Abstract

Background. Visual cue conditioning is a valuable experimental paradigm to investigate placebo and nocebo effects in pain. However, little attention has been paid to the cues themselves and potential variability of effects (their quantity and quality) stemming from the choice of stimuli. Yet, this seemingly methodological question has important implications for the interpretation of experimental findings in terms of their significance for clinical practice.

Methods. We investigated the effect of heat pain conditioning using different types of visual cues (abstract images, faces and pseudo-words) in a group of 22 healthy volunteers. We analyzed conditioning effects calculated as the difference in pain ratings to heat stimuli of identical temperature preceded by conditioned high or low pain cues with 1) subliminal and supraliminal presentation; 2) immediately after conditioning and following extinction. Awareness manipulation and test following indirect, observational extinction allowed us to assess the strength and robustness of the conditioning effects induced with different cue types.

Results. We observed no differences in conditioning effect magnitudes between images, faces and words when all stimuli were presented supraliminally. With subliminal presentation only face stimuli elicited a significant effect; equally only face cue-induced effect withstood extinction.

Conclusions. Our findings indicate that face-related associations to pain might be stronger than other visual cues, as face cues seem to induce stronger subliminal effects and withstand mild extinction.
Original article

In the face of pain: the choice of visual cues in pain conditioning matters

Running head: Cues in pain conditioning

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This work was supported by R01AT006364, R01AT008563, R21AT008707 (NCCIH) to Jian Kong, P01AT006663 to Bruce Rosen / Randy Gollub. There is no conflict of interest for any of the authors.

Significance: We compared different types of neutral cues commonly used in conditioning paradigms and found that faces elicited a stronger, more robust non-conscious effect than abstract images or pseudo-words.
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Methods. We investigated the effect of heat pain conditioning using different types of visual cues (abstract images, faces and pseudo-words) in a group of 22 healthy volunteers. We analyzed conditioning effects calculated as the difference in pain ratings to heat stimuli of identical temperature preceded by conditioned high or low pain cues with 1) subliminal and supraliminal presentation; 2) immediately after conditioning and following extinction. Awareness manipulation and test following indirect, observational extinction allowed us to assess the strength and robustness of the conditioning effects induced with different cue types.

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Conclusions. Our findings indicate that face-related associations to pain might be stronger than other visual cues, as face cues seem to induce stronger subliminal effects and withstand mild extinction.

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Keywords: pain, cue, non-consciously activated conditioning effects, observational extinction.

Introduction

Information about pain intensity conveyed through cues is known to produce an effect on pain perception through conditioning (Atlas et al., 2010; Montgomery and Kirsch, 1997). Cue
conditioning has been successfully used to elicit placebo and nocebo effects (Voudouris et al., 1989; Martin-Pichora et al., 2011; Kirsch et al., 2014). However, little is known about the effect of cue type on conditioning effect, information that could be useful for identifying cues in a clinical setting that are most relevant for inducing placebo and nocebo effects or for developing paradigms inducing such effects, for instance in clinical trials.

The type of cues used for experimental conditioning varies greatly across studies. For example, in the visual domain, previous studies used either symbolic cues with certain connotations or neutral cues. Among the latter, studies predominantly used neutral faces (Jensen et al., 2015) or abstract images (Egorova et al., 2015b). These neutral cues, despite being of different types, are thought to not differ in their conditioning effects, but this has not been tested experimentally. Furthermore, reports of significant non-consciously activated conditioning effects have mostly come from studies that used face stimuli (Olsson and Phelps, 2004; Jensen et al., 2012, 2015). Studies using abstract images showed no (Egorova et al., 2015b) or smaller non-consciously activated conditioning effects (Egorova et al., 2015a). This suggests that the type of stimuli may affect the degree of activation.

In addition, some types of cues potentially relevant for the clinical setting have not been used. For example, although verbal interaction is an important part of medical interaction, neutral word stimuli have not been used for pain conditioning, and little is known about the ability of pseudo-words to induce conditioning effects. At the same time, emotional words have been shown to produce pain modulation, and cue conditioning effects are often directly compared with verbal suggestion effects (Gracely and Wolskee, 1983; Olsson and Phelps, 2004; Benedetti et al., 2007; Sitges et al., 2007; Colloca and Benedetti, 2009).

