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The role of self-reported and physiological stress in nocebo hyperalgesia

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ABSTRACT

Negative expectations can increase pain sensitivity, leading to nocebo hyperalgesia. However, the physiological and psychological factors that predispose individuals to this phenomenon are still not well understood. The present study examined whether stress induced by a social stressor affects nocebo hyperalgesia, and whether this effect is mediated by self-reported and physiological stress responses. We recruited 52 healthy participants (15 men) who were randomly assigned to either the Trier Social Stress Test (TSST) or a control condition (a friendly version of the TSST). Nocebo hyperalgesia was induced using negative suggestions combined with a validated pain conditioning paradigm. We assessed self-reported (anxiety and stress) and physiological (cortisol, alpha-amylase, heart rate, and skin conductance) responses to stress. Both groups exhibited significant nocebo hyperalgesia. The stress group showed higher levels of anxiety, self-reported stress, and cortisol levels compared to the control group while no significant differences were found in other physiological markers. The stress and control groups did not differ in the magnitude of nocebo hyperalgesia, but anxiety levels partially mediated the effects of the stress test on nocebo hyperalgesia. Our findings suggest that an external social stressor does not directly affect nocebo hyperalgesia, but that increased anxiety due to the stressor enhances its magnitude. Thus, it may be worthwhile to investigate whether reducing stress-related anxiety in clinical settings would help alleviate nocebo effects.

1. Introduction

Nocebo effects are negative treatment outcomes that are caused, not by the active components of a treatment itself, but rather by negative expectations that an individual holds about the treatment (Häuser et al., 2012). Side effects of medicines can for example be explained by the occurrence of nocebo effects: when a person expects to experience side effects from a certain medicine, there is a higher chance that they will actually experience them (Faasse & Petrie, 2013). Nocebo effects have been shown in the context of various symptoms: nausea (Levine et al., 2006; Wolters et al., 2019), itch (Bartels et al., 2016; Wolters et al., 2019), and dyspnea (Vlemincx et al., 2021). However, the majority of the research on nocebo effects has been done in the context of pain. Several meta-analyses have demonstrated that pain sensitivity can be increased by nocebo effects (Madden et al., 2016; Petersen et al., 2014).

One of the ways in which nocebo effects may be established is via classical conditioning. During pain conditioning, pain acts first as an unconditioned stimulus (US) that becomes associated with stimuli around it (conditioned stimuli, CS). Once this association is established, the past experience will then cause an individual to react to the CS with an increase in pain sensitivity (Madden et al., 2016). Nocebo effects can also be induced by negative suggestions given, for example, about pain inducing properties of a sham treatment (Benedetti et al., 2007). The most robust way to induce nocebo effects in the laboratory is combining the conditioning procedure with verbal suggestions (Bartels et al., 2014; Skvortsova et al., 2020). Nocebo effects induced in this manner were shown to be robust and subject to slow extinction times (Colagiuri et al., 2015). In clinical practice, the same principles apply: nocebo effects can be induced, for example, by informing a patient about possible side effects of a treatment (Varelmann et al., 2010) or by conditioning by

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repeated exposure to painful procedures (Taddio et al., 2002).

The general assumption is that nocebo effects (much in the same way as their positive counterpart, the placebo effect), are complex interplays between the context and the individual (Benedetti, 2008; Klinger et al., 2017). Nonetheless, experimental paradigms show that, under similar contextual circumstances, some individuals experience nocebo effects whereas others do not. Gaining knowledge on the circumstances that could leave individuals sensitive to experiencing nocebo hyperalgesia is essential for being able to develop effective ways to prevent it. A potentially interesting factor could be whether the individual, prior to pain conditioning, is in a state of being stressed. Stress is a complex concept that is defined in many different ways. Some researchers, for instance, broadly define stress as the product of an interaction between arousal, perceived aversiveness, and uncontrollability of a situation (Fink, 2016). Any of these factors may amplify nocebo responding. Some evidence already exists that stress could play an important role in the development of nocebo effects. To illustrate, it has been shown that diazepam, an anxiolytic drug, prevented the formation of nocebo effects in pain (Benedetti et al., 2006). Another study (Roderigo et al., 2017) used an experimental stress test (Trier Social Stress Test, TSST) together with the induction of nocebo effects on visceral pain. They found that significant nocebo effect was induced in the stressed group and absent in the control group. These results indicate that the conditions (social stress, or being in a state of stress) might create an environment in which nocebo effects may occur in visceral pain. However, it should be noted, that another study did not find effects of progressive muscle relaxation, aimed at decreasing stress levels, on nocebo effect formation in visceral pain (Elsenbruch et al., 2019).

Because of the mixed findings from the previous studies, the link between stress and the nocebo effect remains unclear. Importantly, all the studies mentioned previously used only verbal suggestions to induce nocebo effects. No study has so far looked at the impact of stress on nocebo effects induced by classical conditioning, even though it has been established that conditioning plays a large role in the formation of nocebo effects (Bartels et al., 2016). Moreover, stress might affect nocebo effects induced by conditioning particularly, because it has been shown that social stress accelerates the learning of negative information (Cornelisse et al., 2011; Espin et al., 2013). In a conditioning paradigm, stress therefore, might accelerate acquisition and consolidation of the pain conditioned responses and potentially slow down their extinction. This notion is further supported by some animal studies, which show that conditioning of nocebo effects (i.e., learned allergic responses) results in larger responses when animals are stressed (Irie et al., 2002; Peeke et al., 1987).

Medical encounters are often stress-provoking. Investigating the influence of stress on the nocebo effect is crucial, as stress has been recognized as a significant contributor to the modulation of various physiological processes, potentially exacerbating negative health outcomes and impairing treatment efficacy (Cohen et al., 2007).

In the present study, we investigated the effect of social stress on nocebo hyperalgesia, induced by classical conditioning with verbal suggestions in healthy volunteers. Our primary hypothesis is that participants exposed to the TSST would demonstrate higher nocebo hyperalgesia and slower extinction of the conditioned nocebo responses, compared to participants exposed to a control non-stressful version of the TSST. In the secondary analysis, we investigated whether self-reported stress, anxiety, cortisol, and alpha-amylase levels mediated the effects of the TSST on nocebo hyperalgesia. We further explored potential mediation of the effects of the TSST on nocebo hyperalgesia by heart rate, skin conductance level and personality characteristics.

2. Methods

2.1. Study design

A mixed within-between subject study design was used. The experimental timeline is presented in the Fig. 1. The local research ethics committee (CEP, Institute of Psychology, Leiden University, NL; reference number: 2020–02-03-A.W.M. Evers-V1–2078) approved the protocol and it was preregistered on the Open Science Framework (OSF; https://osf.io/3z4f8). Participants were randomized to one of two groups: 1) a stress group, and 2) a control group, with a 1:1 ratio in blocks of 4–6 people. Instead of randomizing participants one by one, they were grouped into blocks of 4–6 people, and within each block, randomization was applied to determine the group assignment. This was done to ensure that each group had a similar number of participants over time, reducing the risk of imbalance in baseline characteristics. Separate randomization lists were generated for males and females to ensure equal sex distribution across groups. An independent data manager affiliated with the Health, Medical and Neuropsychology Unit created the randomization lists to reduce the impact of potential bias. The study commenced double-blinded until the TSST, where the group allocation was revealed to the experimenter and, to an extent, to the participants in the control arm (see 'stress manipulation: friendly-TSST'). Data collection ran from February 2020 until April 2022. The testing procedures were adjusted over time to comply with government-issued restrictions due to the global Covid-19 pandemic (see Supplementary materials for details).

