Journal of Behavior Therapy and Experimental Psychiatry 64 (2019) 106–112

Contents lists available at ScienceDirect

Journal of Behavior Therapy and Experimental Psychiatry

journal homepage: www.elsevier.com/locate/jbtep

Nocebo hyperalgesia induced by implicit conditioning

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ARTICLE INFO

Keywords:
Nocebo effect
Implicit conditioning
Electrodermal activity
Contingency awareness
Pain perception

ABSTRACT

Background and objectives: Nocebo hyperalgesia (i.e., increased pain sensitivity based on expectations) can be induced by conditioning, but is supposed to be mediated by conscious expectation. Although recent evidence points to the feasibility of subliminal conditioning of nocebo hyperalgesia with masked faces, face processing might be a special case and the practical implications of subliminal conditioning remain questionable. This study aimed to implicitly condition nocebo hyperalgesia using supraliminal cues.

Methods: Implicit differential nocebo conditioning (N = 48 healthy participants) was implemented by coupling high and low painful electric stimuli to varying visual stimuli that only differed in the symmetry/asymmetry of one component (CS+/CS-) and contained further distracting components. In the test phase, only the low painful stimulus followed both CS to test for conditioned nocebo effects in intensity and aversiveness ratings and electrodermal activity. A behavioral contingency test and a post-experimental questionnaire assessed contingency awareness.

Results: A conditioned effect emerged in the aversiveness (p = .036; η^2 = 0.09), but not in the intensity rating (p = .195) while controlling for contingency awareness. Further, increased skin conductance levels in response to CS + emerged, irrespective of contingency awareness (p = .014, η^2 = 0.13). No conditioned responses in skin conductance responses emerged (p = .872).

Limitations: Expected effects only emerged in part of the outcome variables.

Conclusions: The results support the notion that implicit conditioning of nocebo hypoalgesia is feasible using a novel experimental conditioning design with supraliminal stimulus presentation, although further research is needed. So far, implicitly conditioned nocebo effects might have been underestimated despite vast clinical implications.

1. Introduction

The importance of nocebo effects (adverse effects due to anticipation of negative outcomes) in clinical practice and research is beyond dispute (Colloca & Finniss, 2012; Colloca & Miller, 2011b). Yet, nocebo effects remain understudied compared to the better-known placebo effects, though research shows that findings from placebo research cannot simply be transferred to nocebo effects (Colloca, Sigaudo, & Benedetti, 2008; Petrovic, 2008; Reicherts, Gerdes, Pauli, & Wieser, 2016).

According to Colloca and Miller (2011a), placebo and nocebo effects develop through expectation, which can be induced by verbal suggestion, conditioning, as well as observational learning (Montgomery & Kirsch, 1997; van Laarhoven et al., 2011; Vögtle, Barke, & Kröner-Herwig, 2013). Expectation, however, can be described in different ways (Colloca & Miller, 2011a). Whereas some define it as conscious and reportable (Stewart-Williams & Podd, 2004), others argue that expectations also exist on an implicit level unavailable to conscious experience and therefore not reportable (Kirsch, 2004; Kirsch, Lynn, Vigorito, & Miller, 2004). Accordingly, conditioning without additional verbal suggestion led to placebo hypoalgesia (decreased pain sensitivity) and nocebo hyperalgesia (increased pain sensitivity) that was not predicted by expectancy ratings (Babel et al., 2017, but see also; Carlino et al., 2015).

Along these lines, there have been long-lasting debates and heterogeneous results on implicit conditioning (i.e., conditioning without contingency awareness), depending e.g., on the dependent variable, conditioning design, and method of assessment of contingency awareness (Clark, Manns, & Squire, 2002; Lovibond & Shanks, 2002). Yet, accumulating evidence supports the existence of implicitly conditioned effects (Knight, Nguyen, & Bandettini, 2006, 2003; Schultz & Helmstetter, 2010) possibly mediated by implicit expectations.

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https://doi.org/10.1016/j.jbtep.2019.03.006
Received 17 August 2018; Received in revised form 11 March 2019; Accepted 26 March 2019
Available online 29 March 2019
0005-7916/

Conditioned nocebo effects independent from explicit expectation have been shown in the context of immunosuppression (e.g., nausea in response to an inert treatment during chemotherapy; Pacheco-Lopez, Engler, Niemi, & Schedlowski, 2006), as well as for respiratory depressant responses (a typical side-effect of narcotics) after repeated administration of opioids for pain control (Benedetti, Amanzio, Baldi, Casadio, & Maggi, 1999). In contrast, pain perception has been assumed a conscious process, necessitating conscious expectation in order to induce a nocebo effect (Benedetti et al., 2003). Contrary to that, limited evidence suggests that implicit conditioning of nocebo hyperalgesia might be feasible (Bräscher et al., 2017, 2018), especially by using subliminally (i.e., below the threshold for conscious perception) presented masked faces as conditioned stimuli (Jensen, Kirsch, Odmalm, Kaptchuk, & Ingvar, 2015). Subliminal stimulus presentation, however, has certain limitations. Subthreshold stimulus presentation cannot be guaranteed due to interindividual differences, fluctuating perception threshold, and attentional effects (Lahteenmaki, Hyona, Koivisto, & Nummenmaa, 2015), possibly compromising the implicitness of the conditioned effects. Further, evidence indicates that facial cues might be a special case due to particular processing (e.g., neural processing in a specialized fusiform area; Kanwisher & Yovel, 2006) that potentially facilitates conditioning processes. Other subliminally presented cues (e.g., words, images), for example, were not able to activate (explicitly) conditioned effects (Egorova et al., 2015b, 2017), questioning the relevance of the phenomenon for clinical daily routine.

