

## Nocebo hyperalgesia and the startle response

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2 Per M. Aslaksen<sup>1\*</sup>, Ole Åsli<sup>1</sup>, Morten Øvervoll<sup>1</sup>, Espen Bjørkedal<sup>1</sup>.

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5 <sup>1</sup>) Department of Psychology, Research group for Cognitive Neuroscience, The Faculty of  
6 Health Sciences, University of Tromsø, The Arctic University of Norway, 9037 Tromsø,  
7 Norway.  
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11 \*) Corresponding author: Per M. Aslaksen, Ph.D., Professor, Department of Psychology,  
12 Research group for Cognitive Neuroscience, The Faculty of Health Sciences, University of  
13 Tromsø, The Arctic University of Norway, 9037 Tromsø, Norway. Telephone: + 47 776  
14 49234, email: [per.aslaksen@uit.no](mailto:per.aslaksen@uit.no), web:  
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## Abstract

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2 Background: The literature on the effects of nocebo on pain is sparse. The present  
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4 experimental study investigated whether suggestions of nocebo hyperalgesia modified the  
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6 startle response and whether increased startle contributed to the nocebo hyperalgesic effect.  
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8 Methods: A design with four groups was employed; the participants were randomized into  
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10 either a placebo group, a natural history group, or into two nocebo groups. The participants in  
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12 the placebo and nocebo groups received suggestions of pain decrease or pain increase,  
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14 together with a placebo or nocebo cream applied to the lower arm, respectively. Heat pain was  
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16 induced by a PC-controlled thermode before and after the treatment. White noise was used to  
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18 elicit startle responses. Startle was assessed by measuring eye blink electromyographic  
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20 responses recorded from the right orbicularis oculi muscle. Results: The results showed that  
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22 nocebo suggestions increased reports of pain and startle responses. Increased startle was  
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24 significantly associated with the nocebo hyperalgesic response. Conclusions: The results of  
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26 the present study suggest that verbally induced expectations of increased pain engage cortical  
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28 physiological defensive systems that in turn mediate the experience of increased pain.  
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31 Keywords: nocebo hyperalgesia; placebo analgesia; startle response; emotions; pain;  
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## Introduction

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3 Placebo analgesia is well studied in pain research. The opposite of placebo analgesia,  
4 nocebo hyperalgesia, however, has received a limited amount of experimental attention  
5 (Petersen et al 2014), even though nocebo effects in pain might be equally important in  
6 clinical settings (Colloca & Finniss 2012). The few studies that do exist suggest that nocebo  
7 hyperalgesia is caused by expectations of pain increase that induce negative emotions, which  
8 in turn increase pain. Furthermore, only a few studies have compared nocebo hyperalgesia  
9 and placebo analgesia in the same design (Colloca et al 2010; Aslaksen et al 2015; Reicherts  
10 et al 2016). Nocebo hyperalgesia can be reversed by anxiolytic drugs (Benedetti et al 2007).  
11 Cortical areas that are known to be involved in the processing of negative emotions, such as  
12 the amygdala (Schmid et al 2015) and the hippocampus (Kong et al 2008; Bingel et al 2011)  
13 were shown to be affected by nocebo manipulations. However, only one prior study has  
14 directly tested the assumption that nocebo hyperalgesic suggestions increase negative  
15 emotions that, in turn, predict increases in pain (Aslaksen et al 2015). Geuter and Büchel  
16 (2013) revealed that nocebo treatment affected several measures of pain perception and  
17 simultaneously increased pain-related activity in the dorsal horn of the spinal cord, suggesting  
18 that pain facilitation caused by nocebo manipulations might occur in the spinal cord before  
19 cortical processing of pain. Because nocebo hyperalgesia may affect pain modulating  
20 processes in the spine, it can also be assumed that nocebo suggestions modify automatic  
21 cerebral processes, such as the startle reflex amplitude. The magnitude of the startle reflex is  
22 not prone to reporting biases, such as demand characteristics that might interfere with the  
23 effects of placebos and nocebos (Atlas & Wager 2012). The startle reflex, a defense system  
24 response to strong stimuli with abrupt onset (Lee et al 1996), is modulated by the emotional  
25 state of the organism (Lang, Bradley & Cuthbert 1990). The startle response is larger when  
26 the organism is in a defensive state or experiencing negative emotions (Asli & Flaten 2012).  
27 This modulation seems to be produced by priming of the startle circuitry via the amygdala  
28 (Davis, 1992). Hence, expecting a negative event, such as increased pain induced by nocebo  
29 suggestions should increase the startle response. A previous study (Benedetti et al 2006)  
30 demonstrated that nocebo suggestions increase hypothalamic-pituitary-adrenal (HPA)  
31 activity, showing that endocrine stress responses are core features of nocebo responses. Thus,  
32 information that pain will be increased may activate physiological defense systems that in turn  
33 can be measured as increased startle responses. Conversely, placebo analgesic manipulations  
34 have under specific conditions been shown to reduce the magnitude of the startle response  
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1 (Lyby et al 2012). If nocebo hyperalgesia occurs via emotional processes opposite to those  
2 involved in placebo analgesia (Aslaksen et al 2015), it can be expected that the startle  
3 response increases after nocebo treatment. Pain induction combined with emotional threat  
4 potentiates startle (Bublitzky et al 2013; Horn-Hofmann & Lautenbacher 2015), and it is  
5 therefore likely that an expectation of increased pain will increase the startle response. The  
6 aim of the current study was to test whether verbal suggestions of increased pain together with  
7 nocebo treatment with topical cream activates the automatic physiological defense system that  
8 contributes to increased subsequent pain reports on experimental heat pain. Specifically, we  
9 tested whether nocebo suggestions increased the magnitude of the startle response by  
10 comparing the effects of nocebo manipulations with a placebo manipulation and no-treatment.  
11 We hypothesized that pain and startle magnitude would be highest in the groups receiving  
12 suggestions of pain increases, and lowest in the group receiving analgesic suggestions.  
13 Finally, we expected that increases in startle observed after nocebo suggestion should be  
14 significantly predictive of the nocebo hyperalgesic response.  
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## 29 **Experimental procedures**

