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Nocebo hyperalgesia, partial reinforcement, and extinction

Running Head: Nocebo, pain, and partial reinforcement

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Abstract

Many studies have found evidence of conditioning-induced nocebo hyperalgesia. However, these studies have exclusively involved continuous reinforcement schedules. Thus, it is currently unknown whether nocebo hyperalgesia can result following partial reinforcement. 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 continuous reinforcement (CRF), partial reinforcement (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 only experienced the increase on 62.5% of nocebo trials. In the test phase, pain stimulation was equivalent across nocebo and control trials. Partial reinforcement was sufficient to induce nocebo hyperalgesia, however, this was weaker than continuous reinforcement. Interestingly, nocebo hyperalgesia failed to extinguish irrespective of the training schedule. Additional assessment of expectancies indicated strong concordance between these and nocebo hyperalgesia. Overall, these findings suggest that once established, nocebo hyperalgesia may be difficult to disrupt. As such, partial reinforcement may be one method of reducing the intensity of nocebo hyperalgesia in the clinic, which may be particularly important given its persistence.

Perspectives: 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.

Keywords: Nocebo; pain; expectancy; conditioning; partial reinforcement

Nocebo hyperalgesia, partial reinforcement, and extinction

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, 29, 32]. However, while both placebo analgesia and nocebo hyperalgesia are considered to result from the same general learning mechanisms, i.e. instruction and conditioning, some asymmetries exist. For example, nocebo hyperalgesia is more readily induced via instruction than placebo analgesia is [16] and while endogenous opioids have been shown to underlie instruction-induced placebo analgesia [3, 8, 30, 31], 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 currently unknown. One important example is whether the conditioning schedule affects the magnitude of nocebo hyperalgesia. To date, studies investigating conditioned nocebo hyperalgesia have exclusively involved continuous reinforcement training schedules [10, 15, 16, 20, 28, 29], in which presentation of the nocebo was always followed by hyperalgesia during training. Thus, it is currently unknown whether nocebo hyperalgesia can result following more variable conditioning schedules in which the nocebo is only followed by hyperalgesia on some occasions during training, known as partial reinforcement [11, 17], which may be more ecologically valid. Further, only one study to date [16] has examined extinction of nocebo hyperalgesia, i.e. how long the effect lasts once established. Interestingly, that study found evidence suggesting that nocebo hyperalgesia fails to extinguish, which is contrary to what most learning models would predict.

Importantly, the type of training schedule may influence the rate of extinction. Numerous animal conditioning studies indicate a partial reinforcement extinction effect, whereby partial reinforcement produces conditioned responding that is more resistant to extinction than continuous reinforcement [22, 24, 25, 33]. Thus, nocebo hyperalgesia may be even more resistant to extinction than it currently appears when it is established under partial reinforcement.

The current study addressed these gaps in knowledge by comparing the magnitude and rate of extinction of nocebo hyperalgesia following continuous and partial reinforcement schedules using experimentally-induced pain. We recently conducted the only study to date comparing continuous and partial reinforcement on placebo analgesia and found that partial reinforcement produced weaker placebo analgesia than continuous reinforcement, but that the placebo analgesia established under partial reinforcement was more resistant to extinction [4]. If the training schedule affects nocebo hyperalgesia in a similar way, then one would expect weaker initial nocebo hyperalgesia following partial reinforcement that is more resistant to extinction compared with continuous reinforcement. However, given the asymmetries mentioned above, it seemed quite plausible from the outset that partial reinforcement may affect the development of nocebo hyperalgesia differently to 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 following partial reinforcement.

