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Individuals with chronic pain have the same response to placebo analgesia as healthy controls in terms of magnitude and reproducibility

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Editor-in-chief
PAIN

Dear Editor

We would like to thank your reviewers for their in-depth and thorough comments on our previous manuscript PAIN-D-19-01035R1. We hereby submit the revised new manuscript entitled "**Individuals with chronic pain have the same response to placebo analgesia as healthy controls in terms of magnitude and reproducibility"** by Andrea Power, Christopher Andrew Brown, Manoj Sivan, Ann Lenton, Timothy Rainey, Wael El-Deredy, Anthony Kenneth Peter Jones and Alison Watson.

The changes made in the revised manuscript have been highlighted. A separate document on responses to the reviewers' comments has also been included in this new submission.

We declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

We know of no conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

As the corresponding author, I confirm that this manuscript has been read and approved for submission by all the named authors.

Yours sincerely
Dr Manoj Sivan MD FRCP Ed
Corresponding author

***Reviews for Manuscript and author responses

Reviewer #1: Second review of "Experimental placebo analgesia is equivalent and reproducible in individuals with chronic pain when compared to healthy individuals" ms number: PAIN-D-19-01035R1

The revision has significantly improved the manuscript. This is a study with several strong points, especially that it is very well controlled, and that investigates a theoretically and clinically important problem. However, there are still some problems with the manuscript.

Author response: We thank the reviewer for their detailed feedback and have made appropriate updates as summarised below.

The English language in the text is much improved, but should still be looked over and corrected by a native English speaker.

Author response: We have reviewed the manuscript throughout and updated the language for clarity where needed.

Abstract, l. 40: why is the placebo effect a "meaningful" reduction in pain? A clinically significant reduction in pain, if that is what is meant, has in at least two studies been shown to be 1.3 units on a scale similar to the one used here, which is more than the placebo effect reported in this ms. I suggest to delete "meaningful".

Author response: We have removed the term “meaningful” as per the reviewer’s suggestion.

Design: sessions 1 and 2 should be included along with the other within-subject factors.

Author response: This detail has been added to the Design section (P5, Line 9).

Laser stimuli: Pain threshold and intensity can vary between the arms. Were the laser stimuli calibrated individually for each arm? This must be described more precisely.

Author response: This detail has been added to the Laser Stimuli section (P6, Line 5).

Reproducibility: I assume that the participants were in the same groups (control, placebo) in session 2 as they were in session 1, and this should be explicitly stated.

Author response: We have updated the manuscript with this detail as suggested (P9, Line 20).

Please be more specific on what the dressing is that covered the placebo cream?

Author response: More detail of the dressing has been added to the Conditioning Phase (Phase 2) section (P7, Line 20)

How long was the interval from the end of the conditioning phase till the start of the post-conditioning phase? In the studies known to me, the placebo treatment has been re-applied in this interval. Why was the cream not re-applied before the post-conditioning phase?

Author response: The post-phase was almost immediately after the conditioning phase. We have updated the manuscript with this detail in the Conditioning Phase section (P8, Line 20).

P. 9, l. 3: unconscious conditioning is referred to. How many of the participants had used pain-relieving cream previously?

Author response: We did not make a record of participants' previous use of analgesic creams, although this would have been useful data in retrospect. Our assumption is that unconscious conditioning could have potentially occurred, based on the experimental design rather than considerations of the participants' prior experience. We have updated the manuscript text here to clarify that unconscious conditioning could occur "due to the participants' experience of experimental pain reduction. (P9, Line16)"

Results, Placebo effect: the placebo effect was 0.74 points on an 11-point scale, and is described as "strong". Why is 0.74 a strong effect? Please provide the effect size, or compare to other studies (several studies have shown larger effects).

Author response: Unfortunately there are no widely accepted standardised effect size metrics (e.g. equivalent to Cohen's d or eta-squared) from linear mixed models and we cannot therefore use such metrics to compare to other studies. In addition, studies from other groups may not have used the pain scale in the same way (with the same anchors) used in this study. A more comprehensive comparison to other studies (e.g. based on meta-analysis of effect sizes) is beyond the scope of the current article and so we have removed the word "strong" to prevent confusion (P 13 Line 7 and P14 line 3).

Results, treatment x phase x arm interaction: it is stated that the 3-way interaction "modified the pain rating…by 0.26 points" (the beta value in table 3) compared to the treatment x phase interaction. I am not familiar with mixed models, but I do not understand this statement: I guess that pain ratings were modified by adding a third factor, arm, but how was pain modified? Increased or decreased? And where? Mean overall pain is of course the same whether you have two or three factors. And why is the beta-value of 0.26 mixed into this? My understanding of a beta value is not that it is directly translated into changes in a dependent variable (pain intensity).

Author response: We have added the following explanation to the supplementary materials to enable the reader to interpret the beta coefficients (we have also updated the Table 3 legend to summarise these points): "Coefficients in fixed effects models are interpreted in the same way as in ordinary least squares regressions. For the categorical predictors, the model generates dummy variables for the observed categorical predictors, one of which is used as the base/reference category. The regression coefficient is the *partial slope* of the linear relationship between the outcome variable (pain rating) and the part of a predictor variable that is independent of (uncorrelated with) all other predictor variables. The magnitude of the beta represents the change in pain rating associated with a change of value 1 in the predictor variable when all other predictor variables are held constant. Since we are dealing with the interpretation of the beta coefficients from categorical variables, and the betas reported are unstandardized (i.e. not adjusted according to the standard deviation of the variables), the betas have a meaningful interpretation in terms of the original pain scale." (Supplementary Materials).

To further elaborate for the reviewer with some examples: The intercept of the model (value of 6.08) is the value we would predict for the pain rating if all other coefficients (betas) were zero. Categorical predictor variables, for example "treat" in Table 3, are dummy coded such that the levels

change by one unit (for two levels, these values are 0 or 1). The beta for a one unit difference represents switching from one category to the other. The beta for this “treat” effect is then the average difference in pain rating between the category for which treat = 0 (the reference group/condition) and the category for which treat = 1 (the comparison group/condition). Although this is more complex to conceptualise for interaction effects, the beta for the categorical interaction predictor “treat:phase” (for example) is also the unit change in pain ratings explained by this interaction (this is also within the 0-10 range of the pain scale). In this case this means that the interaction changes the pain rating by a value on the 0-10 scale of -0.74. Just as in multiple regression, to interpret the direction of the relationship between variables, the signs (plus or minus) of the beta coefficients are important. If the beta coefficient is negative as in this case, then the relationship of this variable with the dependent variable is negative (e.g., the pain rating decreases due to the interaction).

With this in mind, we can interpret the the 3-way interaction of “treat:phase:arm”. In our original version of the analysis, “arm” was dummy coded such that the “treated arm” was the reference level (coded 0) and “untreated arm” had a value of 1. However, in the new Table 3 you will see that we have re-coded this variable so that untreated/treated have values of 0/1 respectively. This is to maintain consistency with the “treat” predictor variable where the reference level indicates “untreated”. Otherwise, as the reviewer correctly points out, interpretation of this value is potentially confusing. Now, the value of 0.26 has a negative sign - this indicates that adding “treated arm” to the model (specifically, to the “treat:phase” interaction) further decreases the pain rating by a value of 0.26. Please note that the new model has changed the statistics for the intercept term but aside from this and the change in sign for the arm terms, the statistics remain the same.

We have updated the Results test to help readers interpret these values: “This [3-way] interaction indicated that pain ratings changed (with the negative sign indicating a decrease) on average by a further 0.26 points (t = -3.14, p = 0.002, 95% CIs: -0.10 to -0.43, Table 3) due to the additional effect of the treated vs. untreated arm.”

Furthermore, I have a question regarding the 2-way compared to the 3-way interaction: judging from Figure 2, there clearly seems to be an interaction of treatment x phase, and table 3 supports this. Then the significant 3-way interaction is described on p. 12, and it is stated: "this indicates a placebo effect that is evident on both arms (treated and untreated) but is larger on the treated arm ". This statement seems to be wrong. Judging from figure 2, adding the "arm" factor does not seem to explain much more of the variance than the 2-way interaction does, as the results for the treated and untreated arm are almost identical whether the subject was in the control or placebo group. An interaction involving "arm" as factor suggests that these lines are not parallel, although figure 2 indicates the opposite. Please use follow-up tests to provide evidence that placebo effects are larger on the treated arm.

Author response: As shown in Figure 2, the slope of the line for the placebo effect (right plot) on the treated arm (filled circles) is steeper (more negative) compared to the untreated arm; conversely, for the control group the slope for the treated arm is more shallow (less negative) compared to the untreated arm. This is what gives rise the significant interaction effect. Although the relative difference in slopes is subtle by view of the figure, it is nevertheless clear that there is a difference that corresponds to a 0.26 point change as indicated in Table 3. Linear mixed models provide larger statistical power than ANOVA for detecting such effects. Follow-up tests could be conducted on pairs of means (e.g. placebo group, post-conditioning, treated vs. untreated arm) but this would not provide insight into the effect of “arm” on the placebo effect, since the placebo effect is defined by

the “treat:phase” interaction. We believe the 3-way the interaction effect is most easily interpreted by view of the relative relationships showed in Figure 2.

Also, there is no mention of the factor "arm" in the Discussion, although much of the Results is dedicated to this factor. If placebo effects are similar for both the treated and untreated arm, this suggests that the placebo effect is non-specific. In the literature there are data to support bot this view, and the view that placebo effects are specific and can be limited to a single arm or finger. This is theoretically very important and should be included in the Discussion.