2.2. Participants

Healthy participants between 18–35 years of age were recruited with online advertisements on social media, on Leiden university's official participant recruitment system SONA, and with physical flyers spread on the campus. The study was advertised as a study on the effects of glucose on social performance and pain sensitivity. Prior to being invited for a laboratory appointment, volunteers filled out an online survey that assessed eligibility for participation. The in- and exclusion criteria are described in detail in the Supplementary materials.

2.3. Measures

2.3.1. Pain ratings

After each heat stimulus, participants verbally rated how much pain they experienced on a 0 ('no pain') to 10 ('worst pain imaginable') numeric rating scale (NRS) and the ratings were noted by the experimenter to the experimental protocol. To test the effects of prior conditioning on pain as a primary study endpoint, the pain ratings after the first ON and first OFF stimulus in the testing phase of the conditioning task were compared (Skvortsova et al., 2020). Secondarily, the extinction rate of prior conditioning was assessed by evaluating changes in the comparison between the pain ratings for ON and OFF trials over time in the testing phase of the conditioning task.

2.3.2. Self-reported anxiety and stress

Self-reported anxiety was measured with the short state version of the State Trait Anxiety inventory (STAI-Ss, Marteau & Bekker, 1992). This questionnaire consists of 6 statements (e.g., 'I am tense') that are evaluated on a 4-point rating scale (1 'not at all', 4 'very much'). Total scores were calculated by reverse scoring positive statements (e.g., 'I am calm') and summing all items. Self-reported stress was measured by having participants rate a single question ('How stressed are you at this moment?') on a Visual Analog Scale (VAS) that used the anchors '0/not at all' and '10/extremely'.

2.3.3. Salivary cortisol and alpha amylase

Saliva samples were taken with salivettes (Sarstedt, Nümbrecht, Germany) at five time points: at baseline, right before the start of the (friendly) TSST, immediately and five minutes after the (friendly) TSST, and immediately after the conditioning task. The salivettes were stored in a − 20 ℃ fridge until analysis. Analyses were done by the Clinical Chemistry and Laboratory Medicine (KCL) department of the Leiden

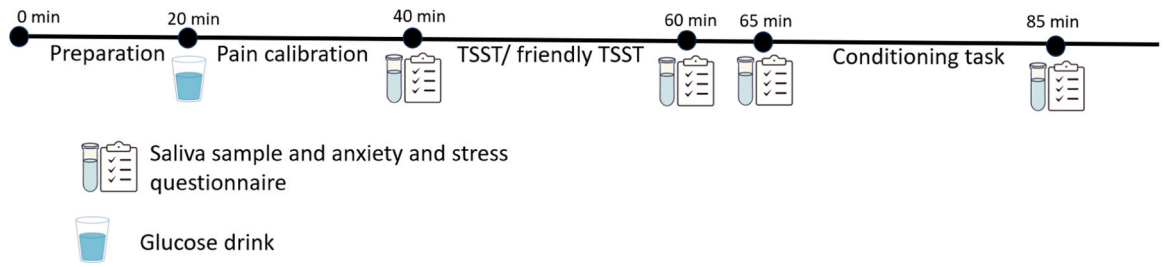

Fig. 1. The timeline of the experiment.

University Medical Center. After defrosting, the samples were centrifuged. All analyses were performed with a Cobas 8000 Modular Analyzer (Roche Diagnostics Nederland B.V., Woerden, the Netherlands). Salivary cortisol enzyme immunoassay with the Elecsys Cortisol II reagent kit was performed on the Cobas 8000 e602l module, according to the manufacturer's instructions. For alpha amylase, 1:100 diluted samples were assessed via immunoassay with the AMYL2 reagent on the Cobas 8000 c502 module.

2.3.4. Heart rate (HR) and skin conductance level (SCL)

Physiological responses to stress and pain were assessed with a noninvasive Biopac© apparatus that consisted of the MP150 Data Acquisition system and the software Acqknowledge 5.0.4 (BIOPAC Systems Inc., Goleta, CA, USA). Physiological data was recorded continuously throughout the session according to standardized procedures (see the Supplementary materials for details**)**. Mean heart rate (HR) in beats per minute (bpm) was extracted from the ECG data for the duration of the (friendly)TSST and the conditioning task separately. Mean skin conductance level (SCL) in microsiemens (μS) was extracted from the data for the duration of the (friendly)TSST and the conditioning task. Visual inspection of the HR and SCL data and extraction of their means was undertaken using the PhysioData Toolbox (Sjak-Shie, 2022).

2.4. Other questionnaires

Participants filled out the Fear of Pain Questionnaire-III (FPQ-III, McNeil & Rainwater, 1998), a questionnaire consisting of 30 items (e.g., 'I fear the pain associated with being in an automobile accident') rated on a 5-point rating scale (1 'not at all', 5 'extreme'), to assess general pain-related fear. We used the total score that is calculated by summing all items. The Pain Catastrophizing Scale (PCS,Sullivan et al., 1995) was used to evaluate the extent of catastrophic thinking about painful events. In this questionnaire, participants are asked to reflect on past painful experiences and indicate the extent to which they experienced 13 thoughts (e.g., 'There's nothing I can do to reduce the intensity of the pain') or feelings (e.g., 'It's awful and I feel it overwhelms me') during these events on a 5-point rating scale (0 'not at all', 4 'all the time'). A total score was calculated by summing all items. Finally, the Body Attention, Ignorance and Awareness Scale (BAIAS, Beugen et al., 2015) was used to assess attention to bodily symptoms. This questionnaire consists of 16 items that pertain to attention ('In general I pay attention to my physical sensations'), awareness ('I notice when my physical sensations are beginning to change') and ignorance ('I have physical sensations that I can't quite identify') of bodily symptoms and that are rated on a 4-point rating scale (1 'not at all', 4 'completely'). For each of these subscales a score is generated by counting the sum divided by the number of items.

2.5. Stress manipulation

2.5.1. Glucose drink

Previous research shows that people exhibit stronger cortisol responses to stressors, including the TSST, after glucose consumption relative to fasting (Kirschbaum et al., 1997). Therefore, participants ingested a drink which consisted of 30 g of pure dextrose powder dissolved in 200 ml of water at the start of the procedure to ensure a minimal available concentration of glucose in the circulating blood, as recommended in the standardized TSST protocol by Labuschagne et al. (2019).

2.5.2. Trier Social Stress Test

Participants in the stress condition underwent the TSST, a validated and common stress induction test that reliably increases cortisol secretion for a short period of time (Kirschbaum et al., 1993; Labuschagne et al., 2019). In particular, we employed the TSST protocol published by Labuschagne et al. (2019), with minor adjustments. Because we measured heart rate and skin conductance, participants stayed in the same room during the preparation of the interview instead of entering a new room. The TSST started with a 5-minutes preparatory phase, during which participants prepared a speech for a (sham) job interview. Participants were told that the speech would be audio- and video-recorded and analysed for nonverbal behaviour. They then had to talk about their personal qualifications and argue why they would be the best candidate for their dream job during a 6-minute interview in front of a judge panel, consisting of two research group members, who were acting as doctors in white lab coats. The judges were trained to not express emotional support, to ask critical questions and to provide standardized negative verbal feedback. The participants also underwent a 4-minute mental arithmetic task (e.g., counting down in steps of 17 from 1965) while under pressure from the judges to calculate as fast as possible. The judges told participants to start over whenever they made a mistake.