In this study, we investigated within-subject differences in response to conditioning with 3 types of neutral stimuli: abstract images, neutral faces and pseudo-words. The purpose of this study was to investigate potential effects of cue choice on the magnitude, automaticity and robustness of conditioning effects. Specifically, we compared consciously and non-consciously activated conditioning effects elicited by different cue types. Identifying whether conscious awareness was necessary to produce a conditioning effect was important, as previous studies varied in reports of non-conscious activation, which we hypothesized was due to the stimulus type.

We also assessed the resistance of conditioning effects induced by different cues to indirect extinction. Our previous study showed no differences in the amplitude of conditioning effects.
between direct and indirect observational conditioning (Egorova et al., 2015a). In this study we chose to use indirect extinction for all participants and cue types in order to test its feasibility.

**Methods**

The study was performed at Massachusetts General Hospital; subjects completed the experiment in about two hours, with no follow-up. Subjects were told that the purpose of the study was to learn about the relationship between memory and the experience of pain. We also mentioned to the participants that we were investigating how seeing visual cues and receiving noxious heat pain stimuli of differing temperatures can affect their pain experience.

**Subjects**

Twenty-six healthy participants with no psychiatric or neurological disorders and not taking any psychotropic medication were enrolled. Four subjects were excluded from the study, two due to inconsistent pain ratings, one due to inattention during stimulus presentation, and another because a different masking image was presented (see the experimental procedures section for more details). The final sample consisted of 22 participants (15 females) with an average age of $25.7 \pm 3.1$ years (Mean ± SD).

The Institutional Review Board at Massachusetts General Hospital approved all study procedures. The study was conducted in accordance with the approved guidelines. All enrolled subjects provided written informed consent before beginning any study procedures.

**Stimuli and equipment**

**Visual Stimuli**

Three types of visual cues were used throughout the experiment. The first type consisted of fractal images. The second consisted of male faces of neutral expression, and the third consisted of single-syllable, three letter words. All cues were in grayscale and presented on a black background; words were grayed to match contrast with the images and faces (Figure 1). Each cue of each type was associated with one of three experimental conditions: in the conditioning sessions, a high pain cue was presented with a high level of heat pain while a low pain cue was presented with a low level of heat pain; in the test sessions, a third, previously unseen and unconditioned cue was introduced as a control stimulus and was presented with a moderate level of heat pain as with all cues during the test phase. The high and low pain cues of all cue types were counterbalanced across subjects. All stimuli were presented using the Presentation® software (Version 16.3, www.neurobs.com).

**Heat pain administration**

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Noxious heat stimuli were delivered using a PATHWAY system (Medoc Advanced Medical Systems, Israel). All heat stimuli were initiated from a baseline temperature of 32 °C and increased to the target temperature. Each stimulus was presented for 2.5 seconds, including approximately 200 ms to ramp up to the target temperature and 350 ms to ramp down to the baseline temperature. Heat stimuli were applied to the left volar forearm during the conditioning phase and right volar forearm during the test phase; the position of the probe was changed for every session. The mean moderate temperature used during the test phase was 46.2 ± 1.6 °C (Mean ± SD). The average difference between the high and low temperatures during the conditioning phase was 3.8 ± 1.2 °C.

In order to calibrate pain stimuli levels to each individual’s tolerance, an ascending heat pain sequence was used first, starting at 35 °C and increasing by 1 °C to 50 °C or the subject’s maximum tolerance. Subjects were asked to rate the level of pain using the 0-20 Gracely Pain Intensity Scale (Gracely et al., 1978). Temperatures that elicited subjective intensity ratings of 5, 10, and 15, which we designated as low, moderate, and high pain, respectively, were selected for each subject. Once the low, moderate, and high heat pain levels had been determined, the subject was tested for rating response consistency. A random sequence of 3 low- and 3 high-intensity pain stimuli was administered. If the subject could consistently rate the high stimulus as more intense than the low stimulus, the experiment was initiated.

**Experimental procedures**

The experiment consisted of five phases: conditioning (3 sessions), conditioning test (2 sessions), observational extinction (1 session), extinction test (1 session), and cue recognition task.