### 30 **Participants**

31 Sixty-four healthy volunteers (female: n = 35) between the ages of 19 to 37 (mean =  
32 21.64, standard deviation = 3.3) were recruited by advertisement at the University of Tromsø,  
33 Norway. Due to abnormal startle responses, three subjects (2 females) were excluded after the  
34 experimental procedure, leaving sixty-one participants for the statistical analyses. Participants  
35 who presently suffered from or who had previously experienced any severe disease (including  
36 chronic pain), pregnancy, those with cutaneous injuries on the arms and hands, and those who  
37 took a prescribed medication (with the exception of oral contraceptives) were not allowed to  
38 participate. All volunteers received a gift certificate worth 250 Norwegian Kroner (NOK) as  
39 compensation. The study was approved by the Regional Committee for Medical Research  
40 Ethics, Region North, Project nr 402/2012.  
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### 54 **Design**

55 A four group mixed design was employed. The groups were as follows: Placebo  
56 (n=16, 10 females), Natural history (n=16, 7 females), Nocebo information (NI) (n=15, 9  
57 females), Nocebo information + temperature manipulation (Nocebo TMAN) (n=14, 7  
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1 females) × five trial (pre-test 1 + pre-test 2 + manipulation trial + post-test 1 + post-test 2).  
2 The number of trials needed for the pain measures was based on the results of a previous  
3 study (Aslaksen et al 2015) where three pain trials were sufficient for detecting valid and  
4 statistically significant placebo responses. In the present study, we chose to increase the  
5 number of trials to five because of smaller group sizes compared to those in the Aslaksen et al  
6 (2015) study. The participants were randomized into the different groups according to their  
7 participant number. All experimenters (2 males, 2 females) were clinical psychology students  
8 with experience in performing experimental laboratory testing. The gender of the  
9 experimenters was balanced to reduce the influence of experimenter gender effects (Aslaksen  
10 et al 2007). The experimenters worked in pairs consisting of one male and one female to  
11 minimize experimenter gender-related effects. Thus, one male and one female experimenter  
12 tested each participant. The experiment was executed according to a double-blind procedure  
13 in the three conditions where the application of placebo cream was required. The  
14 experimenters were unaware of whether a true anesthetic cream or a placebo cream was  
15 applied. The software controlling the pain stimulation was pre-programmed, and the  
16 experimenters were unaware of the actual temperature of the pain stimulation.  
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### 30 **Pain stimuli**

31 Pain was induced by contact heat stimuli (30 × 30 mm aluminum contact thermode,  
32 (Pathway, Medoc, Israel) applied to the right volar forearm. The thermode had a baseline  
33 temperature of 32°C when applied to the arm. The duration of the pain stimuli was 20 s with a  
34 plateau for approximately 15 s at 47°C in both the pre-test and the post-test. During the  
35 manipulation trial, the same duration and plateau for the maximum temperature was used but  
36 with various maximum temperatures according to the group assignments to maximize the  
37 manipulations (Placebo: 46°C, Natural history: 47°C; Nocebo Information: 47°C; Nocebo  
38 Information + temperature manipulation: 48°C).  
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### 49 **Subjective measurements**

50 During each pain stimulus, the participants reported their pain intensity on a  
51 Computerized Visual Analogue Scale (COVAS, Medoc, Israel) ranging from 0–100, where 0  
52 represented “no pain” and 100 represented the “most intense pain imaginable.” Subjective  
53 stress was measured using two adjective pairs, similar to those used in previous studies  
54 (Aslaksen et al 2011, Aslaksen & Lyby 2015, Aslaksen et al 2015, Lyby et al 2011), from the  
55 Norwegian translation of the Short Adjective Check List (SACL) (Mackay et al 1978). The  
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1 adjective pairs were tense-relaxed and nervous-calm. The adjective pairs were converted to  
2 numerical rating scales, where a score of zero indicated complete relaxation/calmness and a  
3 score of ten indicated maximum tension/nervousness. The stress score was expressed as the  
4 mean score for the two adjective pairs. Stress measures were obtained before the pre-test,  
5 immediately after the administration of the placebo cream, and immediately after the post-  
6 test.  
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### 10 11 12 **Placebo cream**

13 The university hospital pharmacy at the University Hospital of Northern Norway  
14 produced 100 ml tubes of placebo cream (E45 Cream; Crookes HealthCare, UK). All tubes  
15 were numbered according to a list of codes and had an identical design. The code list was  
16 created by the university hospital pharmacy and was kept by the supervisor of the study  
17 (PMA), who did not participate in the experimental procedures. The experimenters were told  
18 that half of the participants that received the topical cream received a commonly used local  
19 anesthetic cream; however, all tubes contained the E45 cream. A dose of 3 g of placebo (E45)  
20 was used for each participant, similar to a previous study (Aslaksen et al 2015).  
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29 To test whether previous experience with analgesic creams impacted pain responses,  
30 we asked the participants whether they had used non-prescribed/over-the-counter analgesic  
31 creams during the last ten years. Furthermore, they were asked to rate the efficacy of the  
32 analgesic cream on an 11-point numeric rating scale, where 0 indicated “no analgesic effect at  
33 all”, and 10 indicated “perfect analgesia”. Lastly, we asked all participants about their  
34 expectations of the effect of the analgesic cream in the present experiment. The question was  
35 as follows: “In case you receive an analgesic cream in the present experiment, how effective  
36 do you expect this cream to be to reduce pain? Please indicate a number between 0 and 10  
37 where 0 indicates no analgesic effect, and 10 indicates perfect analgesia”. Both questions  
38 regarding previous experience and expectancy in the present study were presented in written  
39 form. After the questions were answered, the written form was placed in an envelope to keep  
40 this information away from the experimenters.  
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### 52 **Startle measures**