2.5.3. Control condition Friendly-TSST

In the control condition, participants performed tasks similar to the TSST but without the stressful elements. Participants were explicitly instructed that they were in the control group. Instead of a stressful job interview, participants talked about casual topics (e.g., hobbies, favourite books and movies, traveling) with two persons who were introduced as colleagues of the experimenter. Prior to the conversation, participants were asked to prepare a list of topics, and they were told that the conversation would be held to fill the time before the next measurements, and to ensure that physiological data could be compared between-groups with regard to movement and talking. This procedure has been used before as a control condition for the TSST (Wiemers et al., 2013). In addition, participants performed simple math tasks (e.g., counting up in steps of two, counting back in steps of one) as a control for the stressful arithmetic task. Task duration was matched across the TSST and Friendly-TSST.

2.6. Heat pain paradigm

Heat stimuli were applied with a standardized heat pain application device (TSA-II, Medoc Advanced Medical Systems, Ramat Yishai, Israel) on the dorsal side of the non-dominant arm. All stimuli were applied by ramping up an ATS 3×3cm thermode temperature from a baseline level of 32 ℃ to a peak level between 42 and 49.5 ℃, which was held for 4 s.

The ramp up rate was 10 ℃/s.

2.6.1. Warmth and pain thresholds

As a first step, participants indicated the moment when they felt the thermode was getting warmer (warmth detection) and when they felt the applied heat was getting painful (pain detection).

2.6.2. Calibration

The temperatures were then calibrated for each participant individually. In the first part of calibration, ascendingly hotter stimuli were applied in steps of 0.5 ℃ starting from 42 ℃ upwards until the maximum allowed temperature (49.5 ℃) was reached or until pain was rated as 8. Based on the pain ratings given in this first part of calibration, two heat levels were identified, eliciting medium pain (equal to 4 on a numeric rating scale (NRS) with anchors 0 'no pain' and 10 'worst pain imaginable') and high pain (equal to 8 on the 0–10 NRS). Because of high exclusion rates (for details see supplementary materials), of which most (45%) could be attributed to a too high pain tolerance, the procedure was changed on August 12, 2021. The new procedure was to determine temperatures that induced lower (1.5 – 3 on the NRS) versus higher (4 – 8 on the NRS) pain levels, with the criteria that the lower and higher temperatures had to differ by at least 2 ℃ and that the pain ratings needed to differ by at least 2 points on the NRS. In the second part of calibration, participants were presented with 10 heat stimuli of varying intensities, including 2 stimuli of the medium pain and 2 stimuli of the high pain levels that were chosen in the previous part of the calibration. The other 6 stimuli were of the temperatures between these two levels. The second part of calibration was performed to check whether participants could consistently differentiate between the two selected temperatures. All participants were able to differentiate between the two temperatures.

2.7. Nocebo effect induction

2.7.1. Verbal suggestions

Participants were told that the study aimed to test pain following a combination of heat and light electrical stimulation. Instructions were given that a device would be applied that was capable of increasing pain sensations by sending small electrical pulses that stimulate nerves in the skin. The experimenter explained that during the next task, messages on the computer screen would indicate the state of the device. The message ON would indicate that the electrodes are active and consequently increase pain, whereas OFF would indicate that the electrodes are deactivated and will not affect pain sensations. A sham Transcutaneous Electrical Nerve Stimulation (TENS) device was used (Bentrotens T37, manufacturer: Bentronic Germany) as has been done in previous studies (Colagiuri & Quinn, 2018; Yeung et al., 2014). The TENS is a widely used tool for massage purposes and in the current study, its electrodes were attached to the nondominant hand and forearm. To strengthen the suggestions about the device being active, the experimenter momentarily set it to massage mode so that participants experienced light vibrations but no pain. Stimulation was then gradually decreased until participants could not feel it anymore. After 30 s the TENS turned off automatically and remained off during the subsequent procedures without the participants' knowledge.

2.7.2. Conditioning task

The conditioning task consisted of a learning and a testing phase. A screen indicating whether TENS device was ON (more pain) or OFF (less pain) served as a conditioned stimuli (CS). The pain stimuli either of moderate or of high pain served as unconditioned stimuli (UCS). During the learning phase, 10 moderate pain stimuli (calibrated to 4 on the 0–10 NRS) were given preceded by the black OFF screen intermixed with 10 high pain stimuli (calibrated to 8 on the 0–10 NRS) which were preceded by a burgundy ON screen. During the testing phase, participants were given 20 moderate pain stimuli with a random ON (n = 10) or OFF (n = 10) screen. In both phases, a randomized fixed order was used: 4 pseudorandom sequences of stimuli were created prior to the experiment. Each phase in each sequence started with a moderate pain stimulus combined with an OFF screen. After each stimulus, participants were asked to rate their pain experience (see 'Measures: Pain ratings').

2.8. General procedure

Eligible volunteers were invited to the lab for a single laboratory session starting at 14:00 to control for circadian cortisol variations. Upon arrival, the experimenter explained the study procedures and gave the participants a copy of the information letter for reading. Participants gave informed consent, after which the study commenced. A baseline saliva sample was collected, and participants filled out questionnaires about their demographics as well as the PCS, FPQ-III and BAIAS. The heart rate and skin conductance measures were started. Next, participants were asked to drink the glucose drink and to rinse their mouth afterwards with still water. Heat stimuli were then calibrated, and the heat levels needed for the pain tests were identified. The second saliva sample was taken, and participants were asked to fill out the STAI-Ss and stress questionnaire. Next, a 5-minute standing baseline measurement of heart rate and skin conductance level was taken. During this measurement, the experimenter left the room to sit in an observer's room. The envelope containing group allocation was opened and participants underwent either the TSST or the friendly-TSST, depending on group allocation. Before beginning the TSST, the experimenter introduced the two judges who then set up the room according to the TSST protocol: a camera was placed in the room facing the participant, and a voice recorder left on the table. The experimenter instructed the participant on what to do during the social task and asked the participant to start preparing the speech. For the friendly-TSST, the experimenter introduced the two persons as colleagues, and no recording equipment was placed in the room. Immediately and five minutes after completion of the (friendly-)TSST, saliva samples were taken, and participants filled out the STAI-Ss and stress questions again. Next, participants were given verbal suggestions about the upcoming pain conditioning task and underwent the task. Finally, participants were asked to give a saliva sample and to fill out the STAI-Ss/stress questions once more. Heart rate and skin conductance measures were stopped, and participants were debriefed and compensated for their time. If the appointment ended prematurely, e.g., due to high pain tolerance, or necessary and significant protocol deviations during the TSST, participants were compensated for their time up until that point. All study procedures and documents were in English.

2.9. Statistical analysis

A power calculation was performed with software G*Power 3.19 (Faul et al., 2007), and was aimed to detect differences in conditioning of nocebo effects between the stress and control groups. Input for the power calculation was derived from a study on the effects of acute psychological stress on placebo and nocebo responses in a model of pain and urgency in visceroception (Roderigo et al., 2017), which demonstrated an effect of Cohen's d= 0.44 of stress on nocebo responding in urgency to defecate. Considering the differences in the outcome measure (i.e., pain instead of urgency to defecate) and the nocebo induction method (i.e., conditioning combined with verbal suggestions instead of verbal suggestions only) between studies, we as a result adjusted the effect size for power calculation to a more conservative d= 0.40. Thus, based on an effect size of d= 0.40, a sample size calculation for a mixed repeated measures analysis of variance (ANOVA) indicated that with an alpha level of.05 and a power of $\beta = .80$, 52 participants (26 in each group) would be needed to detect differences in nocebo conditioning of pain levels between the stress and control groups. Consequently, the study is not powered to detect small effects.

