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Nocebo Hyperalgesia, Partial Reinforcement, and Extinction

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Abstract: **Many studies have found evidence of conditioning-induced nocebo hyperalgesia. However, these studies have exclusively involved continuous reinforcement (CRF) schedules. Thus, it is currently unknown whether nocebo hyperalgesia can result after partial reinforcement (PRF). We tested this using electrodermal pain stimulation in healthy volunteers. Undergraduates (N = 135) received nocebo treatment under the guise of a hyperalgesic. Participants were randomly allocated to CRF, PRF, or control (no conditioning). Conditioning involved surreptitiously increasing pain stimulation on nocebo trials relative to control trials. During training, the CRF group always had the nocebo paired with the surreptitious pain increase, whereas the PRF group experienced the increase on only 62.5% of nocebo trials. In the test phase, pain stimulation was equivalent across nocebo and control trials. PRF was sufficient to induce nocebo hyperalgesia; however, this was weaker than CRF. Nocebo hyperalgesia failed to extinguish irrespective of the training schedule. Additional assessment of expectancies indicated strong concordance between expectancy and nocebo hyperalgesia. Overall, these findings suggest that once established, nocebo hyperalgesia may be difficult to disrupt. PRF may be a novel method of reducing the intensity of nocebo hyperalgesia in the clinic, which may be particularly important given its persistence.**

Perspective: ***This study provides novel evidence that partial reinforcement results in weaker nocebo hyperalgesia than continuous reinforcement and that nocebo hyperalgesia fails to extinguish, irrespective of the training schedule. As a result, partial reinforcement may serve as a method for reducing the intensity of nocebo hyperalgesia in the clinic.***

Key words: *Nocebo, pain, expectancy, conditioning, partial reinforcement.*

Most research on placebo effects for pain has focused on placebo analgesia. However, increasing evidence indicates that placebo mechanisms can also amplify pain, referred to as nocebo hyperalgesia.[5,7,14,16,28] As with placebo analgesia, evidence of increased pain ratings during nocebo hyperalgesia is supported by neuroimaging studies demonstrating accompanying modulation of activity in brain regions known to be sensitive to pain.[10,20,28,31] However, although both placebo analgesia and nocebo hyperalgesia are considered to result from the same general learning mechanisms, that is, instruction and conditioning, some asymmetries exist. For example, nocebo hyperalgesia is more readily induced via instruction than placebo analgesia,[16] and although endogenous opioids have been shown to underlie instruction-induced placebo analgesia,[3,8,29,30] instruction-induced nocebo hyperalgesia appears to be mediated by cholecystokinin.[5,6]

Given the traditional focus on placebo analgesia, many of the characteristics of nocebo hyperalgesia are unknown. One important example is whether the conditioning schedule affects the magnitude of nocebo hyperalgesia. Studies investigating conditioned nocebo hyperalgesia have exclusively involved continuous reinforcement (CRF) training schedules,[10,15,16,20,28] in which presentation of the nocebo was always followed by hyperalgesia during training. Thus, it is unknown whether nocebo hyperalgesia can result after more variable conditioning schedules in which the nocebo is followed by hyperalgesia only on some occasions

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during training, known as partial reinforcement (PRF),[11,17] which may be more ecologically valid. Furthermore, only 1 study[16] has examined extinction of nocebo hyperalgesia, that is, how long the effect lasts once established. That study found evidence suggesting that nocebo hyperalgesia fails to extinguish, which is contrary to what most learning models would predict.

Interestingly, the type of training schedule may influence the rate of extinction. Numerous animal conditioning studies indicate a PRF extinction effect, whereby PRF produces conditioned responding that is more resistant to extinction than CRF.[22,24,25,32] Thus, nocebo hyperalgesia may be even more resistant to extinction than it appears when it is established under PRF.

The current study addressed these gaps in knowledge by comparing the magnitude and rate of extinction of nocebo hyperalgesia after CRF and PRF schedules using experimentally induced pain. We recently conducted the only study to date comparing CRF and PRF on placebo analgesia[4] and found that PRF produced weaker placebo analgesia than CRF but that the placebo analgesia established under PRF was more resistant to extinction. If the training schedule affects nocebo hyperalgesia in a similar way, then one would expect weaker initial nocebo hyperalgesia after PRF that is more resistant to extinction compared with CRF. However, given the asymmetries mentioned above, it seemed plausible from the outset that PRF may affect the development of nocebo hyperalgesia differently than its effects on placebo analgesia. Understanding the characteristics of nocebo hyperalgesia is important for discovering ways of reducing its contribution to pain in the clinic. To our knowledge, this is the first test of whether nocebo hyperalgesia can result after PRF.

Methods

Participants

One hundred thirty-five (54% female; mean age = 20.3 years, standard deviation [SD] = 4.0) undergraduate students from the University of Sydney participated. One hundred fifteen were first-year psychology students who participated in return for course credit. For these participants, the study was advertised on an internal website where over 2,000 first-year psychology students can select to participate in various studies. The remaining 20 participants were undergraduate students recruited from the general university population via a volunteer website and were reimbursed $15 for their participation. To be included, participants had to be fluent in English, not have any current or previous heart problems, not currently be experiencing pain, and not have participated in any other placebo-related studies within the School of Psychology. All participants provided informed consent, and the study procedures were approved by the University of Sydney's human research ethics committee.