53 The startle-eliciting noise had an intensity of 95 dB (SPL), instantaneous rise time and  
54 a duration of 50 milliseconds (ms). The stimuli were delivered through Audio-Technica ATH-  
55 M50 headphones. A Bruel and Kjaer 2235 Sound Level Precision Meter was used to measure  
56 the intensity of the startle stimuli. The program that controlled the startle procedure was  
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1 written by the second author in the Coulbourn Human Startle System HSW v. 7.500 – 00 and  
2 run on a Microsoft Windows XP based Dell PC that controlled the presentation of the  
3 experimental stimuli and data acquisition.  
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5 Startle eye blink electromyographic (EMG) responses were recorded from the right  
6 orbicularis oculi with two sintered-pellet silver chloride AgCl miniature electrodes (4 mm  
7 diameter) filled with Microlyte electrolyte gel (Coulbourn Instruments). The inter-electrode  
8 distance was 1.5 – 2 cm. The ground electrode was placed centrally on the forehead. The  
9 EMG signal was amplified by a factor of 50,000 and filtered (13-1000 Hz bandpass) by a  
10 Coulbourn V75-04 bioamplifier. The signal was rectified and integrated with a Coulbourn  
11 V76-24 contour-following integrator with a 10 ms time constant, and the output was sent to  
12 the PC via a LabLinc V interface. Sampling on each trial began 100 ms prior to the onset of  
13 the startle stimulus and continued for 200 ms after the onset of the stimulus.  
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### 23 **Startle response scoring and data reduction**

24 Startle blink reflexes were scored as the difference between the maximum amplitude of the  
25 EMG response within a 0–200 ms window after noise onset, and the mean EMG level during  
26 the last 100 ms prior to the onset of the startle-eliciting noise on that trial. To qualify as a  
27 response, the maximum amplitude had to be a minimum of 30 A/D units above the baseline.  
28 Three of the subjects had abnormal startle responses and/or missed responses. These subjects  
29 were therefore excluded from the analyses. No other startle trials from the remaining 61  
30 participants were excluded due to artifacts. Thus, 18 startle trials were used from each  
31 participant. The mean startle responses for each test (6 trials) were transformed into Z-scores  
32 (mean = 0, SD = 1) before the statistical analyses.  
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### 43 **Procedure**

44 The experiment occurred inside a steel cubicle (2.8 × 2.8 m) where the thermode and  
45 startle apparatus were positioned. The steel cubicle was placed inside a larger room  
46 containing the apparatus for controlling the experimental events and response recordings. The  
47 cubicle was shielded from sound and electricity and was maintained at a constant temperature  
48 of 20°C. All instructions during the experiment were provided verbally to the participants.  
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54 Upon arrival at the laboratory, the participants signed an informed consent form. The  
55 participants received written information together with the consent form stating that the aim  
56 of the study was to test the physiological and psychological effects of different medical  
57 creams on heat pain. The participants were informed that they would either receive an  
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1 analgesic cream, a cream that increased pain or no treatment during the pain stimulation  
2 (Natural history group). The participants did not know what treatment they received or  
3 whether they participated in the control group until after the pre-test.  
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5 After the experimenter obtained the signed consent, each participant was seated in a  
6 comfortable chair inside the cubicle. Then, the experimenters instructed the participant on  
7 how to use the COVAS and attached the thermode to the right volar forearm, at the  
8 dermatome corresponding to C8. The electrodes and headphones for startle measurements  
9 were attached. Subsequently, subjective stress was measured. Each participant then received a  
10 5 second pain stimulus at 46°C prior to the pre-test to reduce novelty of the heat pain  
11 experience. Then, the habituation/baseline trials for the startle measures were performed.  
12 Prior to the startle measurements, the participants were instructed to listen carefully to the  
13 sound. Each startle test consisted of 6 trials. The interstimulus interval ranged from 17 to 23 s  
14 (mean 20 s).  
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17 After a two minute break, the experimenter started the first pain stimulations (pre-  
18 tests). Following the pre-tests, the experimenter delivered information regarding the cream,  
19 followed by application of the cream to a 5×5 cm location on the right volar forearm. The  
20 instructions for each cream were as follows. The Placebo group was told, “The cream that will  
21 be applied to your arm reduces pain. The substance in the cream is used as a local anesthetic  
22 in many pain-reducing remedies and is effective against heat pain.” The Nocebo information  
23 and the Nocebo information + temperature manipulation groups were told, “The cream that  
24 will be applied to your arm increases the effect of the heat pain and you will feel more pain.  
25 The substance in this cream is used in many medical remedies. Even though the pain feels  
26 more intense, the cream will not inflict any burn wounds.” In the natural history condition, no  
27 cream was applied, and no information regarding medication was provided. During the break,  
28 the participants in the natural history group were told to relax for a few minutes and wait for  
29 the procedure to continue.  
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32 Following a 20 minute application period, subjective stress was measured. Then, a  
33 startle measure was obtained to measure the effect of the information provided with the  
34 cream. Subsequently, the thermode was again attached to the forearm 1 cm below the site of  
35 the thermode stimulation in the pre-test to avoid possible lesion related hyperalgesia, and the  
36 experimenter initiated the temperature manipulation trial. The last two pain stimulations  
37 (post-tests) were performed two minutes after the temperature manipulation. The interval  
38 between the post-tests was 2 minutes. After the last post-test, the final subjective stress and  
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startle measurements were obtained. The experimental procedure had a total duration of approximately one hour. See Fig. 1 for an overview of the procedure.

