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The Optimal Learning Cocktail for Placebo Analgesia: A Randomized Controlled Trial Comparing Individual and Combined Techniques

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Abstract: **This study investigated for the first time the effects of individual and combined application of 3 learning techniques (verbal suggestions, classical conditioning, and observational learning) on placebo analgesia and extinction.**

Healthy participants (N = 206) were assigned to 8 different groups in which they were taught through either a verbal suggestion, a conditioning paradigm, a video observing someone, or any combination thereof that a placebo device (inactive transcutaneous electric nerve stimulation [TENS]) was capable of alleviating heat pain, whereas one group did not (control). Placebo analgesia was quantified as the within-group difference in experienced pain when the placebo device was (sham) 'activated' or 'inactivated' during equal pain stimuli, and compared between groups.

Placebo analgesia was induced in groups with 2 or 3 learning techniques. Significantly stronger placebo analgesia was induced in the combination of all 3 learning techniques as compared to the individual learning techniques or control condition, underlining the additional contribution of 3 combined techniques. Extinction did not differ between groups. Furthermore, pain expectancies, but not state anxiety or trust, mediated placebo analgesia.

Our findings emphasize the added value of combining 3 learning techniques to optimally shape expectancies that lead to placebo analgesia, which can be used in experimental and clinical settings.

Perspective: ***This unique experimental study compared the individual versus combined effects of 3 important ways of learning (verbal suggestions, classical conditioning, and observational learning) on expectation-based pain relief. The findings indicate that placebo effects occurring in clinical practice could be optimally strengthened if healthcare providers apply these techniques in combination.***

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Placebo effects are beneficial effects for a clinical outcome due to the psychosocial context of clinical treatment and are well-studied in pain (ie, placebo analgesia).[8,9,17,24] Placebo analgesia can significantly impact pain symptoms in a variety of conditions and inducing these effects in clinical practice is, therefore, desired by researchers and clinicians.[46] However, inducing and maintaining placebo analgesia is difficult as a complex interplay of multiple mechanisms (eg, learning, expectancies, emotions, and interpersonal communication) underlie the effect.[8]

Learning plays a central role in establishing placebo effects, and in pain, this is presumed to be mediated through conscious as well as unconscious expectancies.[19] The perceived likelihood of a response (eg, perceived pain reduction) as the outcome of a stimulus (eg, taking painkillers) constitutes true somatic effects (eg, placebo analgesia).[8,47] Various learning techniques can induce these expectancies of which verbal suggestions, classical (Pavlovian) conditioning, and observational learning are most widely studied.[1,4,13,15,19,22,46] Verbal suggestions elicit (conscious) expectancies through specific instructions (eg, *"This medicine lowers pain"*).[46] Classical conditioning creates either conscious or unconscious expectancies by the repeated pairing of a neutral stimulus (eg, a color) with the natural effect of a specific stimulus (eg, a painkiller) on a bodily sensation (eg, analgesia).[22,41] Observational learning induces conscious or unconscious expectancies due to observing behavior (eg, relaxation after pain relief) and subsequently expecting these associations to occur in oneself as well.[4,6,36] Individually, all 3 learning techniques can elicit placebo analgesia, whilst some combinations (eg, classical conditioning with verbal suggestions) have been found to create larger effects that seem less sensitive to extinction.[5,20,35] Unfortunately, it is unknown which exact combinations create more robust placebo effects as no study has compared all learning techniques, and any combination thereof, to one another.[4,18,36,42,46,64] On top of that, the mediation by conscious expectancies for any of these combinations of learning techniques has never been systematically addressed before in a single experiment.

Next to expectancies, the effect of learning on placebo analgesia might be mediated by anxiety or trust. Anxiety seems negatively related to placebo effects; learning to expect lower pain sensations might decrease anxiety, which could alleviate pain perception.[38] Trust could be influenced by the believability of suggested outcome expectancies, which might partially depend on learning and interpersonal communication.[33,45] The role of anxiety and trust is however still unclear.[21,26,38,54,56] Studying them, next to expectancies, as mediators, could expand knowledge on how learning leads to placebo analgesia.

Understanding the influence of learning techniques in isolation and combination on placebo analgesia, and unraveling underlying mediators, could further help to implement placebo strategies in clinical practice.[35] Therefore, we aimed to investigate if the combination of all 3 ways of learning (verbal suggestion, classical conditioning, and observational learning) would induce more placebo analgesia than individual application in experimentally-induced heat pain. Secondly, other comparisons between (combinations of) learning techniques, comparisons between (combinations of) learning techniques and non-specific effects (control), extinction of placebo analgesia for each (combination of) learning technique(s), and mediation by pain expectancies, state anxiety, or trust were explored.[2,20] As previous work has indicated that combining ways of learning leads to more robust placebo effects,[18,22] our hypothesis was that the combination of all 3 different learning techniques would elicit larger placebo analgesia than their individual application.

Methods

This study was conducted at the laboratory sites of the Faculty of Social and Behavioural Sciences, at Leiden University in the Netherlands. The protocol for the study was approved by the Leiden University Psychology Research Ethics Committee (2020-11-26-A.W.M. Evers-V1-2785) and registered in the Netherlands Trial Registry (NL8207, currently findable at the WHO ICTRP search portal: https://trialsearch.who.int/Trial2.aspx?TrialID=NL8207). The study was reported following the CONSORT statement for randomized controlled trials.

Design

This randomized controlled trial had a mixed (between-within) subjects design with 8 groups and 2 types of trials within every group. The 8 study groups consisted of 7 experimental groups, in which the aim was to induce placebo analgesia, and 1 control group, which served to evaluate pain sensation when participants did not expect pain relief. Placebo analgesia in the experimental groups was induced with learning techniques, either administered individually, or in combination with one another: in 3 groups a single technique was used (eg, classical conditioning); in another 3 groups 2 techniques (eg, verbal suggestion and classical conditioning) were used, and in the seventh group the combination of all 3 learning techniques was used. Within every group, participants were exposed to multiple pain stimuli that were combined with either 1 or 2 different visual cues on a screen. This cue indicated that a placebo device (ie, non-functional TENS device) was either working (ie, active trial) or not working (ie, inactive trial). Participants in the experimental groups were led to expect by the group-specific learning technique(s) that activation of the placebo device would attenuate their pain, whereas those in the control group thought it measured pain. The experimental setup for every group was comprised of 3 phases: a calibration phase, a learning phase, and a test phase. In the calibration phase, heat pain temperatures corresponding to a low and moderate pain intensity were determined for use in the learning and test phases. In the learning phase, participants were subjected to the experimental or control versions of 3 different manipulations: a verbal suggestion, a classical conditioning paradigm, or a video observing someone. Experimental manipulations were specifically aimed to induce placebo

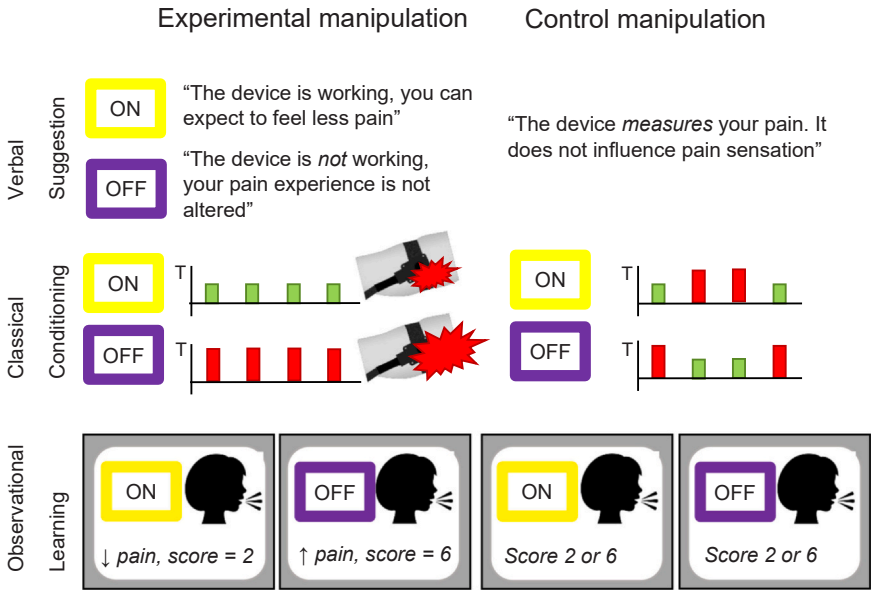

Figure 1. Manipulations in the experiment. During the learning phase, participants were subjected to 3 different kinds of manipulations: a verbal suggestion, a classical conditioning paradigm, or a video observing a model participant in a short part of the test phase. In a classical conditioning paradigm, participants are subjected to 2 levels of heat pain stimuli (low or moderate) that are presented along with a visual cue, which shows the activation or inactivation of a sham device. There were 2 versions of each manipulation: a version aimed at inducing placebo analgesia by means of learning (experimental manipulations) or a version merely used as a control (control manipulations). During an experimental verbal suggestion, participants were instructed that a sham device would lower their pain when activated, whereas, during a control suggestion, participants were told it merely measured pain. In an experimental conditioning paradigm, the different levels of heat pain were congruently coupled to one of the visual stimuli to falsely create the impression that the sham device was lowering the participants' pain perception. In a control conditioning paradigm, heat pain levels were presented at random, to prevent an association between the visual cues and pain perception of a participant. In an experimental video, the model participant scored heat pain significantly lower when a visual cue indicated that a sham device was activated compared to when it was inactivated. In the control video, the heat pain scores from the model participant were not matched to the visual cues. Participants in every group were subjected to a different set and order of manipulations. This set was unique for every group and specifically applied to obtain placebo analgesia by means of the studied learning technique, combinations of learning techniques, or none (control).