Design

The design followed our previous study on PRF and extinction of placebo analgesia.[4] The key exception was that participants were told that they were taking part in a study testing whether transcutaneous electrical nerve stimulation (TENS) could increase pain sensitivity, with participants in the experimental groups receiving conditioning with a surreptitious increase in pain. Table 1 shows the full study design. The TENS was a dummy device with its supposed activation signaled by tactile vibration and a beeping sound. Participants were randomly allocated to 1 of 3 groups. The CRF group received training whereby every time the TENS was activated (nocebo trials), the pain simulation was surreptitiously increased relative to no TENS trials (control trials). The PRF group received training whereby the pain stimulation was surreptitiously increased relative to control trials on only 62.5% of nocebo trials but the pain stimulation remained the same as control trials on the remaining 37.5% of nocebo trials. A 62.5% PRF schedule was chosen to approximate a ratio of reinforcement to nonreinforcement of 2:1 to simulate a treatment setting in which the treatment regularly, but not always, leads to hyperalgesia. A control group was told that they were acting as controls and would not receive any TENS. Instead, they were led to believe that the dummy device measured skin conductance and that it would be activated on only half the trials to ensure that it did not interfere with any of the other equipment. In this group, activation of the device and level of pain stimulation were noncontingent such that the pain was surreptitiously increased on half the trials with the device active and half the trials with the device inactive. This was done in blocked fashion according to Au Yeung et al[4] to avoid any potential superstitious conditioning. For all groups, the training phase consisted of 32 trials: 16 trials

Table 1. Summary of Study Design

Group	Instruction	Conditioning	Test/Extinction
CRF (n = 37)	Told receiving TENS to increase pain	16 nocebo → 100% 16 control → 60%	16 nocebo → 100% 16 control → 100%
PRF (n = 40)	Told receiving TENS to increase pain	10 nocebo → 100% 6 nocebo → 60% 16 control → 60%	16 nocebo → 100% 16 control → 100%
Control (n = 42)	Told no treatment controls	8 active + 8 control → 100% 8 active + 8 control → 60%	16 nocebo → 100% 16 control → 100%

NOTE. In the CRF and PRF groups, the participants were led to believe that the nocebo device was a TENS machine that would increase their pain. In the control group, the participants were told that the same device was a method of measuring skin conductance, with no mention of any potential effects on pain. The device was active on half the trials and inactive on the other half. The active trials in the CRF and PRF groups constituted the nocebo trials. Inactive trials are labeled control trials.

with the device active and 16 control trials. This meant that the CRF group experienced 16 pairings of the nocebo with increased pain stimulation, whereas the PRF group experienced 10 pairings of the nocebo with increased pain stimulation and 6 nocebo trials with no increase in the level of pain stimulation.

The test phase occurred immediately after the conditioning phase, with no break or signal that a new phase had begun. In this phase, all groups underwent a further 16 trials with the device active and 16 control trials with the device inactive, all with the pain stimulation kept at the intensity administered for control trials during training regardless of whether the device was active or not. This provided the test of nocebo hyperalgesia and whether or not it extinguished after the reinforcement was withdrawn. The dependent variable was pain report after each painful stimulus. In a novel addition to the current study, we also assessed participants' expectancies for pain before each individual painful stimulus, which allowed us to test how the conditioning manipulations affected expectancy as well as the relationship between expectancy and nocebo hyperalgesia.

Materials

Verbal Instructions

All participants were given an information sheet on arrival that described TENS only briefly as involving passing an electrical current through the skin, with no suggestion of how this might affect their pain. The 2 conditioning groups received more substantial information on TENS as follows. Before the dummy device was attached, they received a 1-page handout including sections "What is TENS used for?", "How does TENS work?", and "What's so good about TENS?" The handout suggested that TENS was effective for enhancing pain by "enhancing the conductivity of the pain signal being sent to the brain." The conditioning groups were also given oral instructions that supported this as the nocebo device was being attached to their arm. These instructions were as follows:

> This is the TENS electrode *[researcher shows participant the nocebo device].* TENS stands for transcutaneous electrical nerve stimulation. TENS can increase pain by amplifying the pain signals as they travel up your arm and into your brain [*researcher follows the path from the electrode placement up the participant's arm]*. The TENS itself is not painful, but you will feel a small sensation when it's turned on.

The control group were given no additional information about TENS other than the brief mention in the initial information sheet. They did not receive the TENS handout. They received only oral instructions suggesting that a device measuring skin conductance was being attached to their arm. The instructions were as follows:

> You have been allocated to the control group, which means that you will not receive TENS. But, your skin conductance will still be measured. This is the electrode that measures skin conductance *[researcher shows participant the device].* Skin conductance is a measure of autonomic arousal. You will feel a slight sensation when the skin conductance is being recorded, but it won't be painful. Because the skin conductance electrode can interfere with other equipment, we will only turn it on half the time.

Nocebo Device

No TENS was delivered to participants at any stage of the experiment. TENS was simply used as a cover story to explore nocebo hyperalgesia. The device was a stimulus isolator (Model FE180; ADInstruments, Sydney, New South Wales, Australia) that generated tactile stimulation via direct currents sent to electrodes attached on the dorsal forearm on the participant's nondominant hand. A full description can be found in Au Yeung et al.[4]

Pain Stimuli

Pain was induced via electrocutaneous stimulation similar to that used in other studies on placebo analgesia.[4,13,15] Electrically induced pain was chosen because the intensity of the stimulation can easily be manipulated surreptitiously to achieve conditioning and, unlike some other devices, for example, CO_2 laser, it allows repeated stimulation at the same site without risk of tissue damage. Each stimulus consisted of an electrical shock delivered to the dorsum of the participant's nondominant hand via 2 silver chloride electrodes, each approximately 1 cm apart. Stimuli were generated by a pain stimulator (Model SHK1; Contact Precision Instruments, Cambridge, MA). The stimuli were square pulses with a duration of .5 seconds and frequency of 100 Hz.

