is perceived can change on a trial-by-trial basis, because of the use of different cues (i.e. a low or high tone) [27]. While in our experiment the cue itself is altered (the meaning of “DNS on” is changed from part 1 to part 2), this does indicate that pain experience can be subject to sudden changes, which our results would support. Our verbal instructions during nocebo reduction may have been more dominant than the preceding nocebo-conditioning procedure, which could explain why the nocebo effect was reduced right from the start of counterconditioning and extinction. Nevertheless, the reduction of the nocebo effect may have been strengthened by the counterconditioning or extinction procedure that followed. It could be relevant to compare the effects of counterconditioning and/or extinction with and without open-label verbal suggestions, to better disentangle the effects of the individual learning mechanisms

Summarized, in an open-label lab context, counterconditioning and extinction can both reduce nocebo effects, with counterconditioning fully reducing nocebo effects and inducing a placebo effect and extinction only partially reducing the nocebo effect. It is however to be researched whether these findings can be replicated in closed-label and/or clinical settings. In future studies, it would be relevant to investigate whether open-label counterconditioning can also effectively reduce nocebo effects induced by closed-label conditioning, since this better resembles real-world settings, and to compare open- and closed-label procedures. This can give more insight into the separate effects of learning mechanisms (i.e., conditioning and verbal suggestions) and the open-label aspect of the

procedure. While one previous study did not find any differences in efficacy between open- and closed-label conditioning [19], differences between open- and closed-label counterconditioning have not been researched.

Importantly, our findings suggest that counterconditioning can not only reduce a nocebo effect, but even produce as strong a placebo effect as could be induced without prior negative learning experiences. However, the lack of induced fear and the open-label nature of the study could have made it easier to reduce the established nocebo effects, meaning it is important to replicate these findings in a study inducing nocebo effects in a closed-label fashion.

The effectiveness of open-label counterconditioning in an experimental setting is promising for clinical practice, as it could offer a new treatment strategy for reducing nocebo effects, while remaining fully transparent to patients. However, it remains to be researched whether the current findings in healthy participants will also be found in chronic pain patients. Several factors can influence the rise of nocebo effects in patients with chronic pain. Conditioning effects, such as the association of a doctor's white coat with a painful treatment, but also verbal cues, such as information on certain painful side effects, can lead to negative expectations regarding a treatment or (the development of) symptoms and thus a nocebo effect [28]. Additionally, patients have shown to have an attentional bias towards pain information [29], which could further increase the chance of negative expectations. Potentially, open-label procedures could be extra effective in altering these kinds of naturally-occurring expectations, as open-label suggestions are very explicit and may shift the attentional focus of patients towards pain reduction because of specifically mentioning pain will be manipulated. These expectations of pain reductions are then further validated by the counterconditioning procedure itself (during which pain is actually lowered). As mentioned above, closed-label procedures leave some uncertainty regarding pain levels, which may lead patients to focus on the pain they previously experienced, while in open-label procedures this may be less likely. Nevertheless, those suffering from chronic pain might still respond less to counterconditioning than healthy controls, due to multiple negative treatment experiences in the past [30]. Alternatively, patients may have a stronger desire for relief, meaning it is also possible they respond better to counterconditioning, as studies have shown desire for pain relief is associated with placebo analgesia [31,32]. Therefore, the efficacy of this procedure should be tested in individuals with chronic pain.

Additionally, applying such a procedure in a clinical setting can be more challenging than in an experimental setting, where the nocebo effect was induced experimentally. In a clinical setting, nocebo effects are acquired over time and it may prove difficult to

establish which associations induced those nocebo effects. More importantly, in the lab the symptoms experienced can be directly manipulated (i.e., the amount of pressure pain can manually be decreased during counterconditioning), whereas in a clinical setting, this is not possible (e.g., if patients experience nausea upon entering the hospital because of previous treatment experiences in the hospital, this nausea cannot easily be manipulated). Therefore, the procedure may have to be adjusted before clinical application; while the symptom cannot be targeted directly, it is possible to pair the hospital setting to something of a more positive valence than the nausea (e.g., music that makes the patient happy). Alternatively, an association with symptom decrease could be conditioned in a lab environment where symptoms can be manipulated, after which homework exercises can be given to promote generalization of this association to different environments. An example of such a treatment protocol is described in Meijer et al., 2022.

A possible limitation of the study is that valence of the CS has not been measured throughout the experiment. Therefore, it is not possible to judge whether valence regarding the CS changed in the expected direction during conditioning and after counterconditioning or extinction. Several studies demonstrating the superiority of counterconditioning over extinction (using fear or evaluative conditioning) have suggested this might be because counterconditioning effectively changes CS valence, whereas extinction does not [34–37]. However, it could be argued that nocebo counterconditioning does not completely resemble counterconditioning used in fear or evaluative paradigms, as the US is never fully taken away (only lowered to a less intense level). It is therefore important to assess whether this is sufficient for altering CS valence, as the alteration of valence has been found to strengthen the reduction of fear and reduce relapse , suggesting the effects of nocebo reduction of the nocebo effect might last longer when CS valence is successfully altered during counterconditioning.

Furthermore, while this study was only conducted in females to be able to better compare this in future studies with fibromyalgia patients (the majority of which is female), it would be good to compare the current study procedures in males. There may be differences in males and females regarding response to (open-label) suggestions and conditioning.

In conclusion, this study demonstrates that nocebo effects on the pressure pain modality can successfully be induced by a combination of open-label conditioning and verbal suggestions. Moreover, open-label counterconditioning and extinction can effectively reduce these nocebo effects, with counterconditioning leading to a stronger reduction than extinction and even producing a placebo effect similar to placebo conditioning without prior negative-associative learning. While more research is needed on the effectiveness of counterconditioning in chronic pain patients, the current study

demonstrates that open-label counterconditioning in a pain modality that is relevant for many chronic pain conditions may be a promising new strategy for reducing nocebo effects in a non-deceptive and ethical manner.

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CHAPTER 2 SUPPLEMENTARY MATERIALS

A. Formulas used during calibration

Ascending series:

If no score between 4.5 and 5.5 was reported during ascending series, the following formula was used to determine the maximum amount of pain administered during random series:

$$\textit{Maximum pain intensity (in kPa)} = kPa_y + \frac{\overline{kPax - kPay}}{(x - y)} * (5 - y)$$

- x = Lowest NRS score higher than desired range (4.5-5.5/10)
- y = Highest NRS score lower than desired range (4.5-5.5/10)
- kPax = kPa intensity at NRS score x (if multiple intensities were scored identically, the highest intensity was chosen).
- kPay = kPa intensity at NRS score y (if multiple intensities were scored identically, the highest intensity was chosen).

Random series & Calibration check:

No pain:

When none of the stimuli was scored between 0-1, a pressure intensity was used of 50 kPa lower than the lowest intensity during random series/calibration check.

Slight pain:

If no score between 2 and 3 was reported during random series, the following formula was used to determine the slight pain value:

$$\textit{Slight pain intensity (in kPa)} = kPa_y + * (2.5 - y)$$

- x= Lowest NRS score higher than desired range (2-3/10)
- y = Highest NRS score lower than desired range (2-3/10)
- kPax = kPa intensity at NRS score x (if multiple intensities were scored identically, the median of those intensity was taken).
- kPay = kPa intensity at NRS score y (if multiple intensities were scored identically, the median of those intensity was taken).

Moderate pain:
If no score between 4.5 and 5.5 was reported during random series (but there were scores **higher** than 5.5), the following formula was used to determine the slight pain value:

*Moderate pain intensity (in kPa) = kPa_y + * (5 – y)*

- x= Lowest NRS score higher than desired range (4.5-5.5/10)
- y = Highest NRS score lower than desired range (4.5-5.5/10)
- kPax = kPa intensity at NRS score x (if multiple intensities were scored identically, the median of those intensity was taken).
- kPay = kPa intensity at NRS score y (if multiple intensities were scored identically, the median of those intensity was taken).

If no score between 4.5 and 5.5 was reported during random series (but there were scores **lower** than 5.5), the following formula was used to determine the slight pain value:

*Moderate pain intensity (in kPa) = kPa_y + * (5 – x)*

- x= Highest reported NRS score
- y = Second highest reported NRS score
- kPax = kPa intensity at NRS score x (if multiple intensities were scored identically, the median of those intensity was taken).
- kPay = kPa intensity at NRS score y (if multiple intensities were scored identically, the median of those intensity was taken).

If there are no scores in the intended ranges, the same formulas as for random series can be used.

B. Verbal suggestions (translated from Dutch)

Part 1: Nocebo induction

All groups

We know from research that treatment with a placebo pill can cause people to feel less pain. This is known as a placebo effect. A placebo pill can also cause people to feel more pain, which is called a nocebo effect. Research has also shown that these effects are also present when people know they are receiving a placebo. The change in pain is caused by instructions that are given about the effects placebos can have. Placebo and nocebo effects can also be induced by classical.

In our study, instead of a placebo pill, we will use a sham DNS device. DNS stands for Dermal Nerve Stimulation. A normal DNS device can send small electrical pulses and thereby influence pain. This is a sham device, meaning no electrical pulses can be sent.

Nocebo-conditioning group

In this part of the study, we want to teach you, by using classical conditioning, that whenever the DNS device turns on, your pain will get worse. Each time the DNS device is turned on, we will administer a pain stimulus of a higher intensity compared to when the DNS device is turned off. You will then learn that if the device is turned on, you will feel more pain. Eventually, merely turning on the device will lead to you experiencing more pain.

You will now receive a series of stimuli. Before every stimulus you will see “DNS on” or “DNS off” on the screen. Whenever you see “DNS on”, your pain will be higher compared to when you see “DNS off”.

Sham-conditioning group

You will now receive a series of stimuli. Before every stimulus you will see “DNS on” or “DNS off” on the screen. During the tests, you will receive stimuli of different intensities. The intensity of the stimuli is not related to the text on the screen.

Part 2: Nocebo reduction

Counterconditioning group

In this part of the study, we want to teach you that the DNS device will reduce your pain. We will do this by using conditioning again. Each time the DNS device is turned on, we will administer a pain stimulus of a lower intensity compared to when the DNS device is turned off. You will then learn to that if the device is turned on, you will feel less pain. Eventually, merely turning on the device will lead to you experiencing less pain.

Whenever you see “DNS on”, your pain will be lower compared to when you see “DNS off”.

Extinction group

In this part of the study, we want to teach there is no longer a connection between the device being turned on or off and the amount of pain you experience. We will no longer use conditioning, meaning we will not administer higher intensity stimuli whenever the DNS device is turned on. Again, before every stimulus you will see “DNS on” or “DNS off” on the screen.

Continued nocebo conditioning group

We will now repeat the procedure. Before every stimulus you will see “DNS on” or “DNS off” on the screen. Whenever the DNS device is turned on, we will administer a higher intensity stimulus, compared to when it is turned off.

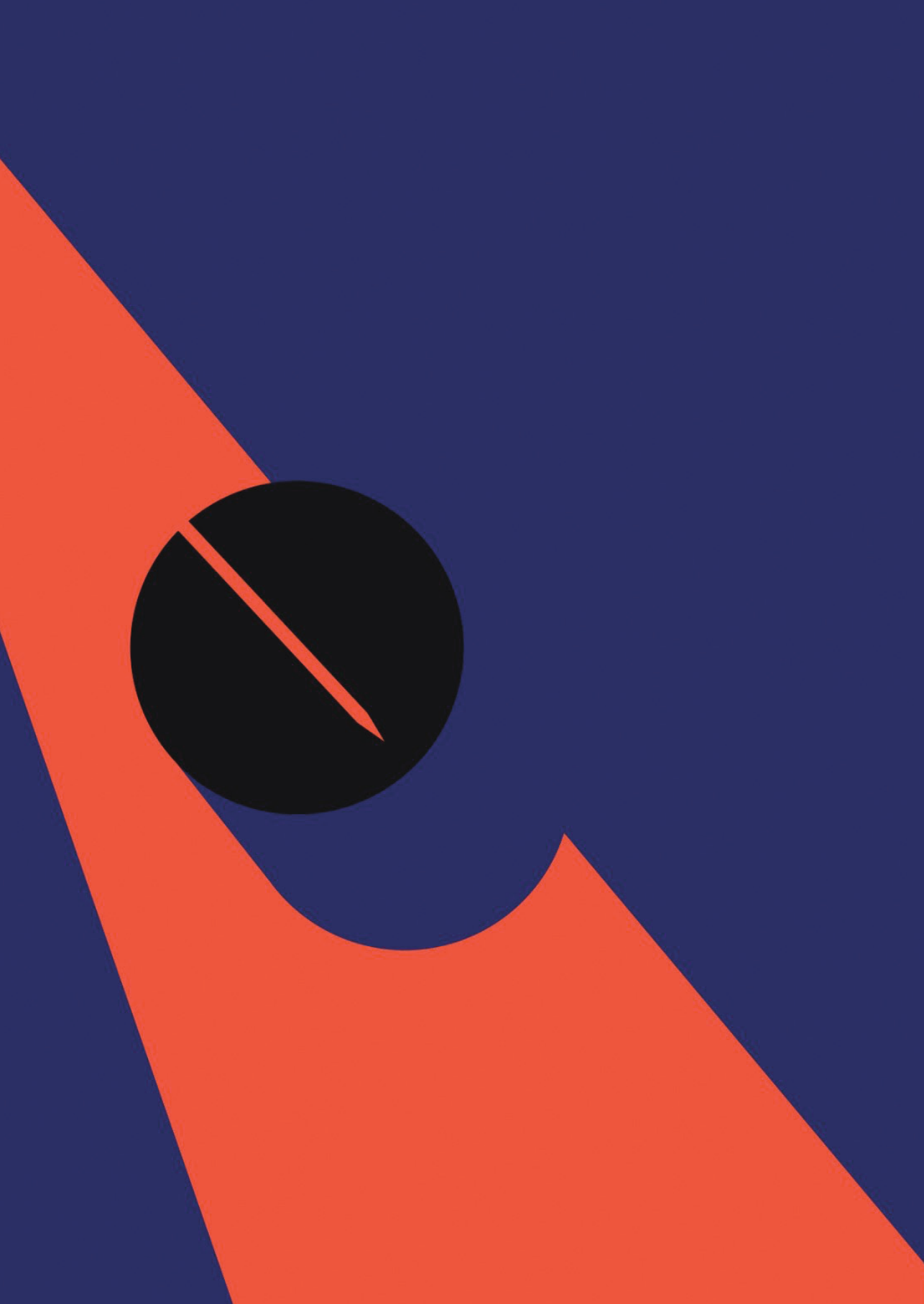
Placebo conditioning group

In this part of the study, we do want to teach you that the DNS device will influence your pain. By using classical conditioning, we want to teach you that when the sham device turns on your pain will reduce. Each time the DNS device is turned on, we will administer a pain stimulus of a lower intensity compared to when the DNS device is turned off. You will that if the device is turned on, you will feel less pain. Eventually, merely turning on the device will lead to you experiencing less pain.

You will receive a series of stimuli again. Before every stimulus you will see “DNS on” or “DNS off” on the screen. Whenever you see “DNS on”, your pain will be lower compared to when you see “DNS off”.

C. Overview of performed analyses

Research Question	Analysis
Nocebo induction; within groups	2 Paired Samples T-Tests (average NRS score experimental trials vs. average NRS score after control trials in testing phase 1)
Nocebo induction: group comparison	Independent Samples T-Test (nocebo effect induced in both groups are compared)
Manipulation check learning phase of: - Nocebo conditioning - Sham conditioning	2 Paired Samples T-Tests (average NRS score experimental trials vs. average NRS score after control trials in learning phase 1)
Manipulation check potential differences in scoring of control trials learning phase vs testing phase nocebo conditionin group	Paired Samples T-Test (average rating final control trial learning phase vs first control trial testing phase
Nocebo reduction; within groups	3 One-Sample T-Tests (comparison of amount of nocebo reduction to test value 0, within each group).
Nocebo reduction; group comparison - Counterconditioning vs extinction - Counterconditioning vs continued nocebo conditioning - Extinction vs continued nocebo conditioning	3 mixed ANOVAs (comparison of the amount of group and time (nocebo effect after part 1 vs nocebo effect after part 2)).
Reduction speed (only for counterconditioning vs extinction)	Mixed ANOVA (the interaction between group (counterconditioning and extinction) and time (all 10 experimental trials in the learning phase of part 2)
Placebo induction; within groups (only for counterconditioning and placebo conditioning)	2 Paired Samples T-Test (average NRS score experimental trials vs. average NRS score after control trials in testing phase 1)
Placebo induction; group comparison	Independent Samples T-Test (placebo effect induced in both groups are compared)



CHAPTER 3

Susceptibility to nocebo hyperalgesia, dispositional optimism, and trait anxiety as predictors of nocebo hyperalgesia reduction

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ABSTRACT

Objectives: The current paper explores the psychological predictors of nocebo hyperalgesia and whether the reduction of nocebo hyperalgesia can be predicted by susceptibility to nocebo hyperalgesia and psychological characteristics.

Methods: To this end, nocebo effects on pressure pain were first experimentally induced in 83 healthy female participants through conditioning with open-label instructions about the pain-worsening function of a sham TENS device, to assess susceptibility to nocebo hyperalgesia. Participants were then randomized to one out of two nocebo-reduction conditions (counterconditioning/extinction), or to continued nocebo conditioning (control), each combined with open-label instructions about the new sham device function. Dispositional optimism, trait and state anxiety, pain catastrophizing, fear of pain, and body vigilance were assessed at baseline.

Results: Results showed that lower optimism and higher trait anxiety were related to a stronger *induction* of nocebo hyperalgesia. Moreover, a stronger induction of nocebo hyperalgesia and higher trait anxiety predicted a larger nocebo *reduction* across interventions. Also, nocebo hyperalgesia and optimism moderated the effects of the nocebo-reduction interventions, whereby larger nocebo hyperalgesia and lower optimism were associated with a larger nocebo reduction after counterconditioning, compared to control, and also compared to extinction for larger nocebo hyperalgesia.

Discussion: Our findings suggest that open-label conditioning leads to stronger nocebo hyperalgesia when trait anxiety is high and dispositional optimism is low, while these psychological characteristics along with larger nocebo hyperalgesia also predict open-label counterconditioning to be an effective nocebo-reduction strategy. Susceptibility to nocebo hyperalgesia, trait anxiety, and dispositional optimism might be indicators of a flexible pain regulatory system.

Keywords: Nocebo effect; pressure pain; hyperalgesia; prediction; counterconditioning

1. INTRODUCTION

Nocebo effects are adverse treatment outcomes that are not attributable to active treatment components[1]. They can be induced via learning processes of classical conditioning and instructional learning[2]. Recently, studies have investigated the learning processes for reducing nocebo effects[3–6]. Amongst these, extinction works by no longer reinforcing[7], or in other words no longer strengthening, the association between pain increase and a (sham) treatment, whereas counterconditioning is a method actively targeting the reversal of painful associations with a (sham) treatment. Findings suggest that counterconditioning is a more successful method for reducing nocebo hyperalgesia than extinction[3,5]. However, research is still lacking for which individual differences predict susceptibility to nocebo effects or, equally importantly, the recovery therefrom.

Individuals differ in the degree to which they are susceptible to learning negative associations that result in nocebo effects[2,8]. Here, susceptibility is a continuous term referring to the tendency to being influenced by an experimental manipulation or a psychological characteristic. Research into psychological characteristics provides some indications for people high on fear or anxiety, and low on optimism to be more susceptible to nocebo effects, while other research shows no associations for these, or shows an even weaker evidence for other expectancy-related traits, such as for higher pain catastrophizing or body vigilance[9–11]. Individual differences may also exist during nocebo reduction. Research is needed to examine whether psychological characteristics and susceptibility towards nocebo hyperalgesia predict the level of nocebo reduction by different learning interventions. Possibly, a larger baseline nocebo hyperalgesia could be associated with more resistance to nocebo reduction[12,13], although the opposite might be true if larger nocebo hyperalgesia leads to a stronger desire for pain relief, which might increase the intervention efficacy[14].

To this end, the current research entails additional exploratory analyses on a study in which the open-label induction and reduction of nocebo effects on pressure pain was investigated in a healthy female sample[5]. Adding onto their findings, the current research aims are four-fold. First, we explore whether any of the six psychological characteristics namely, dispositional optimism, state and trait anxiety, pain catastrophizing, fear of pain, and body vigilance predict the strength of nocebo hyperalgesia after conditioning with open-label instructions about the pain-increasing function of a sham TENS device. Second and third, we investigate the predictive roles of susceptibility to the induction of nocebo hyperalgesia and psychological characteristics in the magnitude of nocebo change after two nocebo-reduction interventions, i.e., counterconditioning and extinction combined with open-label instructions, with continued open-label nocebo conditioning serving as

a control condition. Fourth, we explore whether susceptibility to the induction of nocebo hyperalgesia and psychological characteristics moderate the effects of these nocebo-reduction interventions. This extensive exploration of the predictors of nocebo reduction is novel and can be useful in the future for selecting the most effective nocebo-reduction strategy (either counterconditioning or extinction) based on individual differences.

2. METHODS

2.1 Design

The current research is part of a larger study[5] approved by the Psychology Research Ethics Committee of Leiden University (CEP18-1114/442; pre-registration ICTRP Trial ID: NL8033). In line with the aims of the current research, only a subset of experimental conditions from the larger study was considered for analysis, which entailed the manipulations for inducing and reducing nocebo effects on pressure pain (Figure 1). For further details on all experimental conditions, including the larger study aims and their findings, the readers are referred to a separate publication[5]. Data was used from the same sample, which has a sufficient sample size for conducting the planned analyses of the current research[15]. During the experiment, participants were randomly allocated to a condition where nocebo effects were induced *(nocebo conditioning)* and subsequently, they were further allocated (1:1:1) to either one of the two nocebo-reduction conditions, *counterconditioning* or *extinction,* or to the control group, *continued nocebo conditioning*. The idea behind this two-step design was to create an experimental model that potentially mimics real-life learning events where nocebo effects are induced and then altered by various learning processes. Moreover, in all groups, open-label instructions were provided about the function of a sham Transcutaneous Electrical Nerve Stimulation (TENS) device. Open-label instructions were chosen to allow for a more ethical implementation of this design as a possible nocebo-reduction strategy for future clinical practice. Findings from open-label placebo studies indicate that an inert treatment can be prescribed without the concealment of their non-pharmacological contents, i.e., without deception[16]. The positive treatment outcomes can be still achieved by combining placebo administration with the rationale that placebo mechanisms can lead to the medical improvement of symptoms[16–18]. The current experiment applied this open-label rationale to induce and reduce nocebo effects by using a sham TENS device as *the inert treatment*. As such, participants were informed about the inefficacy of the sham TENS device, i.e., that in reality it cannot send electrical signals, but that through expectation mechanisms, the sham activation of the device can lead to either pain in- or decrease in line with the instructions given about the device.

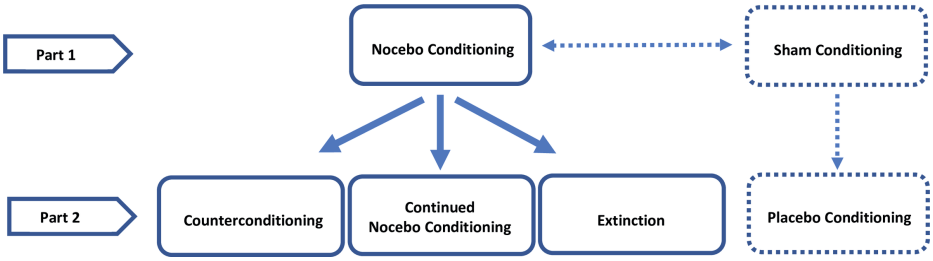

Figure 1. Overview of the full study design. Solid lines indicate the experimental groups that are part of the current study.

2.2 Participants

Healthy females between 18 and 35 years with good understanding of Dutch language were recruited for the study. Since pressure pain is an ecologically valid stimulus type for disorders involving musculoskeletal pain[19,20], in which nocebo effects may play a clinically relevant role, and because adult women report more musculoskeletal problems than men[21], our sample consisted of only female participants to increase the generalizability of current findings to the clinical studies involving pressure pain. The exclusion criteria were: severe physical or psychological disorders, chronic pain complaints (≥ 3 months) in the past or present, pain on the day of the experiment, injuries on the hands, Reynaud's disease, color-blindness, pregnancy or breastfeeding, and current use of medication except for contraceptives. Participants were asked to not drink alcohol and not to use any painkillers, sleep medication, or recreational drugs within the 24 hours prior to the experiment. They were also asked to not wear any nail polish or acrylic nails on the thumbnail of their non-dominant hand. An exclusion criterion during the first phase of the lab session, the pain calibration phase, was the inability to reliably distinguish between different pressure intensities.

Participants were recruited via posters and flyers distributed and handed out at various locations within Leiden University, and via the online participant recruitment platform Sona (Sona Systems, Tallinn, Estonia). The study consisted of a single experimental session of 2 hours, which took place in the Faculty of Social and Behavioral Sciences labs of Leiden University. Participants were compensated with either cash (€15) or study credits for their participation.

2.3 Pressure pain application

Pressure pain was induced on the thumbnail using a custom-made automated, pneumatic, computer-controlled pressure administrator, which was borrowed from the Karolinska Institute in Sweden[22], including a hand-piece borrowed from King's College London. This device is still investigational. The thumb of the non-dominant hand was inserted

into the transparent hand-piece, which applied pressure to the middle of the thumbnail via a piston with a 1 cm^2 probe. Each stimulus lasted 2.5 s, with a 30 s inter-stimulus-interval. The device could only maximally apply 850 kPa (≅ 8.7 kgf/cm^2) pressure on the thumbnail, which is an intensity lower than the average that can be tolerated in healthy participants[23] and was chosen as a safety measure considering the repetitive stimulus administration. Additionally, an emergency stop button was provided so that participants could stop the pressure stimuli at any given moment during the experiment if they could not endure the pressure.

2.3.1. Pain measurement

Participants verbally rated the pain intensity of each pressure stimulus on a Numeric Rating Scale (NRS), with the end points 0 representing no pain and 10 worst pain imaginable. Participants were able to rate their pain up to a decimal point. They were asked to only rate above zero (thus 0.1 and upwards) when they start to feel pain next to feeling a sensation of pressure. The verbally reported NRS ratings were entered into a computer by the experimenter after each trial.

2.3.2 Pressure pain calibration

Pressure pain was individually calibrated to evoke similar pain levels across participants due to expected individual differences in sensitization[24]. Pressure intensities starting from 100 kPA (≅ 1 kgf/cm^2) were administered with 50 kPA (≅ 0.5 kgf/cm^2) increments on the thumb-nail until participants rated ≥ 5.5 on the NRS or until 850 kPA (≅ 8.7 kgf/cm^2) was reached. Based on the highest intensity of pressure scored as zero on the NRS and the highest scored pressure intensity, 3 new intermittent intensities were calculated that were equidistant from each other in magnitude. Together, these five intensities were randomly administered three times to determine the pressure intensities rated between the ranges 0-1, 2-3, and 4.5-5.5 on the NRS to determine non-painful, slightly painful, and moderately painful pressure intensities, respectively. Since participants were allowed to rate using decimal points on the NRS, a barely painful pressure intensity (0-1 on the NRS) was also accepted as non-painful, as it was expected that the repeated administration of pressure stimuli could lead to a slight sensitization, which could increase the non-painful rating higher than zero. When the participants did not rate within the targeted range, standard formulas were used to interpolate the mid-value of the target range based on surrounding ratings[5]. A calibration check followed where the pressure stimuli for non-painful, slightly painful, and moderately painful intensities were randomly administered with slightly painful pressure intensity presented thrice and the rest presented twice. The pressure intensities were adjusted based on the same formulas if they were rated more than once outside of the target range. Five-minute breaks were taken between each calibration step to minimize stimulus sensitization. Breaks were extended by one

minute, up to five minutes, if the participant indicated having pain ≥ 2 on the NRS. No participants asked for a break longer than five minutes.

2.3.3 Sham TENS device

A sham TENS device (BeurerEM80, Beurer GmbH, Ulm, Germany) was used for the conditioning paradigm, which was renamed as a Dermal Nerve Stimulation (DNS) device to avoid possible preconceptions about TENS from interfering with experimental manipulations. Two TENS electrodes were attached vertically on the radial side of the forearm of the non-dominant hand. The device itself was switched on as seeing a light would suggest its activation to the participant, but actually it was never activated, thus electrical signals were not delivered at any stage of the experiment. This device is not labelled for the use under discussion. E-prime version 3.0 (Psychology Software Tools, Pittsburg, PA) was used for presenting the texts "DNS on" and "DNS off" on a monitor screen to indicate the (sham) activation of the DNS device. These texts were presented in purple and yellow, counter-balanced across participants.

2.4 Induction and reduction of nocebo hyperalgesia

2.4.1 Nocebo induction: Nocebo conditioning in Part 1

All participants were given open-label instructions about nocebo effects and how they can be induced by the principles of classical conditioning. They were informed that a sham nerve stimulator, called DNS, would be used for conditioning them to expect pain increase. This would be achieved by administering a moderately painful pressure stimulus to the thumbnail during the sham activation of the DNS device, but in fact the DNS device would not send any electrical signals. The electrodes would remain attached on the arm to mimic the administration of electrical signals, similar to the act of swallowing a placebo pill even when knowing it does not contain any active components. During the 20 trials of the learning phase, participants were conditioned to expect a pain increase in half of the trials, hereafter referred to as experimental trials. For this, the text "DNS on" appeared on the screen 1 second prior to receiving a moderately painful pressure stimulus and the experimenter pressed a button on the DNS to evoke a beep sound to indicate its sham activation. In the other half of the trials, i.e., the control trials, "DNS off" appeared on the screen and a slightly painful pressure stimulus was administered. Directly after the learning phase trials, the testing phase followed. During the testing phase, 3 trials were associated with "DNS on" and 3 trials with "DNS off", where this time both DNS conditions were paired with only slightly painful pressure stimuli. Participants were not informed during the open-label instructions that they would be receiving only slightly painful pressure stimuli during the testing phase.