analgesia through a learning technique (see Fig 1). Control manipulations were adopted to prevent performance bias by equalizing all groups as much as possible in interaction time and the number of manipulations spent between the experimenter and participant. Every group was subjected to different sets of manipulations to study the individual or combined effects of the learning techniques (see Fig 2). In the test phase, the effect of the manipulations on experienced pain intensity was tested by comparing the active and inactive trials while delivering heat pain stimuli of individually calibrated moderate intensity only. Placebo analgesia at the group level was defined as a significant average difference in lower experienced pain intensity in the first 3 active trials than in the first 3 inactive trials of the test phase. A more extensive description of the calibration procedure, experimental and control manipulations, and test phase is provided below in the sections 'Calibration of heat pain', 'Experimental manipulation', 'Control manipulation' and 'Placebo analgesia'.

Participants

Sample size calculation was performed using G*Power version 3.1.9.4.[25] The required effect size was based on the estimated minimal relevant effect size for the primary research question. Considering the effect sizes reported in the literature for the individual learning techniques,[36,46] proving an effect between different combinations of learning techniques was assumed to be difficult. Hence, the effect size (of 3 learning techniques as compared to an individual technique) was estimated to be moderate (η_p^2 = .06). The primary research question was analyzed with a repeated-measures mixed model Analysis of Variance (ANOVA) (featuring group, trial type, and time as independent variables; see 'Main analysis' for more details). Initial G*Power calculations yielded a sample size of 296 for a design with 8 groups, an alpha level of .05 and a presumed power of .80 (see Supplementary Material). To account for drop-outs, the total number of required participants for the study was estimated to be 320 (40 per group). Upon revisiting the original sample size calculation by a statistician, the number of levels for the within-subject factor trial type was not specified appropriately, resulting in an overestimation of the required sample size. The second calculation indicated a total of 232 participants needed for 8 groups, which equals to 29 participants per group (for a further explanation, see Supplementary Material).

Participants were randomly allocated to the 8 different groups using a blocked randomization scheme (size = 8) stratified for sex (3:1 female/male ratio). An independent researcher created a randomization list, printed out the tickets for every participant, and stored these in sealed envelopes to prevent selection bias.

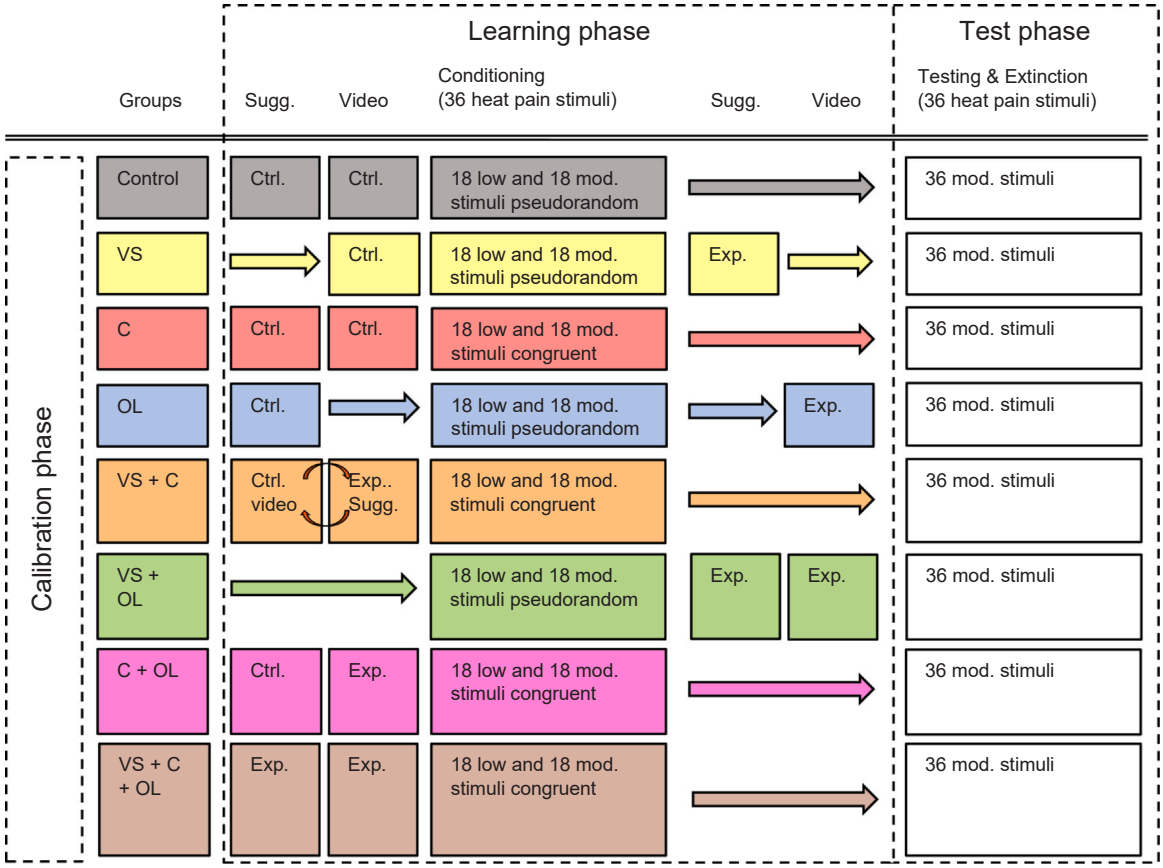

Figure 2. Overview of the study design. The included participants were randomly allocated to 8 different groups after calibration of the heat pain stimuli. Depending on the group allocation, participants received a manipulation in the form of a suggestion, a video, and/or a conditioning paradigm that could be either experimental or control. The participants in some groups received the manipulations in a different order (groups: VS, OL, VS + C, VS + OL) to prevent interference of the control manipulations with the effect of learning on placebo analgesia. The groups receiving a verbal suggestion, a video, or a combination of a verbal suggestion and video were subjected to the experimental manipulation(s) after receiving the control conditioning paradigm to prevent them from learning that the device would not change their pain during conditioning. The group receiving a verbal suggestion and conditioning were subjected to their experimental manipulations after receiving their control video to prevent them from learning through the video that the placebo device would not alter pain perception. In the test phase, all participants received 36 moderate heat pain stimuli that consisted of 18 experimental and 18 control trials. Any difference in experienced pain between the trials was considered evidence for placebo analgesia. The arrows indicate that no manipulation was provided at that specific moment of the experiment. C, classical conditioning; Ctrl, control; mod, moderate; OL, observational learning; Exp, experimental; Sugg, suggestion; VS, verbal suggestion.

Participants were included in the study if they were between 18 and 35 years of age, English or Dutch-speaking, and healthy. Exclusion criteria were: 1) Presence of pain at the moment of testing (this includes chronic pain disorders, but also acute pain, assessed as an NRS score of 1 or higher), 2) Use of prescription analgesics within 24 hours before testing (eg, opioids, 5-HT receptor agonists), 3) Use of over the counter analgesics within 12 hours before testing, 4) Presence of any severe physical or mental disabilities (eg, cardiovascular disease, kidney disease, depression, autism), 5) Use of long-term medication for a physical or mental disability that might interfere with pain perception (eg, anti-epileptics, antidepressants, opioids, cannabinoid oil), 6) Use of >2 alcoholic units within 12 hours before testing, 7) Use of any hard drugs (eg, amphetamines) within 48 hours prior to testing, 8) Use of any soft drugs (eg, marijuana) within 12 hours prior to testing, 9) Experience with previous research regarding placebo-related learning techniques (verbal suggestion, conditioning, and social learning), 10) Pregnancy or currently breastfeeding, 11) Injuries on the wrists or arms that will be used for testing and are at the location of the thermode or TENS electrode, at the time of participation, 12) Presence of a pace-maker or implantable cardioverter-defibrillator, and 13) Unable to reliably detect a difference in pain of less than 2 points on the numeric rating scale (NRS) between low and medium heat pain intensities discovered during the calibration procedure.[44,58] This was adopted to minimize risk of a non-successful conditioning paradigm when a participant cannot reliably rate a difference in pain sensation between active and inactive trials.[59]

Thermal Pain Stimulation

Heat pain stimuli were utilized to induce painful sensations. Heat pain was applied with a TSA-II (Medoc Advances Medical Systems, Rimat Yishai, Israel), by using a 3 × 3 cm probe (thermode) that was attached to the volar side of

the non-dominant lower arm of the participant. The temperature of the stimuli had a baseline of 32 °C and could reach a maximum peak temperature of 50 °C. Throughout the experiment, heat pain stimuli were delivered with a ramp-up and ramp-down rate of 8 °C/seconds. Peak temperatures lasted for 4 seconds and the interstimulus interval was 10 seconds. The thermode was moved every 24 heat stimuli to prevent habituation or sensitization of the skin.