The aims of the present study were to test whether implicit conditioning of nocebo hyperalgesia is effective, indicated 1) by subjective ratings of intensity and aversiveness of electric stimulation (primary outcomes) and 2) by electrodermal activity (secondary outcome), and 3) to introduce a new paradigm for implicit differential conditioning, not depending on subliminal stimulus presentation. Therefore, visual stimuli served as cues contingently differing only in symmetry or asymmetry of one component of the stimulus. Visual stimuli were different in every trial and distracting components were added to prevent the development of contingency awareness.

2. Methods

2.1. Sample

A power analysis estimated a required sample size of $N = 44$ (medium effect $f = 0.25$, alpha = .05, power = 0.90; GPower 3.1.9.2) for the main effect in a repeated measures ANOVA. Thus, the proposed sample size of $N = 48$ will be more than adequate for the main objective of this study and should allow for expected attrition.

Participants were recruited via social media and e-mail. Exclusion criteria (c.f. Supplementary Material) were checked with a screening questionnaire. Forty-eight healthy participants ($M = 25.79$ years, $SD = 4.45$; 25 females) took part in the experiment after giving written informed consent. Participants received monetarily compensation after the experiment. The experimental protocol was conducted in accordance with the Declaration of Helsinki (2008) and approved by the Local Ethics Committee.

2.2. Visual and electric stimuli

Weakly (low pain) and moderately painful electric stimuli (high pain; 500 ms) were applied to the dorsal index finger of the dominant hand by a bipolar constant-current stimulator (DS5; Digitimer, Welwyn Garden City, Hertfordshire, UK; cf. Supplementary Material) and served as unconditioned stimuli (US).

Abstract pictures (black figures on white ground with red lines in the foreground; Fig. 1) served as conditioned stimuli. For example, pictures with a symmetrical black figure were coupled to high pain (CS +), whereas pictures with asymmetrical black figures were coupled to low pain (CS-). This assignment was counterbalanced across participants, i.e., for half of the participants, symmetrical figures serves as CS + and for the other half asymmetrical figures served as CS + . Red lines in the foreground of the pictures served as distractors. There was no other differentiating feature (e.g. shape, complexity, percentage of white or black area, etc.) with regard to the contingency besides symmetry. A new picture was presented in every trial (in total 110 different pictures) in order to hinder the development of contingency awareness. Pictures were presented for 4 s and at a randomized time point within the last two seconds, the electric shock was applied (delay conditioning, i.e., the US is presented during the presentation of the CS).

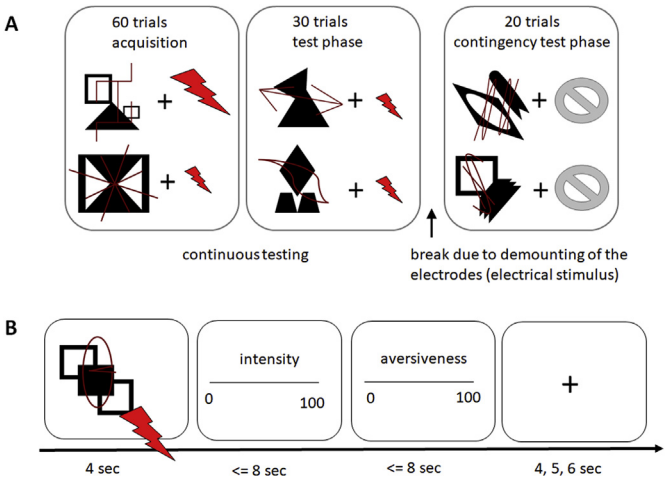

Fig. 1. A Experimental design illustrating the number of trials during acquisition, test, and contingency test phase as well as coupling of the visual stimuli (CS + and CS-) with the high and low painful electrical stimulus in acquisition (illustrated as an example: asymmetrical picture as CS + , symmetrical picture as CS-). During test phase, only the low painful electrical stimulus was applied and during contingency test phase, no electrical stimulus was applied. **B**: Trial sequence. A trial starts with the presentation of the CS (4 s). During the last two seconds of the CS, the electric stimulus is applied (only during acquisition and test phase). Afterwards, participants rate the electric stimulus on two visual analog scales (intensity and aversiveness). A fixation cross (4, 5, or 6 s) is shown before the start of the next trial.

