To remove or not to remove?

Removal of the unconditional stimulus electrode does not mediate instructed extinction effects

Camilla C. Luck[1, 2] & Ottmar V. Lipp[1, 2]

[1]School of Psychology and Speech Pathology, Curtin University, Australia

[2]ARC-SRI: Science of Learning Research Centre

Author Notes

Address for correspondence:

Camilla C. Luck, School of Psychology and Speech Pathology, Curtin University, GPO Box U1987 Perth WA 6845, Australia. Email: camilla.luck@postgrad.curtin.edu.au

Acknowledgements

This work was supported by grant numbers DP120100750 and SR120300015 from the Australian Research Council.

Word count: 7816

Abstract

Following differential fear conditioning, the instruction that the unconditional stimulus will no longer be presented (instructed extinction) reduces differential electrodermal responding to CS+ and CS-, but does not affect differential conditional stimulus valence evaluations. Reductions in differential electrodermal responding have been attributed to the provision of verbal instructions, however during instructed extinction the unconditional stimulus electrode is often removed as well. This removal could reduce the participants' general arousal levels rendering the detection of differential electrodermal responding difficult. The current study examined this alternative interpretation by comparing the electrodermal responses and conditional stimulus valence evaluations of an instruction/electrode on group, an instruction/electrode off group, and a control group who were not instructed. Following instructed extinction, differential electrodermal responding was eliminated in both instruction groups, an effect that was not influenced by the attachment/removal of the electrode. Replicating previous findings, conditional stimulus valence was not affected by instructed extinction. The results suggest that verbal instructions, not unconditional stimulus electrode removal, reduce differential electrodermal responding during instructed extinction manipulations.

Key words: fear conditioning, instructed extinction, electrodermal responses, evaluative learning, conditional stimulus valence.

Fear is not only innate but is also learned – if a neutral stimulus is repeatedly paired with an aversive stimulus it will come to elicit the same fear response as the aversive stimulus. This phenomenon is known as fear conditioning and has been extensively studied to gain an understanding of how fear is acquired and maintained, and how it can be reduced (Craske, Hermans, & Vansteenwegen, 2006). In the laboratory a differential fear conditioning paradigm is often used to study fear learning in humans, involving the presentation of two neutral conditional stimuli and an aversive unconditional stimulus (US). During the acquisition phase, one conditional stimulus (CS+) is paired with the aversive US, whilst the second (CS-), is presented alone. Throughout acquisition, differential responding develops between the conditional stimuli, as the CS+ progressively elicits larger physiological responses and is given lower pleasantness evaluations than the CS- (Lipp, 2006; De Houwer, Thomas, & Baeyens, 2001). During the extinction phase, both CS+ and CS- are presented alone and the differential responding gradually reduces (Lipp, 2006).

Conditioned fear develops and is reduced via associative learning mechanisms – during acquisition, the individual learns that presentations of the CS+ are followed by the US and during extinction the individual learns that the CS+ is presented alone. Instructed extinction is a cognitive manipulation used to examine whether the provision of verbal information alone (in the absence of any explicit learning trials) can reduce differential fear responding. In an instructed extinction manipulation, the experimenter enters the room between acquisition and extinction and informs the participants that the electrodes need to be checked while visually inspecting the electrodermal electrodes. An instruction group is informed that the US will no longer be presented, whilst a control group is not given information about the US occurrence. If the provision of information about the US occurrence is sufficient to change the cognitive representation of the CS-US relationship and thus, reduce conditional responding then the differential physiological responding and differential valence evaluations present on the last trial of acquisition should be reduced or even eliminated at the beginning of extinction in the instruction group, but remain intact in the control group (Lovibond, 2004).

Two recently published studies have reported different patterns of results in response to instructed extinction. Luck and Lipp (2015) report that instructed extinction eliminated differential fear potentiated startle and electrodermal responding at the beginning of extinction, but had no effect on an index of conditional stimulus valence measured continuously and concurrently with the physiological indices of fear learning. Conversely, Sevenster, Beckers, and Kindt (2012) report the elimination of differential electrodermal responding on the first trial of extinction, but a delayed effect of instructed extinction on fear potentiated startle, such that differential startle responding persisted for the first two extinction trials in the instruction group but remained intact over ten trials of extinction training in the control group. Although both Luck and Lipp (2015) and Sevenster et al. (2012) report the immediate elimination of differential electrodermal responding following instructed extinction, inspection of the provided figures suggests that in the instruction group of Luck and Lipp's (2015) study, differential electrodermal responding was eliminated due to a decrease in responding to the CS+, whereas in Sevenster et al.'s study (2012) differential responding was eliminated due to an increase in responding to the CS-.