2.4.2 Nocebo reduction: Counterconditioning in Part 2

Participants allocated to the counterconditioning group were instructed that during this part of the experiment they would be conditioned to expect to receive no pain instead of moderate pain when the DNS device is activated. This would be achieved by administering a non-painful pressure stimulus during the sham activation of the device. The device (de)activation procedure was similar to part 1; except that this time a non-painful pressure stimulus, instead of a moderately painful one, was paired with the text "DNS on" during half of the 20 learning trials and with a slightly painful pressure stimulus during the other half. During all 6 testing phase trials, only slightly painful pressure stimuli were administered unbeknownst to the participants regardless of DNS activation in half of the testing trials.

2.4.3 Nocebo reduction: Extinction in Part 2

In the extinction group, participants received the instruction that this time they would be taught to expect no relation between the sham (de)activation of the DNS device and the amount of pain they receive. Therefore, during the sham activation of the DNS device, moderately painful pressure stimuli would no longer be administered. During all 20 learning trials and 6 testing phase trials, in which DNS was (de)activated in half of the trials, participants received only slightly painful pressure stimuli.

2.4.4 Control condition: Continued nocebo conditioning in Part 2

In the continued nocebo conditioning group, participants were told that this part would be exactly as before and that they would receive higher pain during the sham activation of the DNS device compared to its sham deactivation. Same as in nocebo conditioning, participants received a moderately painful pressure stimulus during the experimental trials and slightly painful pressure stimulus in the control trials of the learning phase. Again, only slightly painful pressure stimuli were administered unbeknownst to the participants during the testing phase trials.

2.5 Operationalization of nocebo hyperalgesia and nocebo change

Nocebo effects were measured by calculating the mean difference between the pain ratings in all 3 experimental trials ("DNS on") and the pain ratings in all 3 control trials ("DNS off") from the testing phase in part 1 or 2. Nocebo hyperalgesia refers to the magnitude of nocebo effects obtained after nocebo conditioning in part 1. Nocebo change refers to changes in the magnitude of nocebo effects between parts 1 and 2. To obtain this variable, nocebo effects calculated in part 2 were subtracted from the nocebo effects in part 1. A larger positive score on this nocebo change variable indicates a larger reduction in nocebo hyperalgesia from part 1 to part 2.

2.6 Questionnaires

Dispositional Optimism. Dispositional optimism is the extent to which an individual believes that future outcomes will be good or positive[25]. Based on a systematic review, lower levels of optimism were relatively consistently related to stronger nocebo responses, whereas higher levels of optimism were relatively consistently related to stronger placebo responses[10]. The Life-Orientation Test-Revised (LOT-R) was used for assessing dispositional optimism[26]. LOT-R is a 10-item measure containing positive items such as *"In uncertain times, I usually expect the best",* negative items such as *"If something can go wrong for me, it will*", and filler items. Respondents rate each item on a 5-point scale from 0 = *Strongly disagree* to 4 = *Strongly agree*. To calculate the optimism score, three negatively worded items are reverse coded and added to the three positively worded items, resulting in a total score from 0 to 24, with higher scores indicating higher optimism.

Trait anxiety. Trait anxiety is regarded a relatively stable personality trait, indicating individual differences in the intensity and frequency of perceiving stressful situations as dangerous or threatening[27]. (Trait) Anxiety has been repeatedly found to correlate with a stronger nocebo response[10,11,28]. The trait scale of the State-Trait Anxiety Inventory (STAI-T) was used for assessing trait anxiety[27]. The scale contains 20 items about how a person generally feels, such as *"I feel pleasant" or "I feel nervous and restless".* Respondents rate each item on a 4-point scale, with the end points 1 = *Almost never* and 4 = *Almost always*. To calculate the trait anxiety score, positively phrased items are reverse coded and then the sum score of all items is calculated. The scores range between 20-80 points, with higher scores indicating greater trait anxiety.

State anxiety. The state scale of the State-Trait Anxiety Inventory short-form (STAI-S-6) was used for assessing state anxiety[29]. STAI-S-6 is sensitive to changes in transitory anxiety and indicates raised levels of anxiety at a given moment[27]. When pain increase is anticipated within an environment, the resulting anticipatory anxiety has been found to lead to nocebo hyperalgesia[30]. The scale consists of 6 items, measuring how respondents feel *"right now, at this moment"* with items such as *"I feel calm"* or *"I feel tense"*. STAI-S-6 is rated on a 4-point scale, with the end points 1 = *Not at all* and 4 = *Very much so*. Positive items were reverse coded and then the sum score of all items was calculated. For comparability with the full STAI-S, scores were adjusted to range between 20-80 points, with higher scores indicating greater state anxiety.

Pain catastrophizing. Catastrophizing is defined as an exaggerated negative mental state brought on by actual or anticipated painful experiences[31]. One study found that pain catastrophizing was highly correlated with stronger nocebo effects on pressure

pain induced by verbal suggestions and observational learning[32]. However, the same group failed to find this correlation in another study with socially induced nocebo effects on pressure pain[33]. The Pain Catastrophizing Scale (PCS) was used for assessing pain catastrophizing[31]. PCS is a multidimensional construct that measures rumination (e.g., *"I can't stop thinking about how much it hurts"*), magnification (e.g., *"I become afraid that the pain will get worse")*, and helplessness (e.g., *"I feel I can't go on"*). It consists of 13 items rated on a 5-point scale, with the end points 0 = *Not at all* and 4 = *All the time*. The sum score of all items was calculated, ranging from 0-52, with higher scores indicating more pain-catastrophizing thoughts.

Fear of pain. Fear of pain is related to the emotional reactions surrounding actual or anticipated pain, leading to avoidance behavior, which may be more disabling than actual pain[34]. Especially higher fear of medical pain was found to mediate the increase in stress levels following a nocebo intervention, where higher stress levels were related to greater nocebo hyperalgesia[35]. Also, fear induced in subjects high in fear of pain was found to abolish the positive effects of placebo analgesia[36]. The Fear of Pain Questionnaire-III (FPQ-III) was used for assessing fear of pain[37]. FPQ-III is a 30-item questionnaire measuring fear related to severe pain (e.g., *"Being in an automobile accident"*), minor pain (e.g., *"Biting your tongue while eating"*), and medical pain (e.g., *"Receiving an injection in your arm"*). The FPQ-III is scored on a 5-point scale, with the end points 1 = *Not at all* and 5= *Extreme*, and the sum score of all items was calculated, ranging from 30-150, with a higher score indicating greater fear of pain.

Body vigilance. The Body Vigilance Scale (BVS) was used for assessing the tendency to attend to bodily sensations[38]. One study showed that the level of body vigilance moderated the increase in symptoms after taking a placebo that participants believed to be an actual drug[39]. The more participants focused on their symptoms, the more symptoms they reported. On the other hand, another study found that increased attention to somatic symptoms reduced pain levels when pain expectancy was high[40]. BVS consists of four main items. Three items assess the degree of attentional focus, perceived sensitivity to changes in bodily sensations, and the average amount of time spent attending to bodily sensations. The fourth item involves rating how much attention is directed to 15 separate sensations such as *"heart palpitations"* or *"feeling detached from self"*. Ratings were made on 0-10 scales with endpoints 0= *Strongly disagree* and 10= *Strongly agree* for items one and two, 0= *Never* and 10= *Always* for item three, 0= *Never* and 10= *Very much* for the sensation ratings in item four. Ratings in the fourth item were averaged to get a single score and afterwards the sum score of all four items was calculated, ranging from 0-40, with higher scores indicating greater focus on bodily sensations.

2.7 Procedure

After arriving to the lab, participants received information about the experiment after which they signed an informed consent form and were screened for in- and exclusion criteria. If eligible, they continued with the experimental steps, starting with filling in psychological questionnaires (Qualtrics, Provo, UT), followed by participating in all measurements involving pressure stimuli. Non-painful, slightly painful, and moderately painful pressure intensities were individually calibrated using the pressure pain device. After a successful calibration procedure, when participants were able to differentiate between the three pressure intensities, the experimenter opened the randomization envelope to randomly allocate participants to their respective experimental condition for parts 1 and 2. Sham electrodes of the DNS device were attached to the arm and further information was provided about the procedural steps in part 1. Twenty learning phase trials and 6 testing phase trials from part 1 followed. After a 10-minute break, participants received further instructions about the procedural steps in part 2. Again, 20 learning phase trials and 6 testing phase trials from part 2 followed. After the end of the experiment, the electrodes were removed from the arm and participants were asked to fill in exit questionnaires, which are reported elsewhere[5]. Afterwards, participants were debriefed and reimbursed for their participation.

2.8 Statistical Analyses

All statistical analyses were conducted using the R[41], version 4.1.0. Normality of study variables was checked and log transformations were performed for the nocebo hyperalgesia score (skewness = 0.92) and nocebo change score (skewness = 0.96) due to a moderate skewness towards the right[42]. However, we did not find any impact of data transformation on study results; therefore, it was decided to only report the results from non-transformed data to ease interpretation of findings. Variance Inflation Factor (VIF) values of independent variables were screened for multicollinearity, and this was not detected as all VIF values were below 10[43]. For the regression analyses, residual scatterplots were visually inspected for the assumptions of normality, linearity, and homoscedasticity, which were not violated. Also, no influential values were detected (Cook's $D < 0.5$). A p-value below 0.05 was considered statistically significant. To assess reliability, Cronbach's alpha levels were calculated for the psychological scales. Cronbach's alpha levels ranged from .77 (LOT-R) to .93 (BVS), corresponding to acceptable to excellent internal consistency[44].

To answer the first research question of whether psychological characteristics were related to nocebo hyperalgesia, their univariate relationships were tested using Pearson's correlation coefficients, and their multivariate relationships were tested using a multiple regression analysis, where the standardized scores from six psychological characteristics

were entered to the model as predictors with nocebo hyperalgesia as the outcome variable. For the remaining research questions, a hierarchical regression analysis was conducted to assess the statistical contribution of each block of predictors to the nocebo change score. All continuous predictors were centered around the mean to facilitate interpretation of interaction effects[43]. The analyses were performed twice. The first time the group variable was dummy coded with the control condition (i.e., continued nocebo conditioning) as reference group. The second time, the extinction group was taken as reference group. This enabled all three groups to be compared with each other. All predictors were entered into the model in 4 steps according to a pre-determined order. In step 1, dummy variables of group were added using force-entry. In step 2, nocebo hyperalgesia was force-entered into the model to answer the second research question, to identify the added value of nocebo hyperalgesia in predicting nocebo change from these experimental groups. Note that by including nocebo hyperalgesia as a covariate, the estimated model effects became identical for all possible outcome measures, i.e., "nocebo change score" versus "raw intervention score", which additionally justifies our decision on choosing "nocebo change score" over the "raw intervention score" as the outcome measure for this model, to facilitate the interpretation of findings[45]. In step 3, six psychological characteristics were force-entered to answer the third research question, to identify the added value of psychological characteristics in predicting nocebo change across groups. For the fourth research question, we investigated whether nocebo hyperalgesia and psychological characteristics moderated the group effects on nocebo change. Therefore, in step 4, two-way-interaction terms between the dummy variables of group and nocebo hyperalgesia as well as between the dummy variables of group and each of the six psychological characteristics were included. To check whether the block of predictor(s) added at each step significantly contributed to an increase in the explained variance, ANOVA comparisons were performed between the nested models created in each subsequent step (i.e., global tests).

To interpret the findings, the model created in step 2 was used for answering the second research question, since the global test of this model represents the effect of the single variable entered in that step. For answering the third and fourth research questions, the effect of the global tests of steps 3 and 4, respectively, represent the effect of a group of variables; therefore, to be able to interpret the individual variables, and to increase the interpretability of the model, we relied on the final model created with stepwise selection. When these global tests for steps 3 and 4 are significant, applying stepwise selection becomes warranted[46]. As the stepwise selection method, we applied forward selection based on the largest decrease in the Akaike Information criterion (AIC) between two models[46]. With this selection procedure the terms within the complex model (i.e., with all possible predictors) were stepwise added to a simple one to obtain the most

parsimonious model. As such, all predictors were stepwise added by the program following an automatic selection procedure. This procedure preserves the principle of marginality. Lastly, interaction plots were created for variables with a significant interaction effect.

3. RESULTS

A total of 166 participants enrolled in the study. Seven participants were excluded during screening for not fulfilling the inclusion criteria, 46 participants were excluded during the pain calibration phase for not being able to reach a moderate pain rating for the highest administered pressure intensity, 3 participants were excluded due to technical problems and 2 after pressing the emergency stop button due to pain sensitization, yielding 108 eligible participants of which 83 participated in the nocebo conditioning group. Therefore, a total of 83 healthy female participants (*Mean age*: 20.46, *SD:* 2.17) were included in the final analysis. Amongst these, 27 were allocated to counterconditioning, 29 to extinction, and 27 to continued nocebo conditioning in part 2.

To summarize the relevant findings from the larger study[5], nocebo effects were successfully induced in part 1, with significantly larger nocebo effects after nocebo conditioning than sham conditioning (i.e., control). In part 2, a larger reduction of nocebo effects was found after counterconditioning compared to extinction and continued nocebo conditioning (i.e., control).

The current analyses showed that nocebo hyperalgesia (M= 1.29, SD= 0.95) ranged between -0.33 and 4.37 points, whereby 95.2% (N= 79) of participants had a positive score, indicating they were nocebo responders. Regardless of nocebo responsiveness, all participants were included in further prediction analyses. Mean nocebo change score across all three groups of part 2 was 0.91 (SD= 1.37), which ranged between -2.17, indicating an increase in nocebo effects, and 5.47 points, indicating a nocebo reduction between part 1 and part 2. Mean change score in the counterconditioning group was 1.98 (SD= 1.5), in the extinction group 0.77 (SD= 0.90), and in the continued nocebo conditioning group -0.01 (SD= 0.90).

3.1 Psychological predictors of nocebo hyperalgesia

An overview of means, standard deviations, and the intercorrelations between nocebo hyperalgesia and six psychological characteristics are displayed in Table 1. Testing for univariate relationships, more trait anxiety (Pearson's r= .28, p< .01) and less optimism (Pearson's r= -.22, p< .05) were associated with larger nocebo hyperalgesia. Next, to test their multivariate relationship, nocebo hyperalgesia was regressed on all psychological

characteristics in a multiple regression analysis (Table 2). Taken together, psychological characteristics did not significantly explain the variance in nocebo hyperalgesia ($F(6,76)= 1.9$, $R^2= .062$, $p= .09$).

Table 1

Means, standard deviations, and intercorrelations of nocebo hyperalgesia and psychological characteristics ($N = 83$).

Variable	*M*	*SD*	*2*	*3*	*4*	*5*	*6*	*7*
1. Nocebo Hyperalgesia	1.29	0.95	-.22*	.28**	.21	.03	-.03	.14
2. Optimism	16.08	3.68	-	-.58***	-.22*	-.25*	-.17	-.09
3. Trait Anxiety	36.71	6.75		-	.66***	.43***	.38***	.27*
4. State Anxiety	32.97	9.80			-	.32**	.27*	.24*
5.Pain Catastrophizing	13.48	7.58				-	.51***	.54***
6. Fear of Pain	71.29	16.18					-	.32**
7. Body Vigilance	19.39	6.96						-

Note. *$p< .05$; **$p< .01$; *** $p< .001$ (two tailed)

Table 2

Summary of multiple regression analysis for psychological characteristics predicting nocebo hyperalgesia ($N = 83$).

	Nocebo Hyperalgesia	
Variable	*β*	*p-value*
Intercept	1.29	< .001
Optimism	-.10	.44
Trait Anxiety	.23	.19
State Anxiety	.07	.62
Pain Catastrophizing	-.14	.32
Fear of Pain	-.13	.27
Body Vigilance	.17	.18
Full Model	Adj. $R^2 = .06$	
	$F(6, 76) = 1.9$, $p = .09$	

Note. *β* is the standardized regression coefficient

3.2 Predictors of nocebo reduction

Table 3 displays an overview of the hierarchical regression steps entered for creating the nested models. The ANOVA comparisons of all nested models, i.e., the global tests, differed statistically from each other, indicating that each block of predictor(s) significantly increased the explained variance of the full model. As these global tests were significant, forward selection was applied for the final model to increase interpretability.

Table 3

Summary of hierarchical regression steps, the explained variance, and the ANOVA tests of the increase in explained variance from one step to the other (*N* = 83).

	Nocebo Change			
Variable	R^2	ΔR^2	*F-statistic*	*df*
Step 1 Group	0.35	-	-	-
Step 2 Nocebo Hyperalgesia	0.61	0.26	52.13***	(79,1)
Step 3 Optimism Trait Anxiety State Anxiety Pain Catastrophizing Fear of Pain Body Vigilance	0.70	0.09	3.76**	(73,6)
Step 4 Group x Nocebo Hyperalgesia Group x Optimism Group x Trait Anxiety Group x State Anxiety Group x Pain Catastrophizing Group x Fear of Pain Group x Body Vigilance	0.79	0.09	1.92*	(59,14)

Note. Only the variables kept in the model after forward selection are presented in the final model. R^2: Explained variance; ΔR^2*:* Change in explained variance from one step to the other; *F-statistic:* F-statistic from one step to the other; *df*: Degrees of freedom.
*p < .05; **p < .01; ***p < .001 (two-tailed)

In step 1, the group variable significantly explained 35% of variance in nocebo change score. In line with the primary findings of the larger study[5], groups differed in nocebo change, with counterconditioning showing a significantly higher nocebo change score, indicating an average larger reduction in nocebo hyperalgesia, compared to both extinction and the control group, and extinction showing a significantly larger reduction in nocebo hyperalgesia compared to the control group (Table 4 and Table 5).

In step 2, nocebo hyperalgesia significantly explained an additional 26% of the variance in nocebo change score, where a larger induction of nocebo hyperalgesia was associated with a significantly larger nocebo reduction (*b*= 0.73, *SE*= 0.10, *t= 7.22, p*< .001). This indicates that those participants who were more susceptible to acquiring nocebo hyperalgesia in part 1 were also more susceptible to learning new associations related to nocebo reduction in part 2.

In step 3, the inclusion of psychological characteristics significantly explained an additional 9% of the variance in nocebo change score and in step 4, the inclusion of moderators significantly explained an additional 9% of the variance. Because multiple variables were entered in steps 3 and 4, their individual contribution was interpreted as part of the final model created with forward selection.

Table 4

Summary of final model after forward selection predicting nocebo change with *continued nocebo conditioning* as reference group (*N* = 83).

	Nocebo Change		
Variable	ΔR^2	ΔAIC	*B step*
Step 1	.35***		
Intercept			-0.01
Group a vs. c			1.99***
Group b vs. c			0.78*
Step 2	.26***		
Nocebo Hyperalgesia			0.73***
Step 3 and 4 (with forward selection)	.15***		
Intercept			-.06
Group a vs. c			1.99***
Group b vs. c			0.82***
Nocebo Hyperalgesia			0.14
Optimism		-42.78	0.13**
Trait Anxiety		-45.40	0.03*
Group a vs. c x Nocebo Hyperalgesia		-41.40	1.07***
Group b vs. c x Nocebo Hyperalgesia			0.55*
Group a vs. c x Optimism		-47.78	-0.14*
Group b vs. c x Optimism			-0.06
Final Model	*Adj.* R^2 = 0.73 $F(73,9)$ = 25.87***		

Note. Only the variables kept in the model after forward selection are presented in step 3 and 4.
Group a: Counterconditioning, Group b: Extinction, Group c: Continued Nocebo Conditioning; ΔR^2*:* Change in explained variance; *AIC*: Change in Akaike's Information Criterion after selecting this variable into the model; *B step* is the unstandardized coefficient for this variable at given analysis.
*p < .05; **p < .01; ***p < .001 (two-tailed)

Table 5

Summary of final model after forward selection predicting nocebo change with *extinction* as reference group (*N* = 83).

	Nocebo Change		
Variable	ΔR^2	*ΔAIC*	*B step*
Step 1	.35***		
Intercept			0.77***
Group a vs. b			1.21***
Group c vs. b			-0.78*
Step 2	.26***		
Nocebo Hyperalgesia			0.73***
Step 3 and 4 (with forward selection)	.15***		
Intercept			0.76***
Group a vs. b			1.17***
Group c vs. b			-0.82***
Nocebo Hyperalgesia			0.69***
Optimism		-42.78	0.07
Trait Anxiety		-45.40	0.03*
Group a vs. b x Nocebo Hyperalgesia		-41.40	0.52*
Group c vs. b x Nocebo Hyperalgesia			-0.55*
Group a vs. b x Optimism		-47.78	-0.08
Group c vs. b x Optimism			0.06
Final Model	*Adj.* R^2 = 0.73 *F*(73,9) = 25.87***		

Note. Only the variables kept in the model after forward selection are presented in step 3 and 4.
Group a: Counterconditioning, Group b: Extinction, Group c: Continued Nocebo Conditioning; ΔR^2*:* Change in explained variance; *AIC*: Change in Akaike's Information Criterion after selecting this variable into the model; *B step* is the unstandardized coefficient for this variable at given analysis.
*p < .05; **p < .01; ***p < .001 (two-tailed)

The forward selection resulted in the selection of group, nocebo hyperalgesia, optimism, trait anxiety, the interaction term of group and nocebo hyperalgesia, and the interaction term of group and optimism as the predictor variables in the final model (see Table 4 and Table 5 for an overview). Together, the final model explained 73% of variance in nocebo change score. Trait anxiety was the only psychological characteristic with a significant main effect (*b*= 0.03, *SE*= 0.02, *t= 2.08, p*= .04) on nocebo change, whereby a higher trait anxiety was associated with a larger nocebo reduction. Aside from this, there was a significant interaction of group and nocebo hyperalgesia. This interaction effect is plotted in Figure 2, where it can be observed that for lower levels of nocebo hyperalgesia, the type of intervention group does not strongly determine nocebo change, whereas for higher levels of nocebo hyperalgesia, counterconditioning results in a higher nocebo reduction than extinction, which in turn results in a higher nocebo reduction than continued nocebo conditioning. Moreover, there was a significant interaction of group and optimism on

nocebo change for counterconditioning and continued nocebo conditioning groups. In line with the significant interaction effect between group (a vs. c) and optimism (Table 4), it can be observed in Figure 3 that at lower levels of optimism, compared to higher optimism, the nocebo-reduction effect of counterconditioning was significantly larger compared to the continued nocebo conditioning group. Based on Figure 3, a similar trend holds for extinction compared to continued nocebo conditioning; however, this interaction effect was not statistically significant. Moreover, optimism levels did not moderate the intervention effect of counterconditioning compared to extinction.

4. DISCUSSION

The current study investigated the predictors of nocebo hyperalgesia and of nocebo change after interventions aimed at reducing nocebo hyperalgesia in a healthy female sample. This study entails additional exploratory analyses on a larger study[5], which can be useful for generating hypotheses for future research. Nocebo hyperalgesia was induced using experimental pressure pain by open-label conditioning, and then reduced by open-label counterconditioning and open-label extinction, with continued open-label nocebo conditioning serving as control group. The role of dispositional optimism, trait and state anxiety, pain catastrophizing, fear of pain, and body vigilance in the induction and reduction of nocebo hyperalgesia were explored. Their multivariate relationship with nocebo hyperalgesia was not significant; however, based on univariate relationships, higher trait anxiety and lower optimism predicted larger nocebo hyperalgesia. Moreover, the main effects showed that larger nocebo hyperalgesia and higher trait anxiety predicted a larger nocebo reduction across groups. Interaction effects showed that for participants with larger nocebo hyperalgesia, compared to smaller, counterconditioning predicted a larger nocebo reduction than extinction and continued nocebo conditioning. For participants with lower optimism, compared to higher, counterconditioning was more effective than continued nocebo conditioning. Our findings provide initial indications that individual differences in nocebo hyperalgesia, as well as dispositional optimism and trait anxiety, could predict changes in nocebo hyperalgesia levels after nocebo-reduction interventions.

Investigation into the psychological differences indicated that higher trait anxiety and lower optimism predicted larger nocebo hyperalgesia. Only trait anxiety was a predictor of nocebo reduction across groups, which suggests that regardless of which nocebo-reduction strategy is selected, as trait anxiety increases not only the induction but also the reduction of nocebo hyperalgesia increases. This appears in contrast to a previous study, which found that higher levels of anxiety, measured by changes in autonomic arousal,

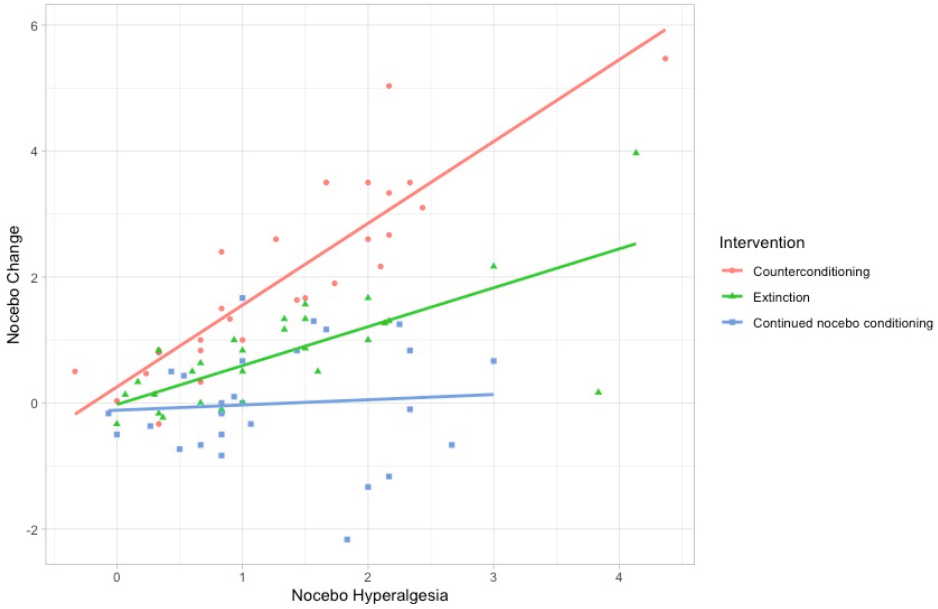

Figure 2. Nocebo hyperalgesia and intervention group as predictors of nocebo change. Note that higher levels on nocebo change indicate a larger reduction in nocebo effects from part 1 to part 2.

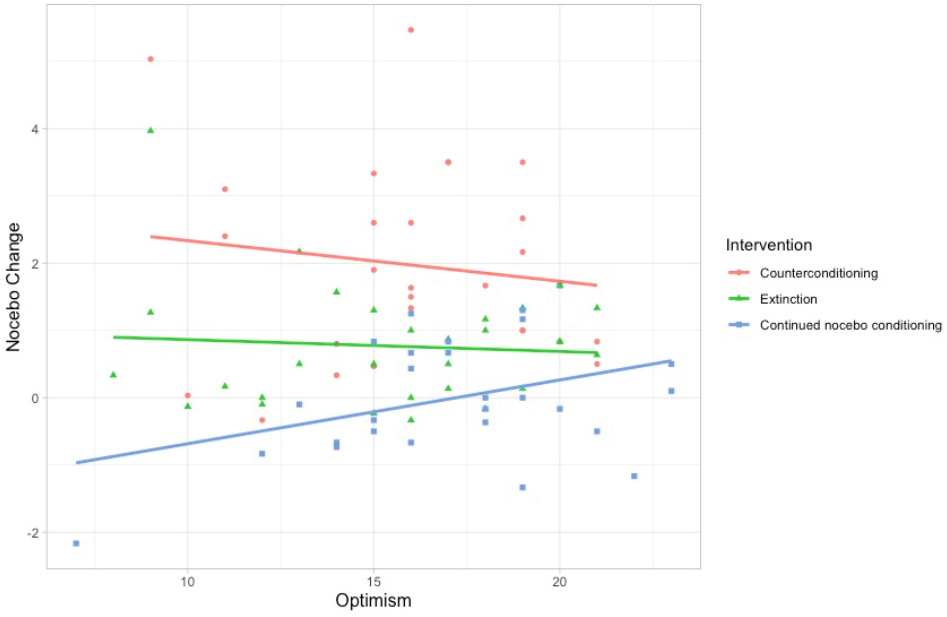

Figure 3. Optimism and intervention group as predictors of nocebo change. Note that higher levels on nocebo change indicate a larger reduction in nocebo effects from part 1 to part 2.

perpetuates nocebo hyperalgesia and leads to resisting extinction[47]. Speculatively, a potential explanation of our findings could be a heightened desire for pain relief experienced during high levels of anxiety, which could have facilitated the efficacy of the given intervention[14]. Moreover, optimism moderated the intervention effects such that when optimism was low, compared to high, counterconditioning was more effective in reducing nocebo hyperalgesia compared to continued nocebo conditioning. It could be hypothesized that for pessimists, an intervention strategy might be more necessary than for optimists in reducing nocebo effects. Note as a limitation that a correction for multiple comparisons was not applied due to the exploratory nature of the current study. Although efforts to identify relevant psychological characteristics are still ongoing, a recent meta-analysis pointed towards consistent findings for the optimism-placebo and anxiety-nocebo associations across the literature[10], which is also largely in line with our current findings on nocebo hyperalgesia. Important to point out here is that the majority of existing studies in the field of placebo and nocebo research are closed-label, with only recent studies investigating less deceptive routes of placebo or nocebo administration[16]. Amongst these, one open-label placebo study has looked into the role of personality characteristics in placebo response, and found that optimism predicted the pain ratings in the deceptive placebo and no-treatment groups, but not in the open-label placebo groups[48]. Taken together, further research is recommended for investigating individual differences in open-label paradigms.