2.3. Psychophysical scales

On the basis of the instructions used in Price, McGrath, Rafii, and Buckingham (1983), the difference between intensity (i.e., how strong the pain is, like the volume of a radio) and aversiveness of a sensation (i.e., how unpleasant or disturbing the pain is, e.g., a sound can be unpleasant independent from the volume) was explained to the participants. Participants were then familiarized with two horizontally oriented visual analog scales (VAS) in order to independently rate intensity and aversiveness of the electric stimuli. The intensity scale was labelled with 0 'not detectable' and 100 'very painful'. At a scale value of 40, an additional anchor was included, labelled 'just painful' (Lautenbacher, Möltner, & Strian, 1992). The aversiveness scale had the descriptors 0, 'neutral' and 100 'very aversive'.

2.4. Experimental design and procedure

Participants took part in one experimental session of approximately 45 min duration and were told that the purpose of the experiment was to investigate the impact of visual stimuli on pain perception. They completed calibration (c.f. Supplementary Material), the conditioning procedure, and the contingency test phase. Afterwards, they answered the post-experimental questions in order to assess second-order contingency awareness (i.e., being aware of being aware).

The conditioning procedure (Fig. 1A) started with the acquisition phase, in which the high pain stimulus was applied during the

presentation of the CS+ and the low pain stimulus was applied during the presentation of the CS- in 60 trials. In the subsequent test phase, only low pain stimuli were applied both during the presentation of CS+ and CS- in 30 trials. After that, the contingency test phase followed with 20 trials, in which no electric stimulus was applied during and the participants were asked to indicate their sensation as if a stimulus had been presented. This sequence served to test first-order contingency awareness (i.e, awareness that does not need introspection, cf. Becker, Kleinböhl, & Hölzl, 2012). The sequence of CS- and CS + trials was randomized within each phase.

Every trial of the acquisition and test phase started with the presentation of a CS (4 s), during which an electric stimulus was applied (the exact time point of the electric shock was randomized within the last 2 s of the presentation of the CS). Then, participants rated their subjective sensation of the electric stimulus on the intensity and the aversiveness VAS. A fixation cross (4–6 s) followed before the start of the next trial (Fig. 1B). In the contingency test phase, no electric shock was applied and the fixation cross was only shown for 2 s, nothing else was changed.

2.5. Post-experimental questionnaire

After the experiment, participants answered a series of questions in a funnel debriefing manner (i.e., asking increasingly specific questions; Chartrand, van Baaren, & Bargh, 2006) in order to assess second-order awareness (cf. Supplementary Material) (Becker et al., 2012).

2.6. Electrodermal activity (EDA)

EDA was analyzed by means of continuous decomposition analysis (CDA) with a response window of 1–4 s after the onset of the respective picture and an amplitude threshold of 0.01 μS (c.f. Supplementary Material for details on data acquisition and preprocessing). This time interval corresponds to the first interval response (FIR), which in differential conditioning designs has shown to be most effective in detecting conditioned responses. Ledalab returns various parameters of phasic and tonic activity, of which CDA.SCR (phasic activity within the response window; skin conductance response) and CDA.Tonic (decomposed tonic component within the response window; skin conductance level) were further analyzed.

2.7. Statistical analyses

Due to equipment failure, one participant's answers to the post-experimental questionnaire were not saved properly, resulting in missing data.

For identification of participants who showed first-order contingency awareness in the contingency test phase, the reliable change index (RCI; Jacobson, Follette, & Revenstorf, 1984), was calculated using Cronbach's α of the respective ratings of the low stimulus during the acquisition phase to determine reliability (r_{tt}; Hiller & Schindler, 2011; RCI = $(X_2\text{-}X_1)/S_{diff}$, whereby S_{diff} is the standard error of the difference score $S_{diff} = \sqrt{2(S_E)^2}$, which can be calculated using the standard error of measurement S_E according to $S_E = S_1\sqrt{1 - r_{tt}}$). Coming from psychotherapy research, the RCI measures whether a change in a person's score from one assessment to the next is statistically significant, i.e., larger than expected by chance, considering the reliability of the measuring instrument. As values > 1.96 indicate a significant change of the individual, participants with an RCI > 1.96 were excluded in those subsequent analyses that tested for the conditioned effect in first-order contingency unaware participants.

Summing the correct answers to the five yes/no questions in the post-experimental questionnaire (Cronbach's α = 0.64), an index of second-order awareness was calculated (awareness index), assuming that a larger index corresponds to a higher degree in second-order contingency awareness.

Repeated measures ANOVAs for the subjective ratings and skin conductance level with the factors 'cue' (CS+, CS-) and 'experimental phase' (acquisition, test phase, contingency test phase) were used to assess differential responding to trials cued with CS+ and CS-. Excluding participants who became contingency aware according to the contingency test phase, repeated measures ANOVAs followed for the test phase, including the factors 'cue' and 'trial' (one to fifteen) in order to test for possible extinction. Then, the centered awareness index was added as a covariate (for the whole sample) and the conditioned effect was tested again assessing whether second-order contingency awareness is a necessary condition for the conditioned nocebo effect.