Calibration of Heat Pain

The pain calibration protocol followed a published standardized protocol.[50] To familiarize participants with the pain, 4 stimuli were delivered to determine warmth detection thresholds (1 practice and 3 calibration trials) and 4 to determine heat pain thresholds (1 practice and 3 calibration trials). Subsequently, participants received 2 distinct series of heat stimuli to detect median temperatures corresponding to low heat pain (NRS ranging from 1 to 3), moderate heat pain (NRS ranging from 4 to 6), or high heat pain (NRS ranging from 7 to 10). High heat pain sensations were determined to evaluate the temperatures corresponding to the total pain range of a participant. In doing so, participants that had difficulty in defining their low or moderate pain could more easily distinguish different pain levels. In the first series, 18 heat stimuli with ascending peak temperatures starting from 36 °C to 50 °C were delivered to select the initial median temperatures corresponding to a low, moderate or high pain. This series was stopped prematurely in case 1) a participant indicated that he/she could no longer handle the pain, or 2) a participant reached a pain level of 9 or higher to prevent them from receiving stimuli above their tolerance level.[59] In the second series, participants received 18 heat stimuli that were plus and minus .5 °C of the selected median temperatures in a pseudorandom order to verify their consistency in scoring. An extra series of 18 pseudorandom heat stimuli were delivered when the temperatures for low and moderate pain could not consistently be determined. In order to prevent that participants could not be conditioned, participants that were unable to sense a minimum difference of 2 NRS points between low and moderate pain stimuli were excluded in this phase.[59] The final 3 median temperatures for low, moderate, and high pain were composed of the temperatures obtained during the first (ascending) and second (ascending and descending) series. Participants were exposed to a maximum of 60 heat pain stimuli in the calibration phase, depending on the number of calibration series.

Heat Pain Stimuli in Learning and Test Phase

Throughout the learning phase, participants received 36 heat stimuli in the conditioning paradigm with temperatures corresponding to a low (18 stimuli) or moderate (18 stimuli) pain intensity. The heat pain stimuli were delivered in a pseudorandom order. In the test phase, participants received 36 equal heat pain stimuli with temperatures corresponding to moderate pain. The thermode was moved every 24 stimuli throughout the learning and test phase to prevent sensitization of the skin. Between the learning and test phase, the thermode remained in the same place to prevent measuring a change in pain sensitivity of participants due to habituation or sensitization when in fact placebo analgesia was tested.

Measures

Placebo Analgesia

Heat pain intensity was assessed on an NRS scale ranging from 0 (no pain) to 10 (worst pain imaginable). Heat pain scores were rated with an accuracy of 1 decimal place (eg, 7.5 or 2.1). Participants were asked to rate the pain from the heat on their arms verbally after every heat stimulus. The scores obtained from the first 6 heat pain ratings in the test phase, during which heat stimuli had the same (moderate) intensity, were subsequently used to assess the occurrence of placebo analgesia. Since placebo analgesia, like many other placebo effects, is often subjected to extinction, the first 6 heat pain ratings were chosen to assess the predominant and most stable effects.[2,34] The size of placebo analgesia was calculated for every group by comparing the pain scores of the first 3 active trials to those of the first 3 inactive trials. This way, the size of placebo analgesia for every group could be calculated, in line with other placebo experimental studies.[3,16,18,55] Since the manipulations in the control group were not aimed at inducing placebo analgesia, any differences between active and inactive trials in this group were defined as non-specific effects.[11] Subsequently placebo analgesia could be compared between groups or compared to non-specific effects of the control group.

Extinction

The extinction of placebo analgesia was analyzed with the difference in heat pain scores that were measured on the same NRS scale across the 18 active trials and 18 inactive trials in the test phase. The 2 pain measurements after the thermode switch (at stimulus 14 and 15 respectively) were excluded from the analysis as the initial heat pain stimulus applied to the skin was often perceived more painful.[55] A significant decline in placebo analgesia over the course of the test phase was considered evidence for extinction (see also section Statistics below).

Pain Expectancies

Pain expectancies were assessed on an NRS scale ranging from 0 (no pain) to 10 (worst pain imaginable) with an accuracy of one decimal place (eg, 7.5 or 2.1). Pain expectancies were rated 4 times, before starting every train of heat-pain stimuli. Participants were shown a cue on a screen indicating that the placebo device was working (active cue) or not working (inactive cue) for the next heat-pain stimulus and were asked to indicate the level of pain they expected to feel during either trial type twice. The size of the pain expectancies was calculated by subtracting the expected pain intensity of the active cue from the expected pain intensity of the inactive cue in the test phase. A higher

score meant that a participant expected more pain relief when the placebo device was supposedly activated.

State Anxiety

State anxiety was measured using the STAI-S-short form.[40] The 6 items (eg, *"I am tense"*) were scored on a 4-point Likert scale ranging from 1 (not at all) to 4 (very much). Final scores were obtained by multiplying the total score with 3.33 (to follow the scoring of the original STAI) and range between 20 and 80, with higher scores indicating more state anxiety at that time. Participants were asked to fill out the STAI-S-s form at the same intervals as the pain expectancies.

Trust

Trust was assessed with 2 items: trustworthiness and honesty (*"How trustworthy do you think the experimenter was?", "How honest do you think the experimenter was?")*. These 2 questions were part of a series of questions assessing trust developed for an experiment in which pain expectancies were manipulated and focused on the trust of the participant toward the experimenters themselves and the instructions they provided.[45] The items were scored on an NRS scale ranging from 0 (not trustworthy/honest at all) to 10 (most trustworthy/honest imaginable). The questions were asked at the same time as the pain expectancies and the state anxiety, right before every train of heat stimuli. The final score for trust was calculated by averaging the scores from both items.

Manipulation Checks

The extent to which participants were convinced that the experimental manipulations had worked, was assessed with 3 questions at the end of the experiment. The questions were: *"Did you feel that the PPT was really working?", "Did you believe the instructions we gave about the PPT?"* and *"Did you believe what you saw in the video?".* PPT stands for 'Physical Pain Transducer' and is the fake name for the placebo device used in the experiment (see also 'Placebo manipulation' below). Participants' answers were categorized into 'yes', 'no' or 'uncertain'. In order to check whether participants assigned to groups with an experimental conditioning paradigm had indeed perceived a difference in pain between the active and inactive trials in the conditioning phase, the NRS scores of all trials from that phase were compared [3,14,55].

All measures were collected using E-prime 3.0 software (Psychology Software Tools, Pittsburgh, PA),Qualtrics software (Qualtrics, Provo, UT), and Microsoft Excel, version 2206 (Microsoft Office).

Experimental Manipulation

The placebo device used for this experiment was an inactive TENS device that is commercially available (Beurer EM 80). The TENS was described as a 'Physical Pain Transducer' or PPT, and attached to the participants' lower arm with 2 electrodes. The participants in the groups with an experimental manipulation were made to believe that whenever the PPT was activated, it would reduce their pain perception. The (sham) activation or deactivation of the PPT device was displayed with visual cues on a computer screen through E-prime 3.0. The cues consisted of the words 'ON', indicating activation, and 'OFF', indicating deactivation, along with a colored (yellow or purple, counterbalanced between participants) border. In groups with the experimental verbal suggestion, participants were specifically instructed that the PPT device could send small, barely detectable, electrical signals that disturbed nerve conductance and thereby lower pain perception when activated. Additionally, a mock calibration procedure of the PPT, in which participants were exposed to short electrical stimuli, was incorporated to increase the believability of the verbal suggestion. Participants were specifically told during the mock calibration procedure that the electrical stimuli they felt were necessary to verify the activation of the device. During the experiment, however, they would not be able to feel the stimuli as these would be very small and barely detectable. After the mock calibration procedure, the electrical stimuli of the PPT were (unbeknownst to the participant) completely reduced in strength whilst the device was still turned on. In other words, the device showed signs of activity (eg, blinking screen), but did not have any electrical output. This way participants were fully deceived that the PPT was working albeit with a barely detectable threshold. In groups that received the experimental classical conditioning paradigm, the 2 distinct visual cues were consistently paired with a low (screen shows 'ON') or moderate (screen shows 'OFF') heat pain stimulus. The repeated pairing of low pain stimuli with the supposed activation of the placebo device served to falsely induce the associative expectation within persons that the device was altering their pain perception. In groups with the experimental observational learning paradigm, participants viewed a 4-minute video of a mock conditioning experiment with a model participant. The video displayed the model participant consistently reporting less pain when the PPT was activated (NRS varying between 2 and 3) as to when the PPT was deactivated (NRS varying between 5 and 6). Participants in these groups learned through observation that the PPT altered pain perception of someone else, thereby convincing them that it could alter their own perception of pain (see Fig 1).