To the best of our knowledge, the current study is the first to suggest that susceptibility to nocebo hyperalgesia is an important predictor of nocebo reduction. A few studies have looked into the influence of prior experiences on subsequent nocebo and placebo effects[12,13]. In these studies, participants' positive or negative treatment expectations were first experimentally manipulated by either classical conditioning[12,13] or observational learning[13], similar to how the current study induced nocebo hyperalgesia with (open-label) conditioning in part 1. Next, the carry-over effect of this manipulation was investigated for the pain ratings after the subsequent placebo or nocebo treatment. Their findings show that positive or negative prior learning experiences carry over to the placebo[12,13] or nocebo response[13] given to the subsequent treatment, respectively. Our findings, on the contrary, show that larger nocebo hyperalgesia predicts a larger nocebo reduction across interventions, although it should be noted that methodological differences exist between the current and previous studies. The current study quantified the amount of experimentally-induced nocebo hyperalgesia, which was used as a predictor of nocebo intervention outcomes, instead of exploring the carry-over effects of nocebo hyperalgesia between interventions. This allowed us to determine whether nocebo-reduction strategies of counterconditioning and extinction are still effective[3,6] when nocebo hyperalgesia is large. Our findings show that the effects of the more active

reduction strategy, i.e., counterconditioning compared to extinction, became stronger for individuals with larger nocebo hyperalgesia. A potential explanation of this finding could be that participants who are more susceptible to nocebo hyperalgesia might be susceptible to learning strategies in general, thereby responding equally strongly to the subsequent nocebo-reduction interventions. Also, the potential influence of ceiling or floor effects occurring in parts 1 and 2 cannot be ruled out entirely for their role in how much individual learning could actually take place during nocebo manipulations. Nevertheless, it seems nocebo hyperalgesia could be harnessed to strengthen the efficacy of nocebo-reduction interventions.

There are several clinical implications of our findings. In more than 95% of our healthy female sample, nocebo effects on pressure pain were successfully conditioned with an open-label suggestion. It is possible that in clinical populations, such as with chronic pain, the conditioning procedure results in more robust nocebo effects than in the healthy population. Potentially in chronic pain populations, increased exposure to negative treatment experiences and persistent pain could be associated with larger nocebo hyperalgesia[49–51] than in healthy populations. Therefore, both nocebo-induction and -reduction parts of our experiment should be investigated in clinical populations to make better inferences about the efficacy of open-label counterconditioning. Moreover, the current study identified a number of prognostic and prescriptive factors related to nocebo reduction. Prognostic factors are related to the general treatment outcomes regardless of treatment choice, whereas prescriptive factors predict individual differences in treatment response that can be used for deciding the most suitable treatment choice[52]. Baseline trait anxiety was identified as a prognostic factor, whereas nocebo hyperalgesia and optimism levels were identified as prescriptive factors. Although open-label counterconditioning resulted in a larger overall mean change in nocebo hyperalgesia compared to other groups, and is therefore always recommended, it remains a good treatment choice especially when nocebo hyperalgesia is strong and when dispositional optimism is low. Moreover, individuals with higher trait anxiety are likely to benefit more than those with lower trait anxiety from any nocebo-reduction intervention; therefore, if treating a highly anxious individual, prescribing any one of the two interventions would likely result in nocebo reduction. Note that the current data is insufficient for making claims or recommendations about who would not benefit from these interventions. The generalizability of these findings should be further investigated in different clinical populations, in older populations, and also using sex/gender balanced designs for more specific treatment recommendations for nocebo reduction.

Several suggestions could be provided for future research directions. First, although the investigation of open-label treatment strategies is desirable due to ethical

considerations[53], learning strategies such as conditioning and extinction likely do not occur as openly in daily life as we have introduced in this experiment. As a study limitation, our results may not be generalizable to daily life or be directly comparable with literature on closed-label paradigms. Future research is recommended to compare the efficacy of learning strategies in different contexts. Second, the nocebo training schedule in the current study was continuous, where the conditioned stimulus was consistently paired with the same pain intensity during the learning phase. In real-life, pain experiences are not consistently encountered in the same treatment contexts; therefore, it would be relevant to also test a more ecological variant of this learning model by including a partial reinforcement group to induce nocebo hyperalgesia and to test the efficacy of open-label counterconditioning also for this group. Third, it would be relevant to compare the efficacy of open-label counterconditioning to the nocebo-preventive strategies. Preliminary findings provide evidence for the efficacy of latent inhibition and overshadowing in inhibiting nocebo effects[54,55], while also contingency degradation is promising[54].

Conclusions

To conclude, lower optimism and higher trait anxiety predict larger nocebo hyperalgesia. Open-label counterconditioning appears to be an especially promising method for reducing (open-label) nocebo hyperalgesia in individuals who are highly susceptible to acquiring nocebo hyperalgesia. Moreover, individuals with high trait anxiety are likely to benefit from either counterconditioning or extinction, whereas for individuals with low optimism counterconditioning, compared to control, is more effective. Our findings suggest that susceptibility to nocebo hyperalgesia, dispositional optimism, and trait anxiety might be indicators of a flexible pain-regulatory system that may shape pain experiences in both a negative and positive direction. Research into nocebo-reduction interventions could help personalize interventions to minimize nocebo effects in clinical practice.

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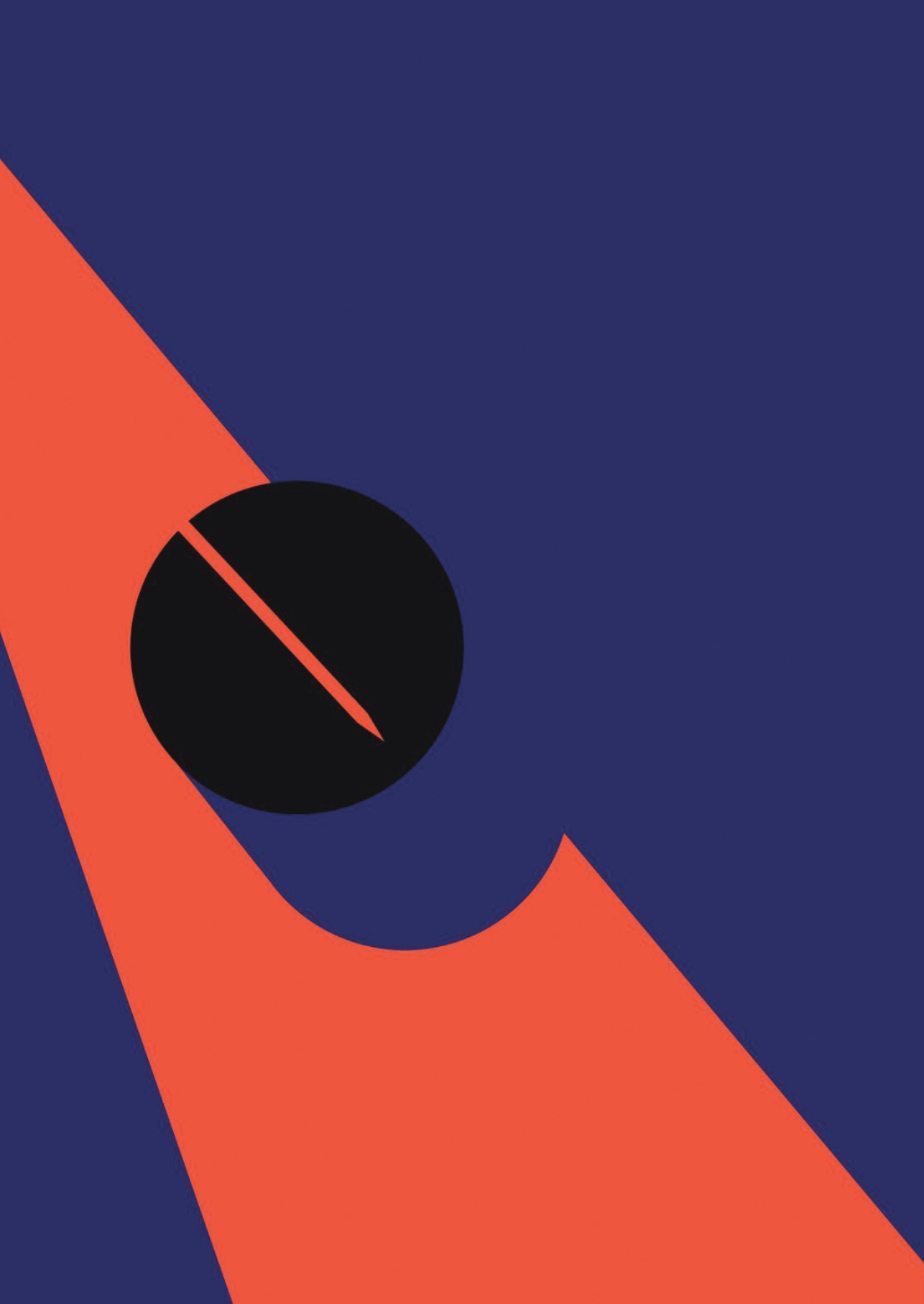

CHAPTER 4

Nocebo hyperalgesia in patients with fibromyalgia and healthy controls: An experimental investigation of conditioning and extinction processes at baseline and one-month follow-up

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ABSTRACT

Nocebo effects are adverse treatment outcomes that are not ascribed to active treatment components. Potentially, their magnitude might be higher in patients with chronic pain compared to healthy controls since patients likely experience treatment failure more frequently. The current study investigated group differences in the induction and extinction of nocebo effects on pressure pain at baseline (*N*=69) and 1-month follow-up (*N*=56) in female patients with fibromyalgia and matched healthy controls. Nocebo effects were first experimentally induced via classical conditioning combined with instructions on the pain-increasing function of a sham TENS device, then decreased via extinction. One month later, the same procedures were repeated to explore their stability. Results suggest that nocebo effects were induced in the healthy control group during baseline and follow-up. In the patient group, nocebo effects were only induced during follow-up, without clear group differences. Extinction was only observed during baseline in the healthy control group. Further comparisons of nocebo effects and extinction indicated no significant changes across sessions; possibly suggesting their overall magnitudes were stable over time and across groups. In conclusion, contrary to our expectations, patients with fibromyalgia did not have stronger nocebo hyperalgesia; instead, they might be less responsive to nocebo manipulations than healthy controls.

Perspective: Current study is first to investigate group differences in experimentally manipulated nocebo hyperalgesia between chronic pain and healthy populations at baseline and 1-month follow-up. Since nocebo effects are common in clinical settings, their investigation in different populations is essential to explain and minimize their adverse effects during treatment.

Keywords: nocebo effect; classical conditioning; pressure pain; hyperalgesia; fibromyalgia

INTRODUCTION

Nocebo effects, which are adverse treatment outcomes unrelated to active treatment components, can occur in clinical or laboratory contexts after receiving an inert or active treatment[1]. They are presumably guided by negative expectations and can be induced and reduced by learning mechanisms[2–5]. An example of nocebo effects is the experiencing of side-effects after disclosing the potential side-effects of a medication, regardless of its pharmacological properties[6]. Various studies have investigated whether nocebo effects can be experimentally induced in healthy or in patient populations (e.g., with chronic back pain, post-operative pain, gastrointestinal disorders, or Parkinson's disease)[7–10]; however, to date, no study has directly compared the magnitude of nocebo effects between a patient and a healthy sample. Research with healthy participants indicates that nocebo hyperalgesia can be induced via classical conditioning and instructional learning, or their combination, with mixed findings on whether nocebo hyperalgesia could be extinguished by extinction[5,11–13]. One study in patients with chronic low back pain has combined conditioning of pain increase with a verbal suggestion that stated both positive and negative effects of a sham opioid treatment and found that placebo, instead of nocebo, effects were induced; possibly due to the ambiguity surrounding the verbal suggestions[10]. Conditioned nocebo effects need to be further investigated using pure verbal suggestions of pain increase, especially in chronic pain conditions such as fibromyalgia where the underlying etiopathogenesis is unclear[14].

Differences may exist in the extent in which patients with persistent physical symptoms, such as fibromyalgia, and healthy individuals are susceptible to nocebo effects. Firstly, patients have a higher cumulative exposure to treatments, which, given the existing challenges in diagnosing and treating fibromyalgia[14] and patients' possible dissatisfaction surrounding disease management[15,16], may have resulted in more negative treatment experiences surrounding treatment failure and patient-doctor exchanges[2,17,18]. Speculatively, along with biological dispositions, repetitive exposure to negative treatment experiences could establish nocebo effects that give rise to the emergence or aggravation of symptoms, and might even propagate symptom chronification over time[18–20]. Resultantly, patients may be more susceptible to acquiring stronger nocebo effects than healthy controls, which may be possibly harder to decrease via extinction[21]. Secondly, fear-conditioning studies have shown learning deficits during pain processing in fibromyalgia[22–25]. In particular, deficits related to contingency learning have been found, where a conditioned stimulus (CS+) paired with an unconditioned stimulus (US) could not be differentiated from another CS that is not paired with the US (CS-)[22]. This could eventually lead to problems with identifying safety cues in the environment that are not predictive of upcoming pain[22,23]. As such, these learning deficits may also

result in (stimulus) generalization of nocebo hyperalgesia, for instance making patients distinguish less clearly between safe and unsafe pain cues. However, the exact underlying mechanisms contributing to nocebo effects in fibromyalgia have not yet been unraveled.

With the goal of elucidating the role of nocebo hyperalgesia in fibromyalgia, the current study is the first to investigate group differences in inducing and decreasing nocebo effects on pressure pain in female patients with fibromyalgia compared to matched healthy controls. Since the majority of nocebo literature is based on findings from healthy participants, this allows us to examine whether patients have a larger magnitude of nocebo effects, which might be harder to decrease. Additionally, we explore whether inducing and decreasing nocebo effects after one month yields comparable findings with the baseline, to examine either the potential stability or progression of these effects over time. Previous literature is limited on the persistence of nocebo effects over time[10,26]. Nocebo effects on experimental pressure pain will be firstly induced by conditioning combined with verbal suggestions on the pain-increasing function of a sham TENS device, and afterwards decreased by extinction. Next, the stability of nocebo effects will be explored at one-month follow-up. Associations between psychological characteristics and the nocebo effect will also be explored for individual differences in the magnitude of nocebo effects.

METHODS

Study Design

This study is part of a larger prospective study on patients with fibromyalgia (ICTRP Identifier: NL8244) and has been approved by the Medical Ethical Committee Leiden-Den Haag-Delft (NL67541.058.18). The current study consists of two experimental sessions taking place at baseline and one-month follow-up (see Figure 1). During the baseline session, nocebo effects on pressure pain were experimentally induced in all participants via classical conditioning combined with verbal instructions about the pain-worsening function of a sham Transcutaneous Electrical Nerve Stimulation (TENS) device. With this procedure, the aim was to condition participants to expect more experimental pain in response to the sham activation of the TENS device. Next, an extinction procedure followed to examine the decrease of potentially induced nocebo effects on pain. All participants were invited to the lab for a second time after one month to take part in nearly the same experimental procedure to investigate the stability of these effects over time. The main difference was that at the 1-month follow-up, the nocebo conditioning and extinction procedures were preceded by a recall testing phase, where we aimed to assess the magnitude of recalled nocebo effects after the baseline session.

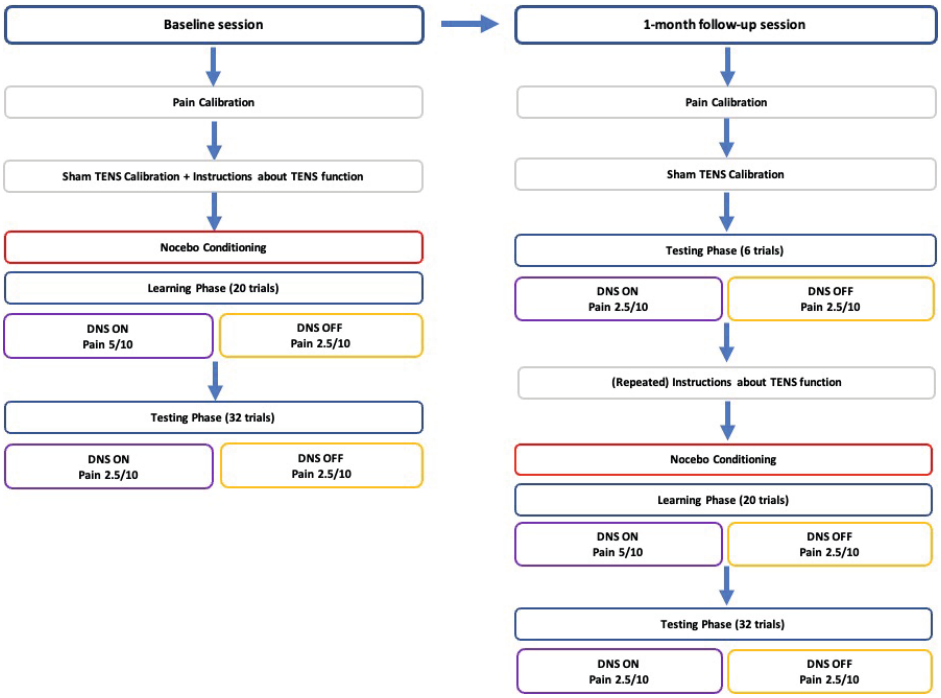

Figure 1. Illustration of the study design. Participants took part in a baseline session and a follow-up session after one month. Both lab sessions consisted of pain calibrations, sham TENS calibrations, instructions about the pain-worsening function of the TENS device, learning phase trials of nocebo conditioning and testing phase trials. The only difference between sessions was that the follow-up session began with a recall testing phase after which instructions about TENS function were repeated. During the learning phase of nocebo conditioning, participants received a moderate pressure pain stimulus when the sham TENS device (labeled as DNS device for participants), was supposedly activated, whereas they received a slight pressure pain stimulus when DNS was supposedly deactivated. In the testing phase, participants received a slight pressure pain stimulus regardless of supposed DNS (de)activation.

Participants

Sample size was calculated using G*Power 3.1[27]. Since to the best of our knowledge previous literature was not detected comparing nocebo effects in healthy and patient populations, it was decided to choose a minimal effect size that is considered clinically relevant[28], i.e., a medium effect size (Cohen's $d = 0.5$, $f = 0.25$) was selected for the planned primary analyses for the baseline and follow-up parts of the study. To conduct a mixed-design ANOVA with two groups and two repeated measurements with an alpha level of .05, a total sample size of $N = 54$ (27 per group) was needed, per session, to demonstrate a power of .95.

All participants were required to be between 18 and 65 years, fluent in the Dutch language, and able to sign an informed consent form. Since fibromyalgia is more prevalent in women than men[29], the current sample consisted of only females to increase the comparability of current findings with existing literature. Healthy controls were matched to patients based on sex, age, and education level. Education level was assessed using the Verhage scale[30], where primary education up to higher general secondary education was categorized as lower education, and higher vocational education up to university education was categorized as higher education. Patients were required to have a fibromyalgia diagnosis by a rheumatologist, which was verified during the telephone screening by patients' self-report of the year, location, and the provider of their diagnosis. Additionally, all participants, including healthy controls, filled in the Fibromyalgia Survey Questionnaire[31] to verify the presence or absence of key fibromyalgia symptoms in each group. Patients were excluded if they received a medical diagnosis other than fibromyalgia explaining their chronic pain symptoms (e.g., rheumatoid arthritis, polyarthritis) or had severe physical or mental co-comorbidities that were not related to fibromyalgia (e.g., cancer, schizophrenia). Patients were allowed to continue treatment as usual and were specifically asked not to make any changes to their usual dose of analgesic medication 24 h prior to the measurements. Healthy controls were excluded if they had chronic pain complaints (≥3 months) in the past or present, a fibromyalgia diagnosis, severe physical or mental co-morbidities that could interfere with the study protocol, current pain on the measurement days (common types of pain such as localized muscle soreness after work-out rated ≤ 3/10 on the Numeric Rating Scale were included), or used analgesic medication within 24 h prior to the measurements. The common exclusion criteria for both groups were: pregnancy or breastfeeding, color blindness, injuries or wounds on the non-dominant hand or arm, refusal to remove possible artificial nails or nail polish covering the thumbnail of the non-dominant hand, an unsuccessful pressure pain calibration procedure, i.e., not being able to stably distinguish between pressure intensities during pressure pain calibration, and as an additional safety measure due to the brief TENS activation: carrying a pacemaker or implanted pumps, or having implanted metals in the non-dominant hand or arm.

Participants were recruited via advertisements, such as flyers shared at various fibromyalgia patient organizations, pain rehabilitation centers, or Facebook. A portion of the healthy control sample was recruited via the Dutch online registry for neuroscience Hersenonderzoek.nl (www.hersenonderzoek.nl). Study participation involved taking part in the telephone screening, filling out baseline questionnaires at home, and attending two lab sessions, one at baseline and one at one-month follow-up. Participants received an ascending share of the total reimbursement in each lab session, in order to provide an extra motivation to complete the study. All participants received €50 compensation for completing all study parts with additional reimbursement of travel costs to the

lab. If a participant dropped out or was excluded during the calibration procedure, the compensation amount was adjusted based on the amount of time spent in the study. Participants gave verbal informed consent for the information collected during the telephone screening, digital informed consent for the online questionnaire, and signed informed consent for the experimental data collection in the lab.

Pressure Pain Application

Pressure pain is an ecologically valid stimulus type for disorders involving musculoskeletal pain[32], such as fibromyalgia[11]. Pressure pain was induced on the thumbnail of the non-dominant hand using a custom-built automatic pressure administrator called Pneumatic Electronic Pressure Pain Administrator (PEPPA) (see Figure 2), engineered by the Support for Research, Laboratory and Education (SOLO) team of Leiden University based on a prototype design from Karolinska Institute in Sweden[33]. To apply pressure pain, the thumb of the non-dominant hand was inserted in a transparent cylinder hand piece built by the Development and Instrumental Affairs department of Leiden University Medical Center (LUMC). Pressure was applied to the middle of the thumbnail via a piston with a 1 cm^2 probe, which automatically moved up and down by the pressured air supplied by an air compressor. Each pressure stimulus lasted 2.5 s, with a 30 s inter-stimulus interval. As a safety measure, the extension air of the cylinder was limited to 80 kPa, corresponding to a thumb force of 15 kgf/cm^2, which is the maximum pain tolerance in healthy participants that is known to be safe for pain administration[34]. Nevertheless, the current study took extra precautions by not exceeding the maximum thumb force of 13 kgf/cm^2 for both patients and healthy participants. Additionally, an emergency stop button was provided for participants to stop the pressure administration if they could no longer endure the pain. E-prime version 3.0 (Psychology Software Tools, Pittsburg, PA) was used for presenting the pressure pain stimuli and for entering participants' pain ratings after each trial.

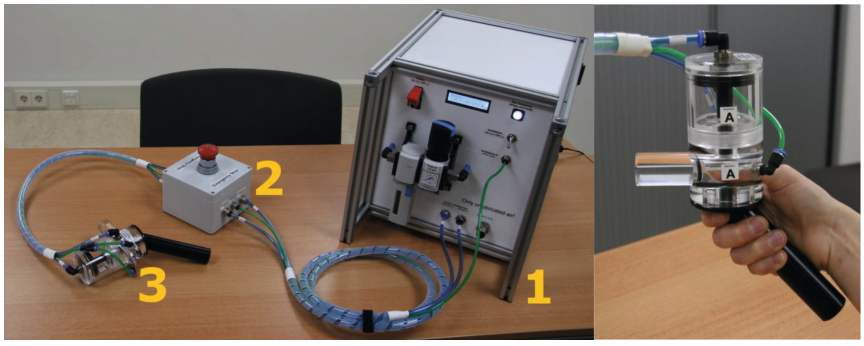

Figure 2. Picture on the left depicts the components of PEPPA. The first is the main device containing the electronics and pneumatics, the second is the emergency stop button, and the third is the hand piece for applying pressure to the thumbnail. Picture on the right demonstrates the thumb insertion into the hand piece.

Pain Measure

Following each experimental pressure stimulus, participants rated their pain intensity on a Numeric Rating Scale (NRS), with the end points 0 representing no pain and 10 the worst pain imaginable. Participants rated their pain by positioning a pointer on a digital horizontal line with anchors, each line representing a decimal on the 0-10 NRS. Participants were instructed to rate above zero (thus 0.1 upwards) when they started to feel pain next to a pressure sensation.

Pressure Pain Calibration

The calibration procedure consisted of three parts, with 5-minute breaks in-between, to minimize sensitization or habituation from repeated stimulus administration. Breaks were extended by one minute, up to five minutes, if the participant indicated still having pain ≥1 on the NRS. No participant has asked for a break exceeding the initial five minutes. Pressure intensities were administered starting from 1 kgf/cm^2 with 0.5 kgf/cm^2 increments until participants rated ≥ 5.5 on the NRS or until 13 kgf/cm^2 was reached. By choosing the highest intensity scored as zero on the NRS and the highest scored pressure intensity, 3 intermittent pressure intensities were calculated that were equidistant from each other in magnitude. Together, these five intensities were then randomly administered three times to determine the pressure intensities rated 0 (ranges 0-1), 2.5 (ranges 2-3), 5 (ranges 4.5-5.5) on the NRS to determine the non-painful, slight, and moderate pain intensities, respectively. Next, a calibration check followed where the pressure intensities for no pain, slight pain, and moderate pain were randomly administered with slight pain presented thrice and the rest presented twice. The experimenter controlled whether the pain ratings were within the targeted ranges; if not, adjusted pressure intensities were based on E-prime's calculations using standard formulas (see Supplemental File I). If manual adjustments were not possible due to the requirement of less pressure than the minimum or more pressure than the maximum amount that PEPPA could safely administer, participants were excluded.

Experimental manipulation of nocebo effects

Sham TENS device

A sham TENS device (Bentrotens T37, Bentronic Gesellschaft fuer Medizintechnik GmbH, Wolnzach, Germany) was used as conditioned stimulus in the conditioning paradigm, wherein a chip was inserted to cease the device from sending any electrical signals after 1 minute. The device was renamed as "Dermal Nerve Stimulation" (DNS) device to prevent possible preconceptions about TENS from interfering with the experimental manipulations. Participants were given a fake device leaflet that read: *"DNS is a device that stimulates nerves via electrical signals. This stimulation helps increase the communication*

between the nerve cells. Nerve cells in the skin communicate with other nerve cells in the spine via electrical signals. The DNS device can influence these signals, for example, by increasing the intensity of the signals coming from a painful stimulus. When these signals are sent from the spine to the brain, you become aware of the sensation of pain. The DNS device applies electrical signals via electrodes attached to your skin. An advantage of DNS is that a light and an (almost) unnoticeable signal is sufficient to influence the communication between the nerve cells; and therefore, to increase your pain sensation."
After participants read the leaflet, the experimenter further explained that the clinical use of DNS is to increase sensations, e.g., to treat numbness that might occur after a surgery or an accident, and that the purpose of the current study is to investigate whether there is a difference in pain sensitivity between patients with fibromyalgia and healthy participants. The real purpose of the experiment, i.e., the investigation of nocebo effects, was not disclosed until the end of the study to not bias any pain-related expectations. A sham calibration procedure followed, where the intention was not to actually calibrate the DNS device but to demonstrate how electrical signals feel on the skin to increase the believability of DNS device function. After cleaning the skin with alcohol, two electrodes were attached vertical to each other on the radial side of the forearm of the non-dominant hand. While the experimenter slowly increased the electrical intensity, participants were asked to indicate the moment that they just noticed a sensation, which was told to be the intensity they would eventually receive throughout the experiment. In reality, all electrical activity stopped after 1 minute. A flashing light allowed the DNS device to appear as if it was still working.

Nocebo Conditioning with Verbal Suggestions

Nocebo effects on pressure pain were induced through conditioning and verbal suggestions using the DNS device. Participants were instructed that when the DNS device was activated, the text "DNS on" would appear on the computer screen, signaling that the device would increase their pain sensitivity, and that the "DNS off" message would appear when the device was deactivated and would not have any influence on their pain sensitivity. DNS on/off messages were presented for 2.5 s using E-prime version 3.0 (Psychology Software Tools, Pittsburg, PA) and were color-coded in either orange or purple, counterbalanced across participants. After the message disappeared, participants received a painful pressure stimulus on their thumbnail for 2.5 s, which was rated on the NRS after each trial with an inter-trial interval of 30 s. The learning phase consisted of 20 trials, where DNS was supposedly activated in half of them. During the experimental trials of the learning phase, i.e., when DNS was supposedly activated, participants received a moderately painful pressure intensity on their thumbnail; during the control trials, i.e., when DNS was supposedly deactivated, they received slight pain. All trials were semi-randomized and not presented more than twice in a row.