METHODS

Participants

One-hundred and thirty-five (54% female; mean age=20.3, SD=4.0) undergraduate students from the University of Sydney participated. One-hundred and 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 partial reinforcement 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 actually a dummy device with its supposed 'activation' signalled by tactile vibration and a beeping sound. Participants were randomly allocated to one of three groups. The continuous reinforcement group (CRF) received training in which every time the TENS was activated (nocebo trials), the pain simulation was surreptitiously increased relative to no TENS trials (control trials). The partial reinforcement group (PRF) received training in which on only 62.5% of nocebo trials the pain stimulation was surreptitiously increased relative to control trials, but on the remaining 37.5% of nocebo trials the pain stimulation remained the same as control trials. A 62.5% PRF schedule was chosen to approximate a ratio of reinforcement to non-reinforcement 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 only be activated on 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 non-contingent such that the pain was surreptitiously increased on half of the trials with the device active and half of the trials with the device inactive. This was done in blocked fashion as per Au Yeung et al [4] to avoid any potential superstitious conditioning. For all groups, the training phase consisted of 32 trials in total: 16 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 not increase in level of pain stimulation.

[TABLE 1 HERE]

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 following each painful stimulus. In a novel addition to the current study, we also assessed participants' expectancies for pain prior to 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 two conditioning groups received more substantial information on TENS as follows. Prior to the dummy device being attached, they received a one 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:

> "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. I'll give you an example of what it feels like now."

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 only received oral instructions suggesting that a device measuring skin conductance was being attached to their arm. The instructions were:

> "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. I'll give you an example of what it feels like now."

Nocebo device. No TENS was actually 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) that generated tactile stimulation via direct currents sent to electrodes attached on the dorsal forearm on the participant's non-dominant hand. A full description can be found in [4].

Pain stimuli. Pain was induced via electro-cutaneous 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, e.g. CO2 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 non-dominant hand via two silver chloride electrodes, each approximately 1cm apart. Stimuli were generated by a pain stimulator (Model SHK1, Contact Precision Instruments). The stimuli were square pulses with duration of 0.5 sec and frequency of 100 Hz.

The intensity of the pain stimuli was calibrated for each participant individually prior to 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 participant was 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 ten on a verbally reported scale. The level of intensity reached at the end of calibration was labelled as the 100% intensity for that particular participant. Intensity of each stimulus during the experiment was determined on the individual's 100% intensity level, their experimental condition, and the particular trial.

Pain ratings. Participants were asked to rate their pain following each painful stimulus on a 100 point computerised visual analogue 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 response meter (model MLT1601/ST, ADInstruments, Sydney, NSW) in which the participant 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 labelled 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 signalled by a 10 second countdown culminating in an "X" appearing on the computer screen, 0.5 seconds after which the painful stimulus was delivered. A prompt appeared on the screen reminding participants to rate their expectancy for 7 secs during the countdown. After each stimulus, participants rated the intensity of the pain on the computerised VAS. In between each trial,

participants had a rest of 10-15 seconds. On nocebo trials, the device was activated for 8sec 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 out of the 16 nocebo trials, as shown in Table 1. The nocebo trials were intermixed with control trials in quasi-randomised order within participants, such that there were no more than two nocebo or control trials in a row. This trial order was employed 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 with the other half at 60% pain intensity. These trials were presented in four blocks of 8 trials (4 trials with the device active and 4 with the device inactive) with two blocks being at 100% intensity and four 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 if they only ever received 100% painful stimulation [4]. The blocked design was intended to prevent potential superstitious conditioning, even though the two events were non-contingent.

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 actual level of stimulation being identical across the two.

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 one-hour session and were tested individually in an isolated testing booth. Upon 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 two conditioning groups were then told that they had been allocated to receive TENS and were given the handout on TENS. The control group was told that they had been allocated to receive no treatment and simply rested for two minutes. The nocebo device was then introduced and attached to the participant, during which each group was given the relevant oral instructions.

The experimenter then explained the trial structure to the participant, left the room, and then initiated the computerised programme 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 email at the completion of the study.