Control Manipulation

Control manipulations were used in the control group as well as in all groups that used one or 2 experimental manipulations to equalize the time and number of manipulations in every group, that is, to prevent performance bias (see Fig. 1 and 2). In groups without verbal suggestion, participants were told that the PPT device was measuring, instead of influencing, physical pain sensation by sensing electrical activity from the peripheral nerves. Participants allocated to groups without conditioning received a control-conditioning paradigm, in which the visual cues (ON or OFF words on

screen with either yellow or purple borders) were delivered at random with low or moderate pain stimuli to prevent any association between cues and pain intensity. In the groups that observed a control video, an experiment was shown in which there was no consistent relation between the activation or deactivation of the placebo device and the pain reduction reported by the model participant.

The order of the manipulations (either control or experimental) was specifically adjusted per group to optimally elicit placebo effects (see Fig 2). Experimental manipulations were always preceded by control manipulations to 1) establish learning-induced placebo analgesia right before the test phase, and 2) minimize inducing counterproductive expectations due to control manipulations that could possibly diminish placebo effects.[10] The only exception to this was the control suggestion, as every participant was initially told that the device would measure pain. Subjecting all participants to this manipulation irrespective of group allocation was necessary due to the cover story of the experiment. Hence, groups that received an experimental verbal suggestion after a control conditioning paradigm were at first told that the PPT device was measuring pain and after completion of the conditioning paradigm, they were told about analgesic properties of the PPT. Importantly, it was explained and subsequently shown that the PPT was only able to lower pain when a different set of electrodes was used and the mode of the device was switched. After the experimenter had switched the electrodes and device mode, these participants underwent the same mock calibration procedure as all other groups with an experimental verbal suggestion. These instructions made sure that participants would not hold on to any contradictory predictions due to the control suggestion and control conditioning paradigm and would be convinced that this different mode of the PPT could actually lower pain. Since the order and content of the manipulations adopted for every group varied, a more in-depth description is supplied in the procedure section below.

Procedure

Participants were recruited through the university's online participant recruitment system, social media, flyers around the university, and personal communication. Recruitment took place from August 2020 up until March 2022. Participants that were interested in the study were informed beforehand that the purpose of the study was to investigate the influence of the mind body interaction on heat pain. The experiment was conducted in a single experimental session that lasted for about 150 minutes. On the day of testing, participants received a detailed instruction about the experiment after which they provided written informed consent. Next, participants filled out several online questionnaires in Qualtrics regarding 1) screening for inclusion, and after eligibility was verified, 2) some demographics, and 3) their psychological characteristics (results published elsewhere). Then the thermode was attached to the lower arm of the participants and their individual pain levels were calibrated. Participants that had difficulty scoring their heat pain consistently were excluded at this stage. They received a partial reimbursement for the study with either financial compensation (€12.75) or study credits. After calibration, the experimenter opened the randomization envelope to reveal the allocation, which was not shown to the participant. In the learning phase, the electrodes of the TENS device were attached to the participants' lower arm and proximal to the placement of the thermode. Participants received an experimental verbal suggestion or control, watched an experimental video or control, and were exposed to 36 low or moderate heat pain stimuli to fulfill an experimental conditioning or control conditioning paradigm. The exact order of the experimental or control manipulations differed depending on group allocation. More specifically, an in-depth description for every group is provided here:

Control Group

Participants in the control group first received the control suggestion. They subsequently viewed the control video in which a model participant provided low or moderate pain scores independent of the visual 'ON' or 'OFF' cues. After this, a control conditioning paradigm followed in which the low or moderate heat pain stimuli were not systematically coupled to the visual 'ON' or 'OFF' cues.

Verbal Suggestion Group

Participants in the verbal suggestion group also first received the control suggestion about the TENS device. Next, they viewed the same control video, followed by the same control conditioning paradigm as the control group. Next, participants received an experimental suggestion about the TENS. More specifically, they were told that the device could also lower their pain experience when activated ('ON' cue shown) yet this required the electrodes and mode of the device to be changed. To explain why this information was provided at that specific time, participants were told that the group they were assigned to also was meant to investigate the analgesic properties of the device. After the suggestion, the experimenter switched the electrodes and mode of the device, and participants underwent the mock calibration procedure to strengthen their believability.

Classical Conditioning Group

Participants in the classical conditioning group received the same control suggestion and control video as the control group. After these 2 control manipulations, participants went through the experimental conditioning paradigm. Throughout the paradigm, low and moderate heat pain stimuli were systematically paired with the 'ON' and 'OFF' cues on the screen, respectively, thereby convincing the participant that activation of the TENS lowered their pain sensation. In order to measure only the effects of conditioning, these participants were specifically not instructed about any pain-influencing properties of the device.

Observational Learning Group

Participants in the observational learning group first received the same control suggestion, followed by the same control conditioning paradigm as described in the control group. Next, they watched the experimental video. The video showed the conditioning phase of the experiment in which a model participant provided low or moderate pain scores congruent with the visual 'ON' or 'OFF' cues, respectively.

Verbal Suggestions and Classical Conditioning Group

Participants in the verbal suggestions and classical conditioning group first received the same control suggestion and viewed the same control video of the experiment as the control group. After the control video, they received the same experimental suggestion as the verbal suggestion group and subsequently underwent the experimental conditioning paradigm as described at the classical conditioning group.

Verbal Suggestions and Observational Learning Group

Participants in the verbal suggestions and observational learning group were initially given the same control suggestion and control conditioning paradigm as the control group. This was followed by the same experimental verbal suggestion as described at the verbal suggestion group and subsequently was shown the same video as the observational learning group.

Classical Conditioning and Observational Learning Group

Participants in the classical conditioning and observational learning group first received the same control suggestion as the control group. Subsequently, the same experimental video was shown as the observational learning group and this was followed by the same experimental conditioning paradigm as described at the classical conditioning group.

Verbal Suggestions, Classical Conditioning, and Observational Learning Group

Participants in the group with all learning techniques combined received right after the control suggestion the experimental verbal suggestion as described at the verbal suggestion and conditioning group. This was followed by the experimental video and experimental conditioning paradigm as described at the classical conditioning and observational learning group.

After completing the conditioning phase, participants continued to the test phase in which they received 36 moderate heat pain stimuli, and analgesia due to placebo effects was examined. The thermode was replaced every 24 stimuli (before the 25th, and 49th stimulus). Additionally, pain expectancies, anxiety, and trust were measured before the 1st, 25th, 37th, and 49th stimulus starting in the learning phase. At the end of the testing phase, participants' saliva cells were collected with a swab to investigate the role of different genetic variations on placebo effects (results published elsewhere). Then, participants were asked to answer the exit questions and subsequently debriefed about the true goal of the experiment. Finally, participants were reimbursed with financial compensation (€18.75) or study credits and thanked for their time.

Statistics

Statistical analyses were conducted using Rstudio version 1.4.1717, with R version 4.0.1.[51] All analyses were verified with a statistician (T.H.). Descriptive statistics were shown as counts and frequencies for categorical variables and means and standard deviations for numerical variables. Demographics were displayed with descriptive statistics. The answers from the manipulation checks were explored with descriptive statistics (counts and frequencies). The difference in pain scores between the active and inactive trials in the conditioning phase was assessed with repeated measures mixed model ANOVA.

Difference Between the Influence of Learning Techniques on Placebo Analgesia

Studying the extent to which the groups consisting of individual learning techniques or different combinations of learning techniques differed in placebo analgesia was done via a three-way repeated measures ANOVA with one between-subject factor (group) and 2 within-subject factors (trial type and time). The pain scores from the first 6 trials of the test phase were entered in the ANOVA. The within-subject factor trial type indicated the presence of an active or inactive trial. Including time as a within-subject factor was done to include all the possible variances from the design in the model. A significant interaction effect of group x trial type would indicate a significant difference in placebo analgesia between any of the groups.

Placebo Analgesia Within Groups

Subsequently, the size of placebo analgesia evoked by the different learning techniques (and combinations thereof) was examined. Studying the extent to which the placebo analgesic effect itself was significant for an individual group (ie, simple effects of the main three-way ANOVA), was done by comparing the average difference between active and inactive trials.