Finally, skin conductance levels (SCL) of CS+ and CS- trials, respectively were predicted with multiple linear regressions using both intensity and aversiveness ratings as predictors in order to confirm the validity of the results of previous analyses showing significant results for aversiveness but not intensity ratings.

Greenhouse-Geisser corrections and Bonferroni-corrected post-hoc tests were applied and measures of effect size (η^2, Cohen's *d*) were reported were applicable. Statistical analyses were performed with JASP 0.84 and IBM SPSS Statistics 23.

3. Results

3.1. Measures of contingency awareness

Contingency test – first-order awareness. Testing first-order contingency awareness on an individual level during the contingency test phase, the RCI identified three participants (6%) who rated the CS + trials as more aversive compared to the CS- trials and thereby showing first-order contingency awareness. Concerning the intensity rating, the RCI identified three participants (6%), as well, who rated the CS + as more intense compared to the CS- in the contingency test phase. One participant was considered first-order contingency aware in both measures.

Post-experimental questionnaire – second-order awareness. In the post-experimental questionnaire, five out of 48 participants answered all five yes/no questions correctly and thus had a second-order contingency awareness index of five (11%). Six participants had an index of four (13%), four participants had an index of three (9%), 18 participants (38%) had an index of two, eleven participants had an index of one (23%), and three participants did not answer any question correctly (6%).

3.2. Behavioral data

Aversiveness ratings. A repeated measures ANOVA of the aversiveness ratings revealed increased aversiveness in trials cued with CS + compared to CS- ('cue': F (1, 47) = 95.78, p < .001, η^2 = 0.671; Table 1). A significant main effect for 'experimental phase' (F (1.7, 80.0) = 10.90, p < .001, η^2 = 0.188; Fig. 2) and an interaction between 'cue' and 'experimental phase' (F (1.4, 68.0) = 85.37, p < .001, η^2 = 0.645) emerged. Bonferroni-corrected post-hoc tests demonstrated that aversiveness ratings in the acquisition phase were increased compared to both the test (t (47) = 4.02, p = .001, d = 0.580) and the contingency test phase (t (47) = 3.26, p = .006, d = 0.471), whereas the ratings of the test and contingency test phase did not differ (t (47) = 2.05, p = .138, d = 0.296). Post-hoc tests of the interaction effect showed an increased aversiveness rating of trials cued with CS + compared to CS- in the acquisition phase (t (47) = 11.16, p < .001, d = 1.611) indicating that participants differentiated between both stimulation levels of the electric shock. In the test phase, trials cued with CS+ were rated as more aversive compared to trials cued with CS- (t (47) = 2.10, p = .042, d = 0.303) meaning that participants showed successful learning, as the actual stimulus intensities were identical during this phase (level of the low pain stimulus). No

Table 1
Mean ratings (and standard deviations) of CS + and CS- trials with regards to stimulus intensity and aversiveness and mean skin conductance response (SCR) and skin conductance levels (SCL) in CS + and CS- trials during the different phases of the experiment.

experimental phase	intensity rating		aversiveness rating	
	CS + trials	CS- trials	CS + trials	CS- trials
acquisition	56.91 (18.27)	35.28 (16.15)	51.70 (21.21)	31.81 (18.04)
test	40.01 (20.80)	39.29 (20.14)	36.32 (21.76)	34.66 (21.24)
contingency test	42.70 (17.20)	42.62 (17.29)	38.36 (18.67)	38.21 (19.21)
	SCR in µS		**SCL in µS**	
	CS + trials	**CS- trials**	**CS + trials**	**CS- trials**
acquisition	0.0021 (0.00375)	0.0022 (0.00370)	0.360 (0.2021)	0.363 (0.2076)
test	0.0016 (0.00299)	0.0017 (0.00337)	0.246 (0.1914)	0.228 (0.1919)
contingency test	0.0025 (0.00471)	0.0033 (0.00563)	0.322 (0.2161)	0.301 (0.2227)

difference in the aversiveness ratings appeared in the contingency test phase (t (47) = 0.19, p = .853, d = 0.027), in which no electric stimulus was applied and participants should indicate their sensation upon an imagined electric stimulus.

Aversiveness ratings in contingency unaware participants. Excluding first-order aware participants when recalculating the learning effect indicated that by trend first order unaware participants still evaluated CS + trials as more aversive than CS- trials during the test phase ('cue': F (1, 44) = 3.26, p = .078, η^2 = 0.069). A significant main effect of 'trial' (F (7.0, 309.3) = 3.30, p = .002, η^2 = 0.070) indicated increased aversiveness ratings over time (although Bonferroni-corrected post-hoc tests did not reach significance). No significant interaction effect emerged between 'cue' and 'trial' (F (7.7, 337.2) = 0.92, p = .500, η^2 = 0.020).