Testing phase: Nocebo Effects and Extinction

Directly after nocebo conditioning, a testing phase including extinction took place. The testing phase consisted of 16 experimental (DNS on) and 16 control (DNS off) trials, which were all paired with only slight pain on the thumbnail regardless of the supposed DNS (de) activation, to no longer reinforce the conditioned nocebo effects. After the first 6 testing phase trials, which were used to determine the magnitude of the nocebo effect after nocebo conditioning[11,35,36], a 10-minute break took place. Following this short break, participants were told that the next part of the experiment would be similar to before and that the DNS on/off text would appear signaling DNS (de)activation. No additional verbal suggestions were provided about extinction. Then, the remaining 26 trials ensued. All trials were semi-randomized and not presented more than twice in a row.

The remaining magnitude of nocebo effects after extinction was determined based on the final 6 testing phase trials (3 experimental and 3 control) [11,35,36].

Stability of nocebo effects and extinction across sessions

The same nocebo conditioning and extinction procedures were repeated at one month follow-up. The main difference was that nocebo conditioning and testing phases were preceded by a recall testing phase, to identify the magnitude of nocebo effects recalled after the extinction procedure in the baseline session. The recall testing phase consisted of 6 trials paired with only slight pain, half of which were experimental (DNS on) trials and the other half control trials (DNS off). All trials were semi-randomized and not presented more than twice in a row.

Self-report measures

The Dutch versions of various questionnaires were used to assess participants' clinical and psychological characteristics, which were filled in once before arriving to the first lab session. The Fibromyalgia Survey Questionnaire (FSQ)[31], which is based on the American College of Rheumatology 2010/2011 diagnostic criteria, was filled in by both groups to assess the presence or absence of key symptoms of fibromyalgia. A Fibromyalgia Severity (FS) score was calculated by summing the symptom severity score, ranging between 0-12, and the widespread pain index, ranging between 0-19; a cut-off score of FS ≥12 was considered reliable to satisfy the diagnostic criteria[37]. Those with FS <12 who had already received a fibromyalgia diagnosis were considered to be improving[37].

The Fibromyalgia Impact Questionnaire (FIQ)[38] was filled in by patients to assess their functional disability related to fibromyalgia (Cronbach's α = 0.85). The first item consists of 11 questions on physical functioning, which is scored by taking the mean of all ratings ranging between 0 (always) and 3 (never). The second item assesses how many days

they felt good in the past week, scored inversely between 0 and 7, and the third item assesses how many days of work they missed in the past week, scored between 0 and 7. Items 4-10 assess the severity of various symptoms, ranging between 0 (no impairment) and 10 (maximum impairment). The first 3 scores are subjected to a normalization procedure, after which all scores are averaged and if a patient didn't answer all questions an equalization calculation was employed. The scores range between 0-100, where an average patient scores 50 and higher scores indicate a larger functional disability[38].

The short version of the Depression Anxiety and Stress Scale (DASS-21)[39] was filled in by all participants to assess the negative emotional states of depression, anxiety, and stress subscales (depression subscale Cronbach's α = 0.83; anxiety subscale Cronbach's α = 0.73; stress subscale Cronbach's α = 0.87). The scale consists of 21 statements that are rated between 0 (did not apply to me at all) and 3 (applied to me very much or most of the time). Scores from each subscale are summed and then adjusted to range between 0-42 per subscale for comparability with DASS-42, with higher scores indicating greater symptom severity.

The Life Orientation Test-Revised (LOT-R)[40] was used for assessing dispositional optimism in all participants (Cronbach's α = 0.73). The LOT-R is a 10-item measure consisting of positive, negative, and filler items rated on a 5-point scale between 0 (strongly disagree) and 4 (strongly agree). To calculate optimism, the negative items were reverse coded and then summed with the positive items, resulting in a total score ranging between 0-24, with higher scores indicating higher optimism.

The Pain Catastrophizing Scale (PCS)[41] was used for assessing pain catastrophizing thoughts in all participants (Cronbach's α = 0.91). PCS is a 13-item measure consisting of rumination, magnification, and helplessness subscales, which is rated on a 5-point scale between 0 (not at all) and 4 (all the time). To calculate a PCS score, a sum score of all items was calculated, ranging between 0-52, with higher scores indicating more pain-catastrophizing thoughts.

The Body Vigilance Scale (BVS)[42] was used for assessing participants' attention to bodily sensations (Cronbach's α = 0.93). The first three items in the BVS are directly rated on an 11-point scale between 0 (never) and 10 (always), whereas the fourth item consists of 15 sub-items that are rated separately. To calculate the BVS score, ratings in the fourth item were averaged and afterwards summed with the first three items, ranging between 0-40, with higher scores indicating greater focus on bodily sensations.

The Pearlin Mastery Scale (PMS)[43] was used for assessing the psychological coping resources of all participants based on self-mastery (Cronbach's α = 0.74). The PMS consists of 7 items rated between 1 (strongly disagree) and 5 (strongly agree). Items are summed up, ranging between 7-35, with higher scores indicating greater levels of mastery.

The state scale of State-Trait Anxiety Inventory short-form (STAI-S-6)[44] was used for assessing state anxiety on the day of experimentation in all participants (session 1: Cronbach's α = 0.77; session 2: Cronbach's α = 0.81). The scale consists of 6-items that are rated on a 4-point scale between 1 (not at all) and 4 (very much so). Positive items were reverse coded and then the sum of all items was calculated. Scores were adjusted to range between 20-80 for comparability with STAI-S.

Patients rated their clinical pain and fatigue levels on the day of experimentation using 11-point scales between 0 (no pain/fatigue) and 10 (worst pain/fatigue imaginable), with higher ratings indicating greater symptom severity. Lastly, exit questionnaires were filled in at the end of the study on the perceived aim of the study, perceived effect of DNS on pain sensitivity, trust in the experimenter, perceived competence of the experimenter, and perceived experiment length. The first item required an open-ended answer, whereas the rest of the items were rated on a 0-10 NRS, with higher scores indicating higher intensity. The perceived experiment length was anchored "exactly long enough" around 5/10 on the NRS.

Procedure

Interested individuals were screened for eligibility via a telephone call, which took approximately 10-20 min. A verbal informed consent was obtained prior to screening. If eligible, participants were invited to the lab sessions, 2-2.5 h each, at the Leiden University Treatment and Expertise Center (LUBEC; Leiden, the Netherlands). Before the first lab appointment, participants were asked to fill in an online battery of questionnaires (Qualtrics, Provo, UT) at home taking about 20-30 min, before which they digitally provided an informed consent. After arriving at the lab, explanations were provided about the upcoming experimental procedures and that the study participation was voluntary. After all questions were answered, the experimenter controlled if the participant fulfilled the eligibility criteria for the day of testing, and then the informed consent form was signed. All participants filled in an online questionnaire to assess their current state anxiety levels, where only patients were asked to additionally indicate their current pain and fatigue levels. A brief demonstration of the PEPPA followed, involving practicing the thumb insertion and pain ratings, and then the pressure pain calibration ensued. Next, written and verbal instructions were provided about the DNS device, after which electrodes were attached on participants' arm and the sham calibration of the DNS device

took place. Directly afterwards, after a non-painful practice trial, the nocebo conditioning and testing phases began. When the experiment finished, the experimenter left the room and participants did a 4-minute relaxation task in the form of a guided breathing exercise instructed via headphones to help recover from the potential stress arising from pain administration. At the end of the session, patients were assisted in downloading an app on their phone for rating their daily pain intensity in the coming 3 weeks, which was a procedure pertaining to the larger patient study and will not be addressed in the current paper.

The follow-up lab session took place one month later at LUBEC. The procedure was the same as during the baseline session, with two exceptions. First, the pressure pain calibration was shorter. The pain ratings from the baseline session were used here to replace the first calibration step, i.e., ascending series, since pain thresholds were not expected to change over one-month. However, the remaining calibration steps, i.e., random series and calibration check, still took place to check whether the pressure intensities from the ascending series were successfully rated again within the targeted pain ranges, and if necessary, adjustments were made using the same formulas. Second, the experimental manipulations now began with 6 additional (recall) testing phase trials to measure the magnitude of recalled nocebo effects remaining from the baseline session. After a 5-minute break, participants were orally reminded again about the function of the DNS device, and then the nocebo conditioning and extinction procedures ensued as before with a 10-minute break halfway into the experiment. At the end of the session, participants completed the relaxation task, filled out exit questionnaires, and were reimbursed for their participation.

Statistical Analyses

Data analyses were conducted using the R software environment, version 4.1.0[45]. ANOVA assumptions of normality, homogeneity of variances, and sphericity were checked with QQ plots, Levene's test, and Mauchly's test of sphericity, respectively. When sphericity was violated, either the Greenhouse-Geisser correction (epsilon < 0.75) or the Huynh-Feldt correction (epsilon > 0.75) was considered[46]. Statistical outliers were detected based on z-scores ($z < -3$ or $z > 3$) of the dependent variable. A *p*-value below 0.05 was considered statistically significant unless indicated otherwise. Partial eta-squared (η_p^2) was calculated as the effect size of ANOVA. A partial eta-squared effect size around 0.01 is considered small, 0.06 considered medium, and 0.14 considered large[47]. Cohen's *d* was calculated as the effect size of pairwise t-tests, where 0.2 is considered small, 0.5 considered medium, and 0.8 considered a large effect size[47]. To check whether groups were successfully matched on age and education level, an independent-samples t-test was conducted on the mean age between groups, and a chi-square test was conducted

on the education level (lower vs higher) between groups, respectively. Independent-samples t-tests were used for analyzing between-group differences in calibration intensities, perceived effect of DNS on pain sensitivity, trust in experimenter, perceived competence of the experimenter, and perceived experiment length. Because of multiple comparisons, a Bonferroni correction was applied such that a *p*-value below 0.01 was considered statistically significant.

As a manipulation check, it was examined whether learning has occurred during nocebo conditioning in both sessions. Four paired-sample t-tests were conducted on the mean pain ratings between experimental and control trials during the learning phase of nocebo conditioning in each session to identify whether the associations of "DNS on" with moderate pain and "DNS off" with slight pain were correctly made by each group. Moreover, open-ended answers describing the perceived aim of the study were checked to see whether any participants identified the DNS as a sham device.

To investigate whether nocebo effects were successfully induced during nocebo conditioning in both sessions and whether this induction of nocebo effects differed between groups, a 2 x 2 mixed-design ANOVA was conducted per session, with group (patient vs healthy control) as between-subjects variable and trial type (experimental vs control) as within-subjects variable on the average pain ratings from the first 3 experimental and first 3 control trials of the testing phase. When a significant interaction effect of group by trial type was detected, Bonferroni-corrected pairwise comparisons were applied to more closely examine the manipulation effects between experimental and control trials in each group.

To examine the change in nocebo effects after extinction in both sessions and whether this extinction in nocebo effects differed between groups, a different analysis plan was chosen including difference scores to facilitate the interpretation of findings. A 2 x 2 mixed-design ANOVA was conducted per session, with group (patient vs healthy control) as between-subjects variable and time (nocebo conditioning vs extinction) as within-subjects variable on the difference scores. The difference score after nocebo conditioning was calculated by subtracting the average pain ratings given to the first 3 control trials from the first 3 experimental trials of the testing phase. The difference score after extinction was calculated by subtracting the average pain ratings given to the last 3 control trials from the last 3 experimental trials of the testing phase. The difference score after nocebo conditioning determined the magnitude of nocebo effects, whereas after extinction, it determined the magnitude of nocebo effects remaining after extinction. By comparing the difference scores after nocebo conditioning and after extinction, we investigated whether the magnitude of nocebo effects was significantly lower after

extinction. When a significant interaction effect between group and time was detected, Bonferroni-corrected pairwise comparisons were applied to determine the manipulation effects between nocebo conditioning and extinction on nocebo effects in each group.

To explore the stability of the induction and extinction of nocebo effects across sessions and whether this differed between groups, a 2 x 5 mixed-design ANOVA was conducted with group (patient vs healthy control) as between-subjects variable and time (nocebo conditioning and extinction from sessions 1 and 2, and the recall testing phase from session 2) as within-subjects variable on the difference scores. The difference score after the recall testing phase was calculated by subtracting the average pain ratings given to the 3 control trials from the 3 experimental trials. If a significant interaction effect was detected between group and time, Bonferroni-corrected multiple pairwise comparisons were computed to determine the time level differences in each group. To examine the stability of induction of nocebo effects across sessions, Bonferroni-corrected pairwise comparisons were applied between the time levels 1) nocebo conditioning in sessions 1 and 2, 2) nocebo conditioning in session 1 and the recall testing phase in session 2. To examine the stability of extinction across sessions, Bonferroni-corrected pairwise comparisons were applied between the time levels 1) extinction in sessions 1 and 2, 2) extinction in session 1 and the recall testing phase in session 2. A *p*-value below .025 was considered to indicate a statistically significant lack of stability in the induction or extinction of nocebo effects across sessions.

To allow for the assessment of extinction efficacy for a subgroup of participants who were observed to be susceptible to learning nocebo effects, sensitivity analyses were conducted for the extinction of nocebo effects after removing nocebo non-responders, i.e., participants with difference scores equal to or below zero, from the analyses. The same analyses were subsequently conducted in the subgroup of nocebo responders for the extinction of nocebo effects and for the stability of extinction across sessions. To allow for the assessment of nocebo and extinction efficacy for participants who could be clearly differentiated in their fibromyalgia symptomatology, another set of sensitivity analyses were conducted after removing patients scoring FS <12 or healthy controls scoring FS ≥12 on the FSQ, using the same analyses for the induction and extinction of nocebo effects in both sessions. Additionally, we checked whether the induced nocebo magnitudes were associated across sessions. This was explored with a repeated measures correlation analysis conducted for the magnitude of nocebo effects between two sessions firstly per group and then after pooling both samples. All sensitivity analyses were reported under Supplemental File II.

Lastly, we conducted Pearson's correlation analyses to examine the relation between the magnitude of nocebo effects in session 1 and depression (DASS-21), trait anxiety (DASS-21), stress (DASS-21), optimism (LOT-R), pain-catastrophizing thoughts (PCS), body vigilance (BVS), and mastery (PMS) in both participant groups, as well as fibromyalgia disability (FIQ) in the patient group. Moreover, for each session, we examined the relationship between the magnitude of nocebo effect induction and the state anxiety (STAI-S-6) and pain and fatigue levels (NRS) on the experiment day.

RESULTS

A total of 81 participants were eligible for participating in the experiment (patients $N = 46$; healthy controls $N = 35$). Of these, 8 participants (6 patients, 2 healthy controls) were excluded during the first session due to problems with pressure pain calibration (i.e., pain ratings were lower than the required pain ranges for moderate and slight pain) and 1 patient dropped out due to misunderstanding the instructions for rating pain intensity. During the second session, 4 participants (1 patient, 3 healthy controls) were excluded due to problems with pressure pain calibration and 8 participants (7 patients, 1 healthy control) dropped out for personal reasons (e.g., scheduling issues, testing positive for COVID-19). Moreover, due to technical and software-related problems, data could not be retrieved from 3 participants (2 patients, 1 healthy control) in session 1 and from another 3 participants (2 patients, 1 healthy control) in session 2. Considering that 28% of patients and 9% of healthy controls dropped out after the baseline session, a total of 69 participants (37 patients and 32 healthy controls) were included in session 1 to also reach a minimum sample size in the follow-up session, which resulted in a total inclusion of 56 participants in session 2 (patients $N = 29$; healthy controls $N = 27$). All included participants per session were considered for statistical analyses.

Descriptive statistics

Table 1 displays the demographic and health-related characteristics of the sample, and Table 2 displays the group means and SDs from psychological characteristics and exit questionnaires. The fibromyalgia severity score in the patient group was between 6 and 26, where 3 patients had scores <12, indicating that they might be in a recovery period; all patients were considered for the main analyses. In the healthy control group, scores ranged between 0-9, where no healthy participant reached the cut-off score. There were no significant group differences in the mean age ($t(67) = 1.64$, $p = .11$) or the education level ($X^2(1) = .31$, $p = .58$) of participants, suggesting a successful group matching. Table 3 displays the means and SDs of calibration values (kgf/cm^2). No significant group differences were observed for the calibration values of slight and moderate pressure

pain intensities in session 1 (slight pain: $t(67) = 1.98$, $p = .053$; moderate pain: $t(67) = 1.98$, $p = .051$) and session 2 (slight pain: $t(54) = .70$, $p = .51$; moderate pain: $t(54) = .92$, $p = .36$). Neither were there any group differences in the perceived effect of DNS on pain sensitivity ($t(59) = 1.38$, $p = .17$), trust in the experimenter ($t(59) = .76$, $p = .45$), perceived competence of the experimenter ($t(59) = .17$, $p = .87$), or the perceived experiment length ($t(59) = 1.35$, $p = .18$). Moreover, Table 3 displays the overall mean pain intensity ratings and Figure 3A and Figure 3B display the trial-by-trial change in mean pain intensity ratings across sessions. Note that in Figures 3A and 3B, an upward trend can be observed in the horizontal lines, which is also reflected in Table 3 by an increase in pain ratings during the extinction phase, both of which potentially illustrating a pain sensitization across trials.

Table 1

Demographic and health-related characteristics of female participants in the study.

	Session 1		Session 2	
Characteristics	Patient (*N* = 37)	Healthy control (*N* = 32)	Patient (*N* = 29)	Healthy control (*N* = 27)
Age (years) [mean (SD)]	37.81(10.47)	33.56(10.97)	34.21(9.96)	33.78(11.31)
Higher education Level [n (%)]	28(76)	26(81)	21(72)	21(78)
Partner [n (%)]	32(87)	20(63)	25(86)	16(59)
Work status [n (%)]				
Student	13(35)	18(56)	13(45)	15(56)
Employed	34(92)	32(100)	27(93)	27(100)
Work (hours per week) [mean (SD)]	24.96(9.99)	26.27(11.67)	26.32(10.11)	27.63(10.74)
Unemployed	1(3)	1(3)	1(3)	1(4)
Volunteer work	9(24)	13(41)	10(34)	11(41)
Run household	16(43)	3(9)	13(48)	3(11)
Disability pension	7(19)	1(3)	4(15)	1(4)
Retired	0	1(3)	0	1(4)
Fibromyalgia Severity (FSQ) [median (IQR)]	18(8)	4(3)	17(9)	4(3)
Fibromyalgia Disability (FIQ) [mean (SD)]	40.95(13.48)	-	41.06(14.04)	-
Fibromyalgia complaints (years) [mean (SD)]	14.51(9.81)	-	14.32(8.59)	-
Fibromyalgia diagnosis (years) [mean (SD)]	6.59(6.16)	-	5.58(3.49)	-

Table 2

Group means and SDs for psychological characteristics and exit questionnaires.

	Patient	Healthy control
Characteristics	*Mean (SD)*	
Depression	7.73(6.83)	1.63(2.98)
Anxiety	5.46(5.07)	1.31(2.25)
Stress	14.11(7.53)	4.81(4.28)
Dispositional Optimism	15.73(3.25)	16.78(2.69)
Pain Catastrophizing	12.27(7.66)	7.28(7.63)
Body Vigilance	13.65(7.16)	10.97(6.18)
Self-Mastery	26.16(3.88)	27.91(3.14)
State Anxiety prior to testing during session 1	34.78(7.19)	29.53(7.67)
State Anxiety prior to testing during session 2	35.64(9.57)	28.35(7.49)
Pain prior to testing during session 1	4.32(1.87)	-
Pain prior to testing during session 2	4.11(2.03)	-
Fatigue prior to testing during session 1	4.59(2.05)	-
Fatigue prior to testing during session 2	4.61(1.91)	-
Perceived DNS effect on pain sensitivity	4.74(2.33)	3.74(2.80)
Trust in experimenter	9.00(1.00)	9.30(0.87)
Perceived competence of experimenter	9.04(0.90)	9.11(1.22)
Perceived length of study	5.67(1.04)	6.11(1.28)

Note. Total sample size for trait and state characteristics in session 1 was 69 (patient *N*=37; healthy control *N*=32) whereas for state characteristics in session 2 and exit questionnaires was 56 (patient *N*=29; healthy control *N*=27).

Table 3

Group means and SDs for pressure intensity levels (calibration) and pain intensity ratings (recall testing phase, nocebo conditioning, extinction) across sessions.

	Session 1		Session 2	
	Patient (*N*=37)	Healthy Control (*N*=32)	Patient (*N*=29)	Healthy Control (*N*=27)
Calibration (kgf/cm2)		*Mean (SD)*		
Slight Pain	4.66(1.90)	5.59(2.00)	5.24(2.28)	5.64(2.25)
Moderate Pain	6.76(2.72)	8.05(2.67)	7.14(2.65)	7.81(2.86)
Recall Testing Phase (0-10 NRS)				
Experimental Trials	-	-	2.73(0.97)	2.77(0.94)
Control Trials	-	-	2.59(0.99)	2.67(1.12)
Difference Score	-	-	0.14(0.67)	0.10(0.55)
Nocebo Conditioning (0-10 NRS)*				
Learning Phase (20 Trials)				
Experimental Trials	5.71(1.37)	5.70(0.90)	5.70(1.22)	5.49(1.22)
Control Trials	3.69(1.41)	3.52(1.05)	3.29(1.35)	3.23(1.20)
Difference Score	2.02(0.50)	2.17(1.03)	2.41(1.11)	2.25(1.13)

Table 3

Continued.

	Session 1		Session 2	
	Patient (*N*=37)	Healthy Control (*N*=32)	Patient (*N*=29)	Healthy Control (*N*=27)
Testing Phase (Trials 1-6)				
Experimental Trials	4.17(1.86)	4.30(1.48)	4.06(1.77)	3.77(1.55)
Control Trials	3.98(1.82)	3.69(1.53)	3.72(1.77)	3.29(1.32)
Difference score	0.19(0.74)	0.61(0.62)	0.34(0.91)	0.48(0.72)
*Extinction** (0-10 NRS)*				
Testing Phase (Trials 26-32)				
Experimental Trials	5.09(1.96)	4.65(1.83)	4.97(1.88)	4.43(1.90)
Control Trials	4.80(1.96)	4.44(1.79)	4.67(1.79)	3.94(1.60)
Difference score	0.29(0.60)	0.21(0.55)	0.29(0.76)	0.49(0.66)

Note for session 1: *patient sample excluding 1 outlier (*N*= 36); **patient sample excluding 2 outliers (*N* = 35)

Assumption checks

The ANOVA assumptions of normality and homogeneity of variances were not violated. In cases where Mauchly's test of sphericity was violated, corrections were made on the degrees of freedom. Notably, two patients were detected as statistical outliers based on the difference scores after nocebo conditioning (z = 4.04) or after extinction (z = -3.72) in session 1. Given the extremity of these statistical outliers and since they had a significant impact on the study findings, they were considered not representative of the sample and were therefore excluded from the corresponding analyses relating to session 1. For more detailed results including these outliers see Supplemental File II. No statistical outliers were detected based on data from session 2.

Manipulation check

Results from the paired-samples t-tests showed that learning had successfully occurred during the learning phase of nocebo conditioning in both sessions for patients (session 1 $t(36) = 14.43$, $p < .001$; session 2 $t(28) = 11.71$, $p < .001$) and healthy controls (session 1 $t(31) = 11.92$, $p < .001$; session 2 $t(26) = 10.35$, $p < .001$). Factors such as having prior knowledge of, or experience with, a TENS device, and in case of experience finding it effective, did not have any significant impact on the magnitude of nocebo effects in either session (for more details see Supplemental File III). Moreover, open-ended answers given to the perceived aim of the study was aligned with the information provided about the study, where no participants suspected that the DNS device was never activated.

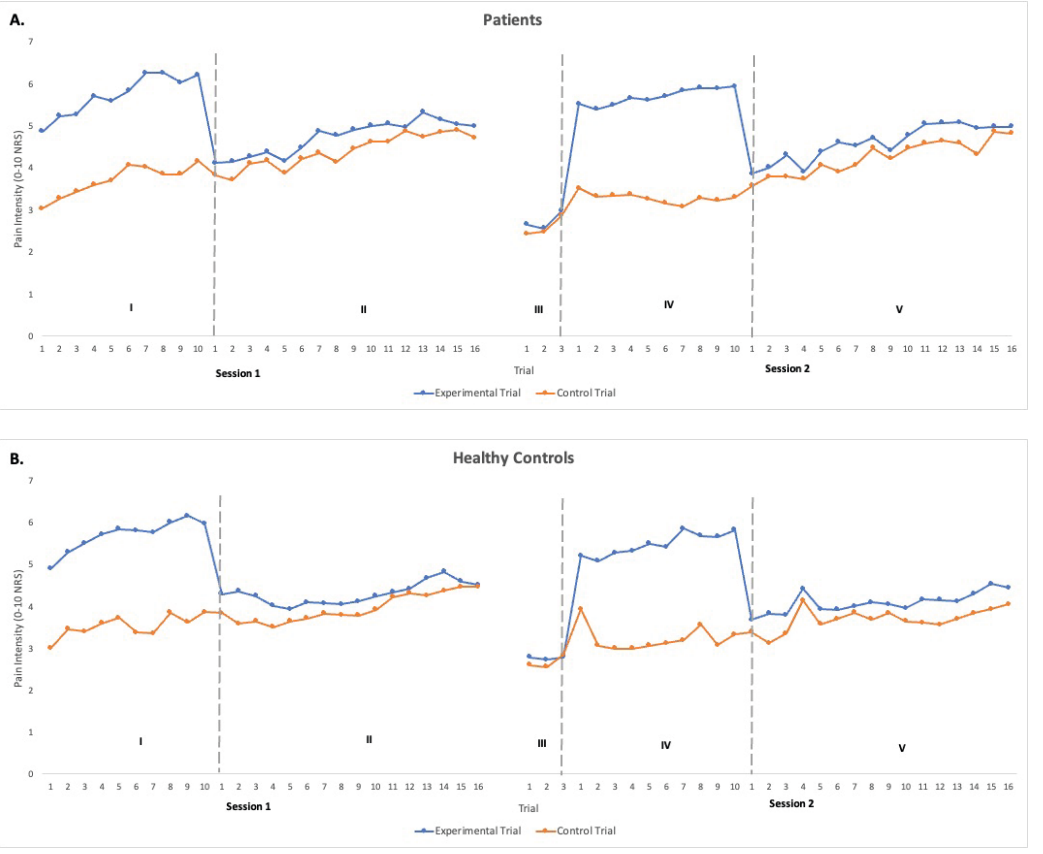

Figure 3. Mean pain intensity ratings across all trials in sessions 1 and 2 in the patient group excluding outliers **(A)** and the healthy control group **(B)**. Experimental and control trials are represented in separate lines. Section I: Trials in the learning phase of nocebo conditioning; Section II: Trials in the testing phase; Section III: Trials in the recall testing phase; Section IV: Trials in the learning phase of nocebo conditioning; Section V: Trials in the testing phase.

Induction of nocebo effects in session 1

A 2 x 2 mixed-design ANOVA showed a significant interaction effect between group and trial type in session 1 ($F(1,66) = 6.36$, $p = .01$, $\eta_p^2 = .08$) and a main effect of trial type ($F(1,66) = 23.43$, $p < .001$, $\eta_p^2 = .27$), but no main effect of group ($F(1,66) = .04$, $p = .84$, $\eta_p^2 < .01$). Bonferroni-corrected pairwise comparisons between the trial type levels at each group showed that the mean pain ratings in experimental trials were significantly higher than control trials in the healthy control group ($p < .001$, $d = .41$). In the patient group, however, the mean pain ratings were not significantly higher in the experimental trials compared to control trials ($p = .13$, $d = .10$). Figure 4 displays the magnitude of induced nocebo effects, across sessions and groups.

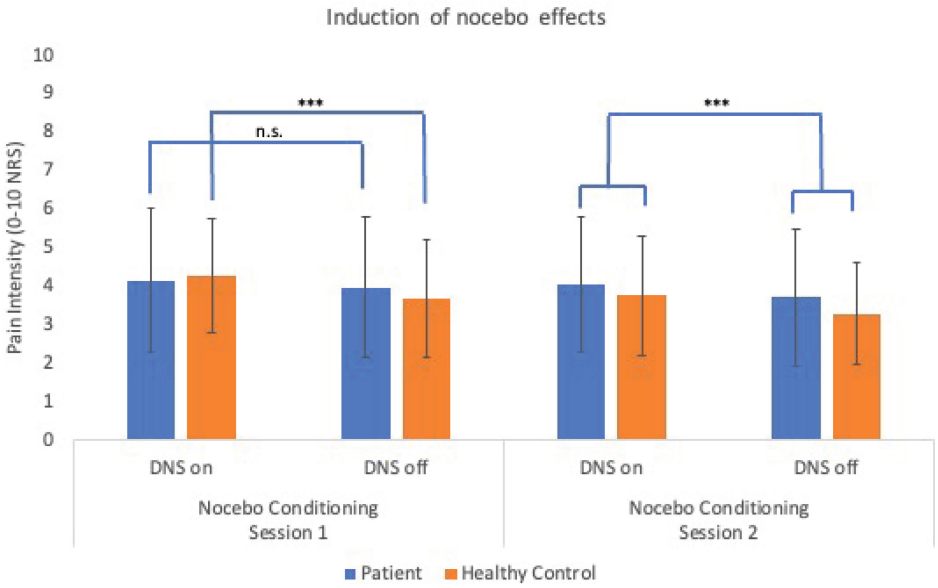

Figure 4. Mean pain intensities from the first 3 experimental (DNS on) and first 3 control (DNS off) trials of the testing phase across groups and sessions. Sample sizes per group are excluding the outliers. If a group x trial type interaction was found, significance levels were presented between groups. If only a main effect of trial type was found, significance levels were presented across groups. Error bars indicate ± SE. ***: $p < .001$; n.s.: not significant.