The intensity of the pain stimuli was calibrated for each participant individually before testing. This was done by initially delivering stimuli at a very low and usually imperceptible level and then increasing the intensity of the stimuli in steps until participants reached a level that they felt was "definitely painful, but tolerable." To ensure this was at least somewhat painful and to avoid potential floor effects, when this level was reached, the participants were asked to verbally report their pain out of 10, with 0 being no pain and 10 being very painful. If their reported pain was less than 6 out of 10, then they were asked whether they felt comfortable trying a higher intensity, such that participants' pain ratings at the end of calibration were at least 6 out of 10 on a verbally reported scale. The level of intensity reached at the end of calibration was labeled as the 100% intensity for that particular participant. Intensity of each stimulus during the experiment was determined on the individual's 100% intensity level, his or her experimental condition, and the particular trial.

Pain Ratings

Participants were asked to rate their pain after each painful stimulus on a 100-point computerized visual analog scale (VAS). Three anchors were used, with 0 (no pain) and 100 (very painful) on the left and right

extremes, respectively, and 50 (moderately painful) in the middle.

Expectancy Ratings

Participants were also asked to rate their expectancy before each painful stimulus. This was done on a response meter (model MLT1601/ST; ADInstruments) in which the participants could move a slider with their dominant hand to rate how painful they expected the next shock to be on a 100-point VAS with the anchors labeled as 0 (no pain), 50 (moderately painful), and 100 (very painful).

Trial Structure and Conditioning Manipulation

Each trial consisted of a single pain stimulus followed by a pain rating. Within a given trial, each pain stimulus was signaled by a 10-second countdown culminating in an "X" appearing on the computer screen, .5 seconds after which the painful stimulus was delivered. A prompt appeared on the screen reminding participants to rate their expectancy for 7 seconds during the countdown. After each stimulus, participants rated the intensity of the pain on the computerized VAS. Between each trial, participants had a rest of 10 to 15 seconds. On nocebo trials, the device was activated for 8 seconds during the countdown. On control trials, the device remained inactive.

The conditioning phase included 16 nocebo and 16 control trials. The CRF group received a surreptitious increase in painful stimulation on all 16 nocebo trials. This was achieved by increasing the pain intensity to 100% on nocebo trials as opposed to 60% on control trials, a similar-sized increase to those previously used in conditioned nocebo hyperalgesia studies.[16] The PRF group received the same surreptitious increase in painful stimulation but only on 62.5% of nocebo trials; that is, pain intensity was increased on 10 of the 16 nocebo trials, as shown in Table 1. The nocebo trials were intermixed with control trials in quasi–randomized order within participants, such that there were no more than 2 nocebo or control trials in a row. This trial order was used to ensure that the different trials were distributed across the test session both within and across participants. For the control group, the conditioning phase also involved 16 trials with the device active and 16 with it inactive. Half of each of these trials were at 100% pain intensity and the other half were at 60% pain intensity. These trials were presented in 4 blocks of 8 trials (4 trials with the device active and 4 with the device inactive); 2 blocks were at 100% intensity and 4 at 60% intensity. This variation ensured that, as with the experimental groups, the control group also had some experience of different levels of pain and using the pain scale as opposed to only ever receiving 100% painful stimulation.[4] The blocked design was intended to prevent potential superstitious conditioning, even though the 2 events were noncontingent.

The test/extinction phase was identical for all groups. It consisted of 32 trials (16 with the device active and 16 with it inactive) in which the intensity of painful stimuli was always set at 60%. This provided the test of whether the CRF and PRF groups would experience greater pain on nocebo trials relative to control trials despite the level of stimulation being identical across the 2 groups.

Exit Questionnaire

An exit questionnaire tested whether participants guessed the true nature of the study, as well as their knowledge of the placebo-shock reduction contingency across groups. The first question asked, "What do you think the study was about?" with an open response. The second and third questions assessed contingency knowledge for the first and second half of the experiment, respectively. The questions read, "In the first [or second] half of the experiment, did you notice any increase in pain when TENS was turned on compared with when it was not turned on?"

Procedure

Participants attended a single 1-hour session and were tested individually in an isolated testing booth. On arrival, they were given an information sheet that described the study as a test of the acute effect of TENS on psychophysiological responses to pain. The 2 conditioning groups were then told that they had been allocated to receive TENS and were given the handout on TENS. The control group members were told that they had been allocated to receive no treatment and simply rested for 2 minutes. The nocebo device was then introduced and attached to the participants individually, during which the participants in each group were given the relevant oral instructions.

The experimenter (V.F.Q. or another) then explained the trial structure to the participant, left the room, and then initiated the computerized program that controlled the delivery of the pain stimuli, activation of the "TENS" device, and pain ratings. The conditioning phase was initiated and was followed immediately by the test/extinction phase without any notification to the participant. At the end of the test/extinction phase, participants completed the exit questionnaire assessing their beliefs about the study. A debrief statement was sent to all participants via e-mail at the completion of the study.

Data Handling and Analysis

Thirteen participants were excluded based on a priori criteria: 6 were not proficient in English, 3 had already completed a study on placebo effects in our laboratory, and 4 rated pain as less than 30 of 100 on trials with 100% pain intensity during the training phase. A further 3 participants were excluded for failing to follow instructions. This left 119 participants with evaluable data.