### **Statistical analyses**

The statistical analyses were performed using SPSS version 23 (SPSS, IBM, USA). The pain, stress and startle data were examined for normality using the Shapiro-Wilk test. The normality test revealed that none of the variables deviated significantly from a normal distribution (all p values were > 0.1).

Group effects and interactions were analyzed using linear mixed models (LMM). LMM was chosen over a repeated measures ANOVA because the data showed systematic changes in the variance for the repeated measures that violated the assumption of sphericity, which is an assumption for the ANOVA analysis. One of the consequences of violating the assumption of sphericity can be inflation of the type I error rate in the ANOVA, especially when using small samples (Clark et al 2012; Smith 2012).

For the startle and stress data, the fixed factors were the Group, Trial and Sex of the participants, with no covariates. The fixed factors included for analysis of the pain data were the Group, Trial and Sex of the participants. To test the hypothesis that increased startle after nocebo suggestions increase pain, the change in startle responses from the pre-test to after the nocebo suggestions were calculated. The same procedure was performed on the stress data to include the change in the stress reports simultaneously with the change in startle in the LMM analysis. Sex was included as a factor in the analyses due to findings in several experimental studies suggesting that males report lower experimental pain compared to females (see Mogil [2012] for an overview). Furthermore, some studies have suggested that sex differences could be found in placebo analgesic responses (Aslaksen et al 2011). Thus, by including Sex as a factor, we controlled whether the sex of the participants influenced the placebo and nocebo responses.

The participants were assumed to induce individual variance regardless of the group alignment. The individual intercepts of the participants were treated as random effects in the repeated LMM analyses. The covariance structure that produced the best fit to the pain and stress data shown by the Akaike's Information Criteria (AIC) was an autoregressive matrix (AR1). For the startle data, an autoregressive moving average structure (ARMA) produced the best fit to data. The results were considered significant if  $p < .05$ . In the LMM, separate post hoc contrast analyses of main factors with more than two levels, and levels within significant

1 interactions were adjusted using Bonferroni pairwise corrections to adjust the p values for  
2 multiple comparisons; an adjusted p-value < .05 was considered significant. The uses of  
3 Bonferroni adjustments are explicitly mentioned in the results where such adjustments were  
4 applied.  
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## 8 **Results**

### 9 **Previous experience with analgesic creams and expectations of analgesia**

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12 In the Placebo group, six of the 16 participants had used a non-prescribed analgesic cream  
13 during the last ten years; the mean efficacy was 5.2 (minimum = 0, maximum = 10, SD =  
14 3.98). In the other groups (Nocebo information n = 15, Nocebo TMAN n = 14, and the  
15 Natural history group n = 16) 18 had used a non-prescribed analgesic cream during the last  
16 ten years; the mean efficacy was 3.7 (minimum = 0, maximum = 7, SD = 1.91). The mean  
17 expectancy of the pain analgesic effect of the cream was as follows: Placebo group: 5.7  
18 (minimum = 0, maximum = 8, SD = 1.62), Natural history group: 5.5 (minimum = 1,  
19 maximum = 7, SD = .8), Nocebo TMAN group: 4.9 (minimum = 0, maximum = 7, SD = 2.25)  
20 and Nocebo information group: 5.15 (minimum = 3, maximum = 8, SD = 1.57). There were  
21 no significant effects between groups in the experienced efficacies of previously used  
22 analgesic creams or the expected efficacies in the present experiment; both F values were <  
23 1.8, and both p values were < .17.  
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### 40 **Startle**

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42 The main effect of Group was non-significant ( $F(1, 51.53) = 1.74, p = .17$ ). The main effect  
43 of Trial was significant ( $F(2, 95.57) = 34.45, p < .001$ ). The Bonferroni-corrected  
44 comparisons revealed higher startle amplitudes after the drug information was provided  
45 compared to the pre-test ( $p < .001$ ) and the post-test ( $p < .001$ ). The Trial x Group interaction  
46 ( $F(6, 95.57) = 2.38, p = .035$ ) revealed no group differences during the pre-test ( $p$  values > .2)  
47 and that startle was higher in the Nocebo TMAN group after receiving the drug-information  
48 compared to the Natural history ( $p = .03$ ) and the Placebo groups ( $p = .04$ ) after Bonferroni  
49 correction (see Fig. 2). During the post-test, the startle response was higher in the Nocebo  
50 TMAN group compared to the Placebo ( $p = .02$ , Bonferroni adjusted) and the Natural history  
51 groups ( $p = .01$ ). The Nocebo information group had higher startle during the post-test  
52 compared to the Natural history group ( $p = .02$ ) after Bonferroni adjustment. The individual  
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variability of the participants was significant for the startle responses, indicated by the significant covariance parameter ( $B = .04$ ,  $Z = 4.82$ ,  $p < .001$ ). See Fig. 2 (panel A) for an overview of the z-transformed startle data.

## Stress

The only significant effect in the stress data was the main effect of Trial ( $F(3, 108.40) = 11.13$ ,  $p < .001$ ). There was a tendency towards significance for the Trial x Group interaction ( $F(9, 108.40) = 1.60$ ,  $p = 0.08$ ), with higher stress reports in the nocebo groups after receiving the drug information compared to the Natural history and Placebo groups. The covariance parameter of the repeated measures suggested that the participants varied significantly across tests ( $B = 2.27$ ,  $Z = 3.27$ ,  $p < .001$ ). The stress data are shown in Figure 2 (panel B).