Primary and Secondary Group Comparisons

To test the primary hypothesis mentioned in the introduction, the placebo analgesia of the group with all 3 learning techniques was compared to the pooled placebo analgesic effect of the 3 groups with individual learning techniques. Placebo analgesia from the individual learning technique groups was pooled to obtain the general effect size of one learning technique. The pooled placebo analgesic effect was examined by

averaging the active and inactive pain scores from all 3 groups and subsequently testing them in a separate two-way (time x trial type) repeated-measures ANOVA. This analysis is referred to as the primary group comparison. Secondly, the difference in placebo analgesia between the group with all 3 learning techniques and pooled placebo analgesic effect of the groups with 2 learning techniques, and the difference between groups with 2 learning techniques and the pooled placebo analgesic effect of the groups with one learning technique was explored. The pooled placebo analgesic scores from the groups with 2 learning techniques were also examined by conducting a two-way (time x trial type) repeated measures ANOVA with the pooled active and inactive scores of all the groups. Post hoc pairwise comparisons between all 8 groups (and all other possible comparisons) were also run to explore any difference in (combinations of) learning (see also Supplementary Material). Although the control group was mainly adopted to study and potentially control for any unintended effects of the control manipulations (ie, non-specific effects), the size of placebo analgesia from the experimental groups was exploratively compared to the effects of the control group. Any significant differences between experimental groups that did not elicit significant placebo analgesia within themselves and experimental groups that did were carefully interpreted. Collectively, these analyses are referred to as secondary group comparisons.

Extinction

Any evidence for the extinction of placebo analgesia over time was analyzed by entering all but 2 measurements of the test phase in the mixed model ANOVA. The 2 pain measurements after the thermode switch (at stimulus 14 and 15 respectively) were excluded from the analysis, as the initial heat pain stimulus applied to the skin was often perceived as more painful.[55] Extinction over all groups was inspected via the trial type x time interaction effect whereas differences in extinction between groups were assessed via the group x trial type x time interaction effect. A significant decline in placebo analgesia over the course of the 36 stimuli was an indicator for extinction.

Mediation by Pain Expectancies, State Anxiety, or Trust

Mediation analyses for pain expectancies, state anxiety, or trust were conducted with step-wise linear regression analyses in distinct simple mediation models. The predicting variable in the mediation models consisted of the 8 different groups. As this was a categorical variable, dummy coding was used to construct the linear regression analysis.[28] The variables were coded in such a way that the control group was considered the reference group. The mediators pain expectancies, state anxiety, and trust were calculated as either difference scores or average scores from 2 measurements during the test phase (before the first stimulus and before the 13th stimulus). The difference in pain expectancy and state anxiety between an experimental trial and a control trial was calculated by subtracting both pain scores, whereas the level of trust was averaged out from the 2 scores as no difference in trust between both trial types was expected. The outcome variable was the size of placebo analgesia observed within a group of the first 6 heat pain stimuli. The difference in the effect of the groups on the different mediators (path a) was tested beforehand with a one-way ANOVA. Mediation was considered to be present if the indirect effect of the predicting variable on placebo analgesia through the mediator (path a x path b = ab) was significant. Significance was inspected non-parametrically by bootstrapping the results with 10,000 samples to construct confidence intervals. The confidence intervals were adjusted to the required alpha level for the analyses (see next section). Furthermore, differences in pain expectancies, state anxiety, or trust between any of the groups were tested post hoc with t-tests.

Assumptions Testing, Outlier Detection, Effect Sizes, and Alpha Levels

The assumptions for the ANOVAs were examined by inspecting the residuals with QQ-plots, residuals plots, Levene's test, and Mauchly's test. The assumptions for the linear models were examined by inspecting the residuals with QQ plots, residuals plots, and Breusch Pagan test. Non-parametric testing was applied when assumptions for normality or heterogeneity of variances were violated. Greenhouse-Geisser corrections were applied when there was a lack of sphericity in the data. Multivariate outliers were detected with Mahalanobis' distance or, in case assumptions for the Mahalanobis were violated, with Z-scores above or below 3.29. The effect size measure used for the mixed model ANOVA was generalized eta squared (η_G^2), with a η_G^2 of .01 considered small, a η_G^2 of .06 considered medium, and a η_G^2 of .14 considered a large effect size. Generalized eta squared was preferred as the main analysis consisted of a between-within-subject design.[43] Unstandardized regression coefficients (b) were displayed as effect measures for the mediation analysis since standardizing regression coefficients with a dichotomous independent variable are problematic for the interpretation of the indirect effect.[27] The alpha level for the main analyses was set at .05 and for planned and post hoc comparisons a Bonferroni correction was implemented.[7] Planned comparisons were conducted with an alpha level of .005 since there were a total of 11 comparisons (see Supplementary Material). Within-subject analyses testing the significance of placebo analgesia in every group subsequently were tested with the same alpha level (.005). Post hoc comparisons between groups were inspected with an alpha level of .002 as there were 22 pairwise comparisons remaining (28 possible pairwise

comparisons minus 6 which were already included as planned comparisons). In the mediation analyses, the placebo groups were merely compared to the control group creating a total of 7 comparisons and thus the alpha level was set to .007.

Results

Demographics

The COVID-19 pandemic impacted participant recruitment in this study. The delay due to the pandemic necessitated an early halt in recruitment despite not meeting the total required number of included participants. The total number of participants assessed for eligibility was 297 of which 208 were eventually randomized across groups. The majority (n = 60 of 89) of the excluded participants was excluded because they were unable to consistently match low and moderate pain levels to a certain temperature; 48 rated the same temperature as low or moderate (15 due to severe habituation, 17 due to extreme sensitization, and 16 at random), and 12 rated the same pain sensation for a large variety in temperatures. Fifteen participants had such a high pain tolerance that the maximum temperature used for this experiment (50 °C) was not able to consistently elicit a moderate pain level. Twelve persons had participated in similar placebo studies and 2 persons had taken drugs prior to the screening. Two randomized participants dropped out of the analysis due to a mechanical failure or a discovered ineligibility after randomization. A total of 206 participants were included in the main analysis; 26 per study group, except for the conditioning + observational learning group, which contained 24 participants. The extinction analysis consisted of 204 participants as a technical malfunction prevented 2 participants from completing all 36 measurements in the test phase. The mediation analysis involving trust consisted of 205 participants because 1 outlier was removed. Twenty-three percent of the participants considered themselves male and the remaining 77% female. The groups were stratified for gender so that each group contained 5 to 6 males and 19 to 20 females. The amount of participants that started the experiment with an 'ON' cue or 'OFF' cue was not perfectly balanced because upon the construction of the randomization table a larger sample size was accounted for. Nevertheless, inspection of the descriptive statistics revealed that the ratio of participants starting with a different cue did not impact placebo analgesia (see Tables 1 and 2). The mean age of participants was 20.7 years old and the overall education level was high, with 90.3% of participants conducting a bachelor of science. The warmth threshold, pain threshold, and intensity (NRS rating) for low and moderate pain stimuli in the conditioning phase were not significantly different between groups (*P*-values ≥ .05), but the average difference between low and moderate NRS ratings was relatively small (overall mean ΔNRS = 2, see also Table 1).

Table 1. Demographic Variables, Temperatures for Thresholds and Pain Intensities, and NRS Scores for Trial Type During Conditioning

Groups	*CTRL*	*VS*	*C*	*OL*	*VS + C*	*VS + OL*	*C + OL*	*VS + C + OL*	*All*	*P-levels*
N§	26	26	26	26	26	26	24	26	206	–
Age§	21.0 (3.4)	20.2 (2.2)	21.2(3.5)	20.4 (2.9)	20.7 (2.9)	21.1 (3.5)	20.2 (2.8)	20.9 (3.3)	20.7 (3.1)	–
Gender (f/m)§	20/6	20/6	20/6	20/6	20/6	20/6	19/5	20/6	159/47	–
Education (high school/bachelor of science/master of science)§	0/25/1	0/22/4	1/23/2	0/24/2	0/25/1	1/21/4	1/22/1	0/24/2	3/186/17	–
Experiment language (EN/NL)§	10/16	12/14	11/15	13/13	12/14	12/14	15/9	15/11	100/106	–
Counterbalance order (ON/OFF)§	12/14	12/14	15/11	10/16	16/10	13/13	15/9	12/14	105/101	–
Warmth threshold (in °C)*	34.1 (.8)	34.0 (1.5)	34.2 (1.2)	33.9 (.8)	33.8 (.9)	34.1 (1.1)	34.1 (1.1)	33.8 (.9)	34.0 (1.0)	*P* = .396
Pain threshold (in °C)*	44.3 (2.6)	43.4 (3.1)	44.0 (3.7)	43.5(2.9)	44.0 (2.7)	44.9 (2.1)	44.0 (2.7)	43.2(3.5)	43.9(3.0)	*P* = .525
Low pain (in °C)*	47.0 (.8)	46.5 (1.2)	46.7 (1.6)	46.6 (1.1)	46.4 (1.1)	46.9 (.9)	46.8 (1.1)	46.7 (1.1)	46.7 (1.1)	*P* = .342
Moderate pain (in °C)*	48.5 (.6)	48.1 (1.0)	48.5 (1.3)	48.2 (.7)	48.2 (1.0)	48.3 (.7)	48.3 (.9)	48.4 (.8)	48.3 (.9)	*P* = .387
NRS active*	3.0 (2.0)	3.0 (2.1)	2.9 (1.7)	2.8 (2.3)	2.9 (2.2)	2.7 (2.1)	3.0 (1.9)	2.8 (2.0)	2.9 (2.0)	*P* = .860
NRS inactive*	4.4 (1.9)	4.2 (2.0)	4.5 (1.6)	4.3 (2.3)	4.7 (2.1)	4.1 (2.1)	4.8 (1.8)	4.7 (1.9)	4.5 (2.0)	*P* = .106

Abbreviations: C, classical conditioning; CTRL, control; OL, observational learning; VS, verbal suggestion.
NOTE. Descriptive statistics for demographics are shown as either counts§ or averages with standard deviations*. Statistical differences between groups for warmth threshold, pain threshold, low pain temperature, moderate pain temperature, and pain perception for active or inactive trials are shown as p-levels.