Extinction of nocebo effects in session 1

A 2 x 2 mixed-design ANOVA showed a significant interaction effect between group and time in session 1 ($F(1,65) = 10.35$, $p = .02$, $\eta_p^2 = .14$), but no main effect of time ($F(1,65) = 2.72$, $p = .10$, $\eta_p^2 = .04$) nor a main effect of group ($F(1,65) = 2.07$, $p = .15$, $\eta_p^2 = .031$). Bonferroni-corrected pairwise comparisons between time levels at each group showed that the mean difference score was significantly lower after extinction compared to nocebo conditioning in the healthy control group, indicating a significant

decrease in nocebo effects ($p < .001$, $d = .68$). In the patient group, the mean difference score was higher after extinction compared to nocebo conditioning; however, this was not significant ($p = .34$, $d = .20$). Figure 5 displays the magnitude of nocebo decrease after extinction, across sessions and groups.

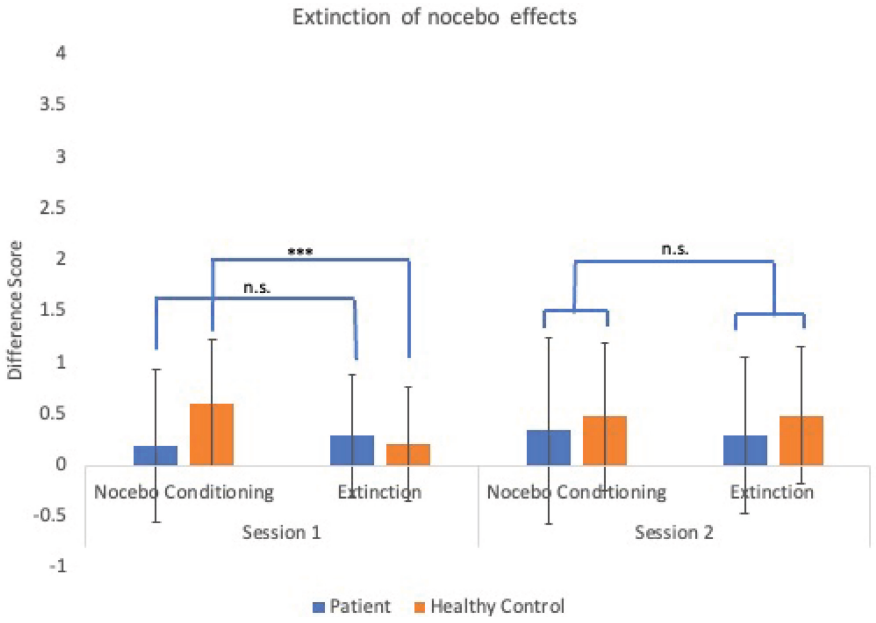

Figure 5. Difference scores based on the first 6 trials (nocebo conditioning) and last 6 trials (extinction) of the testing phase across groups and sessions. Sample size per experimental manipulation consists of all participants in a given session excluding the outliers. If a group x time interaction was found, significance levels were presented between groups. If only a main effect of time was found, significance levels were presented across groups. Error bars indicate ± SE. ***: $p < .001$; n.s.: not significant.

Induction and extinction of nocebo effects in session 2

For the induction of nocebo effects in session 2, the 2 x 2 mixed-design ANOVA showed that there was no interaction effect ($F(1,54) = .41$, $p = .52$, $\eta_p^2 = .01$) nor a main effect of group ($F(1,54) = .75$, $p = .39$, $\eta_p^2 = .01$), but there was a significant main effect of trial type ($F(1,54) = 13.85$, $p < .001$, $\eta_p^2 = .20$), where experimental trials ($M = 3.91$, $SE = .22$) were rated significantly higher than the control trials ($M = 3.50$, $SE = .21$), indicating that nocebo effects were induced across groups. Since this overall finding did not align with the nocebo results from session 1, post-hoc analyses were conducted to get a better insight into the potential group differences in nocebo induction in session 2. Pairwise comparisons of trial type levels at each group showed that the mean pain ratings were significantly higher in experimental trials compared to control trials in the healthy control group ($p = .002$, $d = .33$); however, not significantly higher in the patient group ($p = .054$, $d = .19$).

Moreover, for the extinction of nocebo effects in session 2, the 2 x 2 mixed-design ANOVA showed that there was no interaction effect ($F(1,54) = .06$, $p = .81$, $\eta_p^2 = .001$), nor a main effect of group ($F(1,54) = .92$, $p = .34$, $\eta_p^2 = .02$), or time $F(1,54) = .03$, $p = .87$, $\eta_p^2 = .001$), giving no indication for extinction of nocebo effects across groups.

The stability of nocebo effects and of extinction across sessions 1 and 2

Figure 6 displays the fluctuations in difference scores across all experimental manipulations, with patients showing a relatively more stable trend and lower nocebo effects compared to the healthy control group. The 5 x 2 mixed-design ANOVA showed there was no significant interaction effect ($F(3.59,179.64) = 1.95$, $p = .11$, $\eta_p^2 = .04$) nor a main effect of group ($F(1,50) = 2.25$, $p = .14$, $\eta_p^2 = .04$), but there was a significant main effect of time ($F(3.59,179.64) = 2.54$, $p = .048$, $\eta_p^2 = .05$). Pairwise comparison of the time levels showed that the magnitude of nocebo effects was significantly higher ($M = .33$, $SE = .13$) after nocebo conditioning in session 1 compared to the recall testing phase in session 2 ($p = .01$, $d = .44$). Compared to session 2, the magnitude of nocebo effects after nocebo conditioning in session 1 was not statistically different ($p = .98$, $d = .03$). This indicates that the magnitude of nocebo effects induced during the baseline session were significantly decreased at one-month follow-up, and that the efficacy of the nocebo conditioning paradigm did not significantly differ between sessions. Moreover, pairwise comparisons showed that the difference score after extinction in session 1 did not significantly differ from the difference score after the recall testing phase in session 2 ($p = .05$, $d = .26$). Also, the difference scores after extinction in session 1 and 2 were not significantly different ($p = .65$, $d = .22$). This indicates that the magnitude of nocebo effects observed after extinction at baseline was not different from the magnitude of nocebo effects recalled after one-month, and that the efficacy of the extinction paradigm did not statistically differ between sessions.

Questionnaires

Pearson's correlation analyses indicated that there was no significant relation between the magnitude of nocebo effects during session 1 and each of the nine questionnaire scores (FIQ: $r = -.05$, $p = .79$; DASS depression: $r = -.08$, $p = .49$; DASS anxiety: $r = -.09$, $p = .42$; DASS stress: $r = -.03$, $p = .81$; BVS: $r = .03$, $p = .81$; PCS: $r = -.04$, $p = .78$; PMS: $r = .13$, $p = .28$, LOT-R: $r = .06$, $p = .64$; state anxiety session 1: $r = -.09$, $p = .45$; state anxiety session 2: $r = .06$, $p = .65$). Moreover, patients' pain and fatigue levels on the experiment day were not significantly related to the magnitude of nocebo effects (pain session 1: $r = .20$, $p = .24$; pain session 2: $r = -.08$, $p = .69$ fatigue session 1: $r = .02$, $p = .93$; fatigue session 2: $r = .09$, $p = .66$).

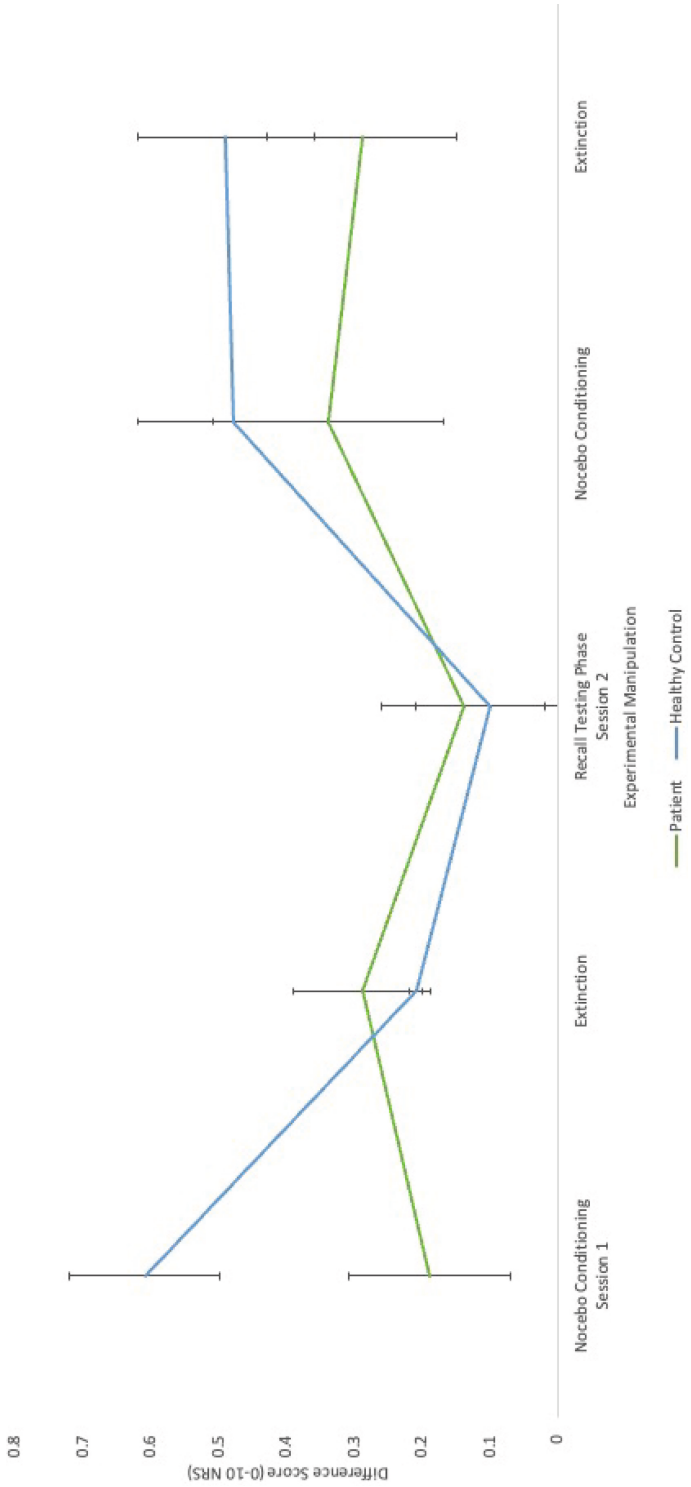

Figure 6. Difference scores per experimental manipulation across groups and sessions. Sample size per experimental manipulation consist of all participants in a given session excluding the outliers. Participant groups are represented in separate lines and the error bars indicate ± SE.

DISCUSSION

The current study investigated potential group differences in inducing and decreasing nocebo effects on experimental pressure pain in female patients with fibromyalgia and matched healthy controls. Additionally, the stability of nocebo effects at a one-month follow-up was examined. Nocebo effects on pressure pain were experimentally induced through classical conditioning with verbal suggestions, and were decreased via extinction. Our results suggest that nocebo effects were induced in the healthy control group, but not in the patient group during the baseline session, although this group difference was not robust. Nocebo effects decreased in the healthy control group after extinction. During the follow-up session, nocebo effects were induced across both groups; however, insights from post-hoc investigations suggest that this effect was primarily observable in the healthy control group, generally aligning with our results from the baseline session. However, unlike the baseline session, extinction was not observed in either group. Moreover, across all participants, the magnitude of nocebo induction and decrease appeared stable over 1-month, although note that only less than half of participants qualified as nocebo responders in both sessions. Contrary to our hypotheses, we did not find stronger nocebo effects, or more resistance to extinction, in the patient group compared to healthy controls. Instead, patients with fibromyalgia might be less responsive towards the experimental manipulation of nocebo effects than healthy controls.

Current literature on the experimental investigation of nocebo effects is largely based on findings from healthy samples[13], with only a number of studies focusing on patients with acute post-operative pain[7] or with chronic pain complaints from irritable bowel syndrome[8]. In these studies, nocebo effects were induced by providing verbal suggestions about the pain-increasing function of a placebo agent[7,8]. The role of classical conditioning in inducing nocebo effects in chronic pain conditions is far less researched[10]. In healthy participants on the other hand, the nocebo conditioning paradigm has been found to successfully induce nocebo effects on a variety of pain modalities, such as heat, electrical, and pressure pain[5,11,12]. In line with previous research, we found that nocebo effects were induced on pressure pain in the healthy control group in both sessions; however, our findings in the patient group were somewhat elusive. Nocebo effects were observed in the patient group only during the follow-up session. However, when including one patient who had an unlikely large nocebo score (i.e., an outlier), significant nocebo effects were observed during baseline, and group difference at 1-month follow-up were not clear. Thus, the group differences found in the current study were not robust. Additionally, it was observed that a lower percentage of patients than healthy controls were nocebo responders in each session. Thus, the current data suggests that patients with fibromyalgia either could be equally or less

responsive to the experimental manipulation of nocebo effects compared to healthy controls. Future studies might consider including equivalence testing or Bayesian statistics in their methodology to better establish whether group differences were not observable.

But how do these findings align with previous literature which suggests that patients could be at risk of developing nocebo effects[1,6,21,48,49]? One methodological explanation could be that the experimental pain intensities administered in the current study may not have been high enough to induce fear in patients, as higher pain intensities have been found to be associated with larger nocebo hyperalgesia, mediated through fear[50]. Patients' ongoing pain experiences in real-life might have been more intense than our administered pressure pain intensities, which might have led patients to experience less fear of pain during nocebo manipulations compared to healthy controls.

Another potential explanation could be related to group differences in pain-reporting variability. A recent study in patients with osteoarthritis of the knee has shown that accuracy in experimental pain-reporting correlates negatively with responsiveness to a placebo[51]. The implication of this finding is that the ability to direct one's attention inwardly, rather than externally, could be related to being able to resist external cues that contribute to placebo responses, and thereby lead to more accurate reporting of pain experiences due to a higher awareness of bodily sensations[51,52]. We did not assess this in the current study, but we speculate that patients' attention towards pain might have been more inwardly-directed compared to healthy controls, potentially due to their ongoing pain experiences in daily-life which might affect the salience networks in the brain[53]. If so, patients might have been less influenced by the sham activation of the TENS device, i.e., the external (placebo/nocebo) cue. However, preliminary findings e.g., on a heartbeat perception task, have shown a reduced awareness in fibromyalgia patients compared to healthy controls[54,55]. Thus, further research is warranted on the interoceptive awareness of pain and attention to placebo/nocebo cues in fibromyalgia.

Moreover, patients with fibromyalgia have been previously found to suffer from contingency learning deficits where safety cues in the environment could not be distinctly identified[22,24]. Potentially, the inability to identify safe pain cues from unsafe ones may have implications for the strength of nocebo hyperalgesia induction, although the current data is insufficient to support this argument. To get a better insight into whether the US-CS contingency awareness plays a role in nocebo learning, future research could consider including additional measurements of contingency awareness between the experimental and control (i.e., safety) cues during the testing phase. This could be useful in identifying whether the ability to learn the predictive cues in the environment (contingency learning) intersects with expectations of adverse treatment outcomes (nocebo effects).

The same experimental procedures were repeated at follow-up. The overall magnitudes of nocebo effects and their extinction did not statistically differ across sessions. However, group differences observed during baseline were no longer clearly present during follow-up, which could be potentially explained by two things. Firstly, nocebo learning might have been more strongly reinforced in patients than healthy controls after repeating the experimental procedure for a second time. Secondly, due to drop-outs, a smaller sample was included in the follow-up analyses than in the baseline analyses, which might have influenced the group effects in the follow-up session. A closer look into the recall testing phase tells us that the magnitude of nocebo effects recalled after one month was comparable to the magnitude of effects remaining after the extinction procedure during the baseline session. The passing of one month probably had no additional influence on the further extinction of nocebo effects. Also, no spontaneous recovery[56], i.e., return of nocebo effects, was detected during recall testing phase. Although the inclusion of the recall-testing phase was necessary in the study design, its potential interference on the subsequent nocebo conditioning procedure cannot be ruled out; nevertheless, our manipulation check indicates that participants did not detect any discrepancy in the DNS device function throughout the experiment and regular breaks were included to reduce any contrast between procedures. A study limitation was that our conclusions on the stability of nocebo induction could not be based on a pure comparison between the nocebo induction procedures in both sessions, as the potential influence of additional procedures which took place in between, i.e., extinction procedure during baseline and recall testing phase, cannot be overlooked. Future studies might consider including a control group without these additional manipulations to purely examine the role of follow-up period length on nocebo stability. Also, longer follow-up periods might present different outcomes in stability, especially if disease progression also occurs on the side.

As a study limitation, the potential influence of floor effects due to small nocebo scores cannot be ruled out entirely. The generalizability of our findings using the nocebo conditioning paradigm on pressure pain requires further replication in healthy and chronic pain populations. Moreover, the pain sensitization observed in the current study was unique, and this issue has not been raised previously in nocebo studies using pressure pain or other pain modalities[5,11,57]. During extinction, an overall increase in pain ratings was observed as a result of pain sensitization; our sensitivity analyses suggest that extinction took place once the nocebo effects were induced in either group. Considering that conditioned nocebo responses are common in clinical practice[6], future research is recommended to take these points into consideration when designing nocebo studies in chronic pain conditions.

To conclude, the current study is first to investigate group differences in conditioned nocebo effects in patients with a chronic pain condition and healthy controls. Contrary to our expectations, we did not find stronger nocebo effects on pressure pain in patients with fibromyalgia compared to healthy controls, if anything, patients might be less, or potentially equally, responsive to the experimental manipulation of nocebo effects as compared to healthy controls. This finding could be related to the current methodological limitations as well as the potential learning differences in patients. Moreover, the overall magnitudes of nocebo effects and their extinction were stable over 1-month. Considering that conditioned nocebo responses are common in clinical settings, further investigation of nocebo effects is essential to minimize their detrimental role during treatment.

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CHAPTER 4 SUPPLEMENTARY MATERIALS

Supplemental File I

Formulas used during pressure pain calibration

Step 1: Ascending Series

During this step, participants receive an increasing amount of pressure stimuli (kgf/cm^2) and rate their subjective pain intensity after each stimulus (0-10 Numeric Rating Scale (NRS)). When a certain pain threshold has been reached (> 5.5 NRS), the Ascending Series stops. Based on the participant ratings, 5 pressure stimulus intensities are calculated that are to be used during Randomized Series. This is done by taking the stimulation intensities on which the lowest (highest stimulus intensity rated between 0-1) and the highest (highest stimulus intensity rated between 4.5-5.5) pain rating was given, and calculate the 3 values that lie in between (with equal distances between each two subsequent values), ending up with 5 intensities in total. If participants did not rate their pain between 0-1 or the 4.5-5.5 ranges, the adjustments were made by E-prime using the following formulas:

- *Lowest pressure intensity:*
  If there are no pressure intensities that are scored 0-1, the lowest possible pressure intensity (1 kgf/cm^2) will be chosen.
- *Highest pressure intensity:*
  Interpolation: When there are no pressure intensities rated between 4.5-5.5, the highest pressure intensity is calculated using the first pain rating higher than the higher bound (5.5) and the first pain rating that is lower than the lower bound (4.5). These ratings are then used to interpolate and calculate the pressure intensity that corresponds to the middle value of the highest pressure intensity (5.0).
  Extrapolation: When there are no pressure intensities rated between 4.5-5.5, and no pressure intensities rated lower than the lower bound (4.5) or no pressure intensities rated higher than the higher bound (5.5) , the two pressure intensities corresponding to the first two ratings higher than the higher bound, or the first two ratings lower than the lower bound, respectively, are used to extrapolate the pressure intensity that corresponds to the middle value of the highest pressure intensity (5.0).

Step 2: Randomized Series

During this step, participants receive each of the 5 pressure intensities 3 times. This results in a total of 15 pressure stimuli. Using ratings of the pressure intensities entered in randomized series, 3 pressure intensities that are directly used in the experiment are calculated. These pressure intensities correspond to: no pain (0-1), slight pain (2-3), and moderate pain (4.5-5.5).

- *No Pain:*
  Extrapolation: When there are no pressure intensities rated between 0-1, the two pressure intensities corresponding to the first two ratings higher than the higher bound (1) are used to extrapolate the pressure intensity that corresponds to the middle value of no pain range (0.5).
- *Slight Pain:*
  Interpolation: When there are no pressure intensities rated between 2-3, slight pain is calculated using the first pain rating higher than the higher bound (3) and the first pain rating that is lower than the lower bound (2). These ratings are then used to interpolate and calculate the pressure intensity that corresponds to the middle value of the slight pain range (2.5).
  Extrapolation: When there are no pressure intensities rated between 2-3, and no pressure intensities rated lower than the lower bound (2) or no pressure intensities rated higher than the higher bound (3), the two pressure intensities corresponding to the first two ratings higher than the higher bound, or the first two ratings lower than the lower bound, respectively, are used to extrapolate the pressure intensity that corresponds to the middle value of the slight pain range (2.5).
- *Moderate Pain:*
  Interpolation: When there are no pressure intensities rated between 4.5-5.5, moderate pain is calculated using the first pain rating higher than the higher bound (5.5) and the first pain rating that is lower than the lower bound (4.5). These ratings are then used to interpolate and calculate the pressure intensity that corresponds to the middle value of the moderate pain range (5.0).
  Extrapolation: When there are no pressure intensities rated between 4.5-5.5, and no pressure intensities rated lower than the lower bound (4.5) or no pressure intensities rated higher than the higher bound (5.5), the two pressure intensities corresponding to the first two ratings higher than the higher bound, or the first two ratings lower than the lower bound, respectively, are used to extrapolate the pressure intensity that corresponds to the middle value of the moderate pain range (5.0).

Step 3: Calibration Check

During this step, participants receive the 3 pressure intensities calculated during the Randomized Series for a final check. No pain intensity (0-1) is presented twice, slight pain intensity (2-3) is presented thrice, and moderate pain intensity (4.5-5.5) is presented twice in a randomized order, where the no pain and moderate pain intensities are never presented subsequently (or other way around). No adjustment is necessary when at least 1/2 no pain intensities, 2/3 slight pain intensities, and 1/2 moderate pain intensities are rated within the correct ranges.

- *No Pain:*
  When none of the no pain intensities were rated 0-1, extrapolation to a pain rating of 0.5 is used to adjust the no pain intensity.
- *Slight Pain:*
  When none of the slight pain intensities were rated 2-3, interpolation or extrapolation to a pain rating of 2.5 is used to adjust the slight pain intensity. When only one of the slight pain pressure intensities were rated in the slight pain range, interpolation or extrapolation is still used, using this one rating within the slight pain range.
- *Moderate Pain:*
  When none of the moderate pain intensities were rated 4.5-5.5, interpolation or extrapolation to a pain rating of 5.0 is used to adjust the moderate pain intensity.

Note on the interpolation/extrapolation: When the first two (or three, however much) **pressure intensities** higher than the higher bound or lower than the lower bound have the same rating, the median of these pressure intensities and the pressure intensity corresponding to the next rating is used for extrapolation. However, when the first two (or three, however much) **pain ratings** higher than the higher bound and lower than the lower bound have the same pressure intensities, nothing is done. When the pressure intensities used for interpolation are the same, the interpolation will result in the same pressure intensity. Same for extrapolation, when the pressure intensities used for extrapolation are the same, the extrapolation will result in the same pressure intensity.

Supplemental File II

Results from the sensitivity analyses

Sensitivity Analyses

It was identified that nocebo effects were induced in 65% of all participants (54% of patients and 78% of healthy controls) in session 1, i.e., the difference scores after nocebo conditioning were above zero. In session 2, this was the case in 66% of all participants (62% of patients and 70% of healthy controls). 43% of all participants (37% of patients and 52% of healthy controls) participating in both sessions were nocebo responders in each session. As part of sensitivity analyses, the same analyses for the extinction of nocebo effects were conducted for only the nocebo responders. To assess the extinction efficacy in session 1, a 2 x 2 mixed-design ANOVA analysis was conducted with 43 nocebo responders (patient N = 18; healthy control N = 25). The results showed no significant interaction effect ($F(1,41) = 1.45$, $p = .23$, $\eta_p^2 = .03$) nor a main effect of group ($F(1,41) = .24$, $p = .63$, $\eta_p^2 = .01$), but there was a significant main effect of time ($F(1,41) = 38.78$, $p < .001$, $\eta_p^2 = .49$). After extinction in session 1, the mean difference score was significantly reduced by 0.43 (SE = .07) NRS points across groups. Moreover, to investigate the extinction efficacy in session 2, the same 2 x 2 mixed-design ANOVA was conducted with 37 nocebo responders from session 2 (patient N = 18; healthy control N = 19). The results showed no significant interaction effect ($F(1,35) = .35$, $p = .56$, $\eta_p^2 = .01$) nor a main effect of group ($F(1,35) = .45$, $p = .51$, $\eta_p^2 = .01$), but there was a significant main effect of time ($F(1,35) = 4.35$, $p = .04$, $\eta_p^2 = .11$). After extinction in session 2, the mean difference score was significantly reduced by 0.28 (SE = .13) NRS points across groups.

Moreover, to investigate the stability of nocebo reduction between groups and across sessions, the same 5 x 2 mixed-design ANOVA analysis was conducted with 22 participants (patient N = 8; healthy control N = 14) who were nocebo responders in both sessions. The results showed no interaction effect ($F(4, 80) = .16$, $p = .96$, $\eta_p^2 = .01$) nor a main effect of group ($F(1,20) = .009$, $p = .92$, $\eta_p^2 = .00$), but there was a significant main effect of time ($F(4, 80) = 8.65$, $p < .001$, $\eta_p^2 = .30$). Similar to earlier findings, Bonferroni-corrected pairwise comparisons between extinction in session 1 and the recall testing phase in session 2 were not statically different ($p = .10$) and neither was the comparison between extinction in session 1 and 2 ($p = .07$).

Moreover, removing 3 patients with FS <12 did not significantly influence the findings on the induction and extinction of nocebo effects in both sessions.

Furthermore, there were no statistically significant associations in the magnitude of nocebo effects between sessions in the patient group ($r(25)= .22$, $p = .27$) nor in the healthy control group ($r(25)= .07$, $p = .74$). After pooling the samples, the results were the same ($r(52)= .18$, $p = .18$).

Results without the exclusion of outliers

Induction of nocebo effects in session 1

Without excluding one patient outlier, the results no longer showed an interaction effect ($F(1,67) = 2.81$, $p = .09$, $\eta_p^2 = .04$), nor a main effect of group ($F(1,67) = .01$, $p = .93$, $\eta_p^2 = .00$), but there was a significant main effect of trial type ($F(1,67) = 21.67$, $p < .001$, $\eta_p^2 = .24$), where mean pain ratings were significantly higher in experiments trials ($M = 4.24$, $SE = 0.20$) compared to control trials ($M = 3.79$, $SE = 0.21$), indicating that nocebo effects were induced across groups.

Extinction of nocebo effects in session 1

Without excluding two patient outliers, the results no longer showed an interaction effect ($F(1,67)= 2.84$, $p = .09$, $\eta_p^2 = .04$), nor a main effect of group ($F(1,67)=1.09$, $p = .29$, $\eta_p^2 = .02$), but there was a significant main effect of time ($F(1,67) = 4.63$, $p = .04$, $\eta_p^2 = .06$), with a significant reduction of nocebo effects of 0.23 NRS points ($SE = .10$) after extinction across groups ($p = .04$).

Supplemental File III

Assessment of prior experience with a TENS device

During baseline questionnaire assessment, all participants were asked to categorically indicate (yes/no) to their prior knowledge of and experience with a TENS device, and in case of experience whether they found it was effective in reducing pain.