## Pain

The LMM revealed a main effect of Trial, with higher pain reports during the pre-tests compared to the post-tests ( $p < .001$ ), whereas there was no difference between post-test 1 and 2 ( $p = .86$ ). The manipulation trial significantly differed ( $p < .001$ ) from all other trials except the first pre-test ( $p = .51$ ) after Bonferroni correction. Males reported less intense pain than females ( $F(1, 54) = 6.78$ ,  $p = .01$ ). The Trial x Group interaction was significant ( $F(12, 138) = 5.98$ ,  $p < .001$ ). The Bonferroni adjusted post hoc testing showed that there were no differences between the groups during the pre-tests (all  $p$  values  $> .85$ ). During the manipulation trial, pain was higher in the Nocebo TMAN group compared to the Natural history ( $p < .001$ ) and Placebo groups ( $p = .001$ ) after Bonferroni correction. During the manipulation, the Nocebo Information group reported more intense pain compared to the Placebo group ( $p < .001$ ), after Bonferroni correction for multiple comparisons. In post-test 1 (all  $p$  values for the post-test contrasts were Bonferroni corrected), pain was significantly higher in the Nocebo TMAN group compared to the Placebo ( $p = .002$ ) and the Natural history groups ( $p = .04$ ). Participants in the Nocebo information group reported more intense pain during post-test 1 than the Placebo group ( $p = .01$ ). In post-test 2, pain was higher in the Nocebo TMAN group compared to the Placebo ( $p < .001$ ) and Natural history groups ( $p = .002$ ). The Nocebo information group reported more intense pain during post-test 2 compared

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2 to the Placebo ( $p = .04$ ) and Natural history groups ( $p = .05$ ). Figure 2 (panel C) provides an  
3 overview of the pain data.

4 The change in startle from the pre-test until after the drug information was provided was a  
5 significant covariate ( $F(1, 54) = 8.17, p = .006$ ) with a positive slope ( $B = 5.57, t(54) = 2.86,$   
6  $p = .006$ ), suggesting that increased startle was associated with increased pain. Increased  
7 change in reported stress had a significant association with increased pain ( $F(1, 54) = 6.22, p$   
8  $= .02$ ) with a positive slope ( $B = 4.37, t(54) = 2.49, p = .02$ ). Figure 3 shows the individual  
9 pain scores and Figure 4 shows the individual predicted pain scores based on the LMM  
10 model.  
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17 To further test the hypothesis that increased startle after placebo information contributed  
18 significantly to placebo hyperalgesia, we performed a univariate LMM analysis with the mean  
19 of the post-test pain scores as the dependent variable, whereas the change in startle from the  
20 pre-test until after the drug-information was provided was used as a predictor along with  
21 group alignment. Both Group ( $F(3, 114) = 6.57, p = .001$ ) and the covariate Change in startle  
22 ( $F(1, 114) = 4.33, p = .043, B = 5.9, t(56) = 2.46, p = .043$ ) were significant predictors of the  
23 level of pain level during the post-tests. After Bonferroni adjustments, the results showed that  
24 pain was higher in the Nocebo TMAN group compared to the Placebo ( $p = .002$ ) and Natural  
25 history groups ( $p = .001$ ), whereas there was no difference between the two nocebo groups ( $p$   
26  $= .19$ ). The Group x Change interaction of the startle response was significant ( $F(3, 114) =$   
27  $6.07, p = .001$ ), where the Nocebo TMAN group x Change interaction of the startle response  
28 had a significantly steeper slope ( $B = 10.71, t(114) = 2.0, p = .04$ ) compared to the Placebo  
29 and Natural history groups ( $B = 8.61, t(114) = 1.9, p = .047$ ), while the slopes of the Nocebo  
30 TMAN and the Nocebo Information groups did not differ ( $B = 1.03, t(114) = .2, p = .84$ ).  
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## 49 **Discussion**