Data handling and analysis

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

ANOVA and Chi-square tests of independence tested for baseline differences in age and gender. For the main analysis on the pain data, conditioning and test phases were analysed 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) that were analysed via mixed ANCOVAs with group and trial as factors, controlling for age and gender. Age and gender were included as covariates as both have been found to influence pain perception in general [21, 38] as well as the placebo effect specifically [37]. However, the pattern of results were 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, i.e. 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 factor. To isolate the effects of the different conditioning schedules we conducted planned pairwise comparisons between each

group. These inter-group 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 gender.

All analyses were conducted in IBM SPSS Statistics 20.0, covariates were mean centred to reduce multicollinearity and 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 gender across the three 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 Figure 1. There was no statistically significant difference in pain ratings averaged across the 60% trials when the nocebo device was inactive during training ($F_{2,114}=0.91$, $p=.41$, $\eta_p^2=.02$), suggesting no differences in overall pain sensitivity between groups. The conditioning manipulation was effective in producing increased pain in both the CRF and PRF group on relevant nocebo trials during training. In the CRF group, pain was rated as 36.6 (SD=13.5) points higher on nocebo trials with the 100% pain stimulation relative to the control trials (always 60% pain stimulation: $F_{1,34}=247.0$, $p<.001$, $\eta_p^2=.88$). 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 ($F_{1,37}=265.0$, $p<.001$, $\eta_p^2=.88$). 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, η_p^2=.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, η_p^2=.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, η_p^2=.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, η_p^2=.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.

[FIGURE 1 HERE]

Test Phase: Pain ratings during the test phase - where all pain stimulation was set to 60% irrespective of whether or not the device was active - are shown in Figure 1. Differences in these pain ratings on nocebo and control trials were compared between groups on the first test trial (where 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, η_p^2=.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, η_p^2=.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, η_p^2=.02), nor was nocebo hyperalgesia in the CRF significantly greater than the PRF group ($F_{1,114}$=2.44, p=.12, η_p^2=.02).

The two-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, η_p^2=.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, η_p^2=.15). There was also a statistically significant nocebo hyperalgesic effect of 4.0 points in the PRF group relative to control ($F_{1,114}$=4.26, p=.04, η_p^2=.04). The strength of the nocebo hyperalgesia was significantly greater in the CRF group relative to the PRF group (mean diff=4.76, $F_{1,114}$=5.57, p=.02, η_p^2=.05). There was, however, no main effect of trial nor a significant group by trial interaction ($F_{10.4,1710}$=1.41, p=.13, η_p^2=.01 and $F_{20.9,1710}$=0.93, p=.55, η_p^2=.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).

[TABLE 2 HERE]

Expectancy

Training Phase: Expectancy ratings for each of the groups are shown in Figure 1. As with the pain ratings, differences in expectancy ratings on nocebo versus control trials were calculated (labelled '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_p^2=.06 and $F_{2,114}$=15.2, p<.001, η_p^2=.21, respectively) as well as a significant time by treatment interaction ($F_{21.0,1710}$=2.70, p<.001, η_p^2=.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_p^2=.21) . Similarly, the PRF group expected an average of 9.3 points more pain than control group on these trials ($F_{1,114}$=9.43, p=.002, η_p^2=.08). Further, 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_p^2=.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_p^2=.15 and $F_{1,114}$=11.4, p=.001, η_p^2=.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_p^2=.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_p^2=.21). Pairwise comparisons revealed that the CRF group expected 26.4 points more pain on nocebo trials relative to control trials than the control group did ($F_{1,114}$=25.1, p<.001, η_p^2=.18). Similarly, the PRF group expected 23.0 points more pain than the control group did on nocebo relative to control trials ($F_{1,114}$=19.9, p<.001, η_p^2=.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 ($F_{1,114}$=0.40, p=.53, η_p^2<.01).