Results of the repeated measures ANCOVA including the awareness index as a covariate confirm the small to medium conditioned nocebo effect for the aversiveness rating ('cue': F (1, 45) = 4.67, p = .036; η^2 = 0.092). Second-order contingency awareness did not significantly explain variance (F (1, 45) = 0.01, p = .925; η^2 < 0.001) and the awareness index did not significantly interact with type of CS (F (1, 45) = 0.93, p = .340; η^2 = 0.018), indicating that the analysis did not find evidence in support of moderation by contingency awareness.

Intensity ratings. A repeated measures ANOVA of the intensity ratings revealed increased intensity ratings for trials cued with CS + compared to CS- ('cue' (F (1, 47) = 133.94, p < .001, η^2 = 0.740, Table 1). A significant main effect for 'experimental phase' (F (2, 94) = 12.80, p < .001, η^2 = 0.214; Fig. 3) and an interaction effect between 'cue' and 'experimental phase' emerged (F (1.4, 67.7) = 118.72, p < .001, η^2 = 0.716). Bonferroni-corrected post-hoc tests showed that ratings were higher in the acquisition compared to both the test (t (47) = 4.61, p < .001, d = 0.666) and contingency test phase (t (47) = 3.14, p = .009, d = 0.453), but ratings during test and contingency test phase only differed by trend (t (47) = 2.29, p = .079, d = 0.331). Further, intensity ratings were increased in trials cued with CS + compared to CS- in the acquisition phase (t (47) = 12.51, p < .001, d = 1.806), but not in the test (t (47) = 1.31, p = .195, d = 0.190), and contingency test phase (t (47) = 0.12, p = .906, d = 0.017). This indicates that participants clearly differentiated between both stimulation levels of the electric shock but did not show a conditioned effect in the test phase when the stimulation intensities were identical after both CS + and CS-.

3.3. Electrodermal activity (EDA)

Skin conductance level. A repeated measures ANOVA on SCL indicated an increased tonic component after trials cued with CS + compared to CS ('cue': F (1, 47) = 8.73, p = .005, η^2 = 0.157; Table 1). Further, a significant main effect of 'experimental phase' emerged (F (1.7, 79.9) = 8.60, p = .001, η^2 = 0.155; Fig. 4) and Bonferroni-corrected post-hoc tests showed that SCL was lower in the test phase compared to acquisition (t (47) = 4.99, p < .001, d = 0.720) and contingency test phase (t (47) = 2.57, p = .040, d = 0.371), whereas no difference in SCL emerged between acquisition and contingency test phase (t (47) = 1.40, p = .503, d = 0.202). A significant interaction effect appeared between 'cue' and 'experimental phase' (F (2, 94) = 4.69, p = .011, η^2 = 0.091). Bonferroni-corrected post-hoc tests suggest that SCL after CS + compared to CS- was increased in the test (t (47) = 2.71, p = .013, d = 0.392) and the contingency test phase (t (47) = 2.50, p = .011, d = 0.361), but not in the acquisition (t (47) = 1.00, p = .389, d = 0.144). This pattern of findings supports the existence of conditioned effects found in the behavioral measure (i.e., aversiveness rating) that remained constant even after an extinction phase.

SCL in contingency unaware participants. Excluding first-order contingency aware participants (n = 3) in the analysis of the conditioned effect shown in the test phase led to similar results as in the whole sample. Increased SCL after trials cued with CS + compared to CS- confirmed the learning effect ('cue': F (1, 44) = 6.60, p = .014, η^2 = 0.130). The significant main effect of 'trial' (F (2.3, 100.2) = 5.50,

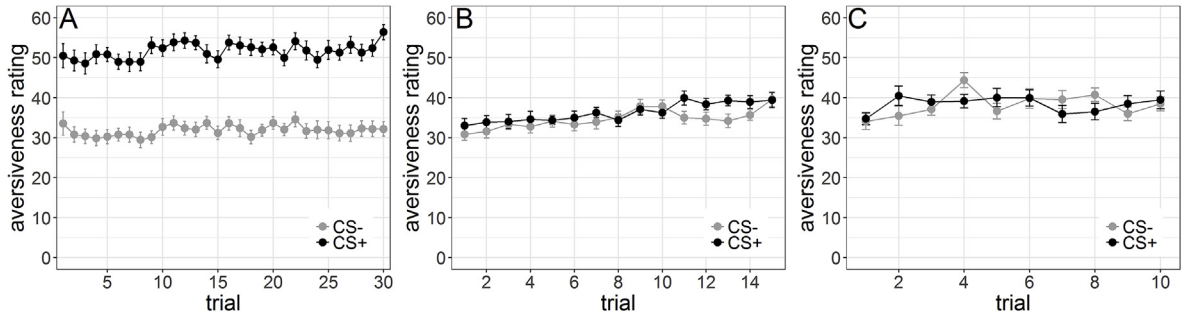

Fig. 2. Mean aversiveness ratings on a visual analog scale of the electric stimuli across trials during acquisition (**A**), test (**B**), and contingency test phase (**C**) with standard error of means.