Analysis of variance and χ^2 tests of independence tested for baseline differences in age and sex. For the main analysis on the pain data, conditioning and test phases were analyzed separately. In each phase, the groups were compared by calculating difference scores between pain with and without the "TENS" device

activated (difference = pain with TENS – pain without TENS; positive scores indicated nocebo hyperalgesia), which were analyzed via mixed analyses of covariance with group and trial as factors, controlling for age and sex. Age and sex were included as covariates as both have been found to influence pain perception in general[21,37] as well as the placebo effect specifically.[36] However, the pattern of results was identical without these covariates included in the model. The critical test of the magnitude of the nocebo hyperalgesia produced by each conditioning schedule was the difference in pain ratings on the first nocebo trial and control trial in the test/extinction phase, that is, trial 17. We expected that the strongest nocebo hyperalgesic effect would occur immediately after the conditioning phase. This is because the first test trial occurs before any extinction has taken place. To explore changes over time and compare rates of extinction across the groups, we tested linear trends whenever trial was included as a factor. To isolate the effects of the different conditioning schedules, we conducted planned pairwise comparisons between each group. These intergroup comparisons were repeated for the expectancy data to test how training influenced the acquisition of expectancies and their time course during the test phase. Multiple linear regression was then used to test the extent to which expectancy predicted nocebo hyperalgesia in each group, controlling for age and sex.

All analyses were conducted in IBM SPSS Statistics, version 20.0 (IBM Corp, Armonk, NY), covariates were mean centered to reduce multicollinearity, the assumptions of covariate-treatment independence and homogeneity of regressions slopes were met, Greenhouse-Geisser corrections were made whenever the sphericity assumption was not met (in which case adjusted degrees of freedom are reported), and results were considered statistically significant when $P < .05$.

Results

There were no statistically significant differences in age or sex across the 3 groups ($F_{2,116}$= 1.63, $P = .20$ and χ^2 [df = 2, N = 119] = 3.09, $P = .21$, respectively).

Pain

Training Phase

Pain ratings during training are shown in Fig 1. There was no statistically significant difference in pain ratings averaged across the 60% trials when the nocebo device was inactive during training ($F_{2,114} = .91$, $P = .41$, $\eta^2_p = .02$), suggesting no differences in overall pain sensitivity between groups. The conditioning manipulation was effective in producing increased pain in both the CRF and PRF groups on relevant nocebo trials during training. In the CRF group, pain was rated as 36.6 (standard deviation [SD] = 13.5) points higher on nocebo trials with the 100% pain stimulation relative to the control trials (always 60% pain stimulation: $F_{1,34} = 247.0$, $P < .001$, $\eta^2_p = .88$). In the PRF group, pain was rated 27.3 (SD = 10.0) points higher on nocebo trials with 100% pain stimulation than on control trials ($F_{1,37} = 265.0$, $P < .001$, $\eta^2_p = .88$). There was also evidence of some nocebo hyperalgesia in the PRF group during training, whereby pain was rated as 11.3 (SD = 11.1) points higher on nocebo trials with 60% pain stimulation than on control trials with 60% stimulation ($F_{1,37} = 33.6$, $P < .001$, $\eta^2_p = .48$). The difference in the magnitude of the pain increase on reinforced nocebo trials during training was significantly greater for the CRF group than the PRF group ($F_{1,77} = 11.9$, $P = .001$, $\eta^2_p = .14$). In the control group, there was no difference in pain ratings when the device was active or inactive at 100% pain stimulation ($F_{1,39} = 1.74$, $P = .20$, $\eta^2_p = .04$). However, at 60% pain stimulation, pain ratings were statistically significantly higher with the device active relative to when it was inactive ($F_{1,39} = 6.89$, $P = .01$, $\eta^2_p = .15$), but this was only by 1.49 (SD = 4.0) points out of 100. Overall, then, the training phase indicated that the conditioning manipulation was effective with only a very slight, if any, unconditioned effect of having the device active on pain ratings.

Test Phase

Pain ratings during the test phase (when all pain stimulation was set to 60% irrespective of whether or not the device was active) are shown in Fig 1. Differences in these pain ratings on nocebo and control trials were compared between groups in the first test trial (in which conditioning should be strongest) as well as over the entire test phase. A summary of the results for the test phase is presented in Table 2. On the first test trial, there was a statistically significant main effect of group ($F_{2,114} = 4.37$, $P = .01$, $\eta^2_p = .07$). Pairwise comparisons revealed statistically significant nocebo hyperalgesia in the CRF group, with the hyperalgesia induced by the nocebo being 8.9 points greater than control ($F_{1,114} = 8.75$, $P = .004$, $\eta^2_p = .07$). There was no statistically significant nocebo hyperalgesia in the PRF group relative to control on the initial test trial ($F_{1,114} = 1.83$, $P = .18$, $\eta^2_p = .02$), nor was nocebo hyperalgesia in the CRF group significantly greater than in the PRF group ($F_{1,114} = 2.44$, $P = .12$, $\eta^2_p = .02$).