Table 2. Average (SD) Placebo Analgesia, Pain Expectancies, State Anxiety, and Trust Average Per Group

Group	*CTRL*	*VS*	*C*	*OL*	*VS + C*	*VS + OL*	*C + OL*	*VS + C + OL*	*P*
Placebo analgesia	.0 (.6)	.5 (1.3)	.1 (1.0)	.3 (1.6)	.6 (1.4)	.7 (1.5)	.7 (1.0)	1.2 (1.5)	< .001
Pain expectancies	.1 (1.4)	.4 (2.0)	.2 (2.0)	1.1 (1.9)	2.1 (1.6)	1.6 (1.5)	2.3 (1.7)	2.6 (1.9)	< .001
State anxiety (STAI)	45.1 (4.5)	44.1 (5.2)	44.2 (4.3)	46.0 (4.0)	44.2 (4.7)	44.5 (5.2)	45.1 (5.6)	45.3 (5.5)	.82
Trust	8.6 (1.6)	8.6 (1.3)	8.7 (1.6)	9.3 (.9)	9.1 (1.4)	9.3 (.7)	8.8 (1.2)	9.2 (1.3)	.17

Abbreviations: C, classical conditioning; CTRL, control; OL, observational learning; VS, verbal suggestion.
NOTE. Descriptive statistics of the placebo analgesia, pain expectancies, anxiety, and trust are shown as averages with standard deviations. Pain expectancies and State Anxiety were calculated as difference scores and larger differences meant that participants expected lesser pain during the experimental trial or were less anxious.

Difference Between the Influence of Learning Techniques on Placebo Analgesia

The mixed model ANOVA revealed a significant interaction effect between the group and trial type ($F[7,198] = 3.69$, $P < .001$, $\eta_G^2 = .019$), which indicated that there were significant differences in placebo analgesia between groups.

Placebo Analgesia Within Every Group

The descriptive statistics of the test phase per group can be found in Table 2. Analyzing the size of placebo analgesia for every group (ie, studying simple effects for trial type within groups) showed significant effects for the groups combining verbal suggestions and conditioning ($t[198] = 3{,}01$, $P = .003$, $\Delta NRS = .62$), verbal suggestions and observational learning ($t[198] = 3{,}31$, $P = .001$, $\Delta NRS = .68$), conditioning and observational learning ($t(198) = 3.38$, $P < .001$, $\Delta NRS = .72$), and the group with the 3 learning techniques combined ($t[198] = 5.92$, $P < .001$, $\Delta NRS = 1.22$). The remaining groups did not display a significant effect.

Primary Group Comparison

The size of placebo analgesia of the group with all learning techniques combined was significantly larger than the pooled placebo analgesic effect of the groups with one learning technique ($t[198] = 3.84$, $P < .001$, $\Delta NRS = .91$) (see Fig 3A). The size of the pooled placebo analgesic effects of the group with all individual learning techniques was borderline significant ($F[1,77] = 7.99$, $P = .006$, $\eta_G^2 = .01$).

Secondary Group Comparisons

The size of placebo analgesia between the group with 3 learning techniques combined did not significantly differ from the pooled placebo analgesic effect of the groups with 2 learning techniques combined ($t[198] = 2.29$, $P = .02$, $\Delta NRS = .54$), nor did the separate groups with 2 learning techniques significantly differ from the pooled placebo analgesic effect at the level of one learning technique (See Supplementary Material). Pooling the groups with 2 learning techniques yielded a significant placebo analgesic effect ($F[1,75] = 27.27$, $P < .001$, $\eta_G^2 = .06$). Pairwise comparisons showed that the group with all learning techniques combined induced significantly larger placebo analgesia than the conditioning group ($t[198] = 3.74$, $P < .001$, $\Delta NRS = 1.09$) and the observational learning group ($t[198] = 3.29$, $P < .001$, $\Delta NRS = .96$), but not the verbal suggestions group ($t[198] = 2.37$, $P = .019$, $\Delta NRS = .69$) (see Fig 3A). The group with all learning techniques combined also differed significantly from the non-specific effects of the control group ($t[198] = 4.32$, $P < .001$, $\Delta NRS = 1.26$), whilst the other experimental groups did not, nor did they differ from each other (P-values $\geq .05$).

Extinction

The assumption for sphericity was not met in the extinction analysis, hence Greenhouse-Geisser corrections were applied to the degrees of freedom. Placebo analgesia in the test phase diminished over the course of all pain trials, as was shown by the significant interaction between trial type and time ($F[9.7,1901.85] = 4.90$, $P < .001$, $\eta_G^2 = .003$). However, no significant difference in the rate of extinction between groups was discovered as was shown by the non-significant interaction between group, trial type, and time ($F[67.92, 1901.85] = .96$, $P = .58$, $\eta_G^2 = .003$). Testing for significant differences between every trial (active versus inactive) over time in the test phase indicated that placebo analgesia was strongest in the beginning for all groups, yet quickly diminished afterward (see Fig. 3B–3D).

In order to verify post hoc if the results from the extinction analysis were significantly impacted by the thermode switch, a sensitivity analysis was run with all heat pain scores from the test phase, including stimulus 14 and 15, entered in the ANOVA. The sensitivity analysis showed a significant interaction effect of trial type x time ($F[8.68,1702.16] = 4.24$, $P < .001$, $\eta_G^2 = .002$), again indicating extinction of placebo analgesia regardless of group. The interaction effect of group x trial type x time was not significant ($F[60.79, 1702.16] = .94$, $P = .618$, $\eta_G^2 = .003$, which was also in line with the results from the original extinction analysis.

Mediation by Pain Expectancies, State Anxiety and Trust

Pain expectancies differed significantly between groups ($F[7, 198] = 8.21$, $P < .001$, $\eta_G^2 = .23$). The linear regression analysis of path a revealed that participants in the groups