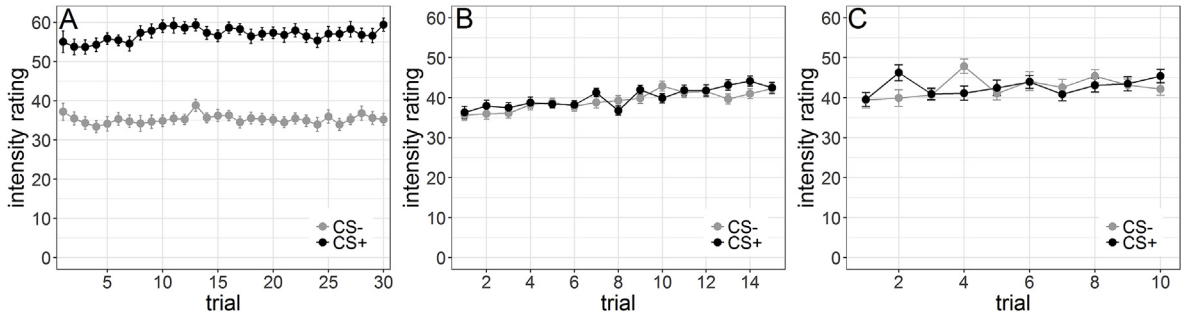

Fig. 3. Mean intensity ratings on a visual analog scale of the electric stimuli across trials during acquisition (**A**), test (**B**), and contingency test phase (**C**) with standard error of means.

p = .004, η^2 = 0.111) along with the Bonferroni-corrected post-hoc tests (trial 1 > trials 4, t (44) = 3.80, p = .046, d = 0.567; trial 1 > trial 6, t (44) = 3.98, p = .026, d = 0.594; trial 1 > trial 7, t (44) = 3.92, p = .033, d = 0.584) indicated a decreasing SCL. The interaction effect between 'cue' and 'trial' (F (5.9, 257.5) = 3.52, p = .002, η^2 = 0.074) and the Bonferroni corrected post-hoc tests (trial 2: t (44) = 2.24,p = .027, d = 0.333; trial 3: t (44) = 3.59, p = .001, d = 0.535; trial 4: t (44) = 2.71, p = .009, d = 0.405; trial 5: t (44) = 3.32, p = .003, d = 0.480; trial 6: t (447) = 2.17, p = .031, d = 0.364; trial 7: t (44) = 1.80, p = .071, d = 0.268) were in accordance with the results of the whole sample.

When including the awareness index as a measure for second-order contingency awareness as a covariate, the conditioned effect remained ('cue': F (1, 45) = 6.52, p = .014, η^2 = 0.126) while neither the covariate (F (1, 45) = 0.27, p = .604, η^2 = 0.006) nor the interaction between 'cue' and covariate (F (1, 45) = 0.14, p = .710, η^2 = 0.003) reached significance. The analysis did not find evidence in support of moderation of the conditioned effect in SCL by contingency awareness.

For the analysis of skin conductance responses, please refer to the Supplementary Material.

Further analyses. In support of the reported results, predicting SCL separately for CS+ and CS- trials in a post-hoc multiple linear regression analyses, only the aversiveness but not the intensity rating was a significant predictor (CS- trials: $\beta_{aversiveness}$ = 0.60, p = .029; $\beta_{intensity}$ = −0.04, p = .889; CS + trials: $\beta_{aversiveness}$ = 0.60, p = .035; $\beta_{intensity}$ = −0.04, p = .887).

4. Discussion

Besides verbal suggestion, learning has proven to be an important factor in the induction of nocebo effects and especially nocebo hyperalgesia (Colloca & Miller, 2011a). Introducing a novel conditioning paradigm, this study investigated whether learning can occur implicitly, i.e., inducing nocebo hyperalgesia in response to a visual cue without the participant's conscious knowledge of this contingency. The results provide preliminary evidence that implicit nocebo conditioning is feasible with a differential conditioning paradigm using supraliminally presented cues. Conditioned effects emerged in the affective but not in the sensory dimension of the pain experience. Further, differential responses in SCL indicate a conditioned effect in autonomic indices.

We are aware of only one study in the context of placebo hypoalgesia that intended to implement an implicit conditioning procedure using supraliminal stimuli, but here only explicit and no implicit learning had taken place (Martin-Pichora, Mankovsky-Arnold, & Katz, 2011). Unconscious activation of previously explicitly conditioned placebo hypoalgesia and nocebo hyperalgesia has been shown before, using subliminally presented cues (Egorova et al., 2015a; Jensen et al., 2012; Tu et al., 2019). Going one step further, implicit conditioning of placebo hypoalgesia and nocebo hyperalgesia was successfully shown using subliminally presented (masked) facial cues (Jensen et al., 2015). However, the condition in question (i.e., acquisition and testing phase with subliminally presented cues) had not been tested separately so that the results remain partly inconclusive. In contrast, the present study uses supraliminally presented visual cues that induced learning but prevented the development of contingency awareness. The results thus confirm and expand evidence for implicit conditioning of Jensen and colleagues as well as others (Bräscher et al., 2017, 2018), and are in line with findings of implicit conditioning in contexts other than nocebo effects (Knight et al., 2006, 2003; Schultz & Helmstetter, 2010).