The data analyses were performed using SPSS Statistics version 23

(IBM Corporation, Armonk, NY) with a 2-tailed significance level of $\alpha < .05$. Before analysis, the data were screened for univariate outliers using Tukey's method. Three outliers were found in the nocebo effect variable and one in cortisol levels. They were brought back to the mean plus 2.5 standard deviations as preregistered. Skewness and kurtosis were used to assess the normality of distribution. Levene's tests indicated homogeneity of variances in the groups. Sphericity was measured with the Mauchly's test of sphericity. In case of violation of sphericity, Greenhouse-Geisser corrections were applied to correct the degrees of freedom and the correction coefficient, ϵ, was reported. Independent samples t-tests tests or nonparametric Mann-Whitney tests (in case a variable was not normally distributed) were used to compare the groups on the baseline and personality characteristics: age, temperature that was rated as medium pain intensity (rated as 4 on the NRS pain scale), temperature rated as high pain intensity (rated as 8 on the NRS pain scale), baseline cortisol and alpha-amylase levels, baseline state anxiety, baseline self-reported stress, fear of pain, pain catastrophizing, behavioural inhibition and behavioural activation.

For our primary analysis, to examine whether the nocebo effects were significant and differed between the groups, a 2×2 factorial ANOVA with group (stress; control) $\times$ cue (on; off) was used with pain scores in the testing phase of the conditioning task as an outcome. Secondarily, to assess whether mean pain scores in response to on and off cues differed in the learning phase of the conditioning task (i.e., during learning of the nocebo effects), a 2×2 factorial ANOVA with condition (stress; control) $\times$ cue (on; off) was used. Furthermore, to examine the extinction process, the nocebo score was calculated as a difference between on and off trials for each of the trials of the test phase. In total, 10 nocebo difference scores were calculated. Next, repeated measures ANOVA was done with the trial number as a within-subject factor, condition (stress; control) as a between-subject factor and nocebo score as a dependent variable to evaluate the effects of the condition and time on the nocebo scores. When interaction or main effects were found, Bonferroni corrected post hoc tests were applied.

Next, the other secondary outcome parameters relating to the stress task were analysed. To investigate whether the stress task affected self-reported anxiety, stress, and cortisol, 3 repeated measures ANOVAs were performed with measurement time (4 levels: baseline, 1 min post-TSST, 5 min post-TSST, post-conditioning) as a within-subject factor and group (stress; control) as a between-subject factor. Alpha-amylase values had a very large variance; therefore, we had to deviate from the preregistered analysis plan and calculate relative change from baseline scores by dividing each measurement moment by a baseline score as advised by the Clinical Chemistry and Laboratory Medicine department of the LUMC. To examine whether the TSST affected alpha-amylase levels, a repeated measures ANOVA was performed with measurement time (3 levels: 1 min post-TSST; 5 min post-TSST; post-conditioning) as a within-subject factor, group (stress; control) as a between-subject factor, and relative change of alpha-amylase from baseline as an outcome variable.

To explore whether the TSST affected physiological responses to stress and pain, two 2×3 repeated measures ANOVAs were conducted with condition (2 groups: stress; control) $\times$ measurement time (3 levels: standing baseline; during (friendly-)TSST; during conditioning task). Mean HR and mean SCL were defined as the dependent outcomes, respectively. Prior to analysis, the ECG and EDA signals were visually inspected and cleaned. Missing data was identified for HR ($n = 18$) and SCL ($n = 13$). The main reason for missing data (both HR and SCL, $n = 12$) was because of technical issues with the TTL port communication for marker placement. For HR, other reasons included systemic extrasystoles and problems with the electrodes or leads (for details: Supplementary materials). After visual inspection of the ECG and SCL signals, data of $n = 19$ was identified as missing for HR, and $n = 14$ for SCL due to technical issues, artefacts such as extrasystoles or otherwise noisy signals. The number of participants in each group remained approximately equal: for HR, group sizes were $n = 17$ each, whereas for SCL, data of $n = 20$ remained in the stress group, and $n = 18$ in the control group.

To investigate whether changes in stress parameters (self-reported stress, state anxiety, cortisol, alpha-amylase, HR and SCL) mediate the effects of the TSST on the nocebo effect, mediation analysis with multiple parallel mediators was conducted. Area under the curve with respect to baseline was calculated for all stress parameters except HR and SCL and used as mediator variables according to standard formulae (Mücke, et al., 2018). For HR and SCL, difference scores were calculated by subtracting the mean during the standing baseline measure from the mean during the TSST. Mean HR and SCL were used before as indices for psychophysiological reactivity to a stressor (Tekampe et al., 2021; Schakel et al., 2019). By subtracting the mean during baseline, we accounted for existing individual differences in physiological parameters at baseline so that we could use the difference scores in exploratory mediation analysis. The mediation analysis was done using the regression-based PROCESS matrix (version 4.2, model 4) for SPSS (Hayes, 2017). For the indirect effect of the TSST on the nocebo effect, bootstrapped 95% confidence intervals were generated with a rate of 5000 samples. Mediation paths were compared by testing the contrasts between indirect effects. To optimally account for missing data on alpha-amylase ($n = 10$), HR and SCL, a mediation model without these variables was tested as a first step (with a sample of $n = 47$). Next, the analysis was repeated with alpha amylase included as mediator ($n = 37$), and finally, a model was tested with all mediators included ($n = 23$). Assumptions for each linear regression included into the mediation analysis were checked. Linearity between independent and dependent variables of the regressions were checked with a visual inspection of a scatterplot. Independence of observations was checked with the Durbin-Watson statistic: the assumption was considered to be met when the statistic values were between 1.5 and 2.5. The normal distribution of residuals was checked by visually inspecting P-P plots. Homoscedasticity was checked by visually inspecting the scatterplot of standardized residuals versus standardized predicted values. Multicollinearity was checked by calculating the variance inflation factor. No violations of assumptions were found.

To explore whether personality factors (fear of pain, pain catastrophizing, behavioural inhibition and behavioural activation) moderate the effects of the stress test on the nocebo effect, a moderation analysis was run for each of these three personality variables separately. Moderation analysis was done using the regression-based PROCESS matrix (version 4.2, model 1) for SPSS (Hayes, 2017) using 5000 bootstrap samples and 95% confidence intervals. Conditional effects of TSST on the nocebo effect were always probed at the low (16th), medium (50th) and high (84th) percentiles of the respective moderating variable. Additionally, we performed a hierarchical regression analysis, to control for the effect of psychological characteristics. In the first step of regression, condition (stress versus control) was added as a predictor. In the second step, pain catastrophizing, fear of pain and behavioural inhibition/activation scores were added as predictors. In the third step, the interaction between the group and each of the psychological characteristics were added as predictors.

Finally, to explore the possible effects of sex on the main outcome variables, several factorial ANOVAs were performed with sex and condition as factors and following dependent variables: nocebo effect in the first trial of the testing phase, area under the curve of state anxiety, area under the curve of self-reported stress, area under the curve of cortisol, and area under the curve of alpha-amylase (separate ANOVAs per each dependent variable).

Partial eta squared was calculated for analyses as an indication of the effect sizes. The effect sizes are interpreted according to Cohen (1977): $\eta_p^2 = .01$ small, $\eta_p^2 = .06$ medium, and $\eta_p^2 = .14$ large effects. The data and the syntax of all analyses are openly available on the Open Science Framework (https://osf.io/b2eqf).