Author response: This point has now been addressed in the Discussion: “Our results showed a placebo effect on both arms, which to a degree makes it a non-specific effect, although with a larger effect seen on the treated arm. In other words, our data suggests we have seen both arm-specific, as well as arm non-specific effects, which is one of the novel findings of this study.” (P20 Line8)

P. 17: Expectations did not predict placebo analgesia in any group of participants. The authors discuss that non-conscious conditioning could be a better predictor. This may be tested by subtracting the pain in the conditioning phase from the pain in the pre-conditioning phase (i.e. the unconditioned response), and correlating this with the placebo effect (the conditioned response). The literature on classical conditioning shows that there is a positive correlation between UR amplitude and CR amplitude, and we have observed the same in some of our studies. I would suggest this should be included in the Results.

Author response: We agree that this would add some insight to the results. The reviewer’s suggestion appears to be to conduct regression analyses of the conditioned response (conditioning phase mean pain ratings minus pre-conditioning phase mean pain ratings) predicting the placebo response (post-conditioning phase mean pain ratings minus pre-conditioning phase mean pain ratings). This would work if we assume no habituation effects over the 3 phases – but since we cannot assume this, we could expect significant regression effects as a result of greater habituation in some participants relative to others. Hence, the analysis would only be insightful if conducted on pain ratings data after subtraction of the data between the treated and untreated arms – this would provide a measure of the site-specific placebo effect that controls for habituation since both arms would be expected to habituate at the same rate.

Another factor to consider is whether the participant was in the placebo-treated group, or alternatively in the control group who went through the same conditioning procedure but were informed that the cream was not an analgesic. An unconscious conditioning effect might be expected to influence both groups equally, whereas a conditioning effect that depends upon congruent expectations (of pain relief) would only impact the placebo treated group.

We have conducted regression analyses (see updated Methods, Supplementary Materials, and Results sections) using linear mixed models to account for dependency in the data between the two study sessions, and including all diagnostic groups pooled together. The basic model included the (arm subtracted) conditioned response as a predictor of the (arm subtracted) placebo response. We also included the treatment group (treated vs. control) as a fixed effect to test for an interaction between the group and the relationship between the decrease in pain ratings during conditioning and the placebo response – this interaction was statistically significant ($p=0.024$). To explore this further two separate models were conducted as follow-up tests – one regression model per group (treated vs. control) with the conditioning pain reductions as the only predictor variable. We found that the conditioned pain decreases significantly predicted the placebo response only in the treated group ($p<0.001$) but not in the control group ($p=0.24$).

Overall, the results show that the change in mean pain ratings from pre-to-post conditioning can be explained by impact of the conditioning procedure only in the treatment group, suggesting that their conscious expectation of pain relief (reinforced by the conditioning procedure) was an important factor in the resulting placebo effect. This has implications for our discussion of conscious vs. unconscious processes and we have updated the discussion accordingly (P19 Line 24).

Reviewer #2: The authors addressed all my comments appropriately.

Author response: Thank you

(END OF REVIEWS)

Abstract

It is unclear whether a diagnosis of chronic pain is associated with an increase or decrease in the placebo response. The aim of this study was to use an experimental placebo conditioning paradigm to test if expectancy for pain relief impacts on acute pain perception in individuals with a chronic pain diagnosis of osteoarthritis (OA) or fibromyalgia (FM), compared to healthy individuals (HI). An inert cream was applied to the dominant forearm of participants (60 OA, 79 FM and 98 HI), randomly assigned to either a placebo or control group. In both groups an inactive cream was applied to the dominant forearm. The placebo group was told this may or may not be a local anaesthetic cream, while the control group was told the cream was inactive. Laser pain was delivered, and numerical pain intensity ratings collected before, during and after cream application, along with expectation of pain relief and anxiety. The procedure was repeated two weeks later to assess reproducibility. There was a significant reduction in pain in the placebo group, independent of clinical diagnosis. Diagnostic groups (OA,FM,HI) did not differ in their magnitude of placebo analgesia or expectancy of pain relief. The results were similar in the repeat session. The results demonstrate that individuals with chronic pain respond to experimental placebo analgesia in a similar and reproducible manner as healthy individuals, despite higher levels of psychological co-morbidity. This has implications for utilising placebo analgesia in the treatment of chronic pain.

Individuals with chronic pain have the same response to placebo analgesia as healthy controls in terms of magnitude and reproducibility

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Individuals with chronic pain have the same response to placebo analgesia as healthy controls in terms of magnitude and reproducibility

All changes in this revised draft have been highlighted

Introduction

Chronic pain carries a huge socioeconomic burden and substantially impacts the quality of life of affected individuals [13]. The lack of effective treatments for chronic pain, combined with our clearer understanding of underlying neurophysiological processes, has led to current research switching its attention from peripheral to central pain processing [41]. There is evidence of dysfunction within the endogenous pain inhibition system in most chronic pain syndromes [22,43]. This has resulted in the investigation of alternative techniques to utilise endogenous pain control mechanisms, for example placebo analgesia, which has been shown to have a significant impact on the subjective perception of pain [30].

The placebo effect, previously considered to be a nuisance variable, has since been shown to have substantial potential to improve patient outcomes [10]. Several commonly investigated techniques are used to induce placebo analgesia, with classical conditioning and manipulation of expectation being the most prominent [29].

In healthy individuals, placebo analgesia can be induced using a conditioning technique whereby the application of a sham anaesthetic cream is paired with the surreptitious lowering of an experimental pain stimulus. We and others have shown measurable and stable physiological and behavioural changes following such placebo (sham) treatments [14,31,46,47,53,54]. Placebo analgesia is reproducible in healthy individuals, and placebo responders tend to display particular cognitive traits, such as higher levels of dispositional optimism and reduced levels of state anxiety [33].

It has been suggested that placebo analgesia may be mediated by reduced negative emotional processing [3,4,35]. Pain normally increases negative emotions, which in turn increases the subjective experience of pain [37]. Conversely, analgesic administration induces the expectation of reduced unpleasant symptoms, which reduces anxiety and, consequently, actual symptoms of unpleasantness [3,4,15,38]. Chronic pain patients have psychological co-morbidities such as anxiety, depression, pain catastrophizing [32,48] and cognitive impairments [8]. For this reason, several models examining the role of expectancy and anxiety in modulation of pain by placebo have predicted reduced placebo analgesia in patients with chronic pain when compared to healthy individuals [33,35,32,48].

Existing data, however, shows that high levels of psychological co-morbidity do not affect the response to placebo in patients with chronic low back pain [5], and one study showed enhancing expectation improved the analgesic effects of acupuncture treatment in individuals with osteoarthritis [24]. In fact, a recent meta-analysis suggests individuals with chronic pain respond better to placebo analgesia than healthy individuals [16]. However, it should be noted that this conclusion was based on comparing average effect sizes from studies containing either pain patients or heathy individuals, but not having both groups within the same study.

In this study we aimed to examine the behavioural response to placebo analgesia in individuals with Osteoarthritis and Fibromyalgia when compared to pain-free healthy individuals, using a standardised, 2-stage conditioning technique (verbal suggestion and the adjunctive procedure of sham analgesic cream application), to optimise the analgesic effect. Furthermore, we explored

the reproducibly of a placebo effect in individuals with chronic pain over two sessions and explored the dependency of the placebo effect on the conditioning procedure.

Methods

Participants

The study was approved by the Greater Manchester West NRES Committee and the University of Manchester Ethics Committee. 60 patients with osteoarthritis (OA) and 79 patients with fibromyalgia (FM) were recruited from the Musculoskeletal Pain Clinics throughout the North West of England and from primary care practices that are part of the North West Primary Health Care Research Network. 98 pain-free healthy individuals (HI) with no history of ongoing pain symptoms were also recruited. Fibromyalgia patients fulfilled the 2010 American College of Rheumatology (ACR) criteria for the diagnosis of FM [53]. OA patients were diagnosed according to the standard ACR criteria [1]. All participants were over the age of 18, with no diagnosed neurological or morbid psychiatric illness, no peripheral vascular disease and no allergies to local anaesthetic creams (such as EMLA). Written informed consent was obtained prior to participation in the study. Handedness was not part of the inclusion criteria as we expected a typical distribution of left verses right handers.

Design

The experimental design included both between-subject factors and within-subject factors. Between-subject factors were the diagnostic categories (OA, FM, and HI) and whether the participant underwent the placebo (sham) treatment or a control experiment with no treatment. Regarding the treatment factor, participants within each of the three diagnostic categories (OA, FM, and HI) were randomized into one of two experimental groups: a Placebo (P) group or a control (C) group, undergoing either an experimental placebo procedure or a control procedure respectively on the right arm (see Figure 1 for details). Within-subject factors included the treatment session (1 and 2, on separate days) and the phase of the experiment (pre-conditioning, conditioning and post-conditioning).

Randomisation: Participants were randomised into treatment and control groups with a 2:1 ratio respectively. This imbalanced design sought to ensure that there were a sufficient number of participants in each of the sham-treated diagnostic sub-groups (minimum 35 per sub-group based on a power calculation) to obtain robust estimates of the intra-cluster correlation coefficient for each, while also allowing for attrition or other causes of data loss.

Laser stimuli

Laser heat stimuli (with a duration of 150 ms and a beam diameter of 15 mm) were applied to the dorsal surface of the both right and left forearms using a CO_2 laser stimulator. Between each pulse there was an inter-stimulus-interval of 10s. After each stimulus the laser was randomly moved over an area of 3 × 5 cm to avoid skin damage, habituation or sensitisation. The participants were asked to rate the stimuli verbally using a 0–10 Numeric Pain Rating Scale

(NRS) with 0 corresponding to no sensation, 4 corresponding to pain threshold, 7 moderate pain and 10 worst pain imaginable. Participants were trained to rate each stimulus prior to starting the experiment. Once the participants reached a moderately painful level (7 on the pain scale) the laser was stopped, and the energy required to elicit a rating of 7 was recorded. This procedure was repeated a minimum of three times for each arm to ensure that ratings remained reasonably consistent. The level 7 was set independently for each arm. Participants were then asked to rate their level of state anxiety using a 0 to 100 visual analogue scale (VAS) (0 no anxiety - 100 extreme anxiety), this was repeated at each treatment phase and at the end of the experiment.