Statistical Analyses

Frequency of responses were calculated. As a manipulation check, 6 One-Way ANOVAs were conducted to investigate whether:

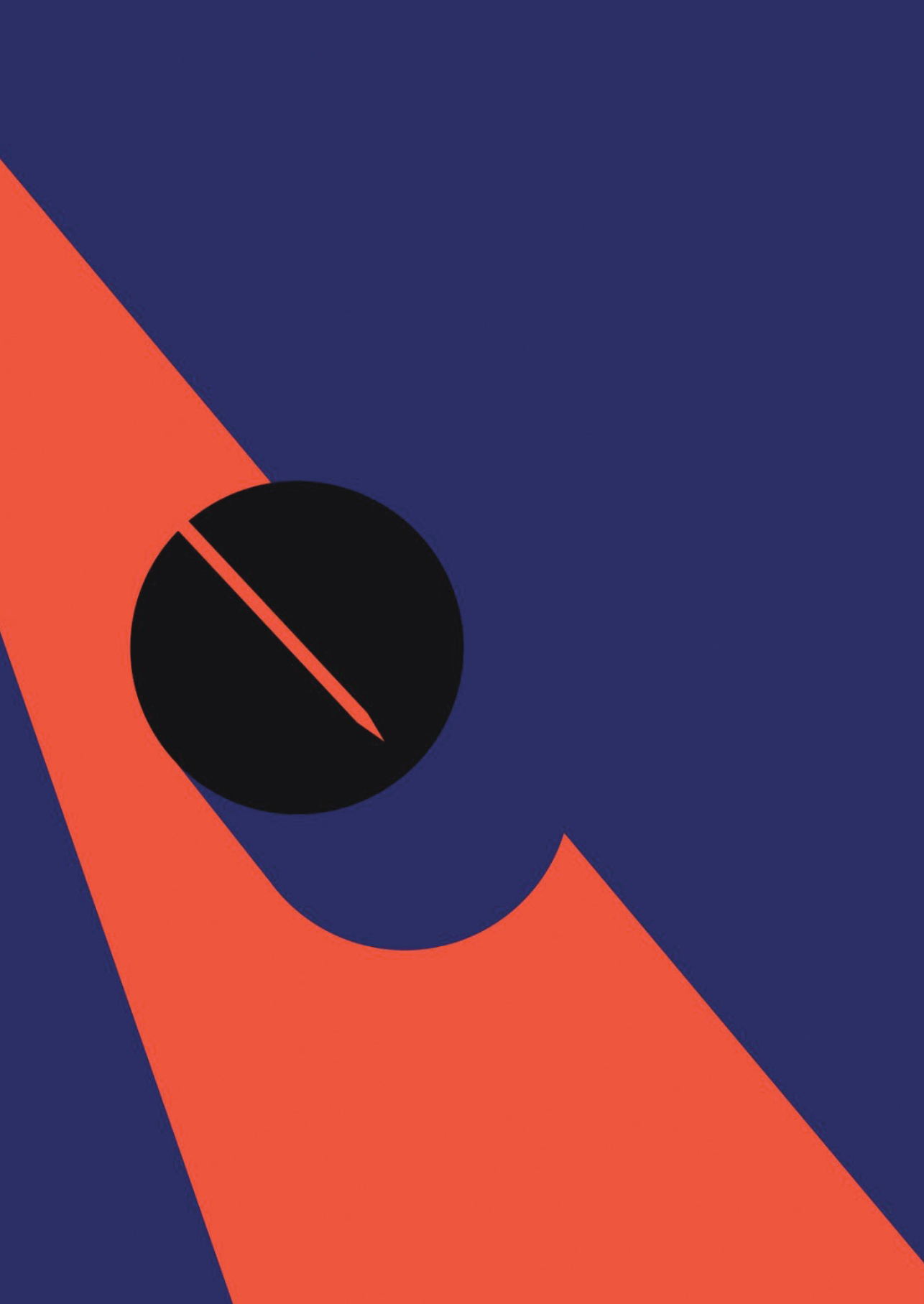
1. Prior TENS knowledge influenced nocebo response (in sessions 1 and 2)
2. Prior TENS experience influenced nocebo response (in sessions 1 and 2)
3. Prior TENS efficacy influenced nocebo response (in sessions 1 and 2)

Bonferroni corrections were applied, where a p-value below .008 was considered statistically significant

Results

Only a small number of participants had prior knowledge of ($N_{patient}$ = 17, $N_{healthy}$ = 3) and experience with ($N_{patient}$ = 8) a TENS device. Amongst those who have experience, 4 patients found that it was effective in reducing pain.

Regarding TENS, neither having knowledge (session 1 $F(1,68) = .22$, $p = .64$; session 2 $F(1,55) = .52$, $p = .47$), experience (session 1 $F(1,68) = .05$, $p = .82$; session 2 $F(1,55) = .03$, $p = .86$), nor perceived efficacy (session 1 $F(1,8) = 4.38$, $p = .08$; session 2 $F(1,4) = 1.86$, $p = .27$) influenced nocebo responses.



CHAPTER 5

Nocebo hyperalgesia and other expectancy-related factors in daily fibromyalgia pain: Combining experimental and electronic diary methods

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ABSTRACT

Objective: Expectancies are known to shape pain experiences, but it remains unclear how different types of expectancies contribute to daily pain fluctuations in fibromyalgia. This combined experimental and diary study aims to provide insights into how experimentally-derived nocebo hyperalgesia and other, diary-derived, expectancy-related factors are associated with each other and with daily pain in fibromyalgia.

Methods: Forty-one female patients with fibromyalgia first participated in a lab procedure measuring nocebo hyperalgesia magnitude, then filled out an electronic diary 3 times a day over 3 weeks regarding the expectancy-related factors of pain expectancy, anxiety, optimism, and pain-catastrophizing thoughts, and current pain intensity.

Results: Our results indicate that experimentally-induced nocebo hyperalgesia was unrelated to diary-assessed expectancy-related factors and did not predict daily fibromyalgia pain. Higher levels of the self-reported expectancy-related factors pain expectancy and pain catastrophizing, but not anxiety and optimism, predicted moment-to-moment pain increases in fibromyalgia, after controlling for current pain, moment-of-day and all other expectancy-related factors.

Conclusion: Our findings indicate that self-reported expectancy-related factors, particularly pain expectancy and pain catastrophizing, are potentially more relevant for predicting daily pain experience than experimentally-induced nocebo hyperalgesia. Further translation of nocebo hyperalgesia is needed from experimental to Ecological Momentary Assessment research. Our findings imply that targeting the decrease in pain expectancy and catastrophizing thoughts e.g., via Cognitive Behavioral Therapy, have potential for improving daily pain levels in fibromyalgia.

Keywords:
diary study; expectancy; pain; nocebo hyperalgesia; fibromyalgia

1. INTRODUCTION

Pain can be shaped by different types of expectancies[1,2]. Expectancies of upcoming pain (i.e., pain expectancies), as well as expectancy-related factors such as anxiety, catastrophizing, and optimism have been found to be associated with pain[1,3–5]. Yet, their combined role in shaping pain experiences in chronic pain conditions, such as fibromyalgia, is fairly unknown. Given that expectancies play an important role for pain modification in fibromyalgia[6], research is needed on how different expectancy-related factors can impact the perceived changes in daily pain.

Experimental and diary studies have shown that higher pain expectancy is associated with increases in (subsequent) pain experiences[4,7–10]. In patients with chronic pain, greater pain expectancy has been found to be associated with increased pain intensity in daily-life[4] and also an indicator of future pain trajectory and quality of life[8,11]. Pain-related fear and anxiety have been associated with overprediction of daily pain, where patients expected to experience more pain than their actual self-reported pain[12,13]. Pain catastrophizing has been also associated with cognitive and emotional aspects of pain that alter pain perception[14]. In fibromyalgia, both maladaptive (pain catastrophizing) and adaptive (pain coping) cognitions have been found to impact changes in daily pain intensity[15]. Morning pain intensity predicted the upcoming pain cognitions in the afternoon, which in turn mediated the end-of-day pain intensity[15]. Moreover, optimism has been found to have a beneficial association with pain experience[16]; as daily pain and fatigue levels increased in fibromyalgia, having higher optimism has been found to facilitate engagement in painful activities and the pursuit of personal goals[17].

Nocebo hyperalgesia (i.e., pain expectancies based on previously-learned associations) can further contribute to pain increase[2]. Given that more established methods of nocebo hyperalgesia induction are lacking outside of the lab, it remains to be investigated whether nocebo hyperalgesia measured in the lab predicts daily pain levels in fibromyalgia. Experimental studies have shown that lower optimism, higher anxiety, and higher pain catastrophizing are associated with stronger nocebo effects[2,18]. However, in these studies, psychological characteristics were assessed as general traits, whereas their assessment via diary methods can provide additional information on daily changes. The magnitude of experimentally-induced nocebo hyperalgesia might reflect a general characteristic for expecting negative pain-related outcomes, which could influence cognitions and emotions underlying the state-like changes in expectancies and pain.

The current study combining experimental and electronic diary study methods investigates the association of both experimentally-induced nocebo hyperalgesia and

diary-derived self-reported expectancy-related factors with each other and with pain in female patients with fibromyalgia. Data on nocebo hyperalgesia were taken from a larger experimental study[19] and the other factors were assessed in the same patient sample via an electronic diary for 3 times a day over 3 weeks. The current study objectives are three-fold and are exploratory given their novelty. First, we explore the relationships of nocebo hyperalgesia with self-reported expectancy-related factors (i.e., pain expectancy, anxiety, pain catastrophizing, and optimism) and pain averaged over 3 weeks. Second, we explore whether each self-reported expectancy-related factor predicts moment-to-moment change in pain, controlling for current pain and moment-of-day. Third, we explore whether nocebo hyperalgesia and self-reported expectancy-related factors together predict moment-to-moment change in pain, again controlling for current pain and moment-of-day. Hereby, we provide a multi-faceted account of the role of both experimental and self-reported expectancy-related factors in fibromyalgia pain.

2. MATERIALS AND METHODS

2.1 Design & Participants

This study is part of a larger prospective study (ICTRP Identifier: NL8244, https://trialsearch.who.int/) approved by the Medical Ethical Committee Leiden The Hague Delft (NL67541.058.18). The current study was carried out in accordance with Declaration of Helsinki. Since fibromyalgia is more common in women than in men[20], the present study included only female patients to ensure that the current results could be better compared to existing research. Forty-one female patients with fibromyalgia (*M* age ± *SD* = 37 ± 10.3, range 20-58) first participated in a lab experiment at the Leiden University Treatment and Expertise Center (LUBEC) to measure the magnitude of experimentally-induced nocebo hyperalgesia[19], and subsequently filled out an electronic diary app on their smartphone for 21 days. All participants provided written informed consent. A monetary compensation up to €100 was awarded for participating in all parts of the study with additional travel costs.

2.2 Nocebo Hyperalgesia Assessment

A well-established nocebo-conditioning paradigm combined with verbal suggestions was used for inducing nocebo hyperalgesia in the lab[21]. Nocebo effects on pressure pain on the thumb nail were induced by leading participants to expect that the activation of a sham Transcutaneous Electrical Nerve Stimulation (TENS) device would lead to pain worsening compared to its deactivation. After repeated pairing of sham activation of the TENS device and a stronger pressure pain applied, a test phase commenced in which pressure pain intensity was similar between sham activation and non-sham activation of

the TENS device. Differences in pain intensity (0-10 Numeric Rating Scale, NRS) reported to the sham-activated and sham-deactivated trials in the test phase were calculated to derive a nocebo hyperalgesia score, with a higher score indicating stronger nocebo hyperalgesia. Detailed descriptions of the lab procedures and the calculation of nocebo hyperalgesia scores are reported elsewhere[19].

2.3 Electronic Diary Assessment

At the end of the lab session, the diary app (Ethica Data Services Inc., Toronto, Canada) was downloaded on participants' smartphones. Participants were prompted to fill in questionnaires in the upcoming 3 weeks for 3 times a day at semi-random moments (morning: 09:00-11:00, afternoon: 14:00-16:00, evening: 19:00-21:00), where a time was randomly selected per time block. Each questionnaire took 2-5 min. The first prompt was sent on the first Monday morning following the lab session. If participants failed to fill in a questionnaire within 30 min after notification, the questionnaire became unavailable to avoid late responses. Participants were able to fill in possible comments via the app or contact researchers for any questions. Researchers monitored participants' response progress and sent standardized weekly motivation messages to increase compliance.

2.4 Measures

In the diary study, the current pain intensity was assessed by the question "How much pain do you experience at the moment?", rated on a 0 ("no pain") to 10 ("worst pain imaginable") NRS. Next moment's pain expectation was assessed with "How much pain do you expect in the {next assessment moment} (morning/afternoon/evening)?", rated on the same NRS as pain intensity. Current anxiety was assessed with "How much anxiety do you feel at the moment?", rated on a 0 ("not at all anxious") to 10 ("extremely anxious") NRS. Current catastrophic thinking related to pain was assessed with three items that each represent a subscale from the Pain Catastrophizing Scale (PCS)[22]. The item "At the moment I am afraid that the pain will get worse" represents the subscale "magnification", "At the moment I am constantly thinking about how much it hurts" represents the subscale "rumination", and "At the moment I feel that the pain overwhelms me" represents the subscale "helplessness". Each item was rated on a 0 ("strongly disagree") to 10 ("strongly agree") NRS. Current optimism was assessed with the item "At the moment I feel optimistic", rated on a 0 ("strongly disagree") to 10 ("strongly agree") NRS.

2.5 Statistical Analyses

All analyses were conducted using the statistical software R (version 4.2.2)[23]. To examine the *zero-order* relationships of experimentally-induced nocebo hyperalgesia with diary-assessed self-reported expectancy-related factors (pain expectancy, anxiety,

pain catastrophizing, and optimism) and pain, Pearson's *correlation* was used. A composite score for pain catastrophizing was first calculated by averaging the scores on the 3 items at each given moment. Diary-assessed variables were averaged across the 63 measurement moments (3 x 21 days), meanwhile skipping missed moments, before calculating Pearson's *correlation*.

Since self-reported diary assessments (level one) are nested within individuals (level two), multilevel analyses were conducted to answer the remaining research questions. The intra-class correlation (ICC) coefficient was calculated for the null model (without entering any variables) to confirm the multilevel structure of the data. Pain expectancy, anxiety, pain catastrophizing, optimism, and pain ratings were shifted 1 assessment moment earlier to investigate their predictive role in next-moment pain (e.g., morning pain expectancy predicting afternoon pain, controlling for morning pain).

To examine whether each of the self-reported expectancy-related factors predicts moment-to-moment change in pain, four multilevel analyses were conducted. Either previous-moment pain expectancy, anxiety, pain catastrophizing, or optimism was entered in each model as fixed effects, controlling for previous-moment pain and next-moment moment-of-day (with two dummy variables). Next moment's pain was modeled as the dependent variable.

To examine whether experimentally-induced nocebo hyperalgesia and self-reported expectancy-related factors together predict moment-to-moment change in pain, another multilevel analysis was conducted. Experimentally-induced nocebo hyperalgesia and self-reported previous-moment pain expectancy, anxiety, pain catastrophizing, and optimism were entered as fixed effects, controlling for previous-moment pain and next-moment moment-of-day. Next moment's pain was modeled as the dependent variable. All models included a random intercept for participants. The mean of nocebo hyperalgesia was centered around the grand mean, given that this was the only time-constant estimate. The mean of each time-varying estimate was centered within-persons[24]. This generates two estimates for the same variable in the model: between-person mean-centered and within-person mean-centered predictor. The former involves taking the mean value of a particular variable for each individual across time and then subtracting this from the overall mean of that variable across individuals. This helps assess how an individual's mean score on a given variable relates to their response on the dependent variable, compared to the overall mean across individuals. The latter relates to how a particular variable fluctuates within an individual over time, relative to their own mean value for that variable. This helps assess the effect of deviations from an individual's own mean score

on the dependent variable. Data was checked for assumptions of linearity and normality using scatterplots and Q-Q plots, respectively.

3. RESULTS

Detailed descriptions of the sample are reported elsewhere[19]. None of the assumptions from any of the analyses were violated. Aggregation of diary data across 41 patients and 63 measurement moments indicated daily pain to be of moderate intensity on average (*M* = 4.81, *SD* = 1.47). Amongst all aggregated diary-assessed variables, pain catastrophizing was the highest-scored item and showed the highest mean variability between patients (see Table 1). Nocebo hyperalgesia was induced experimentally (as the mean score was above 0); however, its magnitude was not large (*M* = 0.23, *SD* = 0.97), while it also showed the smallest mean variability between patients compared to self-reported expectancy-related factors (Table 1)[19].

3.1 Nocebo hyperalgesia's relationship with self-reported expectancy-related factors and pain

Zero-order relationships were assessed using Pearson's *r* as shown in Table 1. Results showed that experimentally-induced nocebo hyperalgesia magnitude did not significantly predict mean self-reported expectancy-related factors or mean pain assessed in the following 3 weeks.

Table 1

Means, SDs, and zero-order correlations of experimentally-induced nocebo hyperalgesia with self-reported expectancy-related factors and pain averaged over 63 measurement moments across patients with fibromyalgia (*N* = 41)

	Nocebo Hyperalgesia[a]				
	M	*SD*	*Pearson's r*	*p-value*	*95% CI*
Nocebo Hyperalgesia[a]	0.23	0.97	-	-	-
Mean Pain Expectancy	5.18	1.47	0.11	0.50	[-0.21, 0.41]
Mean Anxiety	3.42	1.45	0.26	0.12	[-0.06, 0.52]
Mean Pain Catastrophizing	6.42	4.67	-0.07	0.69	[-0.37, 0.25]
Mean Optimism	6.29	1.05	0.24	0.15	[-0.08, 0.51]
Mean Pain	4.81	1.47	0.07	0.69	[-0.25, 0.37]

Note.

[a.] Nocebo hyperalgesia was measured at a single moment, with data missing of 2 patients due to a technical error

Pearson's *r*: refers to Pearson's *correlation* with nocebo hyperalgesia

95% CI: 95% Confidence Interval referring to Pearson's *correlation*

3.2 Self-reported expectancy-related factors predicting moment-to-moment change in pain

Each self-reported expectancy-related factor was entered into a separate multilevel model. Their partial lagged relationships with next-moment pain, after controlling for previous-moment pain and next-moment moment-of-day, are shown in Table 2. The multilevel structure of the data was confirmed by a significant intercept of the null-model (ICC = 0.52). The explained variance of the four models (*Pseudo-R^2*) ranged between 0.59-0.64, with the pain-expectancy model explaining the largest variance in pain (*Pseudo-R^2*: 0.64) with the best model fit (*AIC*: 4784.15). In all models, the moment-of-day covariate was positively related to pain, where patients reported the lowest pain in the morning, compared to afternoon and evening ($p < .001$). Also, the covariate previous-moment pain was a positive predictor of next-moment pain in all models based on between-person ($p < .001$) and within-person ($p < .001$) values. After controlling for these covariates, fluctuations in pain expectancy within a patient predicted their next-moment pain levels ($b = .29$, $p < .001$) based on the within-person mean centered value (denoted as '.cw'). Specifically, if a patient's pain expectancy at a given moment was 1-point higher than their own average pain expectancy across all assessments, this would be associated with a 0.29-point higher pain at the next moment ($p < .001$). Looking at the between-person mean centered value (denoted as '.cb'), a higher pain expectancy at a particular time point did not significantly predict the next-moment pain ($b = .01$, $p = .81$). Moreover, neither the within-person nor between-person values of previous-moment anxiety, pain catastrophizing, or optimism significantly predicted the next-moment pain, after controlling for previous-moment pain and next-moment moment-of-day (see Table 2).

Table 2

Summary of four multilevel models of self-reported previous-moment expectancy-related factors predicting next-moment pain, controlled for previous-moment pain and next-moment moment-of-day, across patients with fibromyalgia ($N = 41$)

			Next-Moment Pain		
Model (Pain Expectancy)	*b*	*SE*	*t-value*	*df*	*95% CI*
Intercept	4.50***	0.06	75.81	1461	-
Afternoon	0.43***	0.08	5.23	1461	[0.01, 0.04]
Evening	0.49***	0.08	5.95	1461	[0.01, 0.04]
Pain.cb	1.02***	0.04	23.48	1461	[0.24, 0.31]
Pain.cw	0.21***	0.03	8.27	1461	[0.03, 0.07]
Pain Expectancy.cb	0.01	0.04	0.24	1461	[0.00, 0.00]
Pain Expectancy.cw	0.29***	0.03	9.86	1461	[0.04, 0.09]
Model	*Pseudo-R^2*: 0.64 *AIC*: 4784.15				

Table 2

Continued.

			Next-Moment Pain		
Model (Pain Expectancy)	*b*	*SE*	*t-value*	*df*	*95% CI*
Model (Anxiety)					
Intercept	4.34***	0.06	73.48	1472	-
Afternoon	0.58***	0.08	6.99	1472	[0.04, 0.09]
Evening	0.78***	0.08	9.62	1474	[0.04, 0.09]
Pain.cb	1.03***	0.02	42.64	1472	[0.45, 0.59]
Pain.cw	0.32***	0.03	12.51	1472	[0.10, 0.13]
Anxiety.cb	-0.01	0.02	-0.30	1472	[0.00, 0.00]
Anxiety.cw	0.01	0.02	0.25	1472	[0.00, 0.00]
Model	*Pseudo-R^2*: 0.62 *AIC*: 4913.10				
Model (Pain Catastrophizing)					
Intercept	4.34***	0.06	71.68	1432	-
Afternoon	0.56***	0.09	6.59	1432	[0.04, 0.09]
Evening	0.77***	0.08	9.42	1432	[0.04, 0.09]
Pain.cb	1.01***	0.03	37.72	1432	[0.40, 0.53]
Pain.cw	0.29***	0.03	9.24	1432	[0.04, 0.08]
Pain Catastrophizing.cb	0.04	0.02	1.42	1432	[0.00, 0.01]
Pain Catastrophizing.cw	0.05	0.03	1.58	1432	[0.00, 0.01]
Model	*Pseudo-R^2*: 0.59 *AIC*: 4792.14				
Model (Optimism)					
Intercept	4.35***	0.06	73.51	1455	-
Afternoon	0.57***	0.08	6.79	1455	[0.04, 0.08]
Evening	0.75***	0.08	9.32	1455	[0.04, 0.08]
Pain.cb	1.02***	0.03	36.36	1455	[0.37, 0.51]
Pain.cw	0.32***	0.03	12.50	1455	[0.07, 0.13]
Optimism.cb	0.00	0.04	0.11	1455	[0.00, 0.00]
Optimism.cw	0.01	0.03	0.27	1455	[0.00, 0.00]
Model	*Pseudo-R^2*: 0.62 *AIC*: 4852.63				

Note.
b is the unstandardized estimate
CI: Confidence Interval
.cb: between-person mean-centered predictor
.cw: within-person mean-centered predictor
Pseudo-R^2: Explained variance of the model
AIC: Akaike's Information Criterion, with a lower score indicating a better model fit
*** $p < .001$ (two tailed)

3.3 Experimentally-induced and self-reported expectancy-related factors predicting moment-to-moment change in pain

Next, we entered all experimentally-induced and self-reported expectancy-related factors into the same multilevel model. Their partial lagged relationships with next-moment pain, after controlling for previous-moment pain and next-moment moment-of-day, are displayed in Table 3. The explained variance of the model (*Pseudo-R^2*: 0.60) was comparable to the four models displayed in Table 2, but with a better model fit (*AIC*: 4370.52). Results show that the moment-of-day covariate was positively related to pain, where patients reported the lowest pain in the morning, compared to afternoon and evening ($p < .001$). Previous-moment pain was a positive predictor of next-moment pain based on between-person ($b = 1.02$, $p < .001$) and within-person ($b = .20$, $p < .001$) values. Controlling for all other variables, nocebo hyperalgesia did not predict next-moment pain ($b = -.01$, $p = .80$). Based on within-person mean-centered values, only the fluctuations in previous-moment pain expectancy within a patient predicted their next-moment pain levels ($b = .30$, $p < .001$). Previous-moment anxiety ($b < .00$, $p = .91$), pain catastrophizing ($b = .01$, $p = .70$), and optimism ($b = .04$, $p = .19$) did not significantly predict next-moment pain. Based on between-person mean-centered values, only previous-moment pain catastrophizing ($b = .06$, $p = .049$) significantly predicted next-moment pain, whereas previous-moment pain expectancy ($b = .01$, $p = .90$), anxiety ($b = -.02$, $p = .54$), and optimism ($b = .05$, $p = .25$) were not significant predictors. This indicates that higher pain catastrophizing than average in the group at a particular time point significantly predicts the next-moment pain.

4. DISCUSSION

The current study examined for the first time the separate and combined predictive value of experimentally-induced and diary-based self-reported expectancy-related factors on fibromyalgia pain variation. Results showed that experimentally-induced nocebo hyperalgesia did not predict mean diary-assessed expectancy-related factors nor pain over 3 weeks in female patients with fibromyalgia. Self-reported expectancy-related factors pain expectancy and pain catastrophizing, but not optimism and anxiety, predicted moment-to-moment changes in pain. Pain expectancy was related to within-person and pain catastrophizing to between-person increases in moment-to-moment pain. When other expectancy-related factors were not taken into account, only pain expectancy was a predictor of within-person fluctuations in moment-to-moment changes in pain. Our results highlight the importance of moment-to-moment changes in expectancy-related factors in understanding moment-to-moment changes in fibromyalgia pain. In particular, pain expectancy and pain catastrophizing seem promising for predicting daily pain fluctuations in fibromyalgia.

Table 3

Summary of multilevel model of both experimental and self-reported previous-moment expectancy-related factors predicting next-moment pain, controlled for previous-moment pain and next-moment moment-of-day, across patients with fibromyalgia (*N* = 41)

			Next-Moment Pain		
Fixed Coefficient	*b*	*SE*	*t-value*	*df*	*95% CI*
Intercept	4.52***	0.06	71.10	1305	-
Afternoon	0.38***	0.09	4.35	1305	[0.01, 0.04]
Evening	0.48***	0.09	5.42	1305	[0.01, 0.04]
Pain.cb	1.02***	0.05	21.30	1305	[0.22, 0.30]
Pain.cw	0.20***	0.03	6.06	1305	[0.01, 0.05]
Nocebo Hyperalgesia.gmc[a]	-0.01	0.04	-0.25	1305	[0.00, 0.00]
Pain Expectancy.cb	0.01	0.05	0.13	1305	[0.00, 0.00]
Pain Expectancy.cw	0.30***	0.03	9.44	1305	[0.04, 0.09]
Anxiety.cb	-0.02	0.03	-0.62	1305	[0.00, 0.01]
Anxiety.cw	-0.00	0.02	-0.12	1305	[0.00, 0.00]
Pain Catastrophizing.cb	0.06*	0.03	1.97	1305	[0.00, 0.01]
Pain Catastrophizing.cw	0.01	0.04	0.38	1305	[0.00, 0.00]
Optimism.cb	0.05	0.04	1.15	1305	[0.00, 0.01]
Optimism.cw	0.04	0.03	1.32	1305	[0.00, 0.01]
Model	*Pseudo-R^2*: 0.60 *AIC*: 4370.52				

Note.

[a:] Nocebo hyperalgesia was measured at a single moment, with data missing of 2 patients due to a technical error

b is the unstandardized estimate

CI: Confidence Interval

.gmc: grand-mean centered predictor

.cb: between-person mean-centered predictor

.cw: within-person mean-centered predictor

Pseudo-R^2: Explained variance of the model

AIC: Akaike's Information Criterion indicating the model fit

*p< .05; *** p< .001 (two tailed)

We examined whether experimentally-induced nocebo hyperalgesia could be a good proxy for predicting expectancy-related factors and daily pain in fibromyalgia. In the lab, small nocebo hyperalgesia effects on pressure pain were induced[19]. Possibly, these effects were only small because experimentally-evoked pressure pain was not fear- or anxiety-inducing enough to generate strong nocebo effects[25,26]. Daily pain experiences of patients are potentially more harmful, unpredictable, and longer in duration compared to safe and controlled experimentally-evoked pain experiences. These differences between experimentally-evoked and daily pain experiences may also partially explain why no associations were found between nocebo hyperalgesia and daily levels of pain expectancy, anxiety, optimism, and pain catastrophizing, nor pain. Future studies are recommended to consider the external-validity of nocebo-conditioning paradigm to better align with daily life and Ecological Momentary Assessment (EMA) studies, for instance by involving patients in the design of clinical research[27].

Our results for pain expectancy are in line with previous studies indicating that pain-related expectancies modify pain intensity, similar to self-fulfilling prophecies[4,8,9,28]. In the current study, we observed that the mean pain expectancy ratings were overall higher than the mean pain ratings assessed over 3 weeks, emphasizing that patients might be overpredicting their upcoming pain intensity. Our findings demonstrated that higher within-person fluctuations in pain expectancy predicts an increase in next-moment pain, after controlling for previous-moment pain and next-moment moment-of-day. Interestingly, additionally taking account of all other expectancy-related factors resulted in almost the same prediction estimate as not taking them into account in the model. Potentially, this could indicate that pain expectancy might share little statistical variance with the other expectancy-related factors. Moreover, pain catastrophizing predicted between-person differences in moment-to-moment pain increase. Although the contribution found is small, the direction of our finding corresponds with the literature[14,15].

To the best of our knowledge, the current study was the first in combining experimentally-induced and self-reported expectancy-related factors in predicting pain in fibromyalgia. However, the external validity of experimentally-induced nocebo hyperalgesia is limited and further research is recommended for translating it to EMA studies. Also, we detected state-like changes in pain expectancy and pain catastrophizing to predict upcoming pain intensity; however, future studies are recommended to also investigate whether state-like measures and trait-like measures, e.g., assessed via questionnaires, provide comparable findings for pain prediction. Moreover, our statistical power was limited when comparing nocebo hyperalgesia measured in a single moment compared to repeated diary assessments of other expectancy-related factors. Future studies could consider supporting the experimental measurement of nocebo hyperalgesia with additional self-report questions assessing previously-learned associations related to pain expectancies. Lastly, we assessed pain based on a general abstraction of patients' specific nature of symptoms, without taking into consideration potential variations experienced due to the widespread nature of pain in fibromyalgia. Future studies examining more fine-grained pain differences could consider incorporating additional questions on pain localization, pain unpleasantness, or the functional impact of pain into their EMA design.

Overall, our findings show that self-reported, but not experimentally-induced, expectancy-related factors, i.e., diary-assessed pain expectancy and pain catastrophizing, are associated with moment-to-moment pain changes in fibromyalgia, highlighting the role of top-down processes in pain modulation. Lab-based nocebo hyperalgesia was unrelated to diary-assessed expectancy-related factors or pain, potentially due to their heterogeneity. Our preliminary findings require further translation from experimental to EMA research. If

replicated, our findings could be useful for interventions targeting pain. More specifically, interventions such as Cognitive Behavioral Therapy (CBT), Acceptance and Commitment Therapy (ACT) or mindfulness could target pain expectancy and catastrophizing thoughts to decrease daily pain levels in fibromyalgia.