Following the standard procedure for instructed extinction studies, Luck and Lipp (2015) removed the US electrode during the manipulation, whereas, Sevenster et al. (2012) left the US electrode attached to enable the re-introduction of the US after extinction in a subsequent reinstatement manipulation and to avoid possible context changes between acquisition and extinction. This difference in procedure may account for the differing pattern of electrodermal responses, reduced electrodermal responses to CS+ vs. increased electrodermal responses to CS-, at the beginning of extinction. Removal of the US electrode has been performed in the majority of instructed extinction studies (Hugdahl, 1978; Hugdahl, & Öhman, 1977; Lipp & Edwards, 2002) to increase the believability of the instructions, however, the US electrode has been suggested to act as powerful contextual cue whose presence alone might be threatening for the participants (Grillon & Ameli, 1998; Lanzetta & Orr, 1986). Removing the electrode could reduce the participants' arousal levels – a reduction which may affect differential physiological responding as physiological indices of positive and negative emotions are enhanced in response to high arousal stimuli (Lang, Greenwald, Bradley, & Hamm, 1993; Cuthbert, Bradley, & Lang, 1996). Removal of the US electrode may also provide an explanation for the differential effect of instructed extinction on physiological fear indices and self-reported CS valence reported by Luck and Lipp (2015), as self-report measures of CS valence do not seem to be influenced by the participants' arousal level.

The current study examined the effect of US electrode attachment/removal on instructed extinction of conditioned fear as indicated by electrodermal responses and self-reported CS valence. These indices were assessed in three groups – a control group who did not receive any information about the US presentation, an instruction/electrode on group who were informed that the US would no longer be presented and had the US electrode attached, and an instruction/electrode off group who were informed that the US would no longer be presented and had the US electrode removed. If the instructional component of the manipulation is responsible for the previously reported instructed extinction effects we would expect an immediate reduction of differential electrodermal responding at the beginning of extinction in both instruction groups, whilst differential responding remains intact at the beginning of extinction in the control group. If on the other hand, removal of the US electrode influenced the results seen in previous instructed extinction studies we would expect to find a difference between the two instruction groups at the beginning of extinction. Consistent with the results reported by Luck and Lipp (2015) we do not expect an effect of instructed extinction on self-reported CS valence regardless of the presence of the US electrode.

Method

Participants

Seventy-eight (47 female) undergraduate students aged 17 - 50 years (*M* = 22.28) volunteered participation in exchange for course credit or monetary compensation. The research protocol was approved by the Curtin University ethics review board. One participant's electrodermal responses were lost due to problems with the recording device.

Apparatus/Stimuli

The conditional stimuli were color pictures of four Caucasian, male adults [NimStim database: images M_NE_C: models 20, 21, 32, 31, Tottenham et al. (2009)] displaying neutral facial expressions. The pictures were 506 × 650 pixels in size and were displayed for six seconds on a 24 inch color LCD screen. The trials were arranged in a pseudo-random sequence such that no more than two consecutive trials were the same. The faces used as the conditional stimuli, the faces used as CS+/CS-, and whether the first trial of each phase was a CS+/CS- were counterbalanced across participants.

A 200 ms electrotactile stimulus, generated by a Grass SD9 Stimulator, pulsed at 50 Hz, was used as the US and delivered to the participant's preferred forearm. Respiration was monitored with a respiratory effort transducer with an adjustable Velcro strap and electrodermal activity was DC amplified at a gain of 5 µSiemens per volt and recorded with two 8 mm Ag/AgCl electrodes filled with an isotonic electrolyte gel. CS valence evaluations were recorded with a Biopac Variable Assessment Transducer with the anchors 0 (very negative) to 9 (very positive). DMDX 4.0.3.0 software (Forster & Forster, 2003) was used to control the stimulus presentation and timing. A Biopac MP150 system, using AcqKnowledge Version 3.9.1.at a sampling frequency of 1000 Hz was used to record the CS valence evaluations, electrodermal responding, and respiration.

Procedure

The participants provided informed consent, washed their hands and were seated in front of a monitor in a separate cubicle. The respiratory effort transducer was attached to the participants' lower torso and the two electrodes were placed on the thenar and hypothenar eminences of their non-preferred hand. A shock electrode was attached with a bandage to the participants' preferred forearm and a shock-work up procedure was employed to set the intensity of the electrotactile stimulus individually to a level that was experienced as 'unpleasant but not painful'. The participants were asked to relax and watch the blank computer screen whilst their baseline electrodermal activity was recorded for three minutes. After this baseline recording, the participants were instructed that they would view faces on the screen and that they should evaluate the faces as pleasant or unpleasant. Participants were asked to rate the faces as soon as they were presented on the screen to avoid contamination by the presence/absence of the electrotactile stimulus and to pay attention to when they received the electrotactile stimulus. The valence ratings were made with the participants' preferred hand ensuring the movement did not interfere with the electrodermal recording and the participant was instructed to move the evaluation dial back to the 'neutral' position after rating the picture. The participant confirmed that they understood what was required and the conditioning experiment, consisting of habituation, acquisition and extinction phases, was started. During habituation, the CS+ and CS- faces were presented four times each, allowing for the habituation of orienting responses. During acquisition, the CS+ was presented eight times with the unconditional stimulus coinciding with the CS+ offset on a 100% reinforcement schedule. The CS- was presented eight times alone. The inter-trial interval was a blank rest screen presented for 11, 13, or 15 seconds.