Pre-experiment questionnaires

Prior to starting the experiment, participants were asked to complete the following questionnaires which were used to assess levels of psychological distress and other psychological variables that predict pain experience: the State-Trait Anxiety Inventory (STAI) [42], the Pain Anxiety Symptoms Scale (PASS) [28], the Hospital Anxiety and Depression Scale (HADS) [57], the Pain Catastrophizing Scale (PCS) [45], the Life Orientation Test (LOT-R) [40] and the Healthy Anxiety Inventory (HAI) [39].

Procedure

The procedure followed a strict script, followed verbatim for both the placebo and control groups: Participants were informed which group they will be in after signing the consent form. Participants in the placebo treatment group were told ‘you may or may not receive a local anaesthetic cream to your right forearm arm’. However, all participants in the placebo treatment group received

an inactive cream on both arms. Whereas participants in the control group were told explicitly that they would receive an inactive cream on both arms. Therefore, the right arm was conditioned within the placebo group and not in the control group. We have previously shown this to be a successful method of inducing placebo conditioning [52,53]. The procedure schematic is represented in Figure 1. In the following, the three experimental phases of the experiment are described as per the placebo (sham-treated) group.

Insert Fig 1 about here

Pre-conditioning phase (Phase 1)

Before application of the cream participants were asked to rate 20 laser stimuli using the NRS 0–10 pain scale - 10 stimuli to the dorsum of the left forearm and 10 stimuli to the dorsum of the right forearm. All pulses were delivered at the individually identified laser energy corresponding to the moderately painful level 7.

Conditioning phase (Phase 2)

Application of sham local anaesthetic (placebo) cream: Aqueous cream, containing paraffin oils was placed on a strip of occlusive, transparent, film dressing (Tegaderm, 3M Healthcare, St Paul, MN, USA). The cream, covered by the dressing, was then placed upon the entire laser stimulation area of both right and left forearms. The occlusive dressing held the cream in position, and this was left in place for 30 min during which time participants were told that the cream would take effect. The appearance of the cream and its application

(using the occlusive dressing), was similar to those commonly used for the local anaesthetic cream EMLA. This was important to reinforce conditioning. Each participant was then asked to rate their expectations regarding pain reduction by indicating on a “Expectation for Pain Relief Scale” using a 0 to 100 VAS (0 = no expectation of pain relief - 100 = high expectation of pain relief) [49]. After 30 min the dressing was removed, and the cream was wiped off.

Following removal of the cream, participants again received 10 laser stimuli to each forearm, but at a surreptitiously reduced energy level just on their right forearm. The reduced energy level corresponded to their individually identified non-painful level 3, while all pulses delivered to the left forearm were still maintained at a laser energy corresponding to the moderately painful level 7. The surreptitious lowering of the laser energy delivered to the right forearm (and not the left forearm) made it easier for participants to compare the sensation directly to their experience of the laser on the other arm. This was a method we had previously used and allowed us to identify site-specific effects vs. non-specific effects that generalise to other regions of the body [52]. This was intended to reinforce the perception that participants had received an active local anaesthetic cream to the right forearm only and that it was having an effect. Participants rated the laser pulses using the 0–10 NRS pain scale. Participants were then asked again to rate their level of anxiety and expectation of pain relief, before entering, almost immediately, into the post-conditioning phase of the experiment.

Post-conditioning phase (Phase 3)

In this post-phase, laser energy applied to the right forearm was surreptitiously raised to the pre-conditioning level, so both arms received laser energy at the participant's original level 7 (perceived to be moderately painful). Participants were asked to rate another 10 pulses on each forearm at this level.

Control group

The intention of the control group was to control for the effects of verbal suggestion on placebo conditioning. Participants in the control group experienced exactly the same procedure in *pre-phase*, *conditioning phase* and *post-phase* as the placebo group, the only difference being was that they were informed throughout the experiment that, i) the cream was inactive and ii) that the laser stimulus would be turned down to a non-painful level just on the right forearm, during the *conditioning phase*. By informing control participants that they were receiving an inactive cream, and that laser energy would be reduced, this controlled for the effects of verbal suggestion, but potentially allowed for unconscious conditioning effects (occurring due to the participants' experience of experimental pain reduction) that matched those of the placebo group.

Reproducibility

A least two weeks after the first session, participants returned for a repeat session. The two sessions are henceforth referred to as Session 1 and Session 2. In Session 2, participants were assigned to the same group and given exactly the same treatment and instructions as in Session 1. To minimise carry-over effects from the first session, all participants were informed that the

treatment they received in the first session would not impact upon the effects of the treatment they received in the second session.

Data analysis

We used linear mixed effects models (LMMs) to test for the presence of placebo effects and to test the hypothesis of group differences in placebo effects. Details of the models used are in Supplementary Materials. The "phase" effect (change in pain ratings from phase 1 to phase 3) was included to test for placebo effects, with "arm" (treated and non-treated) as a separate fixed effect that was expected to interact with phase. We also examined whether there was an effect of session. Analyses adjusted for the potential confounding effect of the pain rating reported during conditioning (phase 2) of the experiment by using the mean pain rating from phase 2 as a participant-level fixed-effect covariate. We also controlled for the laser energy used to elicit pain during the experiment, and participants' age and gender, as fixed effects. Participants were treated as random effects to allow overall pain ratings and placebo effects to vary across participants (see Supplementary Materials for details). Because we used LMMs, we examined the distribution of the residuals of the pain ratings were fitting to the model. Normal probability plots showed that the distribution of the residuals was normal with approximately equal variance over conditions and groups. All statistical tests were 2-sided, with $\alpha = 0.05$. Analyses were performed using both R and Matlab 2019a (MathWorks Inc.) softwares.

Intraclass correlation coefficients (ICCs) were calculated to examine the test–retest reliability of the placebo effects between the two sessions, using

data from the treated arm only. For this calculation we used LMMs to identify variance components for the random effects (participants) and model error term. Further details are in Supplementary Materials.

In addition to investigating placebo effects on pain ratings, we also tested for conditioning-induced changes in anxiety and expectation, primarily focussing on ratings collected both immediately prior and subsequent to conditioning (Anx3 to Anx4, and Exp1 to Exp2 – see Figure 1). Similar LMMs were fitted to this data as per the placebo pain ratings models (model details in Supplementary Materials). We also tested for group differences and trends over time in anxiety ratings over the whole experiment (Anx1 to Anx5). Finally, we tested whether these state variables influenced the response to sham treatment by fitting additional pain rating models that included these state variables (expectation and anxiety) as either main effects or interactions with experiment phase (pre to post-conditioning).

Finally, we investigated whether the conditioning procedure was effective at inducing placebo responses by using LMMs to compare the groups (placebo vs. control) in the extent to which decreases in pain ratings measured during the conditioning procedure predicted decreases in pain ratings in the post-conditioning phase. Importantly, the analysis focussed on site-specific placebo responses by first subtracting mean pain ratings on the untreated arm from those on the treated arm; this also provided control over the confound of habituation effects that might otherwise cause a spurious correlation between conditioning phase and post-conditioning phase pain ratings (caused by different participants habituating at different rates) – since both arms would be

expected to habituate at the same rate. Further details of the models are in Supplementary Materials.

Results

Characteristics of the diagnostic groups

237 participants completed the study (60 OA, 79 FM and 98 HI). A summary of characteristics is shown in Table 1.

Insert Table 1 about here

Laser energy

The energy levels required to elicit a moderately painful subjective level 7 rating were similar between OA and HI groups (HI=17.85±3.1 mj/mm^2, OA=18.8±3.36 mj/mm^2, p=0.06), but significantly lower in FM participants (FM=16.2±4 mj/mm^2, HI vs FM p<0.001, OA vs FM p<0.001). Hence, these values were included as a nuisance covariate in statistical analyses of placebo effects.

Psychometric tests

One-way ANOVA on the diagnostic groups showed a highly significant effect of diagnosis on certain psychological variables that had previously been shown to predict pain experience (p<0.001; Table 2), with OA, FM and HI groups all showing significantly different outcomes. Post-hoc data indicated that FM patients reported significantly increased levels of psychological distress than their OA and HI counterparts in almost all examined outcomes, except in

pain anxiety symptoms where FM and OA displayed similar outcomes (PASS avoidance, p=1; PASS fearful thinking p=0.074).

Insert Table 2 about here

The placebo effect

There was a placebo effect evident from a significant 2-way interaction between treatment group and phase of the experiment, indicating that pain ratings were more decreased, on average, by 0.74 (out of 10 on the numerical rating scale) from pre to post-conditioning in the placebo treatment group compared to the control group ($t = -5.14$, $p < 0.001$, 95% CIs: -0.99 to -0.50, Table 3). Additional but weaker variance in pain ratings was accounted for by a 3-way interaction between treatment group (placebo treatment vs. control), phase of the experiment (pre vs. post conditioning) and arm (treated vs. untreated). This interaction indicated that pain ratings changed (with the negative sign indicating a decrease) on average by a further -0.26 points ($t = -3.14$, $p = 0.002$, 95% CIs: -0.10 to -0.43, Table 3) due to the additional effect of the treated vs. untreated arm. Overall, this indicates a placebo effect that is evident on both arms (treated and untreated) but is larger on the treated arm. These effects are plotted as fitted group means and CIs of the mean in Figure 2. A Likelihood Ratio Test provides strong support for a model of the pain ratings that includes these interactions compared to a control model without these interactions (LR = 152.6, $p<0.001$; comparison of model 2 vs. model 1 in Supplementary Materials).