The 2-way treatment by trial analysis over the entire test phase also revealed a statistically significant main effect of treatment ($F_{2,114} = 10.1$, $P < .001$, $\eta^2_p = .15$). Pairwise comparisons indicated a statistically significant nocebo hyperalgesic effect of 8.9 points in the CRF group versus control when averaged across all test trials ($F_{1,114} = 20.2$, $P < .001$, $\eta^2_p = .15$). There was also a statistically significant nocebo hyperalgesic effect of 4.0 points in the PRF group relative to the control group ($F_{1,114} = 4.26$, $P = .04$, $\eta^2_p = .04$). The strength of the nocebo hyperalgesia was significantly greater in the CRF group relative to the PRF group (mean difference = 4.76, $F_{1,114} = 5.57$, $P = .02$, $\eta^2_p = .05$). There was, however, no main effect of trial nor a significant group by trial interaction ($F_{10.4,1710} = 1.41$, $P = .13$, $\eta^2_p = .01$ and $F_{20.9,1710} = .93$, $P = .55$, $\eta^2_p = .02$, respectively), suggesting that once established, the nocebo hyperalgesic effects did not extinguish. This was confirmed in the pairwise comparisons, with no significant interaction between any of these and the linear trends across trials (all $F < 1$).

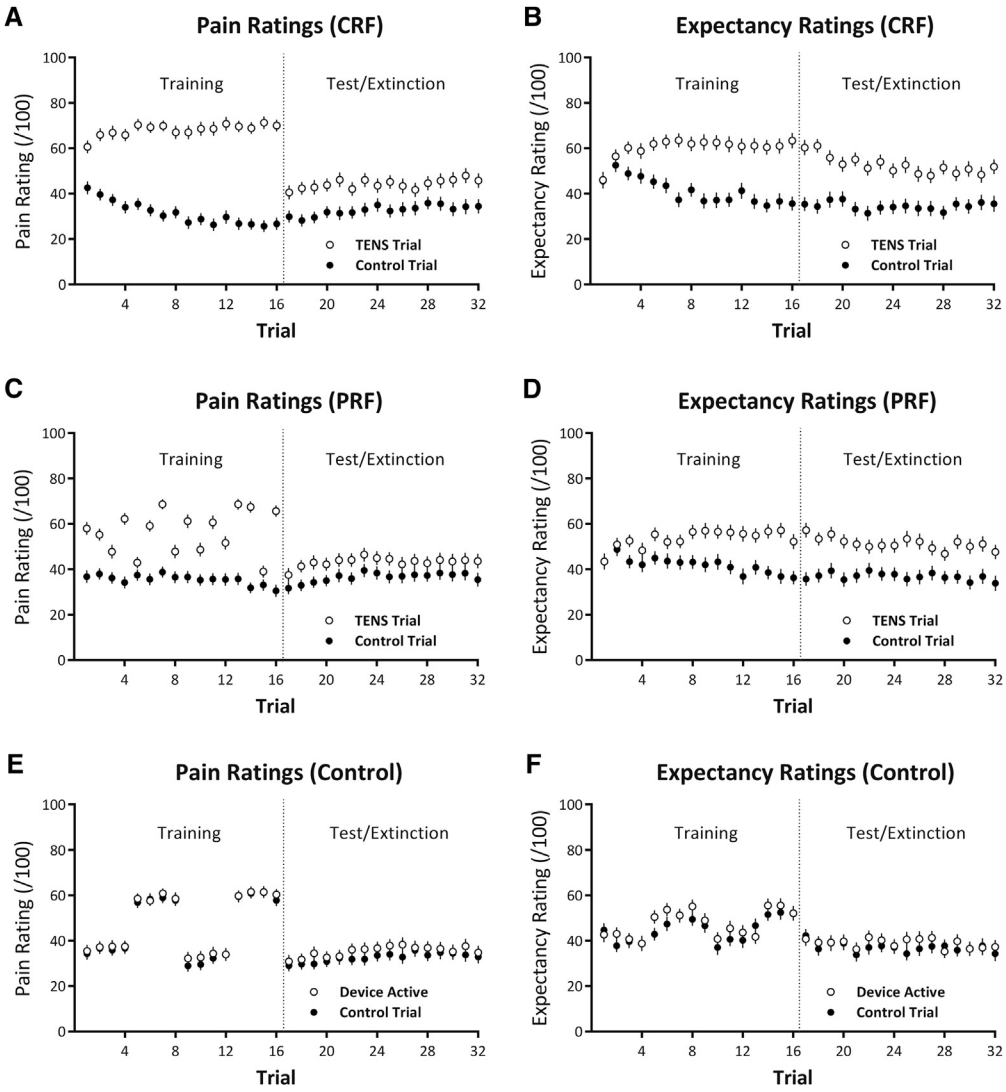

Figure 1. Covariate (age, sex) adjusted mean (± standard error of mean difference) pain **(A, C, E)** and expectancy ratings **(B, D, F)** with the device active versus inactive for the CRF, PRF, and control groups.

Expectancy

Training Phase

Expectancy ratings for each of the groups are shown in Fig 1. As with the pain ratings, differences in expectancy ratings on nocebo versus control trials were calculated (labeled nocebo expectancy) and compared across groups. In the training phase, there were significant main effects of trial and treatment on nocebo expectancy ($F_{10.5,1710}$ = 7.82, P < .001, η^2_p = .06 and $F_{2,114}$ = 15.2, P < .001, η^2_p = .21, respectively) as well as a significant time by treatment interaction ($F_{21.0,1710}$ = 2.70, P < .001, η^2_p = .05). Pairwise comparisons between groups indicated that the CRF group expected an average of 16.7 points more pain than the control group on nocebo relative to control trials during training ($F_{1,114}$ = 30.0, P < .001, η^2_p = .21). Similarly, the PRF group expected an average of 9.3 points more pain than the control group on these trials ($F_{1,114}$ = 9.43, P = .002, η^2_p = .08). Furthermore, the CRF group expected significantly more pain on nocebo relative to control trials than the PRF group during training by an average of 7.3 points ($F_{1,114}$ = 5.49, P = .02, η^2_p = .05). Significant linear interactions across trials between CRF and control as well as between PRF and control ($F_{1,114}$ = 19.9, P < .001, η^2_p = .15 and $F_{1,114}$ = 11.4, P = .001, η^2_p = .09, respectively) indicated that the greater nocebo expectancy in the experimental groups relative to control increased over the course of training, consistent with typical learning acquisition curves. There was no such significant interaction between the CRF and PRF groups ($F_{1,114}$ = 1.16, P = .28, η^2_p = .01), suggesting that despite the higher overall nocebo expectancy for pain in the CRF group, the rate this increased over training was similar in the CRF and PRF groups.