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51 The results of the present study suggest that the placebo hyperalgesic response is  
52 facilitated by an increase in negative emotional activation as measured by the acoustic startle  
53 response. Previous studies have shown that placebo hyperalgesic manipulations heighten HPA  
54 activity (Benedetti et al 2006, Johansen et al 2003), cortical activity in pain-related regions  
55 (Kong et al 2008; Bingel et al 2011; Schmid et al 2015), pain-related activity in the spinal  
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1 cord (Geuter & Buchel 2013), blood pressure and reported negative emotions (Aslaksen et al  
2 2015) combined with increased pain reports. The findings from the present study further add  
3 that successful induction of nocebo expectations increase negative emotions significantly  
4 enough to engage physiological and motivational defense systems (Grillon et al 1991), that  
5 enhance cortical alertness measured by startle responses. Obviously, the expectation of  
6 increased pain is an important warning signal for potential damage to our health (Horn-  
7 Hofmann & Lautenbacher 2015). The results of this study demonstrated that the physiological  
8 response assessed by startle response measurement following nocebo manipulations were  
9 similar to other conditions associated with negative health consequences, such as anxiety  
10 disorders and post-traumatic stress disorder (Davis 2006). Furthermore, the present results  
11 support the existing literature suggesting that emotional factors modify and modulate  
12 physiological outcomes in experimental pain (Flaten et al 2011).  
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22 However, even if negative emotions and expectations of worsening generally increase pain,  
23 differences in the manipulations and treatments in experimental design may result in variable  
24 outcomes (Carlino & Benedetti 2016). For instance, in the present study, the group receiving  
25 the temperature manipulation in the direction of hyperalgesia (TMAN group) exhibited  
26 significantly higher pain scores compared to the placebo and natural history groups, whereas  
27 the nocebo group that did not receive any temperature manipulation exhibited a lower nocebo  
28 effect. These findings suggest that nocebo responses are more efficiently induced if the  
29 expectation of pain increase is paired with an actual experience of increased pain, similar to  
30 findings reported by Reicherts et al (2016). Similar results were reported in studies of placebo  
31 analgesia (Schenk et al 2014), where placebo analgesic responses were more efficiently  
32 induced when combined with a conditioning procedure compared to suggestions of analgesia  
33 alone (Colloca et al 2008). There was a tendency for the nocebo groups to report increased  
34 stress after the manipulation; however, this effect was not significant. However, the startle  
35 data revealed that startle responses were significantly higher in the TMAN group compared to  
36 both the placebo and the natural history group, suggesting that suggestions of hyperalgesia  
37 combined with temperature manipulations in the direction of increased pain produced higher  
38 levels of negative emotion. This result is similar to findings reported by Horn-Hofman &  
39 Lautenbacher (2015), in which startle was potentiated by the threat of increased pain.  
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56 The counterpart of nocebo hyperalgesia, placebo analgesia, has in previous studies been  
57 associated with reduced startle (Lyby et al 2012). However, the results of the present study  
58 found that the placebo did not reduce startle responses, even if both pain and stress tended to  
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be reduced in the placebo group. A possible explanation for the lack of both a significant placebo effect and a reduction in startle responses after placebo administration could be the placebo manipulation itself. A decrease in stimulus temperature by only 1°C after the placebo administration might have been too small of a reduction in pain to induce the experience of an effective painkiller. Furthermore, the temperature manipulations were performed in only one trial. In combination with a small reduction in stimulus temperature, this might have reduced the efficacy of the placebo manipulation. However, the nocebo manipulation combined with an increase in temperature of 1°C significantly affected both pain and startle responses. Previous studies have shown that a single learning trial is sufficient to induce both nocebo and placebo responses, but as learning trials increase, the more persistent the effects of the manipulation become (Colloca et al 2010). Thus, the present results are consistent with previous studies suggesting that verbal suggestions efficiently induce nocebo responses (Aslaksen et al 2015; Aslaksen & Lyby 2015), while placebo responses might require a more robust pain relief experience (Schenk et al 2014). Another, albeit speculative reason for the decreased placebo effects in the present study could be the double-blind procedure. The experimenters did not know whether the cream was inert or an actual painkiller, and their non-verbal behavior in the lab (Czerniak et al 2016) could have been affected by this uncertainty even if they were instructed to perform the experiment according to the written procedure.

Conditioning to obtain a drug or treatment effect is usually not performed in clinical settings. Placebo and nocebo responses during treatment rely on expectations induced by verbal information. However, previous experience with a treatment or a drug might create learning effects that shape clinical outcomes (Benedetti et al 2011). In the present study, we measured the previous experience of analgesic creams, but the experienced efficacies of previously used analgesic creams and the expected efficacies in the present experiment did not differ significantly between groups.

Males reported less intense pain compared to females, but there were no gender-related effects on the placebo or nocebo responses in the present study. This study was not, however, designed to measure gender differences and the distribution of male and female participants was unequal across groups. Thus, future studies with larger samples could explore possible gender-related differences on placebo and nocebo hyperalgesic responses.

## Limitations

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2 One limitation of the present study is the small sample size. Nocebo and placebo effects vary  
3 across individuals (Flaten et al 2011; Carlino & Benedetti 2016), which is also suggested by  
4 the present study (Fig. 3). Small sample sizes might reduce the statistical robustness of the  
5 results. Nonetheless, the nocebo hyperalgesic effects observed in the present study were  
6 associated with increased startle responses and were in line with previous nocebo findings  
7 (Petersen et al 2014). The design employed in the current study consisted of relatively few  
8 trials for the pain and startle measures. The low number of trials may have reduced the power  
9 in the statistical analyses and possibly the reliability of the results. The fact that we asked the  
10 participants to rate their expectancies of pain relief before the experimental pain induction  
11 might have induced a bias in the post-test pain reports, where participants possibly could have  
12 anchored their ratings of the stimulus according to their expectations (Wager 2005).  
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## Conclusions