Insert Table 3 and Figure 2 about here

Although, on average, there was a significant placebo effect, there was considerable variation between participants, evident from the random effect coefficients. The random effect (i.e. participants) for the phase slope (change from pre to post-conditioning) in the model, indicating individual variability in the magnitude of the placebo response, had a standard deviation of 0.89 (95% CIs: 0.80 to 0.98) points on the 0-10 rating scale, even after accounting for the fixed effect of treatment group in the model. This indicates a considerable variability between participants in the placebo response within the treatment and control groups.

However, we did not find evidence that the placebo effect varies significantly across diagnostic groups (OA, FM, HI). Firstly, a Likelihood Ratio Test did not favour strongly enough a model in which diagnostic group interacted with the factors of treatment group, experiment phase, and arm treated (LR = 23.2, p = 0.06). Secondly, results from the model including these interaction terms involving diagnostic group did not reveal any significant interactions. Violin plots (Figure 3) show that all three diagnostic groups who were treated with placebo (i.e. sham treatment groups) showed lower levels of reported pain following application of the placebo cream compared to their control group counterparts, and this was the case in both sessions.

Insert figure 3 about here

Test-retest reliability of the placebo effect

We tested a further mixed model (model 4 – see Supplementary Materials) in which interaction terms were added involving the factor Session. A Likelihood Ratio Test found that this did not improve model fit, suggesting that placebo effects were not depending on the experimental session. To formally investigate test-retest reliability of the placebo effect over sessions, intra-cluster correlation coefficients (ICCs) were calculated on mean pain ratings post-conditioning, after adjusting for pre-conditioning mean pain ratings. The ICC for the sham treatment groups was 0.77, indicating moderately good test-retest reliability, while for the control groups the ICC was weak at 0.21. Breaking down the ICC for the treatment groups into diagnostic sub-groups, the ICCs for the OA, FM and HC groups were 0.78, 0.80 and 0.72 respectively. To visualise the relative difference in placebo effects from session 1 to session 2, Figure 3 plots lines indicating the placebo effect across sessions, for each participant.

Expectation of pain relief

Expectation of pain relief was reported by participants twice during the experiment (pre and post cream application), and the change in expectation ratings was analysed (Exp1-Exp2). There was a clear effect of placebo treatment ($p<0.001$, Figure 4, Table 4), however no effect of session or diagnosis.

Insert Table 4 and Figure 4 about here

Anxiety

Anxiety levels were measured at 5 time-points (Anx1 to Anx5, Table 5) throughout the experiment. There was a clear effect of diagnosis on anxiety ($F=27.47$, $p<0.001$, Figure 4), with FM patients rating higher anxiety levels than both OA and HC participants throughout the experiment (Figure 5). There was also a significant linear trend over time in anxiety ratings from Anx1 to Anx5 ($F=16.98$, $p<0.001$) and anxiety ratings were overall lower in session 2 compared to session 1 ($F=10.55$, $p=0.001$). However, after adjusting for pre-treatment anxiety levels, there was no indication that changes in anxiety during the conditioning phase (i.e. change in anxiety levels between time-points Anx3 and Anx4) were specifically influenced by diagnosis, treatment or session (Figure 4, Table 4).

Placebo effect – Independent of expectation and anxiety

The placebo effect did not appear to be predicted by, or mediated by, expectation or anxiety. Specifically, we found that adjusting for either the baseline levels, or for the pre-post conditioning changes, in expectation or anxiety within the model of placebo effects did not remove the placebo effect. Likelihood Ratio Tests did not favour models that included these variables as either main effects or interacting with terms in the model that included phase (pre vs. post treatment).

Insert Table 5 and Figure 5 about here

Differential prediction of the placebo response by conditioning responses in placebo and control groups

Additional linear mixed models tested for the prediction of site-specific placebo responses (defined by relatively greater decreases in mean pain ratings on the treated vs. untreated arm) from the reductions in pain experience during conditioning (see models 9 to 11 in Supplementary Materials). We initially tested (model 9) whether the linear relationship between conditioning decreases in pain and placebo responses differed between the placebo and control groups. The rationale is that since the control group went through the same conditioning procedure as the placebo group, but were informed that the cream was not an analgesic, this analysis provides further insight into the role of conscious expectation in the efficacy of the conditioning procedure. Specifically, if conditioning required only unconscious processes, the relationship between conditioned and placebo responses would be expected not to differ between groups. However, we found that there was a statistically significant interaction between the fixed effect of group and the conditioning phase decrease in pain in the model (beta = 0.12, 95% CIs: 0.02 to 0.22, t = 2.27, p = 0.024, Table 6). To explore this further, two separate models (models 10 and 11) were conducted as follow-up tests, showing that the conditioning-related decreases in pain significantly predicted the placebo response only in the treated group (beta = 0.20, 95% CIs: 0.13 to 0.27, t = 5.93, p < 0.001, Table 6) but not in the control group (beta = 0.05, 95% CIs: -0.03 to 0.12, t = 1.18, p = 0.241, Table 6). For illustration purposes, the relationships are plotted in Figure 6 using only the data from session 1 (for which there is the largest sample of data). Overall, the results show that the placebo response can be explained by the impact of the conditioning procedure only in the placebo group,

suggesting that the effect of conditioning on the placebo effect depends upon congruent expectations (of pain relief) that were only present in that group.

Insert Table 6 and Figure 6 about here

Discussion

We investigated the reproducibility of placebo analgesia across patients with OA and FM, as well as pain-free HI. We also studied whether expectation of pain relief, psychological distress and changes in anxiety during the procedure were predictors of placebo responses. Lastly, we tested whether placebo responses could be predicted from decreases in pain during conditioning. We found that despite psychological co-morbidities, patients with OA and FM displayed similar experimental placebo analgesic responses to their HI counterparts. This included similar levels of reproducibility and expectations of pain relief. While findings suggested that the placebo response is not predicted or mediated by participants' judgements of their changes in expectation or anxiety, we did find that it was only in the placebo group (who had a conscious expectation of pain relief) that there was a relationship between conditioning and post-conditioning reductions in pain, suggesting that the placebo effect depends upon congruent expectations of pain relief.

Previous evidence for the efficacy and reproducibility of placebo analgesia mainly exists from experimentally evoked pain studies in healthy individuals [2,9,14,51,52,54]. Whereas, evidence for positive placebo analgesic responses and reproducibility in individuals with chronic pain primarily comes from the placebo arm of pharmacological randomised controlled trials

[8,20,21,56]. Here we have shown that placebo responses to experimentally induced pain are as equally reproducible in individuals with chronic pain, as in healthy individuals. As far as we are aware, this is the first study to compare responses between these groups within the same study.

Placebo responders and the placebo response have been previously linked to cognitive traits, such as lower levels of state anxiety [31] and reduced negative emotional processing [3,35]. In contrast, the psychological co-morbidities associated with chronic pain conditions, such as depression and anxiety, have been implicated in reducing the effectiveness of placebo analgesia in chronic pain patients [33,30,46]. Based on this, we anticipated that OA and FM patients would exhibit a reduced placebo response compared to their HI counterparts, but in our study this was not the case.

Correlations between changes in expectation with the magnitude of placebo analgesia have previously been observed [34,36]. This is thought to occur because sensory experiences such as pain are influenced by the interaction between expectations (e.g. of pain relief) and sensory information, such as nociception. Because expectancy is believed to play a significant role in initiating placebo effects, individual differences in placebo analgesia may also be driven by differences in expectancy. Where placebo responses are large for a particular individual or situation, this can be seen as a triumph of expectation over current sensory information [32]. Interestingly, while participant ratings of expectation or anxiety did not predict placebo responses, there was only a relationship between conditioning phase and post-conditioned reductions in pain ratings in the placebo group. This is consistent with the view of previous researchers that conscious expectations may interact with

unconscious conditioning processes for placebo effects to be realised [44]. Insight on the discrepancy between these two analyses comes from recent debate about the role of predictive coding schemes in the brain [7] in which prior experiences (e.g. conditioning) may generate 'unconscious predictions'. If partially unconscious, participants may not accurately report on their expectations but may nevertheless by influenced in their perception and reporting of pain. This may also account for the placebo response observed on the untreated arm in our study: our results showed a placebo effect on both arms, which to a degree makes it a non-specific effect, although with a larger effect seen on the treated arm. In other words, our data suggests we have seen both arm-specific, as well as arm non-specific effects, which is one of the novel findings of this study.

Consistent with our observation of equivalent placebo responses between diagnostic groups, expectation of pain relief, and relative increases in expectation through conditioning, were also similar across diagnostic groups and healthy controls. This suggests that, despite living with chronic pain, individuals with OA and FM still have normal expectancy of pain relief, as well as normal updating of expectancy by conditioning. This is of interest because, although it was not assessed in our study, individuals with chronic pain are more likely to have numerous (positive or negative) healthcare experiences compared to healthy individuals [13], which could affect the expectation of pain relief in future treatments [17]. With our chronic pain cohort, their history of pain, previous treatment experiences, and the refinement of their expectations and beliefs that may result from those experiences, did not appear to interfere with the efficacy of their placebo response.

We also examined anxiety levels during the placebo/sham and control procedures. Unlike expectations of pain relief, we saw a significant difference in baseline levels of anxiety between diagnostic groups. This is consistent with the known psychological comorbidities often experienced by patients with chronic pain, including anxiety and depression [11], pain catastrophizing [19] and cognitive impairments [7,18]. While previous studies suggest that psychological symptomatology can predict the magnitude of the placebo response, but our findings have been contradictory. Wasan et al. found that high levels of psychopathology, including pain-related anxiety, are associated with *heightened* placebo analgesia in chronic lower back pain patients [49], while Lyby showed that increased levels of stress and fear of pain *reduces* the placebo analgesic response in healthy participants [27]. We have previously reported that low state anxiety is a significant predictor of placebo response in healthy individuals [33]. Contrary to these findings, our current data shows that although anxiety levels between diagnostic groups varied significantly, the placebo response did not. Although FM patients showed higher levels of anxiety than both OA and HI participants throughout the experiment, the FM placebo response was no different. The disparity to previous literature could be due to differences in the way participants were selected, differences in the methods used to assess psychological variables (we assessed anxiety whereas Lyby assessed fear of pain and stress), or differences in the placebo paradigm (e.g. our study used conditioning and Wasan's study did not) [49,27].