Test Phase

A summary of the results for the test phase is presented in Table 2. On the first test trial of the test phase, there was a significant main effect of treatment on nocebo expectancy ($F_{2,114}$ = 15.2, P < .001, η^2_p = .21). Pairwise

Table 2. Summary of Analysis of Covariance Models and Relevant Pairwise Comparisons for Pain and Expectancy During the Test Phase

	Group				Trial		Group × Trial			
		Pairwise						Pairwise × Linear Trend		
	Omn	CRF vs CON	PRF vs CON	CRF vs PRF	Omn	Linear Trend	Omn	CRF vs CON	PRF vs CON	CRF vs PRF
Pain										
First test trial										
F	4.37	8.75	1.83	2.44	—	—	—	—	—	—
P	.01	.004	.18	.12						
All test trials										
F	10.1	20.2	4.26	5.57	1.41	1.48	.93	.39	.35	.01
P	<.001	<.001	.04	.02	.13	.23	.55	.54	.55	.98
Expectancy										
First test trial										
F	15.2	25.1	19.9	.40	—	—	—	—	—	—
P	<.001	<.001	<.001	.53						
All test trials										
F	15.6	27.6	17.2	1.21	1.99	7.73	1.70	6.56	1.21	2.02
P	<.001	<.001	<.001	.27	.03	.006	.03	.01	.27	.16

Abbreviations: Omn, the Omnibus Test for that component of the model; CON, control group.

comparisons revealed that the CRF group expected 26.4 points more pain on nocebo trials relative to control trials than did the control group ($F_{1,114}$ = 25.1, P < .001, η^2_p = .18). Similarly, the PRF group expected 23.0 points more pain on nocebo trials relative to control trials than did the control group ($F_{1,114}$ = 19.9, P < .001, η^2_p = .14). The slightly numerically higher nocebo expectancy in the CRF group on the first test trial compared with the PRF group was not statistically significant ($F_{1,114}$ = .40, P = .53, η^2_p < .01).

The 2-way treatment by trial analysis over the entire test phase also revealed a statistically significant main effect of treatment on nocebo expectancy ($F_{2,114}$ = 15.6, P < .001, η^2_p = .22). Pairwise comparisons indicated statistically significantly higher nocebo expectancy of 16.2 points in the CRF group versus control when averaged across all test trials ($F_{1,114}$ = 27.6, P < .001, η^2_p = .20). Nocebo expectancy was also statistically significantly higher in the PRF group by 12.7 points than the control group ($F_{1,114}$ = 17.2, P < .001, η^2_p = .13). As with the first trial, nocebo expectancy averaged across the entire test phase was numerically higher in the CRF group than the PRF group, but this was not statistically significant ($F_{1,114}$ = 1.21, P = .27, η^2_p = .01). There was also a significant main effect of trial ($F_{10.3,1710}$ = 1.99, P = .03, η^2_p = .02) and a significant treatment by trial interaction ($F_{20.6,1710}$ = 1.70, P = .03, η^2_p = .03). An overall negative linear trend across trials suggested that nocebo expectancy decreased over the course of the test phase ($F_{1,114}$ = 7.73, P = .006, η^2_p = .06). There was a significant interaction in this linear trend between the CRF group and the control group ($F_{1,114}$ = 6.56, P = .01, η^2_p = .05), suggesting a sharper decline in nocebo expectancy in the CRF group. There was no significant interaction in linear trends in expectancy between the PRF and control groups or the CRF and PRF groups ($F_{1,114}$ = 1.21, P = .27, η^2_p = .01 and $F_{1,114}$ = 2.02, P = .16, η^2_p = .02). This suggested that there was some extinction of nocebo expectancy in the CRF group relative to control but not for the PRF group relative to control nor between the CRF and PRF groups.

Expectancy and Nocebo Hyperalgesia

Fig 2 shows scatterplots of nocebo expectancy and nocebo hyperalgesia averaged across the test phase for each group. Multiple linear regressions controlling for age and sex indicated that expectancy was a significant predictor of hyperalgesia within each group. For the CRF group, a 10-point (out of 100) increase in expectancy significantly predicted a 3.5-point increase in pain (b = .350, $t_{1,33}$ = 3.94, P < .001, unique R^2 = .307). Despite this already being a relatively large effect size, it was apparent that there was a clear outlier within the CRF group. As can be seen in Fig 2A, this participant had mean nocebo expectancy of –39.2 (ie, expected 39.2 points less pain when the nocebo was activated), which was 3.2 SDs below the mean nocebo expectancy of 17.3 (SD = 17.8) in the CRF group. Removing this participant from this analysis, the unique proportion of variability accounted for by expectancy in the CRF increased substantially, with a 10-point increase in expectancy now significantly predicting an increase of 5.2 points in pain (b = .516, $t_{1,32}$ = 5.91, P < .001, unique R^2 = .507).