The two-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_p^2=.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_p^2=.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_p^2=.13). As with the first trial, nocebo expectancy averaged across the entire test phase was numerically higher

in the CRF group than the PRF, but this was not statistically significant ($F_{1,114}$=1.21, p=.27, η_p^2=.01). There was also a significant main effect of trial ($F_{10.3,1710}$=1.99, p=.03, η_p^2=.02) and a significant treatment by trial interaction ($F_{20.6,1710}$=1.70, p=.03, η_p^2=.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_p^2=.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_p^2=.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 group, nor the CRF and PRF groups ($F_{1,114}$=1.21, p=.27, η_p^2=.01 and $F_{1,114}$=2.02, p=.16, η_p^2=.02). This suggested that there was some extinction of nocebo expectancy in the CRF group relative to control, but not for PRF relative to control, nor between CRF and PRF.

[FIGURE 2 HERE]

Expectancy and Nocebo Hyperalgesia

Figure 2 shows scatterplots of nocebo expectancy and nocebo hyperalgesia averaged across the test phase for each group. Multiple linear regressions controlling for age and gender 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 one clear outlier within the CRF group. As can be seen in Figure 2A, this participant had mean nocebo expectancy of -39.2 (i.e. expected 39.2 points *less* pain when the nocebo was activated), which was 3.2 standard deviations 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 meant that after controlling for age and gender, nocebo expectancy uniquely accounted for 50.7% of the variance in nocebo hyperaglesia 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

The majority of 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, two (<2%) mentioned conditioning, and only one (<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 following partial reinforcement. Second, nocebo hyperalgesia produced by partial reinforcement is weaker than that produced by continuous reinforcement. 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 following partial reinforcement. This extends previous evidence of nocebo hyperalgesia following continuous reinforcement [10, 15, 16, 20, 28, 29], by demonstrating that nocebo hyperalgesia can result even when the contingency between the the nocebo and the nociceptive stimulus is more variable - as may often be the case outside of the laboratory. Thus, the current study increases the ecological validity of laboratory research on nocebo hyperalgesia. It is, however, important to emphasise that the magnitude of the nocebo hyperalgesia produced following partial reinforcement was weaker than that produced following continuous reinforcement. Using Cohen's [12] rules of thumb, the nocebo hyperalgesia induced by continuous reinforcement had a large effects size (η_p^2=.15) whereas for partial reinforcement it was moderate-to-weak (η_p^2=.04). Weaker nocebo hyperalagesia following partial reinforcement is consistent with animal studies that have found evidence of weaker conditioned responding following partial reinforcement compared with continuous reinforcement [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 during the entire test period following training under both partial and continuous reinforcement. This points towards another asymmetry between nocebo hyperalgesia and placebo analgesia in that we recently found that placebo analgesia produced under continuous reinforcement does extinguish [4]. While resistance to extinction of nocebo hyperalgesia following partial reinforcement is consistent with partial reinforcement extinction effects observed in other areas [33], the failure of the nocebo

hyperalgesia established under continuous reinforcement to extinguish could be considered more surprising. This is because conditioned responding following continuous reinforcement typically extinguishs 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 continuous reinforcement fails to extinguish. Specifically, Colloca et al. [16] found that nocebo hyperalgesia was maintained across six extinction trials following continuous reinforcement. Current models propose that nocebo hyperalgesia is at least partially mediated by increased anxiety, one of which's effects is activation of cholecystokinin (CCK) receptors that potentiate pain [18]. Coupled with evidence that people with anxiety disorders, such as PTSD, exhibit impaired extinction of conditioned fear responses [36], 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 and Colloca et al.'s studies involved healthy participants and not ones with anxiety disorders, this may seem at odds with fear conditioning studies on healthy participants, which do show extinction [23]. However, in fear conditioning studies the extinction phase involves the entire removal of the aversive stimulus (i.e. CS→no shock), whereas in nocebo hyperalgesia studies it involves a reduction of the intensity of the painful stimulation, not its entire removal (i.e. 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. Importantly, the current study extends Colloca et al.'s by demonstrating that nocebo hyperalgesia following continuous reinforcement 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. These generally indicated strong concordance nocebo hyperalgesia. Expectancy accounted for between 48-54% of the variance in nocebo hypalgesia 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, 35]. However, while 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 the two involve fairly different types of ratings, that is an appraisal of pain versus an appraisal of a belief, any apparent discordance could be due to differences in the sensitivity of each type of rating to detect changes.