The conditioning phase comprised 3 sessions. The first session presented the cues grouped and ordered by cue type (e.g., images, faces, then words) with the order counterbalanced across all subjects. The second and third sessions presented the cues in a randomly ordered sequence. In all sessions, subjects saw 2 cues of each cue type (6 cues in total), each consistently paired with a high or low level of pain (100% reinforcement). Subjects were informed that they would see the cues on the computer screen, each immediately followed by heat pain stimulation on their arm. Subjects were told that there would be a link between cue and pain intensity. Each cue was presented 6 times per session for 3 sessions, totaling 18 presentations. After each pain stimulus, subjects rated the pain they felt on a 0–100 visual analogue scale (VAS) displayed on the screen as a vertical line with ‘0 No Pain’ and ‘100 Worst Pain Possible’ as anchors referring to pain intensity (Figure 1); the cursor was set in the middle and participants used a computer mouse to indicate their pain level.
For the test phase, subjects were told that they would see the 6 cues again along with 3 new cues (1 per cue type) and that they should rate the level of pain they feel, just as during the conditioning phase. Subjects were also told that while sometimes the cues would appear as before (supraliminally at 200ms), at other times the cue would appear very briefly (subliminally at 33ms) and be immediately followed by a masking image (167ms). Each of the 2 sessions comprised 36 cue presentations, 18 of which were presented supraliminally and 18 subliminally. All 9 cues were presented in a randomly ordered sequence with a moderately painful heat stimulus that was of equal intensity from trial to trial in order to test the effect of cue conditioning on pain perception. Again, subjective pain ratings on a 0–100 VAS were collected. Each session was preceded by three high- or low-intensity pain cue presentations (one of each type) accompanied by the corresponding level of heat pain to boost conditioning effects.

In the observational extinction phase that followed, subjects watched a 10-minute video of a male model (a research assistant in our lab) undergoing the same study but receiving warm, non-painful heat stimuli. The video first showed a side view of the model sitting in the same position as the subject, facing a computer screen with the heat probe attached to the right forearm. This was followed by the cue and then a close-up of the model’s face showing his neutral reaction to the non-painful heat stimulus. Finally, the 0–100 VAS was displayed with the cursor being scrolled down to 0. This sequence was repeated for all cues (3 repetitions per cue). During this phase, subjects did not receive any heat stimuli. All cues were presented supraliminally.

We tested the effects of observational extinction during the extinction test phase by repeating one session from the test phase. Three cue presentations preceded the session as in the test phase but were paired with moderate-intensity pain stimuli and were not included in the analysis, as the first items in a session often feel more painful while participants adjust to stimulation. This way the number of trials also matched that in the test phase.

After the extinction test, subjects underwent a cue recognition test to confirm chance-level awareness of subliminal stimuli. All 9 cues from the test phase were presented supra- and subliminally along with 9 previously unseen cues (3 of each cue type). For each cue, subjects were given a yes/no task to indicate whether they had previously seen the cue and asked to indicate their level of confidence in their response on a 0-100 VAS.

In addition to the pain ratings, we also measured skin conductance levels using PowerLab 4SP GSR Amp with bipolar finger electrodes (MLT116F) placed on the distal phalanx of the index and middle fingers of the subject’s non-dominant (left) hand and LabChart v.8.0 software.
Due to insufficient number of trials for a robust skin conductance analysis of the test phases, only the responses during the conditioning phase were analyzed and reported.

**Statistical analyses**

For the analysis of stimuli type differences, we focused on the behavioral effect of conditioning calculated as the difference between responses to high vs. low cues (high minus low). Given our complex design (3 Stimuli types, 2 Awareness levels, 2 Phases) for statistical analysis, we chose to only focus on the difference between high and low cues, omitting the control cues. Further division into placebo and nocebo (calculated as a difference between low/high and control cues) would increase the number of conditions requiring correction and diminish our ability to detect the effect of the stimulus type as a function of awareness and phase. All statistical analyses were performed using R software (R Core Team, 2014). Reported effect sizes represent generalized eta squared from ezANOVA output (Bakeman, 2005) for ANOVAs and Cohen’s d for t-tests.

**Conditioning analysis**

In order to confirm that all 3 types of cues were learned during the conditioning phase and to compare the effect across cue types during conditioning, we conducted a repeated measures ANOVA on the conditioning effect (high minus low cue) for the 3 cue types (image, face, pseudo-word).