Using subliminal stimuli in order to achieve unconscious conditioning can be problematic. To ensure that masked cues were indeed subliminal, recognition tests were conducted in the above-mentioned studies (Egorova et al., 2015a; Jensen et al., 2012). However, a recognition test does not measure contingency awareness, i.e., conscious knowledge on the contingency of a cue and a painful stimulus in this case (Lovibond & Shanks, 2002). It is therefore conceivable that, although a cue was consciously unrecognized, awareness of the contingency emerged. Further issues of subliminal stimulus presentation concern interindividual differences in the perception threshold and intraindividual fluctuations, e.g., due to attentional effects (Lahteenmaki et al., 2015). Also, the choice of stimulus duration before masking might be critical, as research shows that even with stimulus durations of 10 ms (partial) awareness can occur in part of the

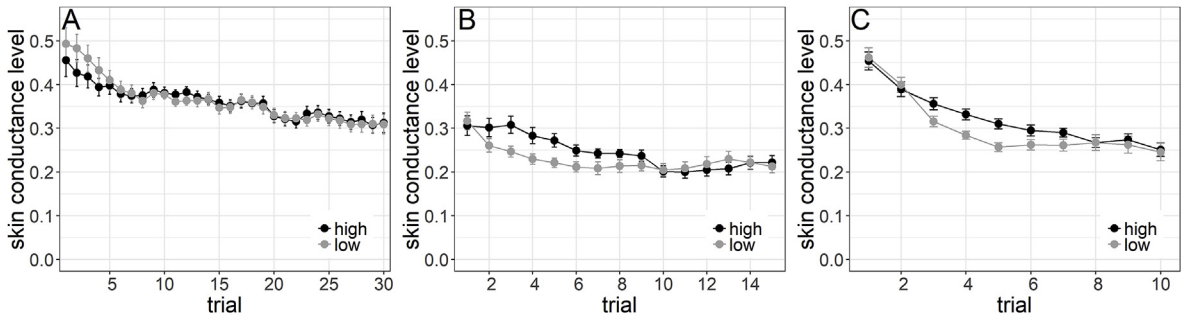

Fig. 4. Mean skin conductance level across trials during acquisition (**A**), test (**B**), and contingency test phase (**C**) with standard error of means.

experimental trials (Lahteenmaki et al., 2015). Finally, evidence indicates that subliminal conditioning of nocebo hyperalgesia with facial cues constitutes a special case because activation of conditioned nocebo hyperalgesia was not successful using subliminally presented images and words (Egorova et al., 2017), restricting the practical relevance of this paradigm. Face processing might be special because of neural processing in a specialized brain area (i.e., fusiform area; Kanwisher & Yovel, 2006) and possible domain-specificity (Kanwisher, 2000).

In this study, we propose a novel conditioning design that allows for implicit conditioning using suprathreshold cues. Instead of showing the same cues repeatedly, a new visual cue was presented in every trial. The feature that distinguished CS+ from CS- was a/symmetry of one component of the visual cue. Further, distractor components were added and prevented the development of contingency awareness in the majority of the participants. To test for contingency awareness, we assessed two different indicators. In a contingency test phase directly following the test phase of the experiment, no pain stimuli were presented, but participants were asked to give a rating as if a stimulus had been presented. Thereby we wanted to test for first-order contingency awareness, i.e., awareness that does not need introspection (Becker et al., 2012; Dienes, 2007). Only three participants were identified as first-order contingency aware using the RCI and excluding these participants reduced the effect of the conditioning procedure only marginally. Tapping a different level of processing, the post-experimental questionnaire assessed second-order contingency awareness, i.e., being aware of being aware (Dienes, 2007) or being able to report awareness. Comprising the answers to five questions, the index of second-order contingency awareness was very liberal. For example, only four participants who assumed a relation between the picture and the intensity of the following electric shock mentioned in their explanation “symmetry” or an associated feature of the picture (e.g., “pictures that were tidied up were followed by less pain”). We thus assume that partially, second-order awareness was the product of active inquiry during the post-experimental questionnaire in several participants. Further, due to the forced-choice format of the answers, participants answered some questions correctly even if they were not aware of the contingency and answered “no” to every question. Second-order contingency awareness was not related to the conditioned nocebo effect, further suggesting that implicit conditioning of nocebo hyperalgesia actually occurred.