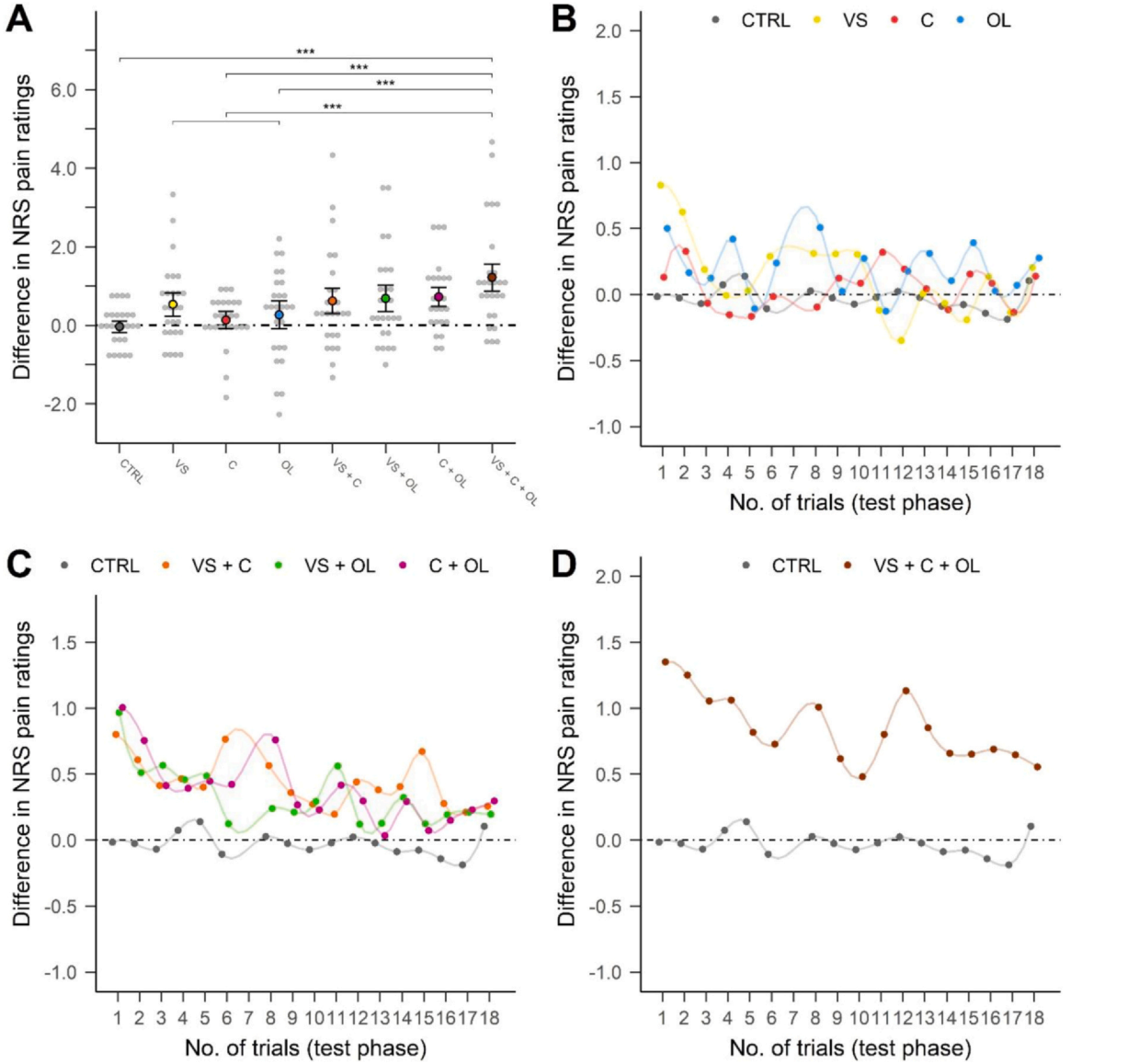

Figure 3. **(A–D)** Group comparisons and extinction analysis. The Y-axis represents the difference in pain ratings between active and inactive trials. The X-axis represents the 18 trials throughout the test phase (either experimental or control). Graph A shows the size of placebo analgesia (mean difference in NRS between the first 6 active and inactive trials ± 95% confidence intervals) in the test phase for every group. The dots depict the placebo analgesic effect per individual. The other 3 graphs **(B, C, and D)** depict the amount of placebo analgesia for every group over time. The extinction of placebo analgesia from the control group is compared to the groups with individual learning techniques **(B)**, the groups with combinations of 2 learning techniques **(C)** or the group with all learning techniques combined **(D)**. Data points for the extinction analysis represent the average size of placebo analgesia and the depicted errorbars are the standard error of the mean. ***= *P* value less than .001. C, classical conditioning; CTRL, control; OL, observational learning; VS, verbal suggestion.

with either 2 or 3 combined learning techniques developed significantly larger expectancies about pain reduction due to activation of the PPT as compared to participants in the control group (verbal suggestions, classical conditioning and observational learning versus control: t(198) = 5.17, $P < .001$, ΔNRS = 2.44, verbal suggestions and classical conditioning versus control: t(198) = 4.07, $P < .001$, ΔNRS = 2.00, verbal suggestions and observational learning vs control: t(198) = 3.21, $P = .0015$, ΔNRS = 1.57, classical conditioning and observational learning vs control: t (198) = 4.50, $P < .001$, ΔNRS = 2.25). The non-parametric bootstrap showed that the effect of the groups with 2 or 3 learning techniques compared to the control group on placebo analgesia was mediated by pain expectancies (verbal suggestions, classical conditioning, and observational learning versus control: ab = .49, 99.3% CI [.09–.89], verbal suggestions and classical conditioning versus control: ab = .39, 99.3% CI [.06–.72], verbal suggestions and observational learning versus control: ab = .31, 99.3% CI [.01–.60], classical conditioning and observational learning vs control: ab = .44, 99.3%CI [.08–.79]). The simple mediation models are visualized in Fig 4. The levels of state anxiety and trust were not significantly different between groups (F[7, 198] = 1.20, $P = .31$, $\eta_G^2 = .0$, and F[7, 197] = 1.50, $P = .17$, $\eta_G^2 = .05$ respectively). Subsequently, the effect of all 3 or 2 learning techniques combined compared to control on placebo analgesia was not mediated by either state anxiety or trust.

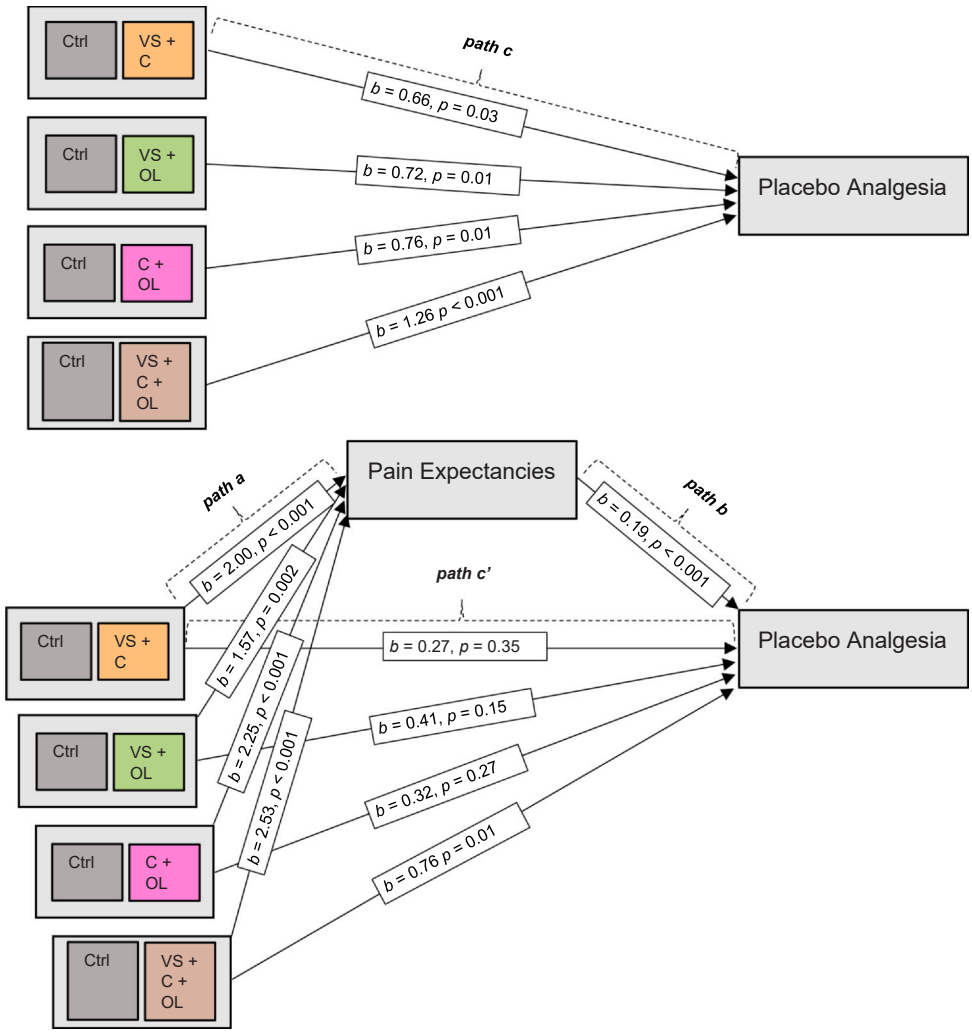

Figure 4. Total effect and mediated effect of groups with 2 or 3 learning techniques on placebo analgesia. Diagram A shows that when mediated effects are not taken into account, the group with all 3 learning techniques has a significant influence on placebo analgesia compared to the control, whereas the other groups do not. The second diagram shows the direct effect (path c') of the groups on placebo analgesia accounted for the indirect effect of pain expectancies (path a * path b). More specifically, all groups now show a significant indirect effect on placebo analgesia compared to the control group, which is mediated by pain expectancies. *P*-levels are adjusted to .007. C, classical conditioning; CTRL, control; OL, observational learning; VS, verbal suggestion.

Manipulation checks

Detailed results from the exit manipulation checks can be found in the supplementary materials. When the results from all groups but the control group were collapsed across conditions, the amount of participants that was convinced of the PPT lowering their pain ranged between 12% and 54%, whereas 29 till 76% of the participants did not think the PPT had any effect on pain, and 4 till 33% was uncertain. In the groups with a verbal suggestion, the amount of participants that believed the instructions ranged between 61% and 85%, whereas 15 till 27% did not, and 0 till 12% were uncertain. In the observational learning groups, the amount of participants that was convinced by the video ranged between 42% and 77%, whereas 8 till 47% of the participants were not, and 0 till 30% were uncertain. In the groups with conditioning, 19 till 54% of the participants had the idea that the PPT was working, whereas 29 till 54% did not, and 0 till 33% was uncertain. The mixed model ANOVA for all trials in the conditioning phase showed that, in every group, pain scores for the active and inactive trials differed significantly from each other (all $P < .001$), providing indirect evidence for successful conditioning in the groups with an experimental conditioning paradigm.