We also analyzed skin conductance data during conditioning to identify any differences between cues in autonomous responses during conditioning. Raw data was acquired at 400/s and smoothed by downsampling it to 10/s; no range correction was performed. Skin conductance response was calculated as the difference between the peak skin conductance level within the 1-4 s window following cue/pain onset and the average skin conductance level during the 1-second interval before the cue/pain onset. As is commonly done for measures of skin conductance reactivity, a square root transformation was applied to the absolute value of the skin conductance response with replacement of the + or − sign prior to statistical analysis (Boucsein, 2012). We performed a repeated measures ANOVA on the mean SCR for the 3 cue types.

**Test analysis**

First we investigated the effect of stimulus type on the conditioning effect with supra- and subliminal presentation by performing repeated measures ANOVA with factors Stimulus type (image, face, word) and Awareness (conscious, non-conscious). We then further investigated
significant interactions and main effects, focusing on the main effects and interactions of the Stimulus type factor.

**Extinction analysis**

In order to study the effect of extinction, we first performed an omnibus repeated measures ANOVA with factors Phase (post-conditioning test, post-extinction test), Stimulus type (image, face, word) and Awareness (conscious, non-conscious). We then specifically investigated the effect of Phase and Stimulus type for supraliminal and subliminal presentation separately. Finally, we studied the effect of Stimulus type following extinction in both conscious and non-conscious conditions.

**Results**

**Conditioning results**

The repeated measures ANOVA between the 3 cue types performed on the conditioning effect (high minus low cue ratings) during the conditioning phase showed no significant differences between the cue types ($F_{(2,42)} = 2.293$, $p=0.113$, generalized $\eta^2=0.008$), see Figure 2A.

The skin conductance also did not differ significantly between the 3 cues, Figure 2B, although there was a slightly increased response to face cues between 1 and 4 s after the cue onset. The analysis of the SCR over this period did not reveal any significant differences between the cue types ($F_{(2,36)} = 1.206$, $p=0.311$, generalized $\eta^2=0.031$), Figure 2C.

**Test results**

The results of the repeated measures ANOVA showed a significant interaction between Stimulus type and Awareness ($F_{(2,42)}=3.858$, $p=0.029$, generalized $\eta^2=0.033$). We then performed separate ANOVAs for supra- and subliminal presentation. In the conscious awareness condition, we observed no main effect of stimulus type ($p=0.6$), suggesting that images, faces and pseudo-words did not differ in their ability to induce a supraliminal conditioning effect. In the non-conscious condition we found a near significant ($F_{(2,42)}=3.097$, $p=0.055$, generalized $\eta^2=0.093$) main effect of Stimulus type. Planned paired t-tests (2-tailed) confirmed that face stimuli produced a significantly greater conditioning effect with subliminal presentation compared with pseudo-words ($t_{(21)}=2.67$, $p=0.014$, Cohen’s $d=0.57$), with near-significant differences between faces and images ($t_{(21)}=1.83$, $p=0.08$, Cohen’s $d=0.61$) and no differences between images and words ($p=0.7$), see Figure 3. We also performed t-tests between subliminal and supraliminal conditions for each stimulus type. There was a significant difference between supraliminal and subliminal effects with images ($t_{(21)}=4.19$,}

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p<0.001, Cohen’s d=0.74) and words (t_{21}=3.2, p=0.004, Cohen’s d=0.73) but not with faces (p=0.71), suggesting that there was no difference between subliminal and supraliminal presentation on the conditioning effect induced with faces.

**Extinction test results**

The results of the repeated measures ANOVA showed a significant interaction between Phase, Stimulus and Awareness (F_{(2,42)}=4.958, p=0.012, generalized η^2=0.02), suggesting that there was a difference between pre- and post-extinction test results affecting both Stimulus type and Awareness.

We then investigated pre- and post-extinction results for each Awareness level separately. For the supraliminal presentation, a repeated measures ANOVA with factors Phase and Stimulus type showed a significant interaction of phase and type (F_{(2,42)}=4.376, p=0.018, generalized η^2=0.029). Planned paired t-tests (2-tailed) for each of the Stimulus types revealed a significant decrease in post-extinction effects for images (t_{21}=4.191, p<0.001, Cohen’s d=0.89) and words (t_{21}=4.197, p<0.001, Cohen’s d=0.89), but not for faces (p=0.71), suggesting that conditioning effects with faces were not extinguished. For the subliminal presentation there were no significant main effects or interactions, suggesting no differences between pre- and post-extinction tests results.