The effect of 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 (i.e. partial reinforcement) 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 following partial reinforcement relative to continuous reinforcement approached a moderate effect size (η_p^2=.05) [12]. Given that the effect size for continuous reinforcement was large (η_p^2=.15), this suggests that using partial reinforcement could lead to substantial benefits to patients in the clinic. Further, reducing the magnitude of nocebo hyperalgesia may be particularly important given its apparent persistence following both continuous and partial reinforcement. If the current failure of nocebo hyperalgesia to extinguish generalises 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 may be observed if we extended the test phase. Importantly, however, the test phase used here involved 16 test trials, which is almost three times longer than in Colloca et al. [16] and the same amount that 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. As such, it would be interesting to test whether the current findings generalise to a clinical setting in which both training and testing occur over multiple days, rather than a single session. At least one study suggests that nocebo hyperalgesia established via instruction alone can be maintained for up to 90 days [34], 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 partial reinforcement. That is, while there was no partial reinforcement extinction effect observed here in the single session employed 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 in order to match the total length of training across the two 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..

Finally, 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 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 in fact 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 then, the current study provides novel evidence that nocebo hyperalgesia can result following partial reinforcement, that this nocebo hyperalgesia is weaker than that produced by continuous reinforcement, and that both are resistant to extinction. The weaker nocebo hyperalgesia following partial reinforcement 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 nocebo hyperalgesia's apparent persistence once established.

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References

[1] Ader R. Conditioned immunopharmacological effects in animals: Implications for a conditioning model of pharmacotherapy. In: White L, Tursky B, Schwartz GE, (eds): Placebo: Theory, research, and mechanisms. New York, Guilford Press, 1985, pp 306-23.

[2] Ader R, Cohen N. Behaviorally conditioned immunosuppression. Psychosom Med 37:333-40, 1975.

[3] Amanzio M, Benedetti F. Neuropharmacological dissection of placebo analgesia: Expectation-activated opioid systems versus conditioning-activated specific subsystems. J Neurosci 19:484-94, 1999.

[4] Au Yeung ST, Colagiuri B, Lovibond PF, Colloca L. Partial reinforcement, extinction, and placebo analgesia. Pain 155:1110-7, 2014.

[5] Benedetti F, Amanzio M, Casadio C, Oliaro A, Maggi G. Blockade of nocebo hyperalgesia by the cholecystokinin antagonist proglumide. Pain 71:135-40, 1997.

[6] Benedetti F, Amanzio M, Vighetti S, Asteggiano G. The biochemical and neuroendocrine bases of the hyperalgesic nocebo effect. J Neurosci 26:12014-22, 2006.

[7] Benedetti F, Lanotte M, Lopiano L, Colloca L. When words are painful: Unraveling the mechanisms of the nocebo effect. Neuroscience 147:260-71, 2007.

[8] Benedetti F, Mayberg HS, Wager TD, Stohler CS, Zubieta J-K. Neurobiological mechanisms of the placebo effect. J Neurosci 25:10390-402, 2005.

[9] Benedetti F, Pollo A, Lopiano L, Lanotte M, Vighetti S, Rainero I. Conscious expectation and unconscious conditioning in analgesic, motor, and hormonal placebo/nocebo responses. J Neurosci 23:4315-23, 2003.

[10] Bingel U, Wanigasekera V, Wiech K, Ni Mhuircheartaigh R, Lee MC, Ploner M, Tracey I. The Effect of Treatment Expectation on Drug Efficacy: Imaging the Analgesic Benefit of the Opioid Remifentanil. Science Translational Medicine 3:70ra14, 2011.

[11] Bouton ME: Learning and Behavior: A Contemporary Synthesis. Sunderland, MA, Sinauer Associates, 2007.