Throughout our experiment we observed a significant linear trend in the reduction of anxiety in all diagnostic groups over time (highlighted in Figure 5). Anxiety levels further reduced in Session 2. Lu et al. also reported similar

reductions in anxiety levels in IBS patients [26], but our study is the first, of which we are aware, that observes equivalent changes in anxiety in OA and FM patients. It is also of interest to note that any decrease in anxiety was not specific to the placebo treatment, as it was also evident in the untreated control groups. Previous research has also failed to elucidate a clear and consistent relationship between changes in anxiety and placebo analgesia. Some evidence suggests changes in anxiety might contribute to the placebo analgesic response [12], while other evidence suggests placebo analgesia is the causal factor in a reduction in state anxiety [25]. While the mechanistic contribution of changes in anxiety are unclear, our results do indicate that changes in anxiety are not dependent on initial levels of anxiety, a finding that has not been previously reported.

A limitation of our study is the possibility of selection bias that might limit generalisation of the findings. Specifically, a meaningful proportion of patients in our study were recruited from the same rheumatology clinic, which means our results apply to a specific demographic. For instance, patients from the same area may have had similar healthcare experiences, which has been shown to alter expectations [17].

Regarding clinical implications, our results suggest that individuals with FM and OA can modulate their responses to experimental pain as efficiently as healthy individuals. In this context, given equally efficient endogenous analgesia across diagnostic groups, our findings suggest potential for the exploration of new treatment strategies that enhance the placebo response, or otherwise utilise endogenous analgesia in these patients, in order to improve treatment outcomes.

The finding that experimental placebo responses in both OA and FM groups were reproducible has some potentially important implications for clinical trial design. The variability of placebo response between individuals is a major problem, particularly for early or small clinical trials of analgesics [12]. One approach to this has been to exclude placebo responders during a pre-screening process (run-in trials) [25]. However, this results in conducting trials on non-representative populations of patients. An alternative strategy is to balance the number of placebo responders in each arm using an experimental placebo procedure to screen for responders/non-responders. However, the validity of the screening procedure depends on how reproducible the placebo response is. Our results show that experimental placebo analgesia is sufficiently reproducible to justify exploring these approaches for balancing placebo responders across arms of a clinical trial.

In summary, by using standardised method for inducing placebo analgesia, we found similarly reproducible changes in expectancy, anxiety and pain experience, as a result of experimental placebo, in individuals with OA and FM compared to pain-free healthy volunteers. Treatment approaches seeking to maximise placebo analgesia may therefore have equal chance of success in both OA and FM populations.

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Conflicts of interest

The authors declare no conflict of interest, including competing financial interests.

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Figure legends

Figure 1:

Study design over time showing the various points at which participants rated laser intensity (Laser Phase 1-3), anxiety levels (ANX1-5) and expectation of pain relief (EXP1-2). The difference between the experimental groups [placebo treated arm (A), placebo untreated arm (B), control treated arm (C), control untreated arm (D)], depended on how the laser energy in phase 2 was manipulated. In all cases inert aqueous cream was applied to both forearms. In the placebo group the right forearm was conditioned to associate the cream with pain reduction by surreptitiously reducing the laser energy level (A), while the left forearm received the painful laser energy (B). The control group were told explicitly that the cream was inert and that the laser energy would be reduced on their right forearm (C), thereby eliminating the effects of conditioning, while their left forearm received the painful laser energy (D). Laser pulses lasted 150 ms, with a beam diameter of 15 mm. Energy levels required to elicit a level 7 response on the pain rating scale ranged between 6.1 and 27.6 mj/mm^2.

Figure 2. Plot showing a change in pain rating due to the interaction between treatment (placebo vs control), phase (pre vs post conditioning phase) and arm (treated vs. untreated). Here we see a clear placebo effect on both arms (treated and untreated) which is greater on the treated arm which received conditioning. Circles are fitted values and error bars are 95% confidence intervals

Figure 3. Violin plots of subject means change in pain rating (phase subtracted P1-P3, arm 1 only) shows reduced levels of reported pain in the placebo group

following cream application than in the control group. This effect was reproducible in both sessions. Group, treatment and session effects are displayed as violins, with session 1 & 2 paired with lines. A perfectly horizontal line for a participant would indicate the same magnitude of placebo response; a slope to the line indicates a change in magnitude. OA = osteoarthrosis, FM = fibromyalgia and HI = healthy individual groups.

Figure 4. Descriptive plots showing change in pain (A), expectation (B) and anxiety (C) following the placebo and control cream in osteoarthrosis (OA), fibromyalgia (FM) and healthy (HI) groups. A. Change in Pain Rating between Phases P1 and P3 shows greater changes in pain across all diagnostic groups following the placebo opposed to the control treatment. (B) Change in Expectation of Pain Relief between EXP1 and EXP2 shows an increased change in expectation following placebo treatment. Here lower values indicate a greater increase in change and (C) the Change in Anxiety Rating between ANX3 and ANX4 time-points shows no difference between Placebo and Control treatment groups. Error bars indicate SEM.

Figure 5. A descriptive plot showing reported VAS anxiety levels at 5 time-points, ANX1-5, during the experiment. Osteoarthrosis patients (OA) and healthy individuals (HI) reported similar levels of anxiety at all 5 time-points during the experiment, while fibromyalgia (FM) patients rated significantly higher anxiety levels throughout. Error bars indicate SEM.

Figure 6. Scatter plots (with least-squares lines) showing site-specific decreases in pain from the conditioning phase as a predictor of the site-specific placebo response in each group (placebo and control). The site-specific placebo response is calculated here as the mean pain ratings (for the treated

arm minus the untreated arm) in the post-conditioning phase minus the pre-conditioning phase (P3-P1). Similarly, the site-specific decrease in pain during conditioning is calculated as the mean pain ratings (for the treated arm minus the untreated arm) in the conditioning phase minus the pre-conditioning phase (P2-P1). A significant prediction is only observed in the placebo group (for statistics, see models 10 and 11 in Table 6).

- We found equivalent and reproducible changes in expectancy, anxiety and pain experience as a result of an experimental placebo procedure in individuals with OA and FM compared to pain-free healthy volunteers.
- The placebo effect did not appear to be predicted by or mediated by expectation or anxiety.
- Treatment approaches seeking to maximise placebo analgesia may have equal chance of success in both OA and FM populations.

Figure

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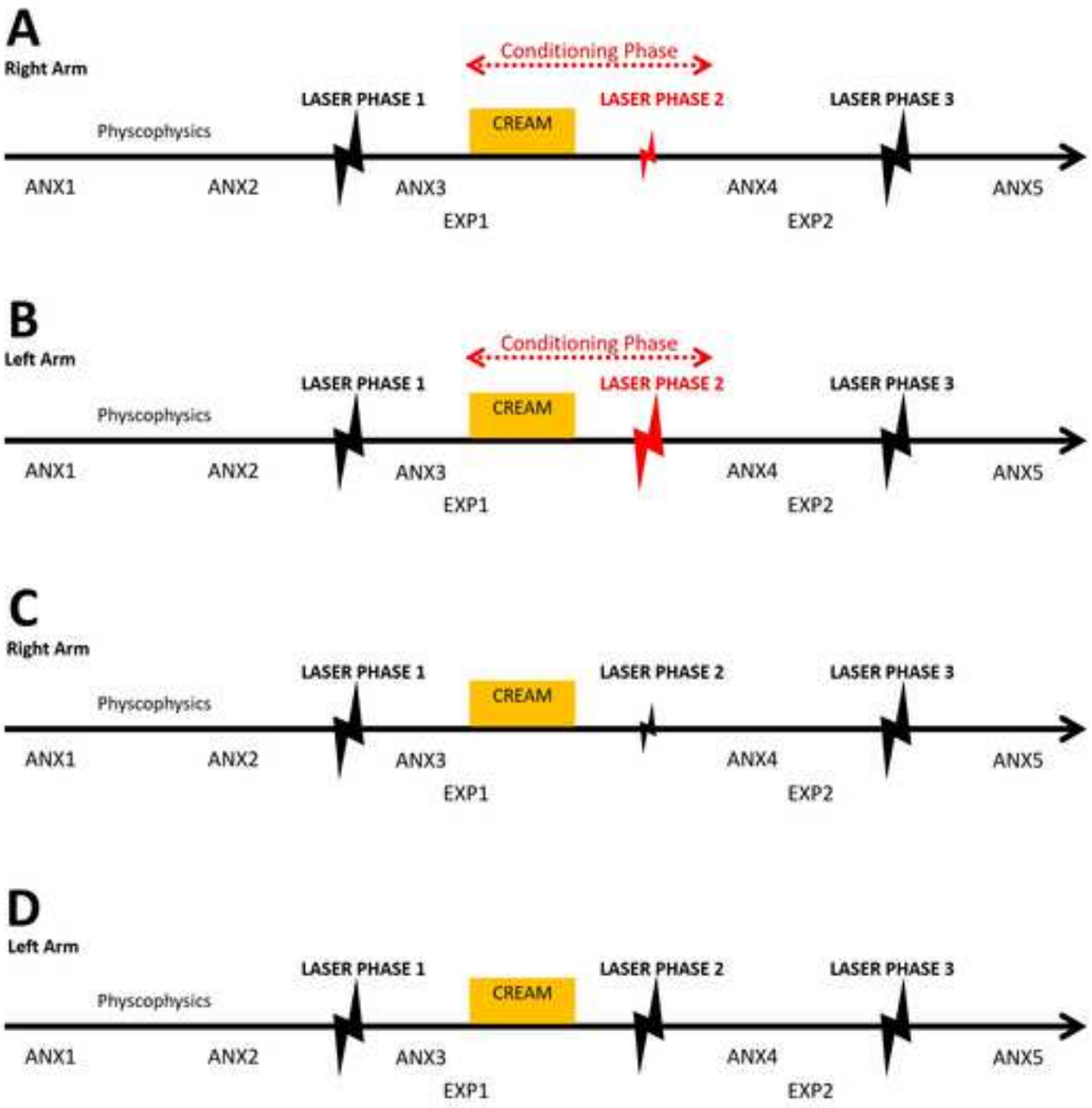