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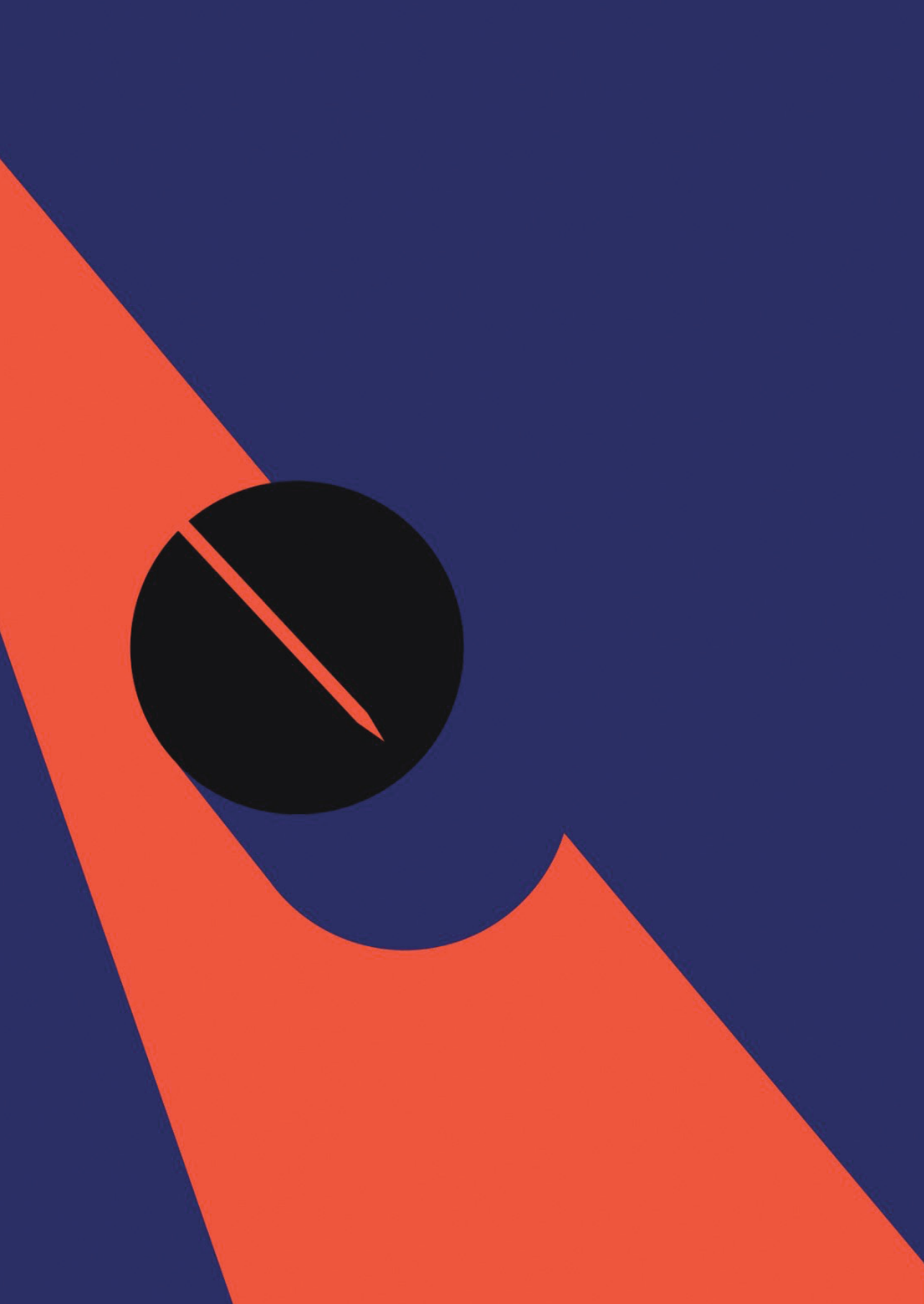


CHAPTER 6

Summary

Nocebo effects are adverse treatment outcomes that are not caused by active treatment components. They can compromise patients' well-being and quality of life, and introduce additional costs on the healthcare system. Previous research shows that nocebo effects are guided by negative outcome expectancies, which can be induced and reduced via learning. Compared to the field of placebo effects, the field of nocebo effects is more recent, with most research conducted with healthy participants. Nocebo research in patients with chronic pain has been scarce, but is important, given that patients' relationship with pain treatment and the surrounding treatment context might be more complex due to the long-term persistence of pain and lack of effective treatments. This could possibly lead to a stronger acquisition of nocebo effects as compared to healthy individuals and also less recovery in patients with chronic pain. Therefore, further investigation is needed for the learning mechanisms behind nocebo hyperalgesia in both healthy individuals and patients with chronic pain. Moreover, identifying not only patients but also healthy individuals who are at risk for acquiring nocebo hyperalgesia is crucial for clinical treatment. Equally important is identifying individuals who are likely to recover from nocebo hyperalgesia for developing learning-based interventions targeting nocebo reduction. Furthermore, investigation into nocebo hyperalgesia in chronic pain conditions, for instance fibromyalgia, could provide additional insights into nocebo-related pain progression in daily life. Insights into the prediction, acquisition, maintenance, and recovery of nocebo hyperalgesia and pain progression could be useful for researchers and clinicians, as targeting expectancy-related factors such as nocebo effects is promising for treating pain in chronic pain conditions.

In the current dissertation, we aimed to investigate the experimental learning mechanisms behind the induction (for example, conditioning, open- and closed-label verbal suggestions) and reduction (for example, extinction, counterconditioning), or in other words the recovery, of nocebo hyperalgesia in healthy individuals and patients with fibromyalgia, and to determine potential differences between groups in the acquisition and recovery of nocebo hyperalgesia. Additionally, we investigated the predictors of nocebo hyperalgesia acquisition and recovery to identify individuals susceptible to these effects. Lastly, in an electronic diary study we aimed to determine whether (experimentally-induced) nocebo hyperalgesia plays a role in daily pain progression in fibromyalgia.

In **Chapter 2**, we aimed to determine novel ways to experimentally induce and reduce nocebo effects on pain. As such, we applied pressure pain, an ecologically-valid pain modality for musculoskeletal disorders such as fibromyalgia, to induce nocebo effects in healthy participants. We also employed open-label, instead of closed-label, verbal suggestions to investigate more ethical ways to manipulate nocebo effects. Participants

were informed about the inert treatment properties of a sham Transcutaneous Electrical Nerve Stimulation (TENS) device and were explained how this could still affect pain through the expectancy mechanisms behind nocebo effects. Moreover, we tested counterconditioning as a novel intervention strategy to reduce nocebo effects. Accordingly, a 2-part RCT was conducted in healthy female participants. After we induced nocebo effects on pressure pain using conditioning combined with open-label verbal suggestions, we compared open-label extinction, counterconditioning, and continued nocebo conditioning (control) for reducing nocebo effects on pressure pain. Our results showed that open-label conditioning combined with verbal suggestions was effective in inducing nocebo effects. Moreover, we found that open-label counterconditioning was more effective in reducing nocebo effects compared to open-label extinction and repeated nocebo inductions. These findings are promising for the future development of more ethical (non-deceptive) learning-based interventions for reducing nocebo effects.

In **chapter 3**, we aimed to identify the predictors of nocebo hyperalgesia acquisition and recovery. Building on the findings in **Chapter 2**, we conducted additional exploratory analyses to determine whether experimentally-induced nocebo hyperalgesia can be predicted by psychological characteristics assessed through questionnaires, such as dispositional optimism, trait and state anxiety, pain catastrophizing, fear of pain, and body vigilance. We also investigated whether the reduction of nocebo hyperalgesia can be predicted by susceptibility to nocebo hyperalgesia and the same psychological characteristics. Our results showed that lower optimism and higher trait anxiety were related to stronger nocebo hyperalgesia induction. Moreover, stronger nocebo hyperalgesia and higher trait anxiety predicted the overall efficacy of nocebo reduction interventions (i.e., counterconditioning and extinction). We also found that participants with stronger nocebo hyperalgesia and lower dispositional optimism had a larger nocebo reduction during counterconditioning than participants with lower nocebo hyperalgesia and higher dispositional optimism. Interestingly, lower dispositional optimism and higher trait anxiety were involved in both stronger acquisition and recovery of nocebo hyperalgesia. Our findings indicate that susceptibility to nocebo hyperalgesia, dispositional optimism, and trait anxiety may shape pain experiences in either direction. Individuals high in trait anxiety are likely to benefit from either nocebo reduction strategy (counterconditioning or extinction) whereas those with stronger nocebo hyperalgesia or lower optimism are likely to benefit the most from counterconditioning.

In **chapter 4**, we aimed to detect the potential group differences in the magnitude of nocebo hyperalgesia induction and reduction in patients with fibromyalgia versus healthy controls. Moreover, we additionally investigated the stability of these effects after a 1-month follow-up. In an experimental study, we accordingly induced nocebo effects on

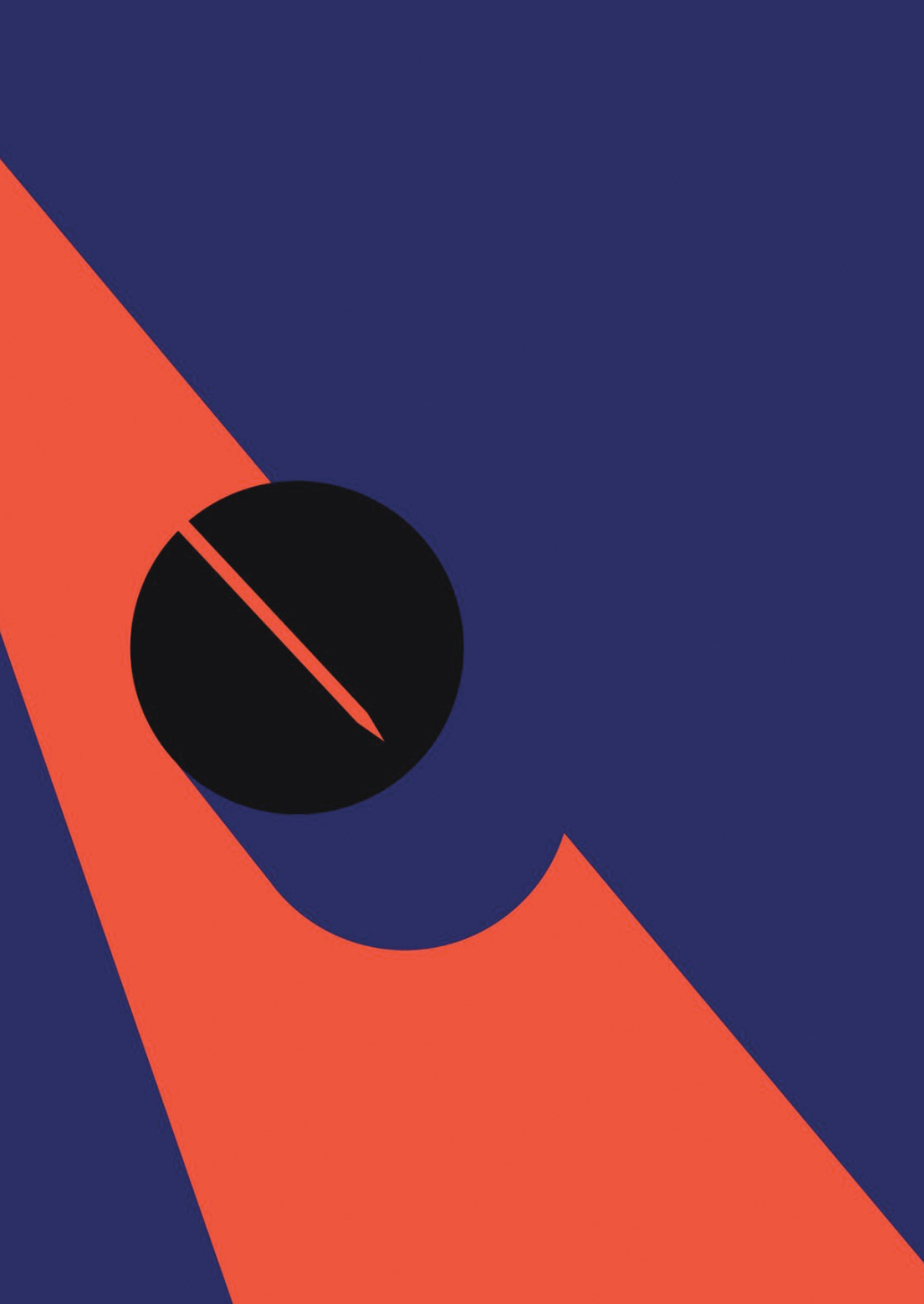
pressure pain using conditioning combined with (closed-label) verbal suggestions about the pain-increasing function of a sham TENS device, and then reduced these effects through extinction. The same experimental procedures were repeated after one month. Our reasoning for this time selecting closed-label instructions, over open-label, and extinction over counterconditioning, was to mimic the acquisition and recovery of nocebo effects as they might occur in daily life.

Contrary to our expectations, we did not find clear group differences in the induction and reduction of nocebo hyperalgesia. Also, across all participants, the magnitude of nocebo hyperalgesia and its extinction was stable after one month. These findings may have positive implications for clinical practice whereby patients with fibromyalgia may not be necessarily at greater risk of nocebo hyperalgesia compared to healthy individuals. However, future replication studies in patients with chronic pain are warranted.

In **chapter 5**, we aimed to identify whether nocebo hyperalgesia magnitude predicts mean pain intensity over 3 weeks in patients with fibromyalgia. We combined our experimental findings from **Chapter 4**, where we additionally assessed expectancy-related factors (i.e., pain expectancy, anxiety, pain catastrophizing, and optimism) and pain intensity in the same patient sample, using an electronic diary three times a day (morning, afternoon, evening) over the three weeks following the baseline experimental session. Our findings indicated that experimentally-induced nocebo hyperalgesia did not predict daily pain, and was unrelated to other expectancy-related factors, in patients with fibromyalgia. Nevertheless, we did find evidence for higher pain expectancy and pain catastrophizing being associated with moment-to-moment increases in pain. Diary-reported factors related to nocebo hyperalgesia, specifically pain expectancy and pain catastrophizing, seem to be promising for future consideration regarding understanding pain progression in fibromyalgia.

Taken together, in the current dissertation we have identified novel strategies for manipulating nocebo hyperalgesia. We found that nocebo effects can be successfully induced on pressure pain, an ecologically-valid pain modality for musculoskeletal disorders such as fibromyalgia. Moreover, open-label counterconditioning seemed promising as a novel intervention strategy for reducing nocebo hyperalgesia. Second, we investigated the predictors of nocebo hyperalgesia acquisition and recovery and found that individuals with lower dispositional optimism and higher trait anxiety might be at greater risk of acquiring nocebo hyperalgesia. These traits were, however, also predictive of better recovery from nocebo hyperalgesia. Moreover, higher susceptibility to nocebo hyperalgesia was also a predictor of recovery from nocebo hyperalgesia. Third, we did not observe stronger nocebo hyperalgesia, nor a stronger resistance to extinction, in

patients with fibromyalgia compared to healthy controls. These effects were also stable across groups after a month. Contrary to our expectations, being a patient, compared to being healthy, was not a risk factor for acquiring nocebo hyperalgesia. Fourth, we found evidence that diary-reported factors related to susceptibility to nocebo hyperalgesia, but not experimentally-induced nocebo hyperalgesia, can predict pain progression in fibromyalgia. All in all, findings in the current dissertation provide insights into the role nocebo hyperalgesia plays in pain. This work suggests that negative expectancies could be targeted via learning-based interventions to minimize nocebo effects and to reduce (chronic) pain in clinical settings.



CHAPTER 7

General Discussion

Pain is a complex phenomenon that can be shaped by top-down factors such as expectancies and learning. Only in the past few decades, expectancies of adverse treatment outcomes, as key factor in nocebo effects, have been considered for their role in aggravating pain-related symptoms. While research on nocebo hyperalgesia is prevalent in healthy individuals, more investigation is needed in ecologically-valid chronic pain modalities and chronic pain conditions, such as fibromyalgia, using different learning strategies (e.g., open- and closed-label verbal suggestions, classical conditioning, counterconditioning, extinction), to identify their potential role in pain progression. In addition, investigation into individual differences in acquiring and recovering from nocebo hyperalgesia could result in useful markers of susceptibility to nocebo-related modulation of pain. To address these topics, the current dissertation first investigated ways to experimentally manipulate nocebo hyperalgesia by administering pressure stimuli to evoke pain (i.e., an ecologically-valid pain modality for musculoskeletal disorders such as fibromyalgia), and by utilizing open-label counterconditioning as novel strategy for manipulating nocebo hyperalgesia in healthy female participants. Moreover, we investigated the predictors of (experimentally-induced) nocebo hyperalgesia and the recovery therefrom in the same healthy female sample. Next, we compared the magnitude of (closed-label) experimental induction and reduction of nocebo effects on pressure pain in female patients with fibromyalgia versus matched healthy controls, while repeating the same experimental procedures at one-month follow-up to assess stability of nocebo effects. Lastly, we employed an ecological momentary assessment (EMA) method in the form of an electronic diary study in the same fibromyalgia sample to investigate whether (experimentally-induced) nocebo hyperalgesia predicts diary-assessed pain.

In this closing chapter, the main findings of this dissertation are summarized in relation to the current literature, while discussing limitations and the implications for research and clinical practice. Moreover, recommendations are provided for future research directions.

Identifying ways to experimentally induce and reduce nocebo effects on pain

In **chapter 2**, our main aim was to study novel ways to induce and reduce nocebo hyperalgesia in a healthy female sample. We aimed to 1) determine whether experimentally inducing pain using pressure stimuli, i.e., an ecologically-valid pain modality for musculoskeletal disorders like fibromyalgia, can be used in the induction and reduction of nocebo hyperalgesia as observed in previous studies using heat and electrical pain modalities[1–4]; 2) test whether providing open-label suggestions, i.e., informing participants on the inert treatment properties and the underlying mechanisms behind nocebo effects, during conditioning is an effective strategy for inducing nocebo hyperalgesia; 3) test the efficacy of open-label counterconditioning as a novel intervention

strategy for attenuating nocebo hyperalgesia. In line with this, we conducted an RCT to investigate the open-label induction and reduction of nocebo effects on pressure pain in a healthy female sample. The pressure pain evocation method tested in the current study was intended to be later used in studies involving patients with fibromyalgia (see **chapter 4** and **chapter 5**).

Our results showed that open-label conditioning combined with suggestions on the pain-increasing function of a sham TENS device was effective in inducing nocebo effects on pressure pain, as compared to sham conditioning. This study was the first to show that open-label induction of nocebo effects on pressure pain is possible, confirming the earlier findings on the efficacy of open-label nocebo conditioning on itch[5]. Important to note here is that in daily life, conditioning more closely resembles the closed-label paradigms, since participants are not deliberately aware of the associations between pain and certain stimuli. Nonetheless, it is relevant to know that open-label instructions on nocebo effects impact hyperalgesia, as this may increase awareness on how to communicate specific treatment information to people. In line with previous (closed-label) counterconditioning studies on other pain modalities[2] and itch[6], open-label counterconditioning of pressure pain was found to be a more effective nocebo-reduction strategy compared to an open-label extinction and control condition in which nocebo conditioning was continued. In particular, counterconditioning did not only reduce nocebo hyperalgesia, but also induced a similar level of conditioned placebo analgesia as placebo conditioning. This indicates that deceptive methods may not be necessary for treating nocebo effects. Open-label counterconditioning of nocebo effects is thus promising for the future design of ethical (non-deceptive) learning-based interventions for chronic pain conditions.

Predicting nocebo-hyperalgesia acquisition and recovery

The aim of **chapter 3** was to identify the predictors of nocebo hyperalgesia acquisition and recovery. More specifically, we conducted additional exploratory analyses on the same experimental study (in **chapter 2**) to identify the psychological predictors of nocebo hyperalgesia. Moreover, we studied whether the reduction of nocebo hyperalgesia can be predicted by susceptibility to (experimentally-induced) nocebo hyperalgesia and psychological characteristics. For this, factors that have been previously shown to be possibly related to nocebo effects (dispositional optimism, trait and state anxiety, pain catastrophizing, fear of pain, and body vigilance) were assessed via validated questionnaires at baseline, prior to the experimental nocebo manipulations. The results showed that stronger nocebo hyperalgesia was predicted by lower optimism and higher trait anxiety. Moreover, larger nocebo hyperalgesia magnitude and higher trait anxiety predicted a larger nocebo reduction across all interventions (i.e., counterconditioning, extinction, and control). In addition, larger nocebo-hyperalgesia magnitude and lower

optimism predicted the largest nocebo reduction after counterconditioning. Our findings indicate that susceptibility to nocebo hyperalgesia, dispositional optimism, and trait anxiety may shape the degree to which nocebo effects on pain are reduced. Individuals high in trait anxiety are likely to benefit from either nocebo-reduction strategy (counterconditioning or extinction) whereas those with larger nocebo hyperalgesia or lower optimism are likely to benefit the most from counterconditioning. Identifying individual differences in the acquisition and recovery from nocebo hyperalgesia can help design more personally-tailored nocebo interventions.

Nocebo hyperalgesia in patients with fibromyalgia versus healthy controls

In **chapter 4**, we aimed to identify potential differences in acquiring and recovering from nocebo hyperalgesia for people with or without chronic pain. Here, we investigated group differences for the magnitude of induced and reduced nocebo hyperalgesia in female patients with fibromyalgia versus female healthy controls that were matched on age and education level. In the lab, nocebo effects on pressure pain were induced via (closed-label) conditioning combined with verbal suggestions on the pain-increasing function of a sham TENS device, which was later decreased via extinction. One month later, the same experimental procedures were repeated in both groups to measure the stability of these effects to identify the role time plays in potential fluctuations (e.g., progression) in nocebo-hyperalgesia levels. In line with previous studies in healthy participants, nocebo hyperalgesia was successfully induced in this group both at baseline and follow-up. However, in the patient group, nocebo hyperalgesia was not significantly induced during the baseline session. During follow-up, nocebo hyperalgesia was observed also in the patient group, while there were no differences in hyperalgesia magnitude compared to healthy controls. Extinction was effective in decreasing nocebo hyperalgesia only in the baseline session of healthy individuals. Post-hoc investigations showed that extinction did take place in those individuals where nocebo effects were induced in either group, in both sessions. Moreover, across all participants the magnitude of nocebo-hyperalgesia induction and decrease was stable after one month. Contrary to our expectations, we did not observe a stronger magnitude of nocebo hyperalgesia, or resistance to extinction, in patients with fibromyalgia compared to healthy individuals. In fact, patients could be either equally or less responsive to the experimental nocebo manipulations compared to healthy controls. As this study was, to the best of our knowledge, the first in comparing differences in nocebo hyperalgesia in people with versus without chronic pain, replication of our current findings is warranted.

Predicting pain progression based on nocebo hyperalgesia

In **chapter 5**, we aimed to predict fibromyalgia pain based on individual differences in nocebo hyperalgesia magnitude. Here, we combined our experimental findings from

chapter 4 with ecological momentary assessment (EMA). Using EMA, we investigated whether experimentally-induced nocebo hyperalgesia magnitude and diary-assessed expectancy-related factors (pain expectancy, anxiety, pain catastrophizing, optimism) predicted changes in daily-pain intensity in the same fibromyalgia sample. Also, the relations between nocebo hyperalgesia and other expectancy-related factors were explored. Following the baseline experimental session (**chapter 4**), the same patient group filled out an electronic diary for 3 times a day for 3 weeks. Our findings showed that particularly higher pain expectancy and higher pain catastrophizing were related to moment-to-moment pain increase. Experimentally-induced nocebo hyperalgesia did not predict pain and was unrelated to diary-assessed expectancy-related factors. Although we did not find evidence for (experimentally-induced) nocebo hyperalgesia being a predictor of fibromyalgia pain progression, pain expectancy and pain catastrophizing (factors related to susceptibility to nocebo hyperalgesia) in particular seem promising for future consideration. This finding could be useful for, for example, future treatment strategies to also target overprediction of upcoming pain (i.e., pain expectancy) and catastrophizing thoughts for reducing fibromyalgia pain.

Differences in nocebo hyperalgesia between people with and without chronic pain

The current dissertation investigated the learning mechanisms behind nocebo hyperalgesia in healthy individuals and in patients with fibromyalgia. Although group differences have not yet been researched before in the nocebo context, previous research exists on how chronic pain populations respond to nocebo hyperalgesia as well as on the group differences inside placebo- and fear-conditioning fields.

Experimental research demonstrates that patients with irritable bowel syndrome (IBS)[7] and with postoperative pain[8] have shown nocebo effects on clinical pain after verbal suggestions of pain increase. One experimental study in patients with chronic low back pain has combined both conditioning and verbal suggestions to induce nocebo effects on clinical pain; however, this study has found placebo effects, potentially due to verbally suggesting both the positive and negative effects of a sham opioid treatment where the placebo suggestions possibly prevailed over the nocebo ones[9]. To the best of our knowledge, our experimental study (**chapter 4**) was the first in combining conditioning with verbal suggestions of (mere) pain increase regarding the activation of a sham TENS device, where we observed no group differences in nocebo hyperalgesia magnitude between patients with fibromyalgia and healthy controls. Also, in the placebo context, a recent brain-imaging study has found no differences between patients with fibromyalgia and healthy controls in their neural response to placebo analgesia[10]. Placebo analgesia induced via conditioning and verbal suggestions led to comparable decreases in both

groups for pain intensity and unpleasantness ratings as well as for the activity in areas related to the neurological pain signature[10]. Similar findings were observed in placebo studies comparing healthy individuals versus patients with the chronic pain conditions temporomandibular disorder[11], episodic migraine[12], and IBS[13].

Research to date suggests that patients with chronic pain respond in a similar manner as healthy controls to placebo and nocebo manipulations. This would suggest that research in healthy populations offers a good proxy for research in patients, as research in healthy individuals may be generalizable to patients. However, although insights into placebo analgesia are useful indicators of expectancies in chronic pain conditions, the psychological and neurobiological mechanisms behind placebo effects do not fully overlap with those of nocebo effects [14–16]. Therefore, further experimental nocebo research is needed in chronic pain conditions to determine whether the lack of group differences observed for placebo effects generalizes to nocebo effects, with our study being the first to provide indications in that direction.

Further informative group comparisons between patients with fibromyalgia and healthy controls come from fear conditioning literature in the context of learning[17–19]. In (pain-related) fear-conditioning paradigms, patients with fibromyalgia were found to have impaired contingency learning (i.e., impairment in learning that one event predicts the presence or absence of another event) and excessive stimulus generalization (i.e., learning of a specific US-CS association is more easily broadened to other stimuli) compared to healthy controls[17-19]. Future research is recommended to examine whether these learning deficits in fibromyalgia play a role in nocebo-learning paradigms. Learning deficits may be also a potential explanation for the inconsistencies we observed across baseline and follow-up sessions for the nocebo hyperalgesia magnitude of patients. For example, not being able to identify the safety cue (i.e., control cue) compared to the experimental cue predicting higher pain could potentially impact the measurement of nocebo hyperalgesia magnitude. Therefore, future studies are recommended to employ additional measurements of contingency awareness between experimental and control cues during the testing phase of nocebo conditioning to gain further insights into group comparisons in nocebo hyperalgesia.

Altogether, our findings demonstrate that patients with fibromyalgia and healthy controls do not show clear group differences in their acquisition of (experimentally-induced) nocebo hyperalgesia and its extinction. However, further replication studies and research considering the previously observed contingency-learning deficits in fibromyalgia are warranted.

Individual differences in nocebo hyperalgesia acquisition and recovery

Accurately identifying individuals at risk of acquiring nocebo effects is vital for the future of clinical treatment, since nocebo effects are detrimental to patients' well-being and quality of life, as well as costly to the healthcare system. Also, identifying individuals who are likely to recover from nocebo effects is important for the design of learning-based interventions for reducing nocebo effects. In terms of predictors, we identified lower dispositional optimism and higher trait anxiety (**chapter 2**) to be related to stronger nocebo-hyperalgesia acquisition in healthy participants. This is in line with previous findings from a systematic review that identified higher optimism to be related to placebo analgesia and higher anxiety with nocebo hyperalgesia[15]. Nevertheless, we were not able to replicate our findings on dispositional optimism and (trait and state) anxiety in our second experimental study (**chapter 4**) in neither a healthy nor patient group. Similarly, the diary-assessed optimism and anxiety levels of patients were found unrelated to (experimentally-induced) nocebo hyperalgesia (**chapter 5**). However, findings should be generally interpreted with some caution given the limited sample sizes and the differences in study methodologies.

Our mixed findings on these predictors could be potentially (partly) explained by our choice of measurement method. A recent meta-analysis points out that trait anxiety, for instance measured via baseline questionnaire, may be limited in predicting the magnitude of nocebo effects, whereas state (or situationally-induced) anxiety appears more critical for the induction of nocebo effects[20]. Therefore, additional to our questionnaire assessments, a trial-by-trial assessment of these psychological factors could have provided additional insights into their situational changes and relevance for the subsequent induction of nocebo hyperalgesia in both participant groups. Moreover, experimentally-induced nocebo hyperalgesia was unrelated to the situational changes (in optimism, anxiety, pain catastrophizing, and pain expectancy) that were assessed via electronic diary in patients. The heterogeneity between these measurement methods could be a potential explanation of our findings. For future studies, it is important to explore additional ecologically-valid ways of measuring potential predictors of nocebo hyperalgesia, such as via electronic diary.