[12] Cohen J: Statistical power analysis for the behavioral sciences. Hillsdale, N.J, Lawrence Erlbaum Associates, 1988.

[13] Colloca L, Benedetti F. How prior experience shapes placebo analgesia. Pain 124:126-33, 2006.

[14] Colloca L, Benedetti F. Nocebo hyperalgesia: how anxiety is turned into pain. Curr Opin Anaesthesiol 20:435-9, 2007.

[15] Colloca L, Petrovic P, Wager TD, Ingvar M, Benedetti F. How the number of learning trials affects placebo and nocebo responses. Pain 151:430-9, 2010.

[16] Colloca L, Sigaudo M, Benedetti F. The role of learning in nocebo and placebo effects. Pain 136:211-8, 2008.

[17] Domjan M: The Principles of Learning and Behavior: Active Learning Edition. 5th ed. Belmont, CA, Thomson Wadsworth, 2006.

[18] Enck P, Benedetti F, Schedlowski M. New insights into the placebo and nocebo responses. Neuron 59:195-206, 2008.

[19] Fitzgerald RD. Effects of partial reinforcement with acid on the classically conditioned salivary response in dogs. J Comp Physiol Psychol 56:1056-60, 1963.

[20] Geuter S, Buchel C. Facilitation of pain in the human spinal cord by nocebo treatment. J Neurosci 33:13784-90, 2013.

[21] Gibson SJ, Helme RD. Age-related differences in pain perception and report. Clin Geriatr Med 17:433-56, v-vi, 2001.

[22] Haselgrove M, Aydin A, Pearce JM. A Partial Reinforcement Extinction Effect Despite Equal Rates of Reinforcement During Pavlovian Conditioning. J Exp Psychol Anim Behav Process 30:240-50, 2004.

[23] Hermans D, Craske MG, Mineka S, Lovibond PF. Extinction in human fear conditioning. Biol Psychiatry 60:361-8, 2006.

[24] Jenkins WO, Stanley JC. Partial reinforcement: a review and critique. . Psychol Bull 47:193-234, 1950.

[25] Kimnle GA: Hilgard and Marquis' conditioning and learning. New York, Appleton-Century-Crofts, 1961.

[26] Kirsch I: How Expectancies Shape Experience. Washington, American Psychological Association, 1999.

[27] Kirsch I. Response expectancy as a determinant of experience and behaviour. Am Psychol 40:1189-202, 1985.

[28] Kong J, Gollub RL, Polich G, Kirsch I, Laviolette P, Vangel M, Rosen B, Kaptchuk TJ. A functional magnetic resonance imaging study on the neural mechanisms of hyperalgesic nocebo effect. J Neurosci 28:13354-62, 2008.

[29] Kong J, Gollub RL, Polich G, Kirsch I, Laviolette P, Vangel M, Rosen B, Kaptchuk TJ. A functional magnetic resonance imaging study on the neural mechanisms of hyperalgesic nocebo effect. J Neurosci 28:13354-62, 2008.

[30] Levine JD, Gordon NC. Influence of the method of drug administration on analgesic response. Nature 312:755-6, 1984.

[31] Levine JD, Gordon NC, Fields HL. The mechanism of placebo analgesia. Lancet 2:654-7, 1978.

[32] Lorenz J, Hauck M, Paur RC, Nakamura Y, Zimmermann R, Bromm B, Engel AK. Cortical correlates of false expectations during pain intensity judgments--a possible manifestation of placebo/nocebo cognitions. Brain Behav Immun 19:283-95, 2005.

[33] Pearce JM, Redhead ES, Aydin A. Partial Reinforcement in Appetitive Pavlovian Conditioning with Rats. The Quarterly Journal of Experimental Psychology Section B 50:273-94, 1997.