In the PRF group, a 10-point increase in expectancy significantly predicted an increase of 4.7 points in pain (b = .474, $t_{1,36}$ = 6.77, P < .001, unique R^2 = .547). In the control group, a 10-point increase in expectancy significantly predicted an increase of 5.2 points in pain (b = .517, $t_{1,38}$ = 4.84, P < .001, unique R^2 = .370). This

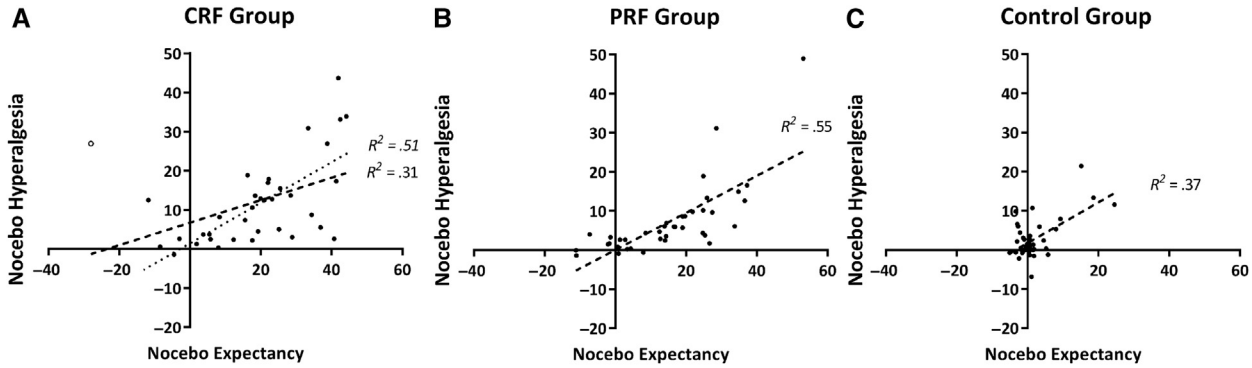

Figure 2. Covariate (age, sex) adjusted scatterplot of nocebo expectancy and nocebo hyperalgesia averaged across the test phase separately for CRF **(A)**, PRF **(B)**, and control **(C)** groups. Lines reflect the slope of expectancy predicting hyperalgesia in the multiple linear regression controlling for age and sex. In the CRF group **(A)**, there was a clear outlier who had averaged expectancy more than 3 SDs below the mean for that group (hollow circle); the lighter dashed line shows the regression slope with that participant excluded.

meant that after controlling for age and sex, nocebo expectancy uniquely accounted for 50.7% of the variance in nocebo hyperalgesia in the CRF group, 54.7% in the PRF group, and 37.0% in the control group. These are substantial effect sizes, especially in the experimental groups, and demonstrate strong concordance between expectancy and nocebo hyperalgesia.

Exit Questionnaire

Most participants appeared to find the cover story credible. Only 26 (22%) made reference to any placebo-related effects. Specifically, 23 (19%) mentioned the effect of expectancy, anticipation, or thoughts on pain, 2 (<2%) mentioned conditioning, and only 1 (<1%) specifically mentioned the placebo effect. The rates of these responses were highest in the control group (33%), followed by the PRF group (22%) and the CRF group (8%).

Discussion

The current study tested the effect of different reinforcement schedules on nocebo hyperalgesia. Four key findings emerged. First, nocebo hyperalgesia can result after PRF. Second, nocebo hyperalgesia produced by PRF is weaker than that produced by CRF. Third, nocebo hyperalgesia is resistant to extinction independently of the training schedule. Fourth, there is strong concordance between expectancy and nocebo hyperalgesia. These findings have a number of important theoretical and practical implications.

First, this study provides novel evidence that nocebo hyperalgesia can result after PRF. This extends previous evidence of nocebo hyperalgesia after CRF[10,15,16,20,28] by demonstrating that nocebo hyperalgesia can result even when the contingency between the nocebo and the nociceptive stimulus is more variable (as may often be the case outside the laboratory). Thus, the current study increases the ecological validity of laboratory research on nocebo hyperalgesia. However, it is important to emphasize that the magnitude of the nocebo hyperalgesia produced after PRF was weaker than that produced after CRF. Using Cohen's[12] rules of thumb, the nocebo hyperalgesia induced by CRF had a large effect size (η^2_p = .15), whereas for PRF it was moderate to weak (η^2_p = .04). Weaker nocebo hyperalgesia after PRF is consistent with animal studies that have found evidence of weaker conditioned responding after PRF compared with CRF.[1,2,19]

Perhaps most interestingly, the nocebo hyperalgesia we induced failed to extinguish independently of the training schedule. That is, the higher pain on nocebo trials compared with control trials remained constant throughout the test period after training under both CRF and PRF. This points toward another asymmetry between nocebo hyperalgesia and placebo analgesia in that we recently found that placebo analgesia produced under CRF does extinguish.[4] Although resistance to extinction of nocebo hyperalgesia after PRF is consistent with PRF extinction effects observed in other areas,[32] the failure of the nocebo hyperalgesia established under CRF to extinguish could be considered more surprising. This is because conditioned responding after CRF typically extinguishes in humans and animals both in general and in fear-conditioning studies specifically, which also involve delivery of electrodermal shocks.[23]