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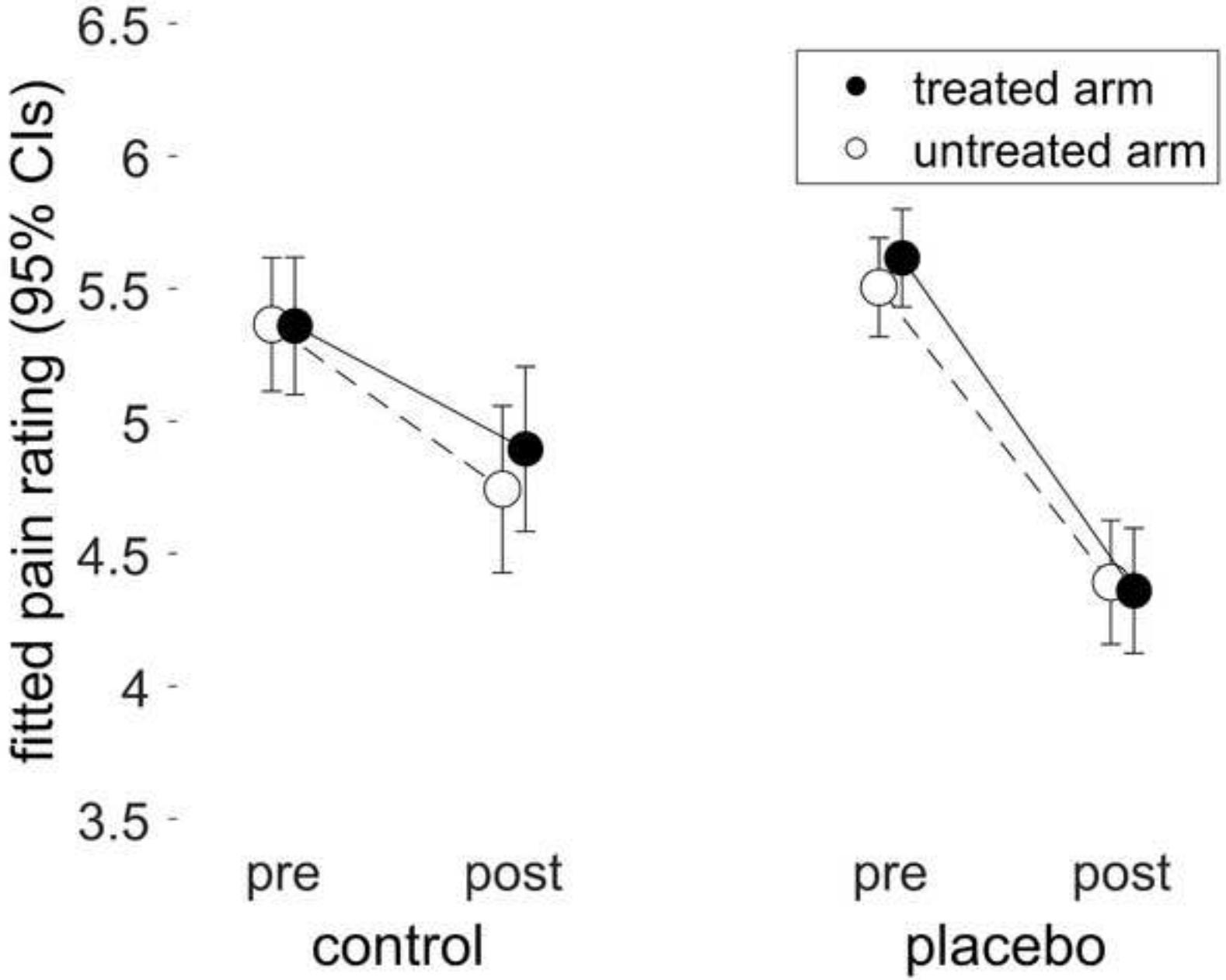

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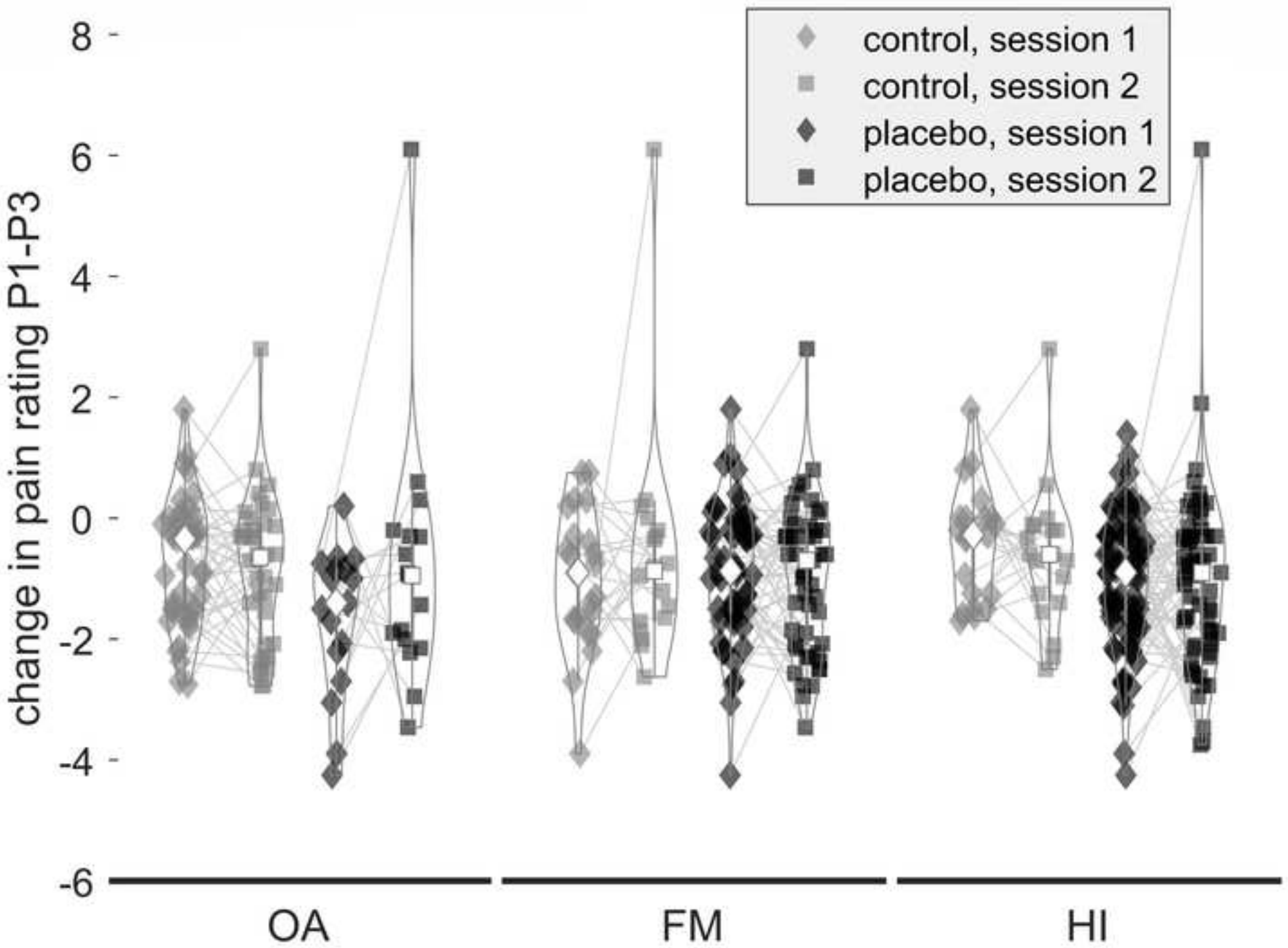