[34] Rodriguez-Raecke R, Doganci B, Breimhorst M, Stankewitz A, Büchel C, Birklein F, May A. Insular Cortex Activity Is Associated with Effects of Negative Expectation on Nociceptive Long-Term Habituation. The Journal of Neuroscience 30:11363-8, 2010.

[35] Stewart-Williams S, Podd J. The placebo effect: Dissolving the expectancy versus conditioning debate. Psychol Bull 130:324-40, 2004.

[36] VanElzakker MB, Kathryn Dahlgren M, Caroline Davis F, Dubois S, Shin LM. From Pavlov to PTSD: The extinction of conditioned fear in rodents, humans, and anxiety disorders. Neurobiol Learn Mem 113:3-18, 2014.

[37] Weimer K, Colloca L, Enck P. Age and sex as moderators of the placebo response - an evaluation of systematic reviews and meta-analyses across medicine. Gerontology 61:97-108, 2015.

[38] Wiesenfeld-Hallin Z. Sex differences in pain perception. Gend Med 2:137-45, 2005.

Tables

Table 1. Summary of study design. In the CRF and PRF groups the participants were led to believe 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 labelled control trials.

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%

Table 2. Summary of ANCOVA models and relevant pairwise comparisons for pain and expectancy during the test phase. Omn. refers to the omnibus test for that component of the model.

	Group				Trial		Group by Trial			
		Pairwise						Pairwise x 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										
Frist test trial										
F	4.37	8.75	1.83	2.44	-	-	-	-	-	-
p	.01	.004	.18	.12						
All test trials	10.1	20.2								
F	<.00	<.00	4.26	5.57	1.41	1.48	.93	.39	.35	.01
p	1	1	.04	.02	.13	.23	.55	.54	.55	.98
Expectancy										
Frist test trial	15.2	25.1	19.9							
F	<.00	<.00	<.00	.40	-	-	-	-	-	-
p	1	1	1	.53						
All test trials	15.6	27.6	17.2							
F	<.00	<.00	<.00	1.21	1.99	7.73	1.70	6.56	1.21	2.02
p	1	1	1	.27	.03	.006	.03	.01	.27	.16