Discussion

In this study, the individual or combined influences of 3 different learning techniques (verbal suggestions, classical conditioning, and observational learning) on placebo analgesia were for the first time studied. More specifically, we aimed to study if combining different techniques would yield larger placebo analgesia than individual application. Placebo analgesia was significantly induced in the groups with 2 or 3 learning techniques combined, though this was not the case in the groups with an individual technique. The primary group comparison revealed that the combination of 3 learning techniques induced significantly larger placebo

analgesia than the pooled placebo effect by individual techniques. Exploratory follow-up analyses separating the individual techniques showed that this held for classical conditioning and observational learning, but not for verbal suggestions. Furthermore, the combination of 3 learning techniques did not induce significantly larger placebo analgesia than the pooled effect of 2 learning techniques, nor did any combination of 2 learning techniques when compared to the pooled effect of the individual learning techniques. Extinction of placebo analgesia occurred in every group regardless of the learning technique(s) and this was independent of the inclusion of 2 trials after the thermode switch. Moreover, results demonstrated the mediating role of pain expectancies (but not for state anxiety or trust) in the association between learning and placebo analgesia observed in the groups with 2 or 3 learning techniques. Our results provide important theoretical insights for experimentally and clinically-induced placebo-analgesic effects, showing for the first time the added value of combining 3 different learning techniques. Yet, some aspects require further evaluation.

In this study, the sizes of placebo analgesia observed in groups with 2 learning techniques were significant, yet they did not differ significantly from the (non-specific) effects observed in the control group or from the placebo effects in the groups with an individual technique. These results do not follow previous experimental placebo studies that discovered significant placebo effects when 2 learning techniques were compared to a control group or an individual learning technique group [5,14,49]. The null findings potentially demonstrate a lack of power and might have resulted from the insufficient sample size, although the current sample was able to detect a minimal significant effect of $\eta_p^2 = .07$. Furthermore, the variability in placebo analgesia among participants was relatively large compared to similar experimental studies [3,55]. This could have been the result of various factors including limited conviction toward the experimental manipulations as was indicated by the manipulation checks or inconsistent scoring of heat pain intensity which increases measurement error. A well-validated calibration method was however used to minimize inconsistent scoring of heat pain[12,55,59,63] and participants responding to placebo analgesia do not necessarily rate their heat pain more consistently than non-responders.[32] Although this study provides a decent indication of the fact that combining different learning techniques increases the magnitude of placebo analgesia, improving on experimental manipulations might help to strengthen the current results and translate them to a more clinical representation.

In order to explore the different components underlying the effect of learning on placebo analgesia, pain expectancies, state anxiety, and trust were studied as possible mediators. The significant mediating effect of pain expectancies on placebo analgesia discovered in the groups with 2 learning techniques or the group with all learning techniques contributes to earlier evidence that placebo effects arise due to the expectancy that a pain sensation is going to be decreased.[10,37,46,60–62] Interestingly, the group with verbal suggestions and classical conditioning, and the group with verbal suggestions and observational learning-induced significant indirect effects of pain expectancies on placebo analgesia when compared to the control group, whilst their total effects were non-significant. Such a finding is possible whenever there are undetected mediators that have a negative influence on the total effect of the group on placebo analgesia.[29] One of these undetected mediators, which has previously shown to negatively influence placebo analgesia, could have been anxiety.[26,39,57] However, in the current study no negative or positive mediating effects of state anxiety were discovered. One explanation could be that participants in all groups received verbal instructions in a calm way and were reassured that the pain stimuli would not cause any somatic damage throughout the experiment. This might have mitigated any group differences in state anxiety (ie, caused floor effects) regardless of the differences in content between the learning techniques (or their combinations).[53] Other studies previously found that related constructs, such as fear of pain, did predict nocebo effects or reduced placebo effects,[39,59] which warrants further research into this mediator. Regarding the other studied mediator of trust, the calming and reassuring suggestions among groups might also have impacted how much trust participants felt toward the experimenter. The results from the mediation analysis confirm that, like state anxiety, trust levels were similar in all groups and any effect of the learning techniques on placebo analgesia did not indirectly come about through trust. Overall, participants scored high levels of trust (8.5 points on average), which, apart from the earlier explanation, might also have been the result of response bias.[48] Participants filled out the trust scale in the presence of the experimenter, which could have persuaded them to score a socially-desirable answer. Regardless of these biases, findings from a recent experiment were in line with the current study, showing that trust did not seem to be involved in expectation effects on pain.[45] The results from this study indicate that placebo effects in behavioral experiments come about through expectancies, whereas the extent of mediation by certain negative emotions (ie, anxiety) or interpersonal relationship factors (ie, trust), which are always assumed to be involved in clinical practice, have to be further evaluated.

Enhancing placebo effects in clinical practice might be a desired outcome by clinicians and researchers to increase the efficacy of healthcare treatments.[24] Many studies have tried to unravel ways of eliciting these effects by studying the mechanisms behind them and looking for implementation strategies by means of different learning techniques.[2,3,5,14,16,18,20,23,30,36,52,57,61,62] The current study expanded knowledge about the interaction between the learning techniques and placebo analgesia by showing that combinations of particularly 3 ways of learning create more effect than merely studying the effects of individual techniques. Combining learning techniques does interestingly not make it more resistant to extinction, although this null

effect could also be due to a lack of statistical power. The results emphasize that the combination of different psychosocial aspects of clinical treatment, for instance, doctor-patient communication (verbal suggestions), patients' previous experiences (conditioning), and patients' peer groups (social learning) all have an important contribution to placebo-analgesic effects.[35] Humans involved in clinical treatments develop expectancies due to these different psychosocial aspects, yet combining these aspects helps to create even stronger expectancies.[46] Results of our mediation analyses eventually suggest that these expectancies play an important mediating role in the shaping of pain experiences through learning and deserve attention from clinicians in order to optimize their pain treatment.[47]

Limitations

As this study aimed at unraveling psychological mechanisms behind placebo effects, only healthy and mainly highly-educated participants were recruited. The necessary consistency in scoring heat pain for the conditioning paradigm further resulted in the pre-allocated exclusion of a considerable amount participants, although these were comparable in gender ratio ($P = .41$) and age ($P = .65$) to the included participants. Since placebo responders seem to vary considerably in their pain perception,[31] excluding inconsistent scoring participants could have impacted the generalizability of the results to clinical practice. Apart from the characteristics of the study population, the included size was lower than anticipated, which influenced the required power for the planned comparisons. Furthermore, blinding of the experimenter and the participant in this experiment was not possible, as both are aware of the fact that the participant receives an intervention that should have some effect on pain sensation. Finally, the control manipulations could have interfered with the experimental manipulations by creating opposing expectancies and thereby altering any placebo-analgesic effects. For instance, participants that underwent a control conditioning paradigm before receiving an experimental manipulation might have become more skeptical toward any analgesic properties of the placebo device. However, implementing control manipulations within this extensive experiment was carefully considered to prevent performance bias and has been successfully applied in previous placebo studies.[2,3] As such, the current extensive research design with the implementation of control and experimental manipulations is also one of the strengths of this experiment.

Conclusions

Overall, the results from our study significantly contribute to the current understanding of how to optimally induce placebo analgesia. Verbal suggestions, classical conditioning, or observational learning are ways of learning that can individually evoke expectancies leading to pain reduction. The current study showed for the first time that combining all these learning techniques significantly enhances placebo analgesia by boosting pain-relieving expectancies. Notwithstanding, it does not seem to make placebo analgesia more robust to extinction.

CRediT authorship contribution statement

J.P.A. van Lennep drafted the study protocol, conducted the majority of the experiments, extracted the study data, conducted the analyses, and drafted and finalized the manuscript, H. van Middendorp supervised J.P.A. van Lennep in the process, reflected on the research questions, and reviewed the study protocol and manuscript, D.S. Veldhuijzen contributed to the setup of the experimental procedure and reviewed the study protocol as well as the manuscript, K.J. Peerdeman reviewed the study protocol as well as the manuscript, M.A. Thomaidou contributed to the setup of the experimental procedure, assisted in data extraction procedures, and reviewed the manuscript, J.S. Blythe contributed to the setup of the experimental procedure, assisted in data extraction procedures and reviewed the manuscript, T. Heyman evaluated data analysis with J.P.A. van Lennep, reviewed the study syntax in Rstudio, and reviewed the manuscript,

A.W.M. Evers was the principle investigator, conceptualized the study aims, reviewed the study protocol, and reviewed the manuscript.

Disclosures

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Conflict of interest: The authors have no conflicts of interest to declare.

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Category

Randomized controlled trial.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jpain.2023.07.009.

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