3. Results

3.1. Baseline characteristics

We collected and analysed full data on the primary outcome for fifty-two volunteers (mean age ± SD: 22.1 ± 3.1, 15 males), who were equally assigned to either stress or control groups. A flowchart of participants' in- and exclusion can be found in the supplementary materials. The baseline and personality characteristics are presented in Table 1. There were no differences between the groups on any of these variables, except for pain catastrophizing and HR: the stress group had a higher pain catastrophizing score than the control group ($t(50) = 2.29$, $p = .026$) and had on average higher mean HR than the control group ($t(33) = 4.76$, $p = .036$).

There was no difference between the sexes on the baseline characteristics (all p's < .15) except for BAIAS attention: females (M= 2.53, SD= 0.42) had a higher BAIAS attention score than males ($t(50) = 2.68$, $p = .01$; M= 2.2, SD= 0.36).

3.2. Primary outcome

3.2.1. Effects of the TSST on the conditioning task

An overview of the pain ratings during the conditioning task is presented in Fig. 2. A pre-analysis check indicated that the calibration procedural change did not affect pain ratings for the high and medium stimuli in the learning phase ($F(1,50) = 0.14$, $p = .709$, and $F(1,50) = 0.26$, $p = .612$, respectively) or the testing phase ($F(1,50) = 0.02$, $p = .888$, and $F(1,50) = 0.09$, $p = .765$, respectively). Therefore, we did not control for this factor in the subsequent analyses. The primary analysis indicated that a significant nocebo effect was present: the first ON trial of the testing phase (M±SD = 3.09 ± 1.69) was rated as more painful than the first OFF trial (M±SD = 2.45 ± 1.63; $F(1, 49) = 70.85$, $p < .001$, $\eta_p^2 = .59$). In addition, a significant extinction of the nocebo effect was found: the trial number significantly affected the magnitude of the nocebo difference score, which decreased over time ($F(9, 423) = 9.89$, $p < .001$, $\eta_p^2 = .17$, $\epsilon = .705$). At the same time, the nocebo effect did not completely extinguish, as the last ON trial of the testing phase (M= 2.80, SD= 1.86) was still rated as significantly more painful than the last OFF trial (M=2,41, SD= 1,71;$F(1, 50) = 232.36$, $p < .001$, $\eta_p^2 = .820$). Stress, by the TSST affected neither the magnitude of the first nocebo trial ($F(1, 49) = 0.12$, $p = .91$, $\eta_p^2 < .001$), nor the extinction of nocebo hyperalgesia ($F(1, 47) = 0.57$, $p = .810$, $\eta_p^2 = .001$).

In the learning phase of the conditioning task, participants rated ON cues (M±SD = 6.77 ± 1.27) as more painful than OFF cues (M±SD = 3.14 ± 1.23; $F(1, 50) = 482.08$, $p < .001$, $\eta_p^2 = .906$). There was no effect of the TSST on the pain ratings during the learning phase ($F(1,50) = 0.04$, $p = .842$, $\eta_p^2 = .001$).

Because there were significant between-group baseline differences in pain catastrophizing and standing rest heart rate, we performed additional analyses for our main outcome of interest (effects of the TSST on the conditioning task), in which we included these variables as covariates. These are described in the supplementary materials. Briefly, adding pain catastrophizing as covariate did not affect any of the outcomes. However, after inclusion of baseline heart rate, significant group (stress, control) x cue (ON, OFF) interactions were found. During conditioning, higher pain ratings were observed in the control group relative to the stress group for ON cues (but not OFF cues). Note that, due to missing data, this analysis was conducted in a smaller sample (n = 34).

3.3. Secondary outcomes

3.3.1. Effects of the TSST on self-reported and physiological stress parameters

Changes in self-reported and physiological parameters over time are depicted in Fig. 3. The TSST significantly affected anxiety levels: there was a significant effect of the measurement time-group interaction on anxiety ($F(3, 41) = 15.43$, $p < .001$, $\eta_p^2 = .247$, $\epsilon = .743$). Pairwise comparisons demonstrated that anxiety levels were higher in the stress groups than in the control group at 1 min (stress group: M±SD = 14.92 ± 3.06; control group: M±SD = 10.16 ± 2.17), 5 min (stress group: M ±SD = 12.25 ± 3.09, control group: M±SD = 9.08 ± 2.34) after the TSST (all $p < .001$) as well as after conditioning (stress group: M±SD = 11.08 ± 2.55; control group: M±SD = 9.4 ± 2.63;($p = .028$).

TSST significantly affected self-reported stress levels: there was a significant effect of measurement time-group interaction on self-reported stress ($F(3, 141) = 11.13$, $p < .001$, $\eta_p^2 = .191$, $\epsilon = .676$). Self-reported stress levels were higher in the stress group than in the control group at 1 min (stress group: M±SD = 4.17 ± 2.33; control group: M ±SD = 1.8 ± 1.61) ($p < .001$) and 5 min after the TSST (stress group: M ±SD = 2.58 ± 1.98; control group: M±SD = 1.16 ± 1.43) ($p = .006$).

A significant effect of the measurement time-group interaction was also found on the cortisol levels ($F(3, 147) = 3.14$, $p = .049$, $\eta_p^2 = .060$, $\epsilon = .654$). Pairwise comparisons demonstrated that there was a significant increase in cortisol levels in the stress group between the baseline (M±SD = 3.93 ± 2.08), 1 min after TSST (M±SD = 4.98 ± 2.72, $p = .035$), and 5 min after TSST (M±SD =6.24 ± 3.45, $p = .003$). In the control group, there was no difference between the baseline (M±SD = 3.76 ± 1.627), 1 min post-TSST (M±SD = 4.18 ± 2.37, $p = .99$), 5 min post-TSST (M±SD = 4.81 ± 2.85, $p = .500$), and post-conditioning (M ±SD =3.62 ± 1.69, $p = .99$) measures. Between groups, cortisol levels differed significantly at the post-conditioning level ($p = .013$).

TSST condition did not affect the alpha-amylase levels ($F(1, 38) = 1.17$, $p = .29$, $\eta_p^2 = .030$). Alpha-amylase levels did not change with time in both groups ($F(1, 38) = 4.11$, $p = .050$, $\eta_p^2 = .101$, data not shown).

No significant group-time interaction effect was found for HR ($F(2, 64) = 1.26$, $p = .292$, $\eta_p^2 = .04$) or SCL ($F(2, 72) = 0.20$, $p = .82$, $\eta_p^2 = .01$). A main effect of time on HR was observed ($F(2, 64) = 5.14$, $p < .001$, $\eta_p^2 = .62$): HR was higher during the standing baseline and stress phase than during the conditioning task (both $p < .001$). Moreover, a significant main effect of group was found: the stress group had a higher heart rate than the control group throughout the entire

Table 1
Means of the baseline and personality measures across the groups with standard errors.