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30 The present study showed that nocebo hyperalgesic suggestions increased pain and elevated  
31 the levels of physiological arousal measured by the acoustic startle response. The increased  
32 magnitude of the startle response facilitated nocebo hyperalgesia, lending further support to  
33 the notion that an increase in negative emotions is necessary for a nocebo hyperalgesic  
34 response to occur.  
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53 analysis or interpretation of the data, writing of the article or the submission process.  
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5 Figure captions:  
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7 Figure 1: Overview of the procedure. Green arrows: Subjective stress measure. Blue arrows:  
8 Startle measure; each startle test consisted of six startle trials. Dashed blue arrow: Startle  
9 habituation trials. P = Placebo. NH = Natural History. NI = Nocebo Information. NTMAN =  
10 Nocebo Temperature Manipulation. VAS = Visual Analog Scale. Pain level was measured  
11 during each pain stimulation.  
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19 Figure 2: Panel A: Startle amplitude shown as Z-scores. The error bars represent the standard  
20 error of the mean. Panel B: Stress reported on numerical rating scales (0-100). The error bars  
21 represent the standard error of the mean. Panel C: Pain intensity reported on 100 mm visual  
22 analog scale. The error bars represent the standard error of the mean. All panels: Tman =  
23 Temperature manipulation. Inf = Information. Dashed vertical lines indicate when the  
24 nocebo/placebo manipulation was performed.  
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33 Figure 3: Individual (ID 1-61) observed pain ratings in each group. Trials 1-2 = Pre-test. Trial  
34 3 = Temperature manipulation trial. Trial 4-5 = Post-tests.  
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41 Figure 4: Individual (ID 1-61) predicted pain ratings in each group based on the linear mixed  
42 model with Group, Trial, and Sex as factors.  
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45 The change in startle and stress (pre-test – drug information) were used as covariates. Trials  
46 1-2 = Pre-test. Trial 3 = Temperature manipulation trial. Trial 4-5 = Post-tests.  
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Figure 1  
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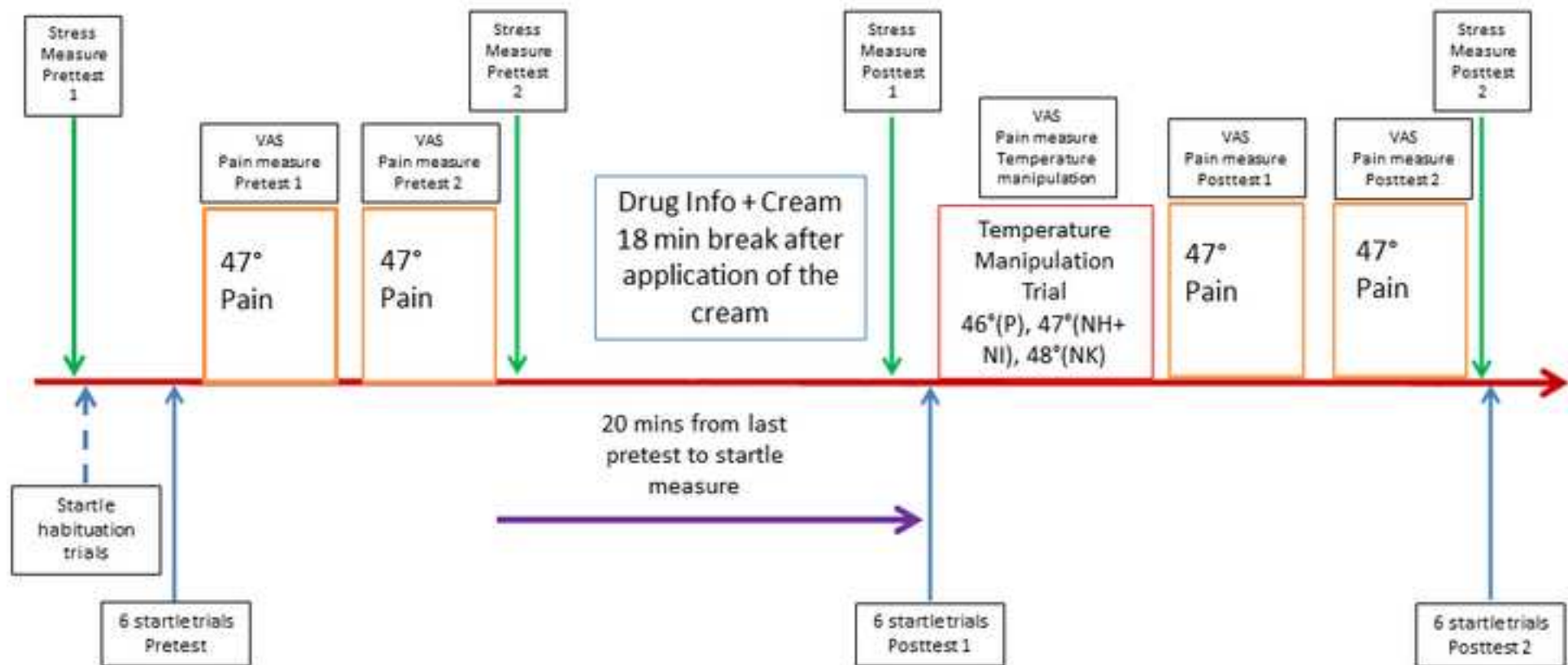