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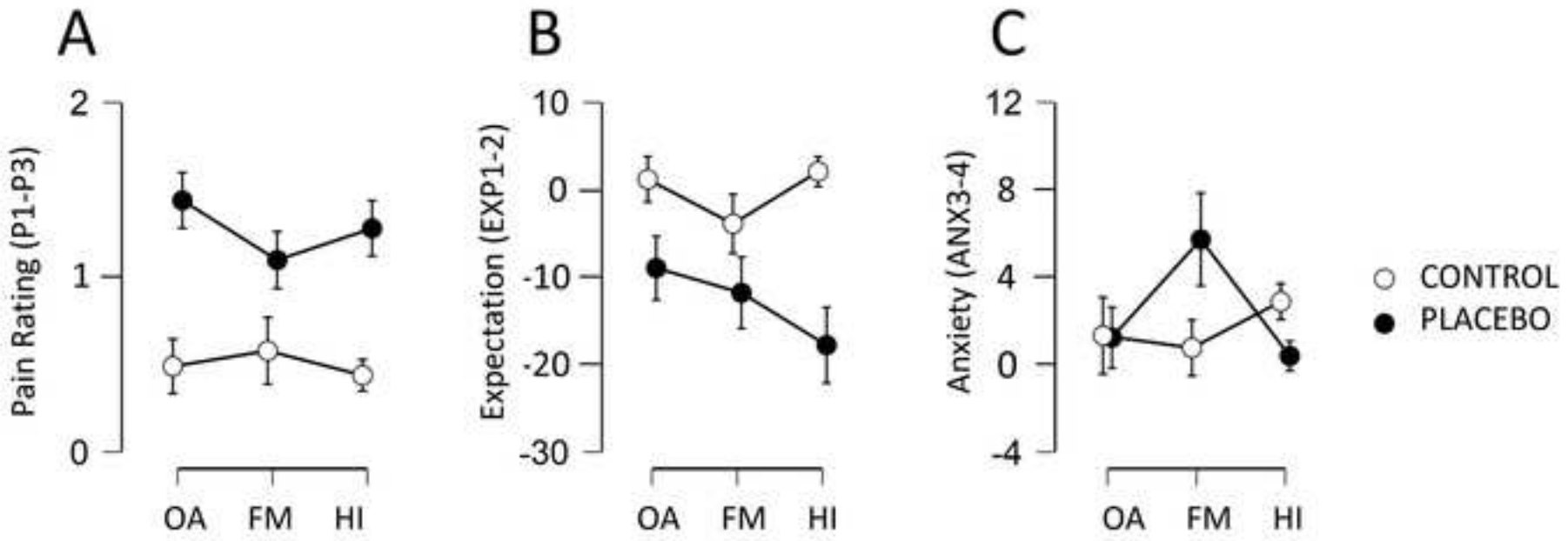

Figure

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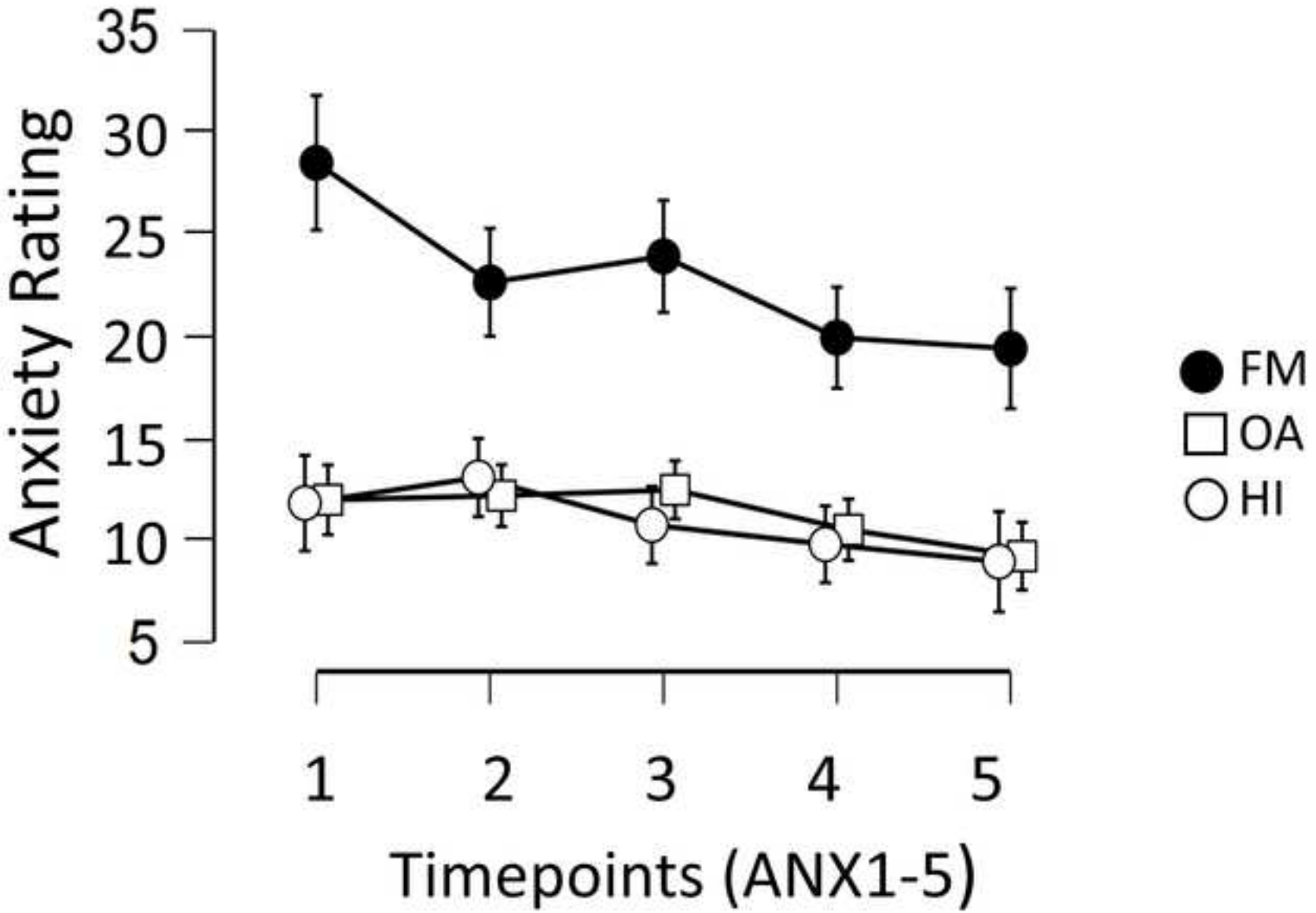

Figure

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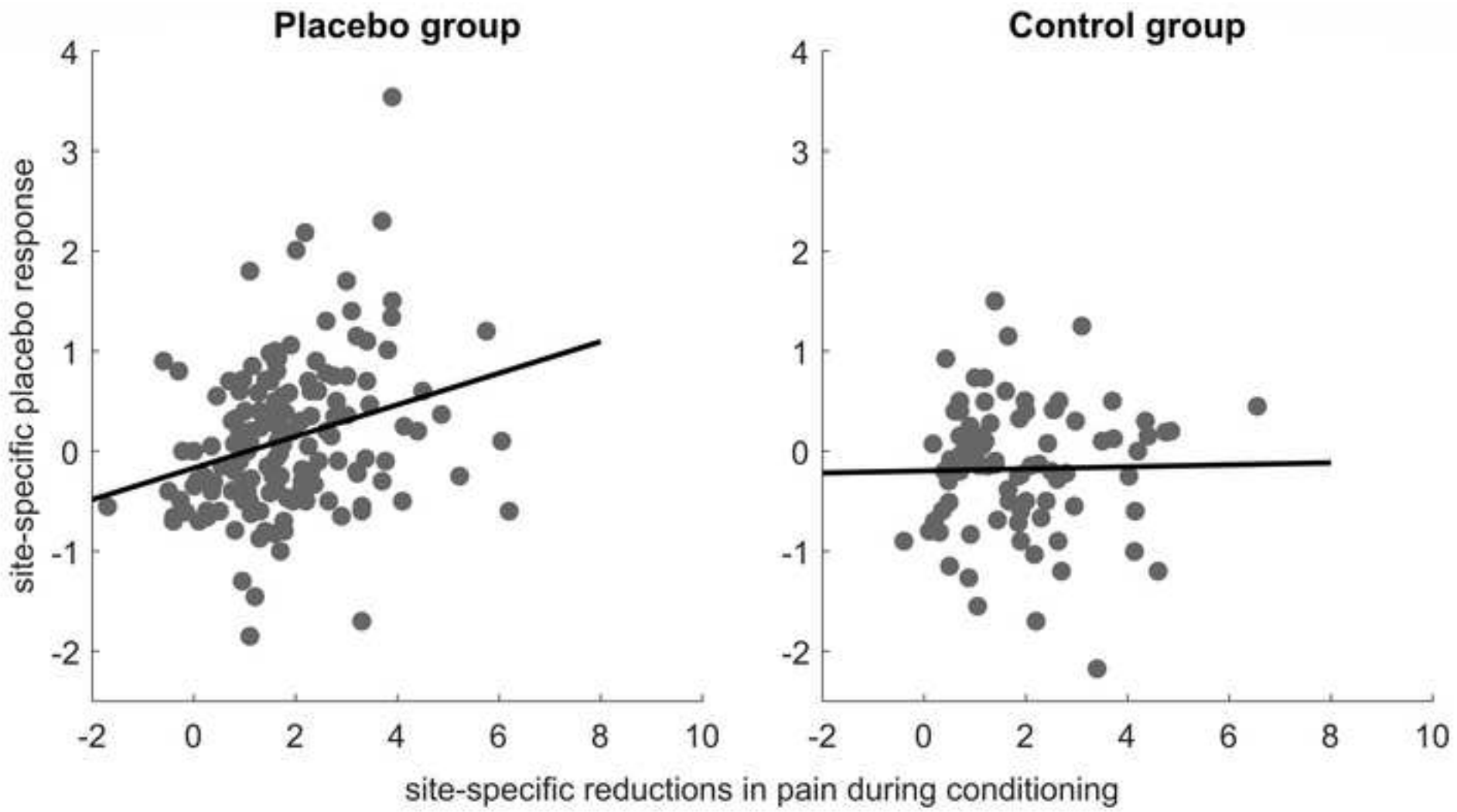

Table

All changes in this revised draft have been highlighted

Table 1: Characteristics of the osteoarthritis (OA), fibromyalgia (FM) and pain-free healthy (HI) participants. Participants were assigned into Placebo and Control groups. N: numbers of participants in each group. SEM: Standard Error of the Mean.