	Stress group (19 females; 7 males)	Control Group (18 females; 8 males)	t/U	p
Age	22.91 (0.70)	21.38 (0.47)	1.83	.074
Baseline state anxiety[a]	10.30 (0.41)	9.88 (0.42)	302.50	.508
Baseline stress[a]	1.46 (0.30)	1.31 (0.28)	315.50	.669
Baseline cortisol[a]	3.93(0.41)	3.76 (0.33)	323.00	.970
Pain catastrophizing	17.27 (1.58)	12.69 (1.22)	2.29	.026
Fear of pain	82.50 (2.88)	76.58 (3.49)	1.31	.196
BAIAS ignorance	2.23 (0.07)	2.26 (0.07)	-0.36	.724
BAIAS awareness	2.14 (0.10)	2.20 (0.08)	-0.47	.638
BAIAS attention	2.42 (0.08)	2.45 (0.09)	-0.19	.847
Medium pain temperature[a]	46.09 (0.29)	45.98 (0.37)	336.50	.978
High pain temperature[a]	48.46 (0.20)	48.42 (0.29)	305.50	.542
Heart rate (bpm) at rest[ab]	94.24 (12.38)	83.69 (8.15)	233.00	.007
Skin conductance level (μS) at rest[b]	3.71 (2.11)	2.86 (1.63)	1.58	.121

μS = microsiemens, BAIAS = body attention, ignorance and awareness scale (Beugen et al., 2015); bpm = beats per minute

[a] Mann-Whitney U test is presented instead of t-test, as nonparametric test was applied to non-normally distributes variables. Mann-Whitney U tests indicated that the temperature level of the medium and high stimuli was not affected by the change in the calibration procedure (both $p \geq .40$)

[b] missing data at baseline were n = 17 for heart rate (n = 9 in the stress group, and n = 8 in the control group), and n = 13 for skin conductance level (n = 6 in the stress group, n = 7 in the control group).

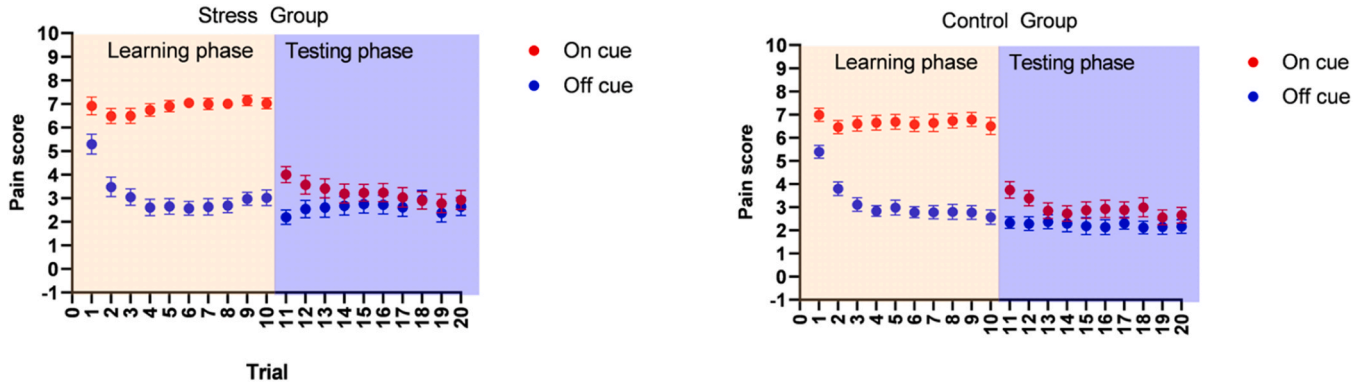

Fig. 2. Mean (with SE) pain scores for each trial of the learning and testing phases across two groups.

experiment including the baseline measure ($F(1, 32) = 5.64$, $p = .024$, $\eta_p^2 = .15$). SCL increased significantly over time ($F(2, 72) = 82.90$, $p < .001$, $\eta_p^2 = .70$) but no main effect of group was observed ($F(1, 36) = 1.64$, $p = .209$, $\eta_p^2 = .04$).

3.3.2. *Mediation of nocebo effects by stress responses*

The mediation model with anxiety, self-reported stress and cortisol as mediators was significant ($R^2 = 0.480$, $F(4, 42) = 3.151$, $p = .024$). It demonstrated that changes in anxiety, but not in self-reported stress or cortisol, mediate the effects of the stress task on the nocebo effect. The stress task significantly increased participants' anxiety (path a1: $b_{x->m1} = 563.00$, SE=195.09, $p = .006$; Fig. 4). Increased changes in anxiety levels were significantly and positively associated with increases in the size of the nocebo effect (path b1: $b_{m1->y} = 0.008$, SE = 0.0003, $p = .012$). Changes in self-reported stress or cortisol did not directly affect the nocebo effect (both p's > .068). The total indirect effect of group on the magnitude of the nocebo effect was significant (path c′: $b_{indirect} = 0.556$, SE = 0.264, 95% CI bootstrap (0.141, 1.163)). Looking at the indirect effects of group through individual mediators, only the indirect effect through anxiety was significant (path c1: $b_{indirect} = 0.433$, SE = 0.264, 95% CI bootstrap (0.082, 0.972)). This indicates that the TSST indirectly increased the magnitude of nocebo, primarily through state anxiety. The direct effect of group on the nocebo effect was significant when controlling for all mediators (path c′: $b_{x->y} = -0.855$, SE= 0.384, $p = .032$). Interestingly, the direction of the direct effect was opposite to the mediated effects: controlling for all mediators, the TSST appears to blunt the nocebo effect magnitude, which rendered the total effect of group nonsignificant (b= −0.299, SE= 0.379, $p = .434$). Overall, the model suggested that when stress leads to increased state anxiety, this enhanced the nocebo effect. For individuals who do not respond to the TSST with anxiety, the experience of stress may blunt nocebo responding. However, note that inclusion of alpha amylase, HR and SCL, in the second and third mediation model rendered the whole model nonsignificant, which may be because of the effects of these mediators, but also due to a change in the study sample and a lack of power to detect effects. The results of these models are demonstrated in the Supplementary materials.

3.4. *Exploratory outcomes*

3.4.1. *The effect of psychological characteristics*

The moderation models with psychological characteristics (fear of pain, pain catastrophizing and behavioural activation/inhibition) were all non-significant, indicating that psychological characteristics did not affect the relation between stress and nocebo effect.

The hierarchical regression models with a the magnitude of nocebo effect in the first trial as a dependent variable, a group as a predictor and psychological characteristics and interactions between group and psychological characteristics were statistically insignificant (first step: group predictor: $F(1,49) = 1.387$, $p = .245$, $R^2 = 0.028$; second step: fear of pain, pain catastrophising, behavioural activation/inhibition predictors: $F(6,49) = 1.103$, $p = .377$, $R^2 = 0.133$; third step: interactions between personality characteristics and group: $F(11, 49) = 0.878$, $p = .569$, $R^2 = 0.203$). None of the psychological characteristics significantly predicted the magnitude of nocebo effect (all p's > .192) or affected the relation between stress and nocebo effects (all interaction p's > .174).

3.4.2. *Sex differences in main outcomes*

No effect of sex and sex-stress interaction was found on the magnitude of nocebo, cortisol response, alpha amylase response and self-reported stress (all p's > .300). There was a significant effect of stress on the state anxiety indicating that males reported lower state anxiety than females ($F(1, 49) = 4.37$, $p = .042$, $\eta_p^2 = .089$).

4. Discussion

The present study did not confirm our main hypothesis and we found no direct effect of social stress on the magnitude of the nocebo effect and its extinction. However, one of our secondary hypotheses was confirmed as the effect of stress on the nocebo effect was partially mediated by state anxiety: social stress enhanced self-reported anxiety, which in turn enhanced the magnitude of nocebo.

First, the TSST in our study was successful in inducing increases in psychological (state anxiety and self-reported stress) and physiological (cortisol) stress responses, confirming the success of the stress manipulation. The nocebo induction manipulation was also successful: conditioning with verbal suggestions induced a significant nocebo effect, which demonstrated an extinction pattern but remained significant after 20 pain trials. These findings correspond to a large body of literature showing that conditioning combined with verbal suggestions is a powerful method to induce nocebo hyperalgesia (Petersen et al., 2014; Skvortsova et al., 2020).