In terms of predictors of nocebo recovery, we found preliminary evidence for susceptibility to (experimentally-induced) nocebo hyperalgesia, dispositional optimism, and trait anxiety being related to nocebo hyperalgesia reduction (**chapter 2**). This finding could be possibly explained by a stronger desire for pain relief when perceived pain is higher during greater nocebo hyperalgesia and lower dispositional optimism, which could in turn increase the efficacy of upcoming interventions[21]. Another possible explanation could be related to the fact that participants who are more susceptible to nocebo hyperalgesia might be

also susceptible to learning strategies in general. This could be potentially facilitated by showing more selective attention towards conditioned stimuli in the environment, and thereby responding equally strongly to upcoming nocebo-reduction interventions[22].

Altogether, we found preliminary evidence for individuals with lower dispositional optimism and higher trait anxiety to be more susceptible to (experimentally-induced) nocebo hyperalgesia. Moreover, individuals with larger baseline nocebo hyperalgesia, lower dispositional optimism, and higher trait anxiety seemed to benefit the most from nocebo-reduction interventions. Our findings provide promising insights into how individual susceptibility to nocebo hyperalgesia, dispositional optimism, and trait anxiety might be modulating pain in either direction. Future replication studies could consider selecting these predictors while investigating the acquisition and recovery of nocebo hyperalgesia in healthy and clinical populations.

Limitations

There are several limitations related to the research questions in the current dissertation. One important limitation in all studies was the inclusion of only female participants. The main argument behind this selection was that fibromyalgia is reportedly more common in women than in men with a proportion up to 9:1 based on epidemiological and population studies[23]. Although research shows that female sex is a risk factor in fibromyalgia[24], some studies argue that this could be a result of women likely consulting their physician more frequently than men[23]. A recent study also highlights the fact that fibromyalgia is not exclusively observed in women, since widely varying estimates of female ratio might be due to participant selection bias in clinical studies, lowering the actual female predominance from 90% to less than 60%[25]. In our studies, we chose female participants to increase the comparability of current findings with existing literature. Also, we aimed to avoid introducing potential bias into our data collection, such as sex differences in pain sensitivity[26]. Moreover, a systematic review has found sex differences in nocebo effects, with females responding stronger than men, potentially due to a larger increase in stress and anxiety after nocebo induction in females[27]. Given that the current patient studies (**chapters 4** and **5**) were the first in the nocebo field, we chose not to introduce sex-related bias. However, the generalizability of our findings to males is limited and requires further investigation. Similarly for our findings with healthy participants (**chapters 2** and **3**), who were university-educated young females, further investigation in males, older populations, and vocation-educated individuals are warranted.

Moreover, small participant samples require a point of attention in placebo and nocebo studies, especially for the statistical analyses involving predictors. Since power calculations were conducted in the current studies to estimate sample sizes required for testing our

primary research questions, our effect sizes especially for secondary research questions involving predictors had wide confidence intervals and/or small effect sizes were not detected. A potential solution other than running large-scale studies, could be a meta-analysis of different studies in the field to assess predictors from a cumulatively larger sample.

Furthermore, the external validity of experimental nocebo hyperalgesia paradigms is a common issue in the field. Although we tried to select an ecologically-valid pain modality for inducing nocebo hyperalgesia, the extent in which pressure pain relates to daily pain experiences in fibromyalgia is debatable. We speculate that experimentally-evoked pressure pain may not have been sufficiently fear- or anxiety-inducing to generate strong nocebo effects in the lab, considering that daily pain experiences of patients might be in comparison more harmful, less predictable, and longer in duration than a safe and controlled experimentally-evoked pain experience. To gain a better insight into this, our experimental procedure could have benefited from additional trial-by-trial measures of fear[28] or anxiety[29]. In our lab studies, we only measured self-reported pain intensity after sham TENS-cue presentation in each trial. Important to note here is that the self-reported evaluation of multiple measures before and after cue presentation could become confusing for participants as this requires a rapid cognitive abstraction of different somatic changes. Moreover, also the external validity of the nocebo conditioning paradigm may have been limited in reproducing learning experiences as they appear in daily life. For example, when exposed to daily pain, pain-related associations might generalize to other cues than only sham treatment (i.e., the conditioned stimulus). Our reasoning for preferring closed-label over open-label instructions in our patient study (**chapter 4**) was to decrease the predictability of pain outcomes to better mimic nocebo effects and extinction as they occur in daily life. To strengthen our research, we have consulted patient partners during the design process of our study, for example to more extensively test the nocebo procedures in the lab. Future studies could consider involving them in other ecologically valid paradigms, such as diary studies.

Another limitation was that for safety purposes the experimenter was present in the lab at all times, which could have influenced the reporting of pain ratings in **chapter 2,** given that the experimenter logged these values into the computer after participants verbally indicated their pain intensity. In **chapter 4**, the experimenter was also present at all times, but the participants themselves logged their pain-intensity ratings into the computer. Moreover, double blinding was not possible for the study with the open-label paradigm due to its non-deceptive nature, which made sure participants and the experimenter were consciously aware of the experimental learning manipulations. Participants were only blinded to the surreptitious change in pressure intensity during the testing phase of

conditioning. For the study with the closed-label paradigm, both healthy participants and patients underwent the same experimental procedures, thereby eliminating the need for blinding of the experimenter. Participants were blinded to the real study aims, along with the surreptitious change in pressure intensity during the testing phase of conditioning. However, participants could not be blinded to the experimental manipulations during the learning phase of conditioning since the pressure intensity administered in this part was aligned with the verbal suggestions provided about the sham TENS function.

Lastly, the COVID-19 pandemic overlapped with studies in the current dissertation. For studies in **chapters 2** and **3**, experimental data collection ended prematurely since data possibly collected during the pandemic was considered incomparable to previously collected data, due to additional safety measures. For studies in **chapters 4** and **5**, additional safety measures were introduced in the lab (e.g., participant and researcher wore mouth masks, a lab set-up was created to ensure sufficient distance between the researcher and the participant). We do not expect this to influence our experimental manipulations; however, the potential impact of the pandemic on participants' expectations and psychological well-being, and its subsequent effects on the outcomes relevant for our study, remains unknown.

Implications and future research directions

There are several implications of our findings for future research and clinical practice regarding group differences in nocebo hyperalgesia, methodological recommendations surrounding experimental procedures, predictors of nocebo hyperalgesia acquisition and recovery, and the potential role of nocebo hyperalgesia in pain progression.

First, we found that it was possible to experimentally induce nocebo hyperalgesia through pressure pain, an ecologically-valid pain modality for musculoskeletal disorders, in both healthy participants and patients with fibromyalgia. The current findings indicate no clear group differences in nocebo hyperalgesia magnitude. The fact that we did not find evidence for group differences could be interpreted as a positive finding. Potentially, patients with fibromyalgia might be under less risk of nocebo hyperalgesia than previously anticipated[30–33]. However, future studies are recommended to also take into account the Bayesian framework for statistical analysis compared to the traditional statistical framework applied in the current studies[34], to better disentangle any latent group differences in experimental settings.

Second, certain methodological issues regarding the nocebo conditioning paradigm are recommended to be revisited by future studies. For example, it would be worthwhile to additionally account for contingency-learning deficits and stimulus overgeneralization

previously observed in patients with fibromyalgia, which could impact learning processes, such as nocebo conditioning[17–19]. Moreover, the selection of conditioned stimuli (CS), which was a sham TENS device in our studies, could be customized by selecting a more personalized CS that represents patients' treatment expectations on pain outcomes. To tackle this, first, a consultation could take place to understand which aspects of the treatment are experienced as harmful, e.g., including contextual factors such as the treatment procedure or aspects of the patient-doctor alliance. Then those aspects could be targeted via positively/negatively framed verbal suggestions to manipulate placebo and nocebo effects, respectively. Although the use of personalized measures is characterized by additional statistical challenges, such as in terms of standardization of outcome measures, this could provide a more ecologically-valid translation of a CS in the lab.

Third, the studies in this dissertation have provided indications to decrease the occurrence of nocebo effects or reduce them and for potential individual tailoring of healthcare. Our research has shown that as a predictor of recovery, nocebo hyperalgesia could be harnessed to increase the efficacy of nocebo-reduction interventions. Counterconditioning, in particular may most benefit patients with higher nocebo hyperalgesia, higher trait anxiety, and lower optimism. Moreover, open-label induction and reduction of nocebo hyperalgesia provided additional insights that deception is not required for their experimental manipulation. This has implications for clinical practice, such that physicians can be honest while employing nocebo-reduction strategies in their patients. Future studies are recommended to further investigate the generalization of our findings to more heterogeneous samples, including males and older individuals of different education levels.

Fourth, we did not find evidence for (experimentally-induced) nocebo hyperalgesia to predict pain progression in fibromyalgia in the diary study. Considering existing methodological limitations surrounding the nocebo conditioning paradigm, this research question could be revisited after employing more externally-valid methods of measuring nocebo hyperalgesia. Also, the role of nocebo hyperalgesia magnitude in pain progression is recommended to be investigated at different fibromyalgia stages, for example at onset as well as at later stages, since this could provide insights into the longitudinal effects of nocebo hyperalgesia on disease progression. Further investigation into the association between central sensitization (i.e., pain-processing abnormalities in the central nervous system[36]), and nocebo hyperalgesia is warranted, given that central sensitization is a characteristic of fibromyalgia and is a framework commonly used for explaining the mismatch in perceived pain intensity during minimal physical impairment or in the absence of clear pathophysiology or injury[36]. This mismatch in pain processing leads to exaggerated processing of bottom-up sensory signals and has

implications for correctly predicting upcoming pain[31]. According to the predictive coding[34] and Bayesian brain[31] frameworks, the brain constantly generates top-down predictions about incoming bottom-up sensory data, where this incoming data serves as a corrective feedback mechanism on top-down predictions. During this process, the brain corrects for possible prediction errors arising between the top-down predictions and bottom-up signals by updating the top-down prediction model, for example by modulating the sensory input to match the prediction model[31,34]. In the context of nocebo hyperalgesia, negative expectancies are thought to directly modulate top-down predictions[31]. This mechanism mimics the bias observed in chronic pain during central sensitization, which unproportionally shifts sensory bottom-up data into top-down painful predictions[31]. Therefore, it could be hypothesized that nocebo effects can further strengthen the top-down prediction bias observed during amplified pain experiences in fibromyalgia. Future prospective research is recommended to examine the long-term impact of nocebo hyperalgesia on pain progression.

Conclusion

The field of nocebo research is rapidly growing, with an increasing number of studies focusing on chronic pain. The current dissertation aimed to find answers to the questions of whether the magnitude of nocebo hyperalgesia is comparable in patients with fibromyalgia and healthy controls, what the predictors are of nocebo hyperalgesia acquisition and recovery through extinction and counterconditioning, and whether nocebo hyperalgesia predicts fibromyalgia pain progression in daily life. Our findings have shown that both open- and closed-label strategies were promising for the experimental manipulation of nocebo effects on pressure pain. Patients with fibromyalgia and healthy individuals did not differ with regard to nocebo hyperalgesia. We identified dispositional optimism and trait anxiety as possible predictors of nocebo hyperalgesia; where these and also susceptibility to nocebo hyperalgesia were identified as predictors of nocebo-reduction interventions, such as counterconditioning. Lastly, diary assessments of nocebo-related pain expectancy and pain catastrophizing, but not experimentally-induced nocebo hyperalgesia, predicted moment-to-moment increases in fibromyalgia pain. These insights are useful for the future design of personalized learning-based interventions for targeting chronic pain. Targeting nocebo hyperalgesia and related factors, such as pain expectancies, pain catastrophizing, anxiety, and lack of optimism, might be promising for attenuating nocebo-induced pain during clinical treatments.

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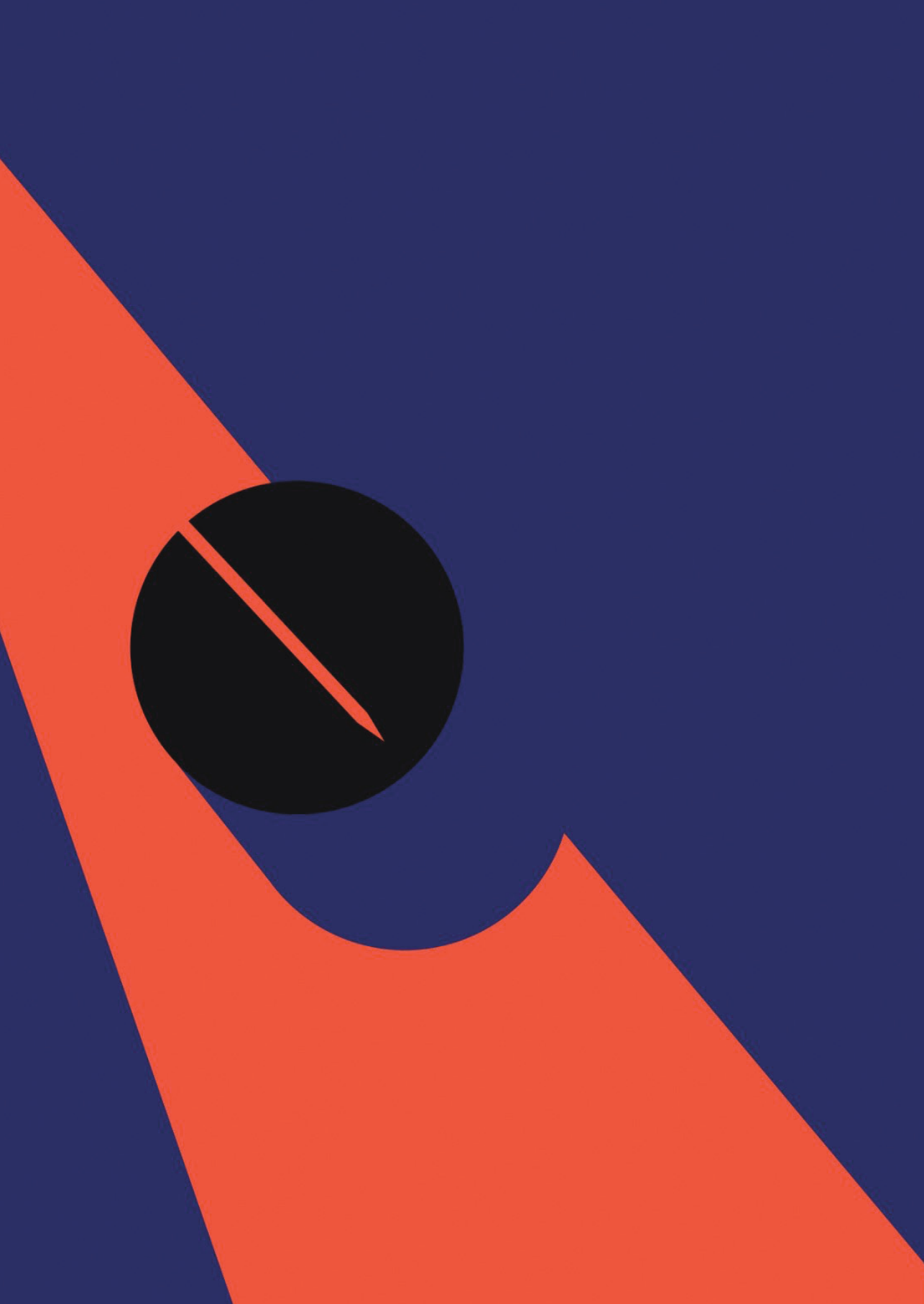

CHAPTER 8

Nederlandse Samenvatting
(Dutch Summary)

Curriculum Vitae

Publications

Acknowledgements

NEDERLANDSE SAMENVATTING (DUTCH SUMMARY)

Nocebo-effecten zijn negatieve behandeluitkomsten die niet door actieve behandelingscomponenten worden veroorzaakt, bijvoorbeeld het ervaren van bijwerkingen na het lezen van een bijsluiter, zelfs als een placebo-pil werd toegediend. Deze kunnen het welbevinden en de levenskwaliteit van patiënten ondermijnen en extra kosten voor het gezondheidszorgsysteem met zich meebrengen. Eerdere onderzoeken tonen aan dat nocebo-effecten worden gestuurd door negatieve verwachtingspatronen, die zowel opgewekt als verminderd kunnen worden via leerprocessen. In vergelijking met de veel onderzochte placebo-effecten zijn nocebo-effecten pas meer recentelijk uitgebreider onderzocht, waarbij de meeste studies uitgevoerd zijn met gezonde deelnemers. Onderzoek naar nocebo-effecten bij patiënten met chronische pijn is beperkt, maar zeer belangrijk, gezien de mogelijke invloed van nocebo-effecten op het effect van behandelingen, evenals op de ervaringen van patiënten met de behandeling. Deze nocebo-effecten worden waarschijnlijk versterkt door de langdurige aanwezigheid van pijn en het gebrek aan effectieve behandelingen. Nocebo-effecten spelen daarom mogelijk een grotere rol bij patiënten met chronische pijn in vergelijking tot gezonde individuen. Om deze reden is verder onderzoek naar de leermechanismen achter nocebo-hyperalgesie (d.w.z. een verhoogde gevoeligheid voor pijnlijke prikkels door nocebo-effecten) bij zowel gezonde individuen als patiënten met chronische pijn noodzakelijk. Bovendien is het van groot belang om niet alleen patiënten, maar ook gezonde individuen te identificeren die risico lopen op het verwerven van nocebo-hyperalgesie voor klinische behandeling. Even belangrijk is het identificeren van personen die waarschijnlijk zullen herstellen van nocebo-hyperalgesie, gezien dit kan helpen bij het ontwikkelen van op leren gebaseerde interventies gericht op het verminderen van nocebo-effecten. Pijn is een complex fenomeen dat kan worden gevormd door top-down processen zoals verwachtingen. Daarom kan onderzoek naar nocebo-hyperalgesie in chronische pijncondities, zoals fibromyalgie, aanvullende inzichten bieden in nocebo-gerelateerde pijnprogressie in het dagelijks leven. Inzichten in de voorspelling, verwerving en herstel van nocebo-hyperalgesie en pijnprogressie kunnen nuttig zijn voor onderzoekers en clinici, aangezien het richten op verwachtingsgerelateerde factoren, zoals nocebo-effecten, veelbelovend is voor de behandeling van pijn bij chronische pijncondities.

In het huidige proefschrift hebben we als doel gesteld om de experimentele leermechanismen achter de inductie (bijvoorbeeld conditionering, open- en closed-label verbale suggesties) en reductie (bijvoorbeeld extinctie, counterconditionering) van nocebo-hyperalgesie te onderzoeken en om potentiële verschillen tussen groepen in de verwerving en het herstel van nocebo-hyperalgesie te bepalen. Daarnaast hebben we onderzocht welke voorspellers er zijn voor de verwerving en het herstel van nocebo-

hyperalgesie om individuen te identificeren die gevoelig zijn voor deze effecten. Tot slot hebben we in een elektronische dagboekstudie als doel gesteld om te bepalen of (experimenteel geïnduceerde) nocebo-hyperalgesie een rol speelt in de dagelijkse pijnprogressie bij fibromyalgie.

In Hoofdstuk 2 hebben we als doel gesteld om nieuwe manieren te bepalen om nocebo-effecten op pijn experimenteel te induceren en te reduceren. Hiervoor hebben we drukpijn toegepast, een ecologisch valide pijnmodaliteit voor musculoskeletale aandoeningen zoals fibromyalgie, om nocebo-effecten bij gezonde deelnemers te induceren. We hebben hierbij open-label in plaats van closed-label verbale suggesties gebruikt, om op een meer ethische manier nocebo-effecten op te wekken. Deelnemers werden geïnformeerd over een niet daadwerkelijk werkzaam Transcutane Elektrische Zenuwstimulatie (TENS) apparaat en aan hen werd uitgelegd hoe dit nog steeds pijn kon beïnvloeden als gevolg van nocebo-effecten en hun verwachtingen. Daarnaast hebben we counterconditionering onderzocht als een nieuwe interventiestrategie om nocebo-effecten te verminderen. Daartoe werd een tweedelig gerandomiseerd gecontroleerd onderzoek (RCT) uitgevoerd onder gezonde vrouwelijke deelnemers. Nadat we nocebo-effecten op drukpijn hadden geïnduceerd met behulp van conditionering gecombineerd met (open-label) verbale suggesties, hebben we open-label extinctie, counterconditionering en herhaalde nocebo-conditionering (controle) vergeleken voor het verminderen van nocebo-effecten op drukpijn. Onze resultaten toonden aan dat open-label conditionering gecombineerd met verbale suggesties effectief was in het induceren van nocebo-effecten. Bovendien vonden we dat counterconditionering effectiever was in het verminderen van nocebo-effecten in vergelijking met extinctie en herhaalde nocebo-conditionering. Deze bevindingen zijn veelbelovend voor de toekomstige ontwikkeling van op leren gebaseerde interventies voor het verminderen van nocebo-effecten.

In hoofdstuk 3 was het doel om voorspellers van het ontstaan en herstel van nocebo-hyperalgesie te identificeren. Voortbouwend op de bevindingen in hoofdstuk 2 voerden we aanvullende verkennende analyses uit om te bepalen of experimenteel geïnduceerde nocebo-hyperalgesie voorspeld kan worden door psychologische kenmerken, zoals dispositioneel optimisme, algemene angst en angst in het moment, pijncatastroferen, angst voor pijn en lichaamsbewustzijn, gemeten met vragenlijsten. We onderzochten ook of de reductie van nocebo-hyperalgesie voorspeld kan worden door de gevoeligheid voor nocebo-hyperalgesie en dezelfde psychologische kenmerken. Onze resultaten toonden aan dat lager optimisme en hogere algemene angst gerelateerd waren aan sterkere inductie van nocebo-hyperalgesie. Bovendien voorspelden sterkere nocebo-hyperalgesie en hogere algemene angst de effectiviteit van nocebo-reductie-interventies (d.w.z. counterconditionering en extinctie). We vonden ook dat deelnemers met sterkere nocebo-

hyperalgesie en lager dispositioneel optimisme een grotere nocebo-reductie lieten zien tijdens counterconditionering dan deelnemers met zwakkere nocebo-hyperalgesie en hoger dispositioneel optimisme. Interessant is dat zowel lager dispositioneel optimisme als hogere algemene angst betrokken waren bij zowel een sterkere inductie als reductie van nocebo-hyperalgesie. Onze bevindingen wijzen erop dat gevoeligheid voor nocebo-hyperalgesie, dispositioneel optimisme en algemene angst pijnervaringen in beide richtingen kunnen beïnvloeden. Individuen met hoge algemene angst hebben waarschijnlijk baat bij verschillende nocebo-reductiestrategieën (counterconditionering of extinctie), terwijl diegenen met sterkere nocebo-hyperalgesie of lager optimisme het meest zullen profiteren van counterconditionering.

In hoofdstuk 4 was het doel om de potentiële groepsverschillen in de mate van inductie en reductie van nocebo-hyperalgesie bij patiënten met fibromyalgie versus gezonde controles te onderzoeken. Daarnaast onderzochten we de stabiliteit van deze effecten na een follow-up van 1 maand. In een experimentele studie hebben we daartoe nocebo-effecten op drukpijn geïnduceerd met behulp van conditionering gecombineerd met (closed-label) verbale suggesties over de pijnverhogende functie van een niet-werkzaam TENS-apparaat en vervolgens deze effecten gereduceerd door extinctie. Dezelfde experimentele procedures werden na één maand herhaald. Onze redenering voor deze keer het selecteren van closed-label suggesties boven open-label suggesties en extinctie boven counterconditionering, was om de verwerving en het herstel van nocebo-effecten na te bootsen zoals die in het dagelijks leven kunnen voorkomen. Tegen onze verwachtingen in vonden we geen duidelijke groepsverschillen in de inductie en reductie van nocebo-hyperalgesie. Ook was de sterkte van nocebo-hyperalgesie en de extinctie ervan stabiel na één maand bij beide deelnemers groepen. Deze bevindingen kunnen positieve implicaties hebben voor de klinische praktijk, waarbij patiënten met fibromyalgie mogelijk niet noodzakelijkerwijs een groter risico lopen op nocebo-hyperalgesie in vergelijking met gezonde individuen. Toekomstige replicatiestudies bij patiënten met chronische pijn zijn gewenst voordat definitieve conclusies hierover mogelijk zijn.

In hoofdstuk 5 was het doel om te identificeren of de mate van nocebo-hyperalgesie de gemiddelde pijnintensiteit over 3 weken bij patiënten met fibromyalgie voorspelt. We combineerden onze experimentele bevindingen uit hoofdstuk 4 met een elektronische dagboekstudie. In deze dagboekstudie rapporteerden dezelfde patiënten drie keer per dan (ochtend, middag, avond) verwachtingsgerelateerde factoren (d.w.z. pijnverwachting, angst, pijncatastroferen en optimisme) en pijnintensiteit gedurende de drie weken volgend op de baseline experimentele sessie. Onze bevindingen gaven aan dat experimenteel geïnduceerde nocebo-hyperalgesie de dagelijkse pijn niet voorspelde en niet gerelateerd was aan andere verwachtingsgerelateerde factoren bij patiënten

met fibromyalgie. Desalniettemin vonden we bewijs dat hogere pijnverwachting en pijncatastroferen geassocieerd waren met moment-tot-moment toenames in pijn. Dagboekgerapporteerde factoren gerelateerd aan gevoeligheid voor nocebo-hyperalgesie - specifiek pijnverwachting en pijncatastroferen - lijken veelbelovend voor toekomstige onderzoeken met betrekking tot het begrijpen van pijnprogressie bij fibromyalgie.

Samengevat hebben we in dit proefschrift nieuwe strategieën geïdentificeerd voor het beïnvloeden van nocebo-hyperalgesie. Allereerst hebben we laten zien dat nocebo-effecten succesvol geïnduceerd kunnen worden met behulp van drukpijn, een ecologisch valide pijnstimulus voor musculoskeletale aandoeningen zoals fibromyalgie. Bovendien vonden we open-label counterconditionering veelbelovend als een nieuwe interventiestrategie voor het verminderen van nocebo-hyperalgesie. Ten tweede hebben we de voorspellers van de verwerving en het herstel van nocebo-hyperalgesie onderzocht en vastgesteld dat individuen met lager dispositioneel optimisme en hogere algemene angst mogelijk een groter risico lopen op het verwerven van nocebo-hyperalgesie. Deze kenmerken waren echter ook indicatief voor een beter herstel van nocebo-hyperalgesie. Een hogere gevoeligheid voor nocebo-hyperalgesie was eveneens een voorspeller voor herstel van nocebo-hyperalgesie. Ten derde observeerden we geen sterkere nocebo-hyperalgesie noch een sterkere weerstand tegen extinctie bij patiënten met fibromyalgie vergeleken met gezonde controles. Deze effecten bleven ook stabiel over groepen heen na een maand. Het zijn van een patiënt met chronische pijn blijkt op basis van dit eerste onderzoek dus geen risicofactor voor het verwerven van nocebo-hyperalgesie. Ten vierde vonden we bewijs dat dagboekgerapporteerde verwachtingsfactoren gerelateerd aan gevoeligheid voor nocebo-hyperalgesie, maar niet experimenteel geïnduceerde nocebo-hyperalgesie, pijnprogressie bij fibromyalgie kunnen voorspellen. Al met al bieden de bevindingen in dit proefschrift inzichten in de rol van nocebo-hyperalgesie bij pijn. Het onderzoek in dit proefschrift heeft daarnaast aanwijzingen geboden dat op leren gebaseerde interventies in de toekomst mogelijk ingezet zouden kunnen worden om nocebo-effecten te minimaliseren en (chronische) pijn in klinische settings te verminderen.

CURRICULUM VITAE

Merve Karacaoğlu was born on November 16th, 1991 in Ankara, Turkey. Growing up, she lived in Turkey as well as in California, USA and Koblenz, Germany. She graduated from Ayrancı Anatolian High School in Ankara in 2010. Afterwards, she was granted a comprehensive scholarship to study Psychology Bachelor of Arts (B.A.) at Bilkent University, Ankara, Turkey. During her time there, she did an internship at the National Magnetic Resonance Research Center (UMRAM) in Ankara and also participated in the Erasmus exchange program at Tilburg University.

Upon obtaining her Psychology B.A. diploma in 2014, she moved to Amsterdam, the Netherlands. She was awarded a scholarship from the VU Fellowship Program to pursue a two-year research master in Cognitive Neuropsychology at Vrije Universiteit (VU) Amsterdam. For her master thesis, she conducted EEG research on the priority switches in the visual working memory. Upon receiving her MSc. diploma in 2016, she worked as a research assistant at the Netherlands Aerospace Center (NLR) in Amsterdam while conducting Virtual Reality (VR) research to improve the soundscape quality of urban areas exposed to aircraft noise. On November 2017, she started her PhD at the Health, Medical and Neuropsychology unit of Leiden University. Her research focused on the prediction of nocebo effects and particularly, its role in pain progression in patients with fibromyalgia.

On November 2022, she began working as a software engineer at SkyLeague Services BV in Amsterdam. Currently, she lives in Leiden, where she completed her PhD dissertation.

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*: shared first co-authorship

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