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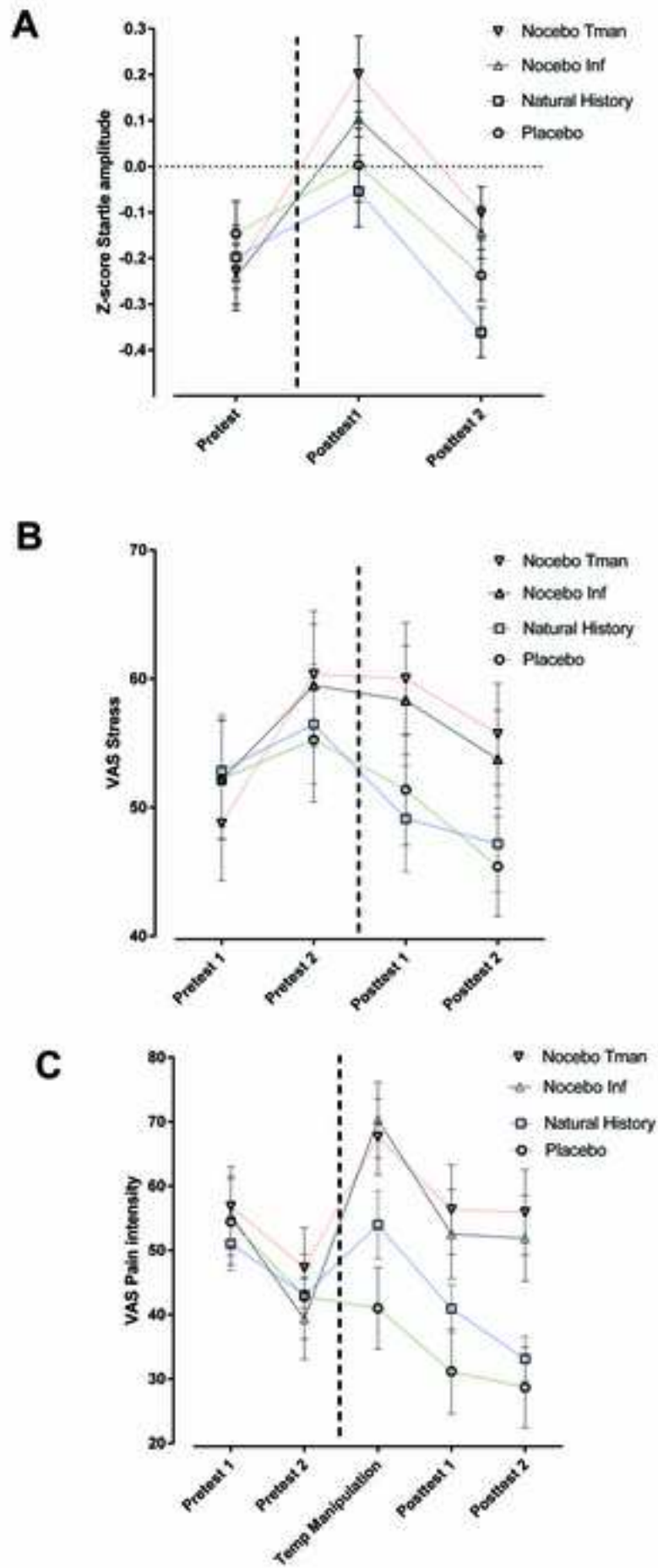


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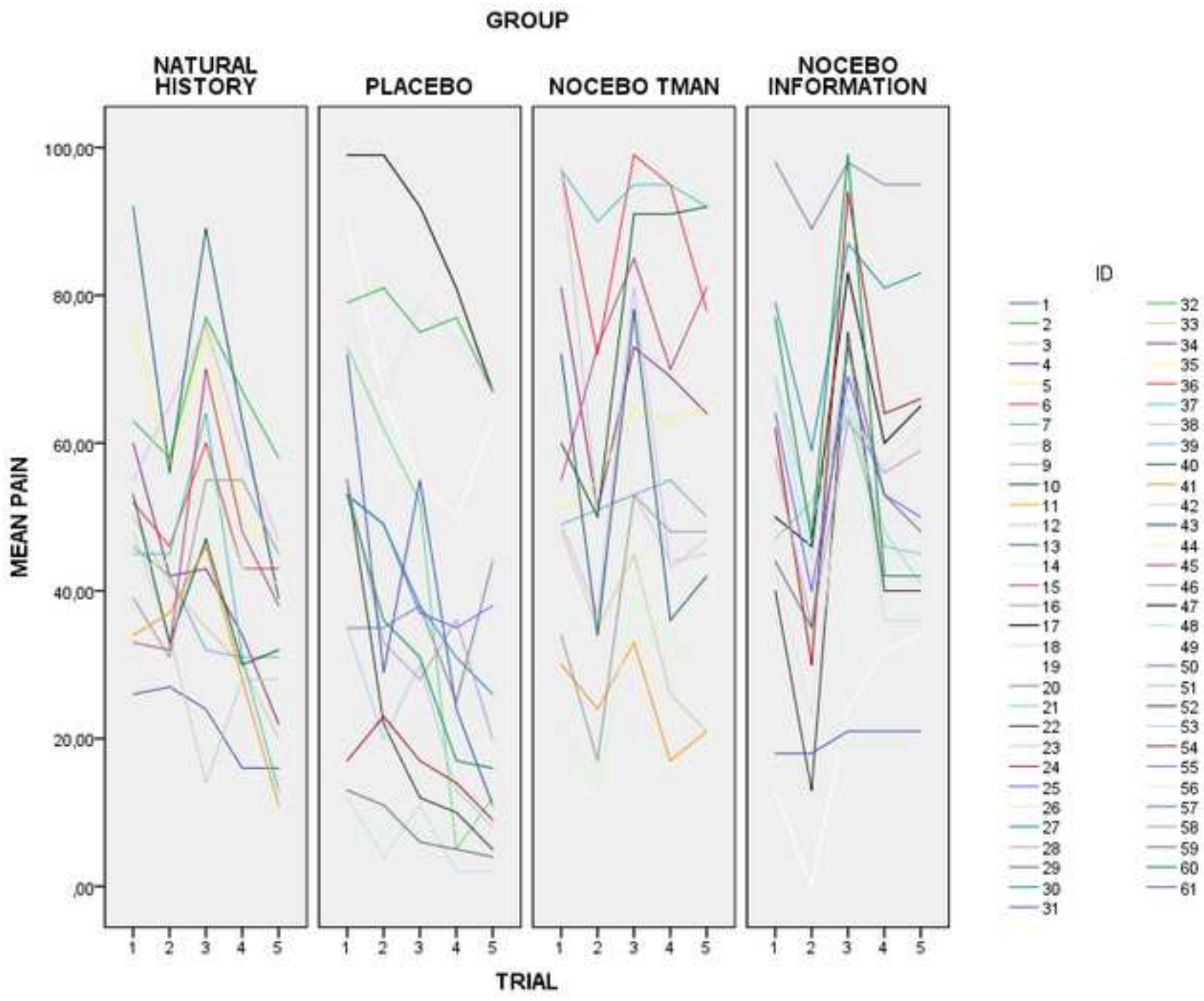


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