Findings concerning the tonic component of the EDA support the results of the aversiveness ratings and indicate that conditioned responses in SCL were not related to contingency awareness. Most importantly, the conditioned effect in SCL remained stable even during the contingency test phase, suggesting prolonged extinction on a psychophysiological level and showing a dissociation from subjective ratings during this stage. While participants subjective ratings’ did not differ after CS+ and CS- indicating a lack of contingency awareness, the constant conditioned effect in SCL provides further evidence of implicit conditioning. SCL represents the tonic component of the EDA and is generally assumed to reflect arousal of the participant, whereas SCR constitutes the phasic component and usually responds to event-related stimuli. Although conditioned responses are frequently reported in SCR, the present findings are in line with previous research reporting conditioned SCL (Schneider, Palomba, & Flor, 2004). Further, SCL is known as a valid indicator of autonomic conditioning for long conditioned stimuli (Lovibond, 1992) and reflects emotional arousal, which fits well to the conditioned effect that was found in the subjective aversiveness but not in the intensity rating. This consideration was further strengthened by the prediction of SCL by the aversiveness but not the intensity rating.

That the conditioned nocebo effect was found only in the aversiveness but not in the intensity rating is in line with a study measuring neural correlates of nocebo hyperalgesia using subliminally and supraliminally presented cues after conscious (i.e., explicit) conditioning. Here, increased activation in thalamus, amygdala, and hippocampus with nonconscious compared to conscious cues for the nocebo condition was interpreted to reflect the processing of perceived threat related to the nocebo cue (Jensen et al., 2014). Similarly, a recent behavioral study found effects after expectation manipulation and conditioning only in affective but not sensory pain ratings (Reicherts et al., 2016), leading to the assumption that the affective component of the pain experience might be more prone to nocebo effects, especially when induced implicitly.

4.1. Limitations

The assessment of contingency awareness with a post-experimental questionnaire or interview has some pitfalls (Dawson, 1973; Lovibond & Shanks, 2002), which we tried to minimize, e.g., by using recognition instead of recall questions. However, alternative methods to assess contingency awareness are difficult to establish and post-experimental interviews have been used successfully (Clark & Squire, 1998; Manns, Clark, & Squire, 2001; Tabbert, Stark, Kirsch, & Vaitl, 2006). As a complementary assessment of contingency awareness, we introduced the contingency test, which was supposed to assess contingency awareness that does not need introspection. Yet, it is possible that participants did not use their knowledge on the contingency in this test. Reliability and validity of this assessment should be confirmed in future studies. Both measures of contingency awareness were assessed retrospectively and after an extinction phase, which can lead to impaired memory or other effects impeding the detection of contingency awareness.

The effect sizes in this study were small to moderate, which is less compared to the moderate to large effect sizes of nocebo hyperalgesia that were reported by a previous meta-analysis (Petersen et al., 2014). It is important though, to consider that nocebo studies were included only if a nocebo treatment (e.g., a sugar pill) had been applied, which was not the case in the present study in order to prevent the development of explicit expectations. Whereas research shows that the combination of conditioning and verbal suggestion leads to larger effects compared to verbal suggestion alone (Petersen et al., 2014), it is conceivable that conditioned nocebo effects are smaller when implicit compared to explicit cues are used (Egorova et al., 2015a, 2017; Jensen et al., 2012, 2015). Yet, the results of this study should be replicated with larger samples in order to verify the present conclusions.

4.2. Conclusions and clinical implications

To summarize, we introduced a novel nocebo conditioning paradigm that enabled implicitly conditioned nocebo hyperalgesia as mirrored in subjective aversiveness ratings and SCL. Although replication is needed, these results support the notion that nocebo hyperalgesia can be induced by implicit conditioning and therefore contradict the idea that nocebo effects in pain necessarily rely on conscious processes such as explicit expectations (Benedetti et al., 2003). We argue that at least part of the multidimensional pain experience, i.e., in the present case the affective component represented by aversiveness rating and SCL, can be modulated by unconscious processes such as implicit nocebo conditioning.

These results have major clinical implications because they suggest that in certain instances, pain increase in patients might be the consequence of an unnoticed association to a cue that had previously been coupled to a painful experience or pain increase. This means that in case of an implicitly conditioned nocebo effect, for example a patient might not be able to consciously identify the trigger of the pain increase, potentially leading to ongoing pain and a feeling of uncontrollability, helplessness, or anger. Generalization effects cannot be excluded, possibly facilitating the occurrence of pain episodes. It also naturally impedes extinction, as deliberate confrontation with the cue cannot be implemented. Further, it has been shown that implicitly conditioned effects can be resistant to extinction (Egorova et al., 2015a, 2017), implying a continued distress for persons concerned.

Disclosures

The authors declare that there is no conflict of interest. This research was supported by research funding of the Johannes Gutenberg University of Mainz.

Author contributions

AKB and MW designed the study, AKB and MB collected the data, AKB analyzed the data, AKB and MW interpreted the data, drafted and revised the manuscript and approved the final version.

Acknowledgements

The authors want to thank M.Sc. Martin Brehmen for his help in collecting the data and Dr. Stefan Schulz for helping to prepare the EDA data for further analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbtep.2019.03.006.

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