Contrary to our expectation, the TSST however did not have a direct effect on the magnitude of the nocebo effect. The mediation model demonstrated that the effect of TSST on nocebo was nevertheless partially mediated by state anxiety. This means that the TSST significantly increased state anxiety in participants, and this increase in anxiety enhanced nocebo hyperalgesia. The reason why the stress test did not have a direct effect on nocebo could be due to the large variability in individual responses to TSST. Possibly, the TSST was not effective in eliciting anxiety in all participants who underwent it. In this case, mediation would indicate that, only in participants who responded to TSST with elevated anxiety, the nocebo effect was increased as well.

The link between anxiety and nocebo has been discussed a lot in literature (Colloca & Benedetti, 2007). Particularly evidence demonstrates that negative expectations about pain trigger anxiety increase and cortisol release, which in turn can cause nocebo (Benedetti et al.,

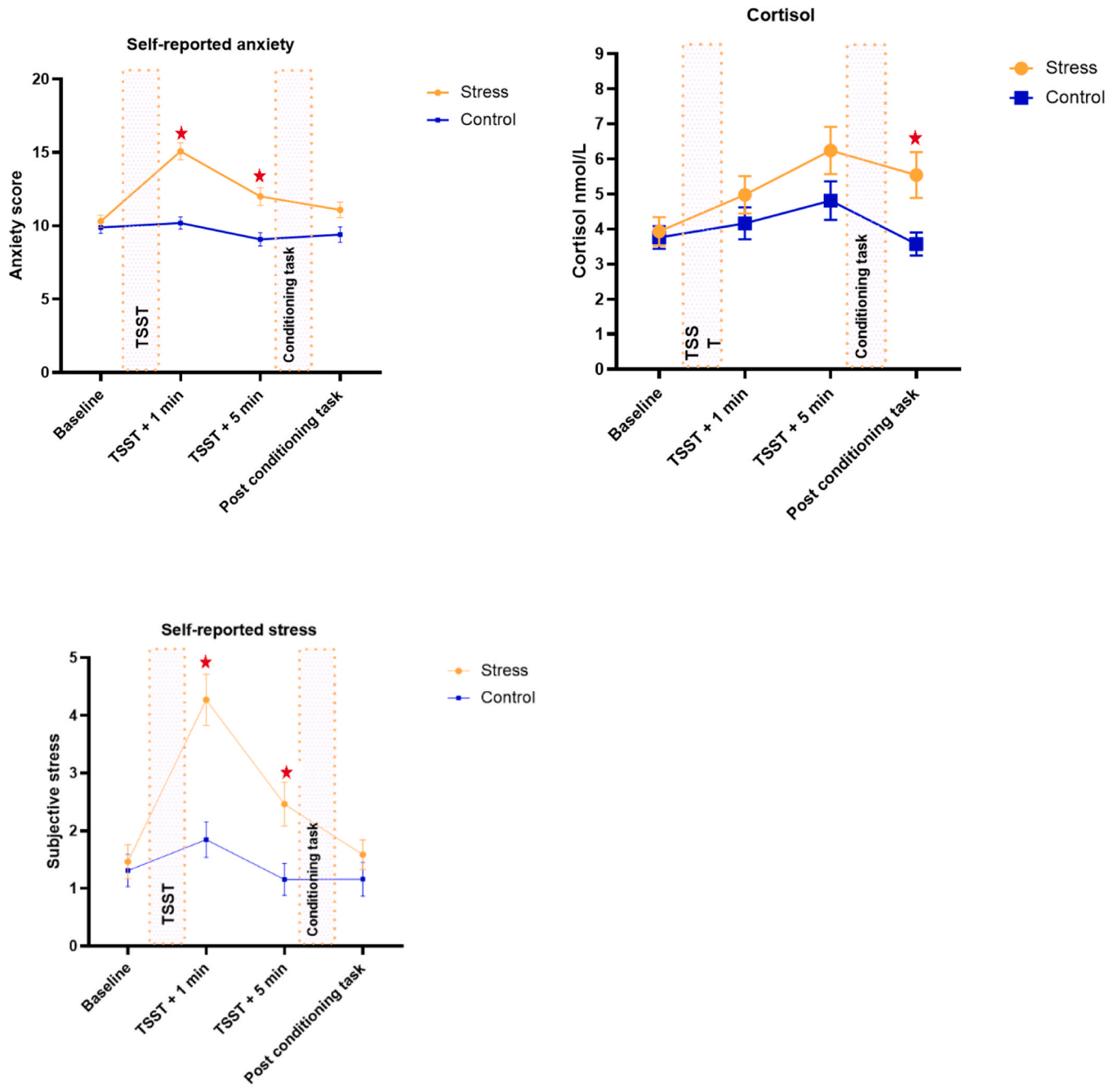

Fig. 3. The timeline of the changes in cortisol, state anxiety and self-reported stress levels (with SE) during the session.

2006). Also, accumulating evidence demonstrates that pain anxiety and fear of pain might be related to higher nocebo effects (Kern et al., 2020). Our research demonstrates that anxiety triggered by an external social stressor rather than by expectations of pain can also enhance nocebo hyperalgesia.

In a sense, these findings fit with theories on stress as well as nocebo effects. The combination of unpleasantness and uncontrollability of the situation created by the TSST, and arousal may have created feelings of anxiety in some, but not all individuals (Fink, 2016). This state may then have amplified nocebo effects only for those individuals, as evidenced by the observed mediation effect. In addition, the findings are in line the idea that the nocebo effect is the result of an interaction between the context (the stressful circumstances created by the TSST preceding pain conditioning) and the individual (being sensitive and responsive to these specific circumstances), which would suit nocebo theories. In literature on nocebo (or placebo effects, for that matter), it is rarely defined what is meant with 'stress' in a clear and concise manner. As a result, there are studies that propose stress as a moderating state which may precede nocebo responding (as in our study, and Roderigo et al., 2017), studies that suggest that being stressed is a potential (psychoneurobiological) mediating mechanism underlying nocebo effects (e.g., Johansen et al., 2003), or that exclusively study stress as a consequence of the placebo or nocebo induction (for review, e.g., Daniali & Flaten, 2020). Interestingly, controlling for baseline heart rate in our analysis revealed a significant interaction between group and cue type, with participants in the control group reporting higher pain for the ON cues. This finding could potentially reflect larger nocebo effects in the control group, which would directly contradict our hypothesis that stressed individuals demonstrate increased nocebo responding. However, this additional analysis needs to be interpreted cautiously given the significant drop in