However, there is already some empirical evidence suggesting that nocebo hyperalgesia established under CRF fails to extinguish. Specifically, Colloca et al[16] found that nocebo hyperalgesia was maintained across 6 extinction trials after CRF. Current models propose that nocebo hyperalgesia is at least partially mediated by increased anxiety, one of the effects of which is activation of cholecystokinin receptors that potentiate pain.[18] Coupled with evidence that people with anxiety disorders, such as posttraumatic stress disorder, exhibit impaired extinction of conditioned fear responses,[35] it may be the case that nocebo stimuli induce heightened anxiety that impairs extinction and results in persistent nocebo hyperalgesia. Given that both the current study and Colloca et al's study involved healthy participants and not those with anxiety disorders, this may seem at odds with fear-conditioning studies of healthy participants, which do show extinction.[23] However, in fear-conditioning studies, the extinction phase involves the entire removal

of the aversive stimulus (ie, cue → no shock), whereas in nocebo hyperalgesia studies, it involves a reduction of the intensity of the painful stimulation, not its entire removal (ie, nocebo → reduced shock). Thus, it may be that experiencing painful, albeit weaker stimuli throughout extinction phases in nocebo hyperalgesia studies induces sustained heightened anxiety that impairs extinction. Irrespective of the mechanism, the current study extends Colloca et al's by demonstrating that nocebo hyperalgesia after CRF fails to extinguish even after substantially longer extinction testing involving a total of 16 test trials, suggesting that its persistence is more than temporary.

Another novel aspect of the current study was our trial-by-trial expectancy. This generally indicated strong concordance with nocebo hyperalgesia. Expectancy accounted for between 48 and 54% of the variance in nocebo hyperalgesia in the CRF and PRF groups, a very large effect and one that is consistent with prominent models of the placebo effect that view expectancy as a key mechanism.[9,26,27,34] However, although there was no evidence of extinction of nocebo hyperalgesia in either experimental group, expected hyperalgesia did decrease over the course of the test phase in the CRF group relative to the control group. This suggests some level of discordance between expectancy and nocebo hyperalgesia at least in terms of extinction. However, because they involve fairly different types of ratings, that is, an appraisal of pain versus an appraisal of a belief, any apparent discordance could be caused by differences in the sensitivity of each type of rating to detect changes.

The effect of the training schedule on nocebo hyperalgesia and its failure to extinguish have some important clinical implications. The current result suggests that interspersing delivery of an active treatment that produces hyperalgesia with a placebo (ie, PRF) could reduce the strength of any nocebo hyperalgesia developed during treatment and thereby reduce the overall pain experienced by the patient. The reduction in nocebo hyperalgesia after PRF relative to CRF approached a moderate effect size (η^2_p = .05).[12] Given that the effect size for CRF was large (η^2_p = .15), this suggests that using PRF could lead to substantial benefits to patients in the clinic. Furthermore, reducing the magnitude of nocebo hyperalgesia may be particularly important given its apparent persistence after both CRF and PRF. If the current failure of nocebo hyperalgesia to extinguish generalizes to clinical settings, then, once established, nocebo hyperalgesia may be difficult to disrupt. If so, then clinicians should make every effort to prevent the development of nocebo hyperalgesia in the first place; otherwise, it may persevere indefinitely. Furthermore, nocebo effects that failed to extinguish in perpetuity could call into question the ethicality of conducting nocebo research.

Some potential limitations to the current study are worth considering. First, although we did not observe any extinction of nocebo hyperalgesia, it is possible that extinction might have been observed if we extended the test phase. However, the test phase used here involved 16 test trials, which is almost 3 times longer than in Colloca et al's[16] study and the same amount in which we observed clear extinction of placebo analgesia.[4] Thus, any potential eventual extinction of nocebo hyperalgesia would likely require substantially longer testing and may not necessarily eventuate under those circumstances. Second and related, extinction was tested in a single session. It would be interesting to test whether the current findings generalize to a clinical setting in which both training and testing occur over multiple days, rather than a single session. At least 1 study suggests that nocebo hyperalgesia established via instruction alone can be maintained for up to 90 days,[33] but we are unaware of any studies testing the effects of conditioned nocebo hyperalgesia over multiple days. Given that there was some evidence of a decrease in expectancy over the test phase in the CRF group but not the PRF group, it could be the case that if testing over multiple days does lead to extinction of nocebo hyperalgesia, then this nocebo hyperalgesia may be more resistant to extinction under PRF. That is, although there was no PRF extinction effect observed in the single session used here, such an effect may exist with chronic pain and treatment outcomes. Third, there was an asymmetry between the CRF and PRF groups in terms of the total number of reinforced nocebo trials they experienced during training. This was an intentional decision to match the total length of training across the experimental groups. Nonetheless, it would be interesting for future studies to compare the effects of different reinforcement schedules on nocebo hyperalgesia matched on the number of reinforced trials compared with matched on training length. A major strength of the current study was the inclusion of trial-by-trial expectancy assessment. However, it is possible that assessing expectancy may have provided participants with clues about the true nature of the study, with a higher number of participants reporting that the experiment was concerned with expectancy and pain in the exit questionnaire than in our previous study on placebo analgesia that did not assess expectancy.[4] This is a potentially difficult problem to overcome and is the reason that we have previously avoided asking participants to report their expectancies. It would be interesting for future studies to experimentally test the extent to which assessing expectancy does influence placebo responding and/or participants' beliefs about the purpose of a study.

Overall, the current study provides novel evidence that nocebo hyperalgesia can result after PRF, that this nocebo hyperalgesia is weaker than that produced by CRF, and that both are resistant to extinction. The weaker nocebo hyperalgesia after PRF suggests that it could be used in the clinic to reduce the development of nocebo hyperalgesia during active treatments, which may be particularly important given the apparent persistence of nocebo hyperalgesia once established.

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