Characteristic	**Control Group (n = 87)**			**Placebo/Sham Treatment Group (n = 150)**		
	N (%) of control group)	Age (yrs ± SEM)	N (%) females	N (%) of placebo group)	Age (yrs ± SEM)	N (%) females
OA	22 (25%)	58.8±1.47	9 (39%)	38 (25%)	60.9±1.8	18 (47%)
FM	24 (28%)	50.9±1.9	20 (83%)	55 (37%)	51.3±1.37	48 (87%)
HI	41 (47%)	34.8±1.76	27 (66%)	57 (38%)	38.3±1.59	32 (56%)
Age (yrs ± SEM)	46±1.6			49±1.2		
N (%) females	56 (64%)			98 (65%)		

Table 2: One-way ANOVAs on the diagnostic groups for psychometric measures.

Questionnaire	OA	FM	HI	ANOVA (p-value)	Post hoc (p-value)		
					OA vs FM	OA vs HI	FM vs HI
STAI2	29.25 ±0.13	38.59± 0.13	27.94± 0.07	p<0.001**	p<0.001**	p=0.948	p<0.001**
STAI1	30.24 ±0.19	39.29± 0.14	27.92± 0.08	p<0.001**	p<0.001**	p=0.391	p<0.001**
STAI1V2	28.31±0. 14	38.44± 0.19	27.37± 0.09	p<0.001**	p<0.001**	p=1.000	p<0.001**
HADS Anxiety	5.66 ±0.06	10.5±0 .06	5± 0.036	p<0.001**	p<0.001**	p=0.889	p<0.001**
HADS Depression	3.96 ±0.062	9.37±0 .06	2.57±0 .027	p<0.001**	p<0.001**	p=0.055	p<0.001**
HAI	12.34±0. 12	19.5±0 .126	9.56±0 .06	p<0.001**	p<0.001**	p=0.071	p<0.001**
LOTR V1	14.0±0.0 7	11.23± 0.07	14.68± 0.05	p<0.001**	p=0.002*	p=1.000	p<0.001**
LOTR V2	14.8 ±0.08	12.39± 0.06	15.75± 0.05	p<0.001**	p=0.007*	p=0.620	p<0.001**
PCS Rumination	5.304±0. 06	8.014± 0.07	4.022± 0.04	p<0.001**	p<0.001**	p=0.190	p<0.001**
PCS Magnification	2.339±0. 03	4.181± 0.05	1.699± 0.02	p<0.001**	p<0.001**	p=0.392	p<0.001**
PCS Helplessness	5.732±0. 07	11.27± 0.09	3.473± 0.04	p<0.001**	p<0.001**	p=0.0024*	p<0.001**
PASS Avoidance	10.77±0. 10	11.51± 0.08	7.389± 0.06	p<0.001**	p=1.000	p<0.001**	p<0.001**
PASS fearful thinking	5.196±0. 09	7.11±0 .08	3.1±0. 04	p<0.001**	p=0.074	p=0.029*	p<0.001**
PASS cognitive anxiety	9.21±0.0 99	14.24± 0.08	7.09±0 .06	p<0.001**	p<0.001**	p=0.070	p<0.001**
PASS physiological response	4.43±0.0 7	9.04±0 .08	4.01±0 .05	p<0.001**	p<0.001**	p=1.000	p<0.001**

Mean ± SEM. Bonferroni post hoc tests highlight differences and similarities between the diagnostic groups.
STAI, State-Trait Anxiety Inventory; HADS, Hospital Anxiety and Depression Scale; HAI, Healthy Anxiety Inventory; LOT-R, Life Orientation Test; PCS, Pain Catastrophizing Scale; PASS, Pain Anxiety Symptoms Scale.
Significance at the 95% level, *p<0.05, **p<0.001.

Table 3: Fixed effects from Model 2 (for models, see Supplementary Materials), including interactions between treatment (placebo vs control), phase (pre vs post conditioning phase) and arm (treated vs. untreated) to test for the presence of a placebo effect. Beta coefficients are unstandardized and categorical predictor variables are dummy coded, such that beta coefficients are interpretable in terms of the original 0-10 pain scale (e.g. a beta value of 1 indicates a change of 1 on the pain scale). Significance at the 95% level, *p<0.05, **p<0.001, ***p<0.0001

	Coefficients						**ANOVA contrasts**			
Parameter	**beta**	**SE**	**t**	**p value**	**CI-95%, lower**	**CI-95%, upper**	**F**	**DF1**	**DF2**	**p value**
(Intercept)	5.60	0.46	12.25	p<0.0001***	4.70	6.49	150.17	1	23882	p<0.0001***
group	-0.10	0.16	-0.61	p=0.544	-0.41	0.22	0.96	2	23882	p=0.358
	-0.25	0.18	-1.37	p=0.171	-0.61	0.11				
treat	0.25	0.13	1.89	p=0.059	-0.01	0.51	3.56	1	23882	p=0.059
session	0.02	0.11	0.17	p=0.866	-0.19	0.23	0.03	1	23882	p=0.084
phase	-0.49	0.10	-4.83	p<0.0001***	-0.69	-0.29	23.37	1	23882	p<0.0001***
arm	0.48	0.06	7.39	p<0.0001***	0.61	0.35	54.67	1	23882	p<0.0001***
age	-0.02	0.01	-2.90	p=0.004*	-0.03	0.00	8.39	1	23882	p=0.0038*
gender	-0.13	0.13	-1.03	p=0.304	-0.37	0.12	1.06	1	23882	p=0.304
laser energy	-0.18	0.17	-1.08	p=0.278	-0.50	0.14	1.18	1	23882	p=0.278
phase2_mean	0.25	0.01	18.15	p<0.0001***	0.22	0.28	329.33	1	23882	p<0.0001**
treat:phase	-0.74	0.13	-5.86	p=0.0002**	-0.99	-0.49	34.36	1	23882	p=0.0001***
treat:arm	0.09	0.07	1.24	p=0.217	0.24	-0.05	1.53	1	23882	p=0.207
phase:arm	0.14	0.07	-2.14	p=0.032*	0.27	0.01	4.58	1	23882	p=0.032*
treat:phase:arm	-0.26	0.08	-3.07	p=0.002*	-0.09	-0.42	9.43	1	23882	p=0.002*

Table 4. Factorial (two-way) ANOVAs with factors for Treatment (Placebo group, Control group) and Diagnosis (fibromyalgia, osteoarthritis, and healthy). 3 ANOVAs were conducted, on Change in Expectation of pain relief, Change in Anxiety, and the subsequent Change in Pain rating (after the conditioning procedure).

	Change in Expectation	Change in Anxiety	Change in Pain
Treatment	F=17.059; p<0.001**	F=0.385; p=0.536	F=31.74; p<0.001**
Diagnosis	F=0.70; p=0.498	F=0.826; p=0.44	F=0.296; p=0.744
Treatment x Diagnosis	F=1.503; p=0.226	F=3.06; p=0.049*	F=0.819; p=0.442

Significance at the 95% level, *p<0.05, **p<0.001, ***p<0.0001

Table 5. VAS Anxiety levels measured at 5 time-points (Anx1-Anx5) throughout the experiment. Osteoarthrosis (OA), fibromyalgia (FM) and healthy (HI) groups.

Anxiety Rating	OA	FM	HI	P value	F values	df
Anx1	12.17±15.83	28.481±24.82	12±14.87	p<0.0001***	19.36	2,30
Anx2	12.69±12.62	22.86±19.93	12.29±13.03	p<0.0001***	11.39	2,27
Anx3	10.19±12.41	24.01±20.90	12.26±12.33	p<0.0001***	16.69	2,29
Anx4	9.53±12.34	20.62±19.34	10.38±12.89	p<0.0001***	12.45	2,29
Anx5	8.84±16.04	19.05±22.25	9.29±14.1	p=0.0003**	8.24	2,31

Significance at the 95% level, *p<0.05, **p<0.001, ***p<0.0001

Table 6: Effect of pain reductions during the conditioning phase ("conditioned" in the table) on placebo responses. Results are from the linear mixed models 9 to 11 in Supplementary Materials, which controlled for pre-conditioned pain ratings and session. Placebo and conditioning changes are site-specific (i.e. mean pain ratings from the treated arm minus the untreated arm), which also controls for habituation effects over time. Beta coefficients are unstandardized and the categorical predictor variable (group: placebo vs. control) are dummy coded.

Interaction between group and conditioned responses (model 9)						
Parameter	**beta**	**SE**	**t**	**p value**	**CI-95%, lower**	**CI-95%, upper**
(Intercept)	-0.29	0.10	-2.85	p=0.005*	-0.50	-0.09
conditioned	0.07	0.04	1.74	p=0.082	-0.01	0.15
treat	0.04	0.12	0.34	p=0.735	-0.20	0.28
treat:conditioned	0.12	0.05	2.27	p=0.024*	0.02	0.22
session	-0.01	0.07	-0.18	p=0.858	-0.15	0.13
pre-conditioned	0.74	0.06	13.14	p<0.0001***	0.63	0.85
Conditioned responses in the placebo group (model 10)						
Parameter	**beta**	**SE**	**t**	**p value**	**CI-95%, lower**	**CI-95%, upper**
(Intercept)	-0.27	0.09	-3.05	p=0.0025*	-0.44	-0.09
conditioned	0.20	0.03	5.93	p<0.0001***	0.13	0.27
session	-0.05	0.09	-0.56	p=0.573	-0.23	0.13
pre-conditioned	0.64	0.07	8.69	p<0.0001***	0.49	0.78
Conditioned responses in the control group (model 11)						
Parameter	**beta**	**SE**	**t**	**p**	**CI-95%, lower**	**CI-95%, upper**
(Intercept)	-0.26	0.10	-2.57	p=0.011*	-0.47	-0.06
conditioned	0.05	0.04	1.18	p=0.241	-0.03	0.12
session	0.03	0.11	0.28	p=0.776	-0.19	0.25
pre-conditioned	0.94	0.08	11.07	p<0.0001***	0.77	1.10

Significance at the 95% level, *p<0.05, **p<0.001, ***p<0.0001

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