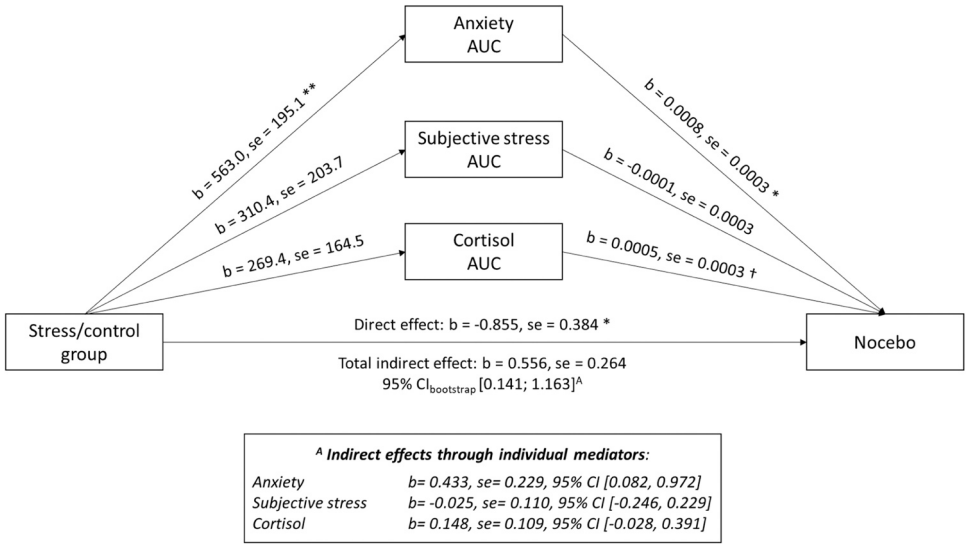

Fig. 4. The effects of the stress test on conditioned nocebo hyperalgesia are mediated by increases in self-reported anxiety, but not cortisol, or subjective stress. The nocebo effect is expressed as the difference between the first ON and first OFF trial in the conditioning task's testing phase. AUC = area under the curve. * ** $p < .001$, * * $p < .01$, * $p < .05$, † $p < .10$.

sample size. Moreover, the findings may also reflect alternate processes, such as acute stress-induced antinociception (Geva et al., 2023). Much remains unclear about how stress, conditioning, and nocebo effects in pain are associated, and which neurobiological mechanisms underlie these effects. Linking the different concepts may become more complex when we consider knowledge generated outside of the nocebo field. For instance, it may be possible that the conditioning procedure and pain stimuli served as specific and additional stressors. Indeed, many stress experiments nowadays incorporate pain cues in their procedures to elicit a state of stress (e.g., the socially evaluated cold pressor task, Schwabe & Schächinger, 2018). Moreover, stress has been found to directly affect both memory (Rudland et al., 2020; Sandi & Pinelo-Nava, 2007) and pain (Butler & Finn, 2009; Ferdousi & Finn, 2018; Jennings et al., 2014). Findings from these areas also show complexity. For instance, stress can result in either hypoalgesia or hyperalgesia depending on the circumstances and individuals (e.g., Geva & Defrin, 2018). Stress may also affect memory in multiple ways, either enhancing it or impairing memory formation depending on the circumstances (Rudland et al., 2020; Sandi & Pinelo-Nava, 2007). Studying stress in the context of conditioned nocebo effects may result in unique insights may be relevant for many of these research areas. Future studies are therefore crucial. In addition, stress and nocebo responses may share resources. Research for instance shows that higher blood glucose level amplifies both cortisol production in reaction to stressors (Kirschbaum et al., 1997) and conditioning and extinction of fearful reactions (e.g., Glenn et al., 2014; Hauck et al., 2023). Similarly, glucose may also amplify nocebo hyperalgesia conditioning. Such shared resources could mean that less glucose was available following the TSST, which speculatively may have resulted in less efficient conditioning. As a consequence, glucose depletion could have negated the effects of stress on nocebo hyperalgesia in the TSST group. Future studies may investigate how glucose can affect nocebo conditioning.

Several limitations of the study must be mentioned. First of all, we powered the study to detect moderate effect sizes (Cohen's d = 0.40). It may be possible that smaller direct effects of stress on nocebo effects exist but were statistically nonsignificant because of a lack of power. Replicating the study in a larger sample may therefore be useful. Secondly, no effect of the social stress test on alpha-amylase was found, which contradicts previous literature that indicated an increase of alpha-amylase in response to TSST (Seddon et al., 2020). Alpha-amylase data in our sample had a large variance which was the reason for the need to deviate from the pre-registered analysis and use the relative change from baseline scores instead of the raw values. Possibly, this variability in the data was caused by the fact that participants were asked to drink a glucose drink before the experimental session, as glucose consumption has been shown to amplify cortisol responses to psychosocial stress (Zänkert et al., 2020) and is recommended to be used as a part of a standard TSST protocol (Labuschagne et al., 2019). At the same time, food consumption has been shown to significantly increase alpha-amylase and it is recommended to ask participants to fast before testing (Strahler et al., 2017). However, as cortisol is the most commonly measured endocrine marker of stress (Chapman et al., 2008), we made a choice for an optimal protocol for cortisol, rather than alpha-amylase. Thirdly, there was a significant difference in the baseline heartrate between the groups with the stress group having higher baseline heartrate than the control group. This could indicate heightened sympathetic nervous system activity in the stress group prior to the stress test that potentially could have affected both the effects of TSST and the conditioning task. While analysing the effects of TSST on the stress markers, we used area under the curve analysis, that takes into the consideration the baseline levels and, at least, statistically controlled for any baseline differences. However, the effect of the heightened heartrate could still have indirectly affected our outcomes. Furthermore, in this study we did not control for the phase of menstrual cycle or the oral contraception use in our female participants. Oral contraception use has been demonstrated to lead to a more blunted cortisol response in previous research (Gervasio et al., 2022). The effects of oral contraception and menstrual cycle phase on sensitivity to experimentally induced pain is inconsistent and seem to be negligible particularly for heat pain (Sherman & LeResche, 2006; Teepker et al., 2010). Not controlling for these factors, our study may have introduced variability into our results that is unrelated to the stress manipulation. But at the same time, the fact that we did not preselect participants based on their hormonal status, made the results of our study more generalizable to the general population. Another study limitation is the fact that men were underrepresented in our sample. The sensitivity analysis also demonstrated, that females had higher state anxiety levels than males during the experimental session. It might have affected the results of the study. However, we can assume that these effects are minimal for several reasons. First, we ensured that the randomization is done in the way sexes are distributed equally between the stress and control condition, therefore, minimizing the interference of sex with the effects of the experimental condition.

Secondly, there was no sex differences found in nocebo effect as well as response to the stress task, which were our main outcomes. Finally, our study was done during the COVID-19 pandemic. During this time, the general population experienced higher levels of stress and anxiety (Salari et al., 2020). Possibly, our participants in both groups had increased levels of stress, in particular because both tasks required social contact which may have been stressful during the pandemic. This could potentially also be the reason why we did not detect any differences in alpha amylase, heart rate and skin conductance levels between the groups, although we did find effects of the stressor on cortisol.

Our results might have important clinical implication. Unlike other studies that looked at the effects of pain-triggered stress on nocebo hyperalgesia (Benedetti et al., 2006), we demonstrated that stress that is not related to pain (socially provoked stress) can also enhance nocebo effect through state anxiety. Therefore, to avoid nocebo effects in clinical practice, it is not only essential to reduce the stress related to the symptoms of patients, but also support patients to be stress free in general. It can be recommended that clinicians pay attention to the general mental state of their patients and possible social stressors. Decreasing state anxiety in the clinical settings might lead to a better health outcome and reduce nocebo effects.

In sum, this study demonstrated that there is no direct effect of social stress on nocebo hyperalgesia, but rather this effect is mediated by state anxiety. These results indicate that special attention has to be paid to social stress and state anxiety of patients to limit the possible nocebo effects.

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Declaration of Generative AI and AI-assisted technologies in the writing process

Statement: The author(s) did not use generative AI technologies for preparation of this work.

Declaration of Competing Interest

The authors declare no conflicts of interests.

Data Availability

The data is shared on https://osf.io/b2eqf.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopsycho.2024.108818.

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