Figures

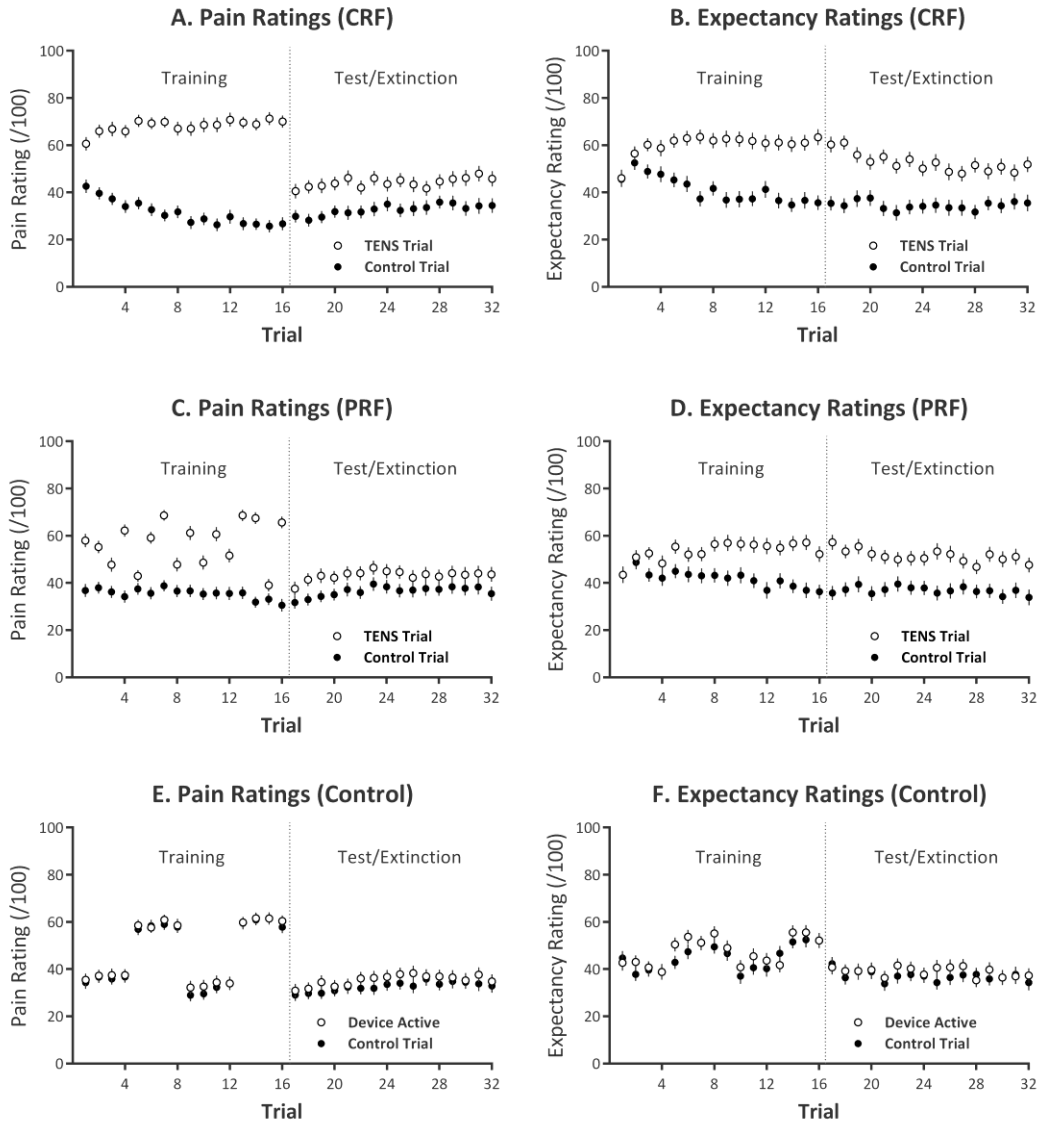

Figure 1. Covariate (age, gender) adjusted mean (±SE of mean difference) pain (A, C, E) and expectancy ratings (B, D, F) with the device active versus inactive for the continuous reinforcement group.

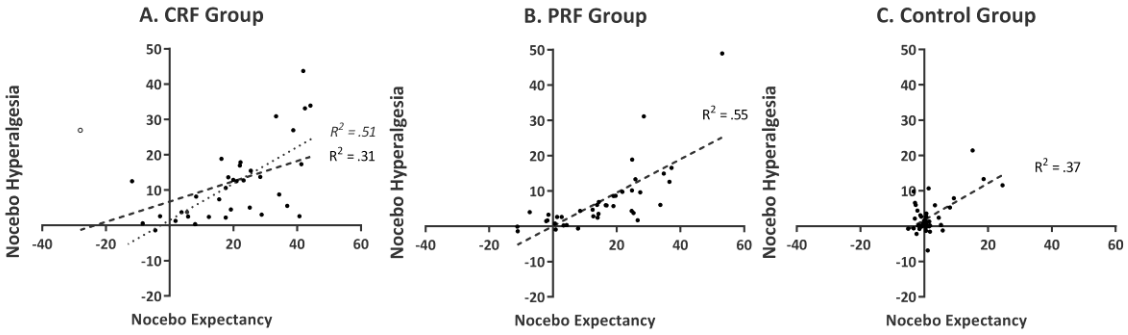

Figure 2. Covariate (age, gender) adjusted scatterplot of nocebo expectancy and nocebo hyperalgesia averaged across the test phase separately for each group. Lines reflect the slope of expectancy predicting hyperalgesia in the multiple linear regression controlling for age and gender. In the CRF group (A), there was one clear outlier who had averaged expectancy more than three standard deviations below the mean for that group (hollow circle), with the lighter dashed line showing the regression slope with that participant excluded.