To be consistent with the analysis of the main effects, we also repeated the analysis only for the extinction phase, for subliminal and supraliminal presentations separately. For the supraliminal condition, there was a main effect of Stimulus type (F_{(2,42)}=3.662, p=0.034, generalized η^2=0.072). Planned paired t-tests between Stimulus types showed a significant difference between word and face (t_{21}=2.72, p=0.01, Cohen’s d=0.57), near-significant difference between image and face (t_{21}=1.83, p=0.081, Cohen’s d=0.39) and no difference between images and words (p=0.75). The differences between conscious and non-conscious conditions following extinction were not significant for any of the stimulus types (image: p=0.33, face: p=0.1 (Cohen’s d=0.36), word: p=0.84), see Figure 3. However, the result in the face condition (p=0.1 in combination with a small to medium effect size) reveals a trend suggesting that there was a conscious but no non-conscious post-extinction effect.

**Cue recognition test**

At-chance recognition of subliminal stimuli was observed for all cue types (image: 55% correct; face: 52% correct; word: 55% correct), with no significant differences between cues types.

**Discussion**

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In this study we investigated whether the choice of visual cues used for conditioning influenced conditioning effects. We specifically manipulated the level of conscious awareness required to elicit conditioning effects during the test phase (supraliminal vs. subliminal), as well as studied the robustness of the effects by trying to extinguish them. While image, face and word stimuli elicited comparable conscious conditioning effects, faces induced greater subliminal effects, as well as withstood mild extinction, unlike the other two cue types. This finding revealing the specific advantage of face stimuli in eliciting conditioning effects helps reconcile the variability in reported non-consciously activated conditioning results. This study also suggests that face stimuli could be more appropriate for use in clinical settings when robust conditioning effects are desired (e.g., in inducing therapeutic placebo effects), whereas word cues could be used for experimental paradigms that attempt to limit placebo/nocebo effects (such as clinical trials).

Conscious conditioning effects in pain paradigms have been reported in studies using various types of cues (Lui et al., 2010; Yu et al., 2014), including neutral faces and abstract images (Jensen et al., 2012; Egorova et al., 2015a) but the magnitudes of effects did not seem to vary considerably. By directly comparing three types of neutral visual cues in a within-subject design, we confirmed that there is no effect of Stimulus type on the magnitude of conscious response. This suggests that when the stimulus is neutral prior to conditioning and the cue is consciously retrieved, the amplitude of the conditioning effect is independent of the type of cue. In addition to the previously shown effects with faces and images, we report significant conditioning effects with word stimuli. This type of stimuli is important to study, as it mimics conditioning effects related to clinically relevant placebo/nocebo cues such as drug names, treatment descriptions or even doctors’ names. We here demonstrate that pseudo-word cues can indeed induce positive and negative conscious expectations.

While no differences between cues were observed with supraliminal presentation, non-consciously activated conditioning effects differed between Stimulus types. Studies that used face stimuli consistently reported no main effect of Awareness, suggesting that there is no difference between subliminal and supraliminal activation (Jensen et al., 2012, 2015). On the other hand, effects of Awareness were observed with abstract image cues (Egorova et al., 2015a, 2015b). The current within-subject study confirmed these previous findings by showing no effect of Awareness for the face stimuli but a decrease in effect amplitude for the image stimuli between different presentation types. Similarly, word stimuli also showed a decrease with subliminal presentation.

Numerous studies and reviews have focused on why and how faces could be special (Farah et al., 1998; Tovee, 1998), including the neural specificity of face processing in the fusiform face area.
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directed toward the subjects showed significant resistance to extinction, while the conditioning
effects of faces directed away were extinguished easily. In our study, the faces were neutral in
expression but were directed toward the subjects. This again suggests that face stimuli are special
compared to words and images. Non-conscious conditioning with angry faces has also been shown
to resist extinction (Esteves et al., 1994).

While previous studies used direct extinction, we here presented extinction trials indirectly,
i.e., subjects observed actors experience ‘no pain’ on all trials. We previously reported that
observational conditioning could lead to comparable conditioning effects as direct conditioning
(Egorova et al., 2015a). These preliminary study results suggest that extinction through observation
could also be possible at least for some types of stimuli (words, images). Interestingly, in our
previous study (Egorova et al., 2015a) the conditioning effect induced with abstract images was not
extinguished following direct extinction. This could be due to the difference in the design (3 vs. 1
type of cue used) or presence/absence of direct exposure to heat. Direct comparison of observational
extinction with direct extinction and no (natural) extinction is needed to specifically demonstrate the
effect of observational extinction.

Limitations

While the current study revealed a special nature of face stimuli in pain conditioning, it could
not address the specific reasons for it. It remains unclear whether faces were faster to be learned and
accessed, or whether familiarity with face stimuli and specifically the relationship between face
processing and pain made conditioning with face stimuli more robust. With regard to learning, it did
not seem to differ across stimulus types during the conditioning phase (no significant differences in
the amplitude of the behavioral effect or skin conductance response). Although effort was made to
make stimuli matched (grayscale images with matched intensity were used, a single backward mask
for all cues was created and experimentally tested/adjusted to mask all cue types equally, etc.) it is
possible that face stimuli were processed faster. Future studies employing time resolved methods
(EEG, MEG, eyetracking) could clarify the nature of face specificity in pain conditioning.

This study focused on the influence of various visual cues on the amplitude of conditioning
effect (high vs. low), as in our previous study (Egorova et al., 2015a). While employing both high
and low cues allowed us to investigate placebo and nocebo effects separately, with the higher
number of experimental conditions (3 cue types, 2 awareness levels, 2 phases), we did not have
sufficient power to investigate differences in the effect type. Our preliminary analyses, however,
indicate that the cue differences reported here are not drastically different between placebo and nocebo effects. This research question, however, warrants further investigation.

In addition, while we put a lot of effort into determining an optimal subliminal presentation latency and masking so that all stimuli were perceived non-consciously through extensive piloting, the average threshold of 33 ms was set rather than individually adapted. The use of 33 ms is typical of many subliminal studies (Olsson and Phelps, 2004, Ng et al., 2012), including a number of our own studies employing this paradigm (Egorova et al., 2015a, 2015b). This chosen latency also resulted in an adequate at-chance stimuli recognition level, as tested at the end of the study. Yet, a recent study (Lähteenmäki et al., 2015) suggested that partial awareness could be present after display durations of 40 ms. Whether completely non-conscious activation was achieved or some awareness was present, only face stimuli induced a significant subliminal effect, suggesting that the chosen 33 ms presentation was effective in differentiating between Stimulus types. Nevertheless, studies carefully controlling individual subliminal thresholds with eye-tracking and adaptive thresholding are needed to ensure the required level of non-conscious perception.

Acknowledgements
We would like to thank Kalliopi Retzepi and Daniel Ott for help in video preparation and Rongjun Yu and the Karolinska Institutet for the visual stimuli.

Author Contributions
N.E. and J.K. designed the study; N.E. and J.P. performed the study; N.E. and J.K. analyzed data; N.E. and J.P. prepared figures; N.E., J.P., and J.K. wrote the manuscript.

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**Figures and legends**

Figure 1. Experimental procedures. A. Succession of experimental phases. B. Cues of each cue type—face, abstract image, word—were presented with heat pain of either high or low intensity. C. During the test phase, all six conditioned cues along with a new, unconditioned cue of each cue type (3 control cues in total) were presented with heat pain of identical, moderate intensity. The cues appeared either supraliminally (200 ms) or subliminally (33 ms + 167 ms backward mask). Subjects rated each pain stimulus on a 0-100 VAS.

Figure 2. A. Behavioral ratings – conditioning effect (pain rating differences high minus low). B. Baseline-corrected skin conductance during conditioning time-locked to the onset of the cue (-1 to 7 seconds). C. Mean SCR calculated as the square root of the maximum over 1-4 s after the stimulus onset minus the average value in the -1-0 s period. In all plots error bars represent standard errors of the mean (SEs).

Figure 3. Main results. Conditioning effects (pain rating differences high minus low) during test (conscious, non-conscious) and extinction (conscious, non-conscious).
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Title:
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Date:
2017-08-01

Citation:

Persistent Link:
http://hdl.handle.net/11343/292897