At the end of the acquisition phase, the experimenter entered the participants' cubicle and informed all participants that the mid-way point had been reached and that the electrodes needed to be checked, before appearing to visually inspect the electrodermal electrodes. For participants in the control group, the experimenter told the participants the shock electrode needed to be checked, before removing and reattaching it. For participants in the instruction/electrode on group, the experimenter removed and reattached the electrode, before informing the participants that they would not receive the electrotactile stimulus anymore. Participants in the instruction/electrode off group were informed they would not receive the electrotactile stimulus anymore and the shock electrode was removed. After this interruption, all participants were informed that the experiment would continue and the extinction sequence was started. Extinction consisted of the presentation of both the CS+ and the CS- eight times, but the electrotactile stimulus was not presented. After the last extinction trial, the electrodes were removed and the participant was led into the control room where they completed the post-experimental questionnaire. The questionnaire included an assessment of contingency awareness, requiring the participants to identify (from a set of four) which two faces they had seen in the experiment and which of these faces had been followed by the electrotactile stimulus.

As a manipulation check, participants were required to indicate whether they had believed the instructions (instruction groups only; yes or no question).

Scoring and Response Definition

The CS valence evaluations were recorded as the largest positive or negative voltage deviation during the six second CS presentation from a one second pre-CS baseline (‘neutral’ position). Any discernible electrodermal response during the three minute baseline was counted to provide a measure of spontaneous electrodermal responding (Dawson, Schell & Filion, 2007). Tonic electrodermal responding, defined as the mean electrodermal level one second prior to CS onset, was examined to provide an index of general arousal (Dawson et al., 2007). Phasic electrodermal responding was scored in multiple latency windows as recommended by Prokasy and Kumpfer (1973). First Interval Responding (FIR) was defined as responses starting within 1-4 seconds of CS onset and Second Interval Responding (SIR) was defined as responses starting within 4-7 seconds of CS onset. Responses to the US were scored during acquisition as responses starting within 7-10 seconds of the CS+ onset (1-4 seconds from US onset). The largest response starting within the latency response window was scored and the magnitude was calculated as the difference from response onset to peak (Prokasy & Kumpfer, 1973). Respiration traces were examined to identify cases where the electrodermal responding was contaminated by deep breaths or excessive movement, however, no such cases were identified and no responses were excluded. The phasic electrodermal responses were square root transformed to reduce the positive skew of the distribution (Dawson et al., 2007), and then range corrected to ensure that each participant was given an even weight in the analyses, reducing the influence of outliers (Boucsein et al., 2012; Dawson et al., 2007). The reference used for the range correction was the largest response displayed by the participant, typically the response to the first or second presentation of the US. Prokasy and Kumpfer (1973) recommend scoring electrodermal responses in multiple windows as there is evidence that first interval responding is more sensitive to orienting and second interval responding is more sensitive to anticipation effects (Lockhart, 1966; Stewart et al., 1959). During habituation, only first interval responses were scored as they reflect orienting to novel stimuli (Öhman, 1983) and anticipation of the unconditional stimulus would not be expected. Prior to analysis, CS valence evaluations and phasic electrodermal responding were averaged into blocks of two consecutive trials to reduce the influence of trial by trial variability.

Statistical Analyses

First and second interval electrodermal responding and conditional stimulus valence evaluations were subjected to separate 3 × 2 × n (Group [control, electrode on, electrode off] × CS [CS+, CS-] × Block [habituation = 2, acquisition = 4, extinction = 4]) factorial ANOVAs for habituation, acquisition, and extinction. As the influence of the instructional manipulation is expected between the last trial of acquisition and the first trial of extinction, additional 3 × 2 × 2 (Group [control, electrode on, electrode off] × CS [CS+, CS-] × Phase [last trial of acquisition, first trial of extinction]) factorial ANOVAs were performed. Unconditional electrodermal responding during acquisition was subjected to a 3 × 4 (Group [control, electrode on, electrode off] × Block [4]) factorial ANOVA. Multivariate F values (Phillai's Trace) and partial eta-squares are reported for all main effects and interactions. All main and simple effect comparisons were conducted using Bonferroni adjustments to protect against the accumulation of α –error and adjusted *p* values are reported for these follow-up analyses. IBM SPSS Statistics 22 was used to conduct all analyses, and the significance level was set at .05.

Results

Preliminary Checks. The male to female sex ratio did not differ between groups (control: 8:16, electrode on: 14:16, electrode off: 9:15), $\chi^2(2) = 1.06$, $p = .588$, however the groups did differ in age, $F(2, 77) = 3.70$, $p = .029$, $\eta p^2 = .090$. The electrode off group (M = 25.50 years, SD = 10.93 years) was older than the electrode on group (M = 20.17 years, SD = 2.53 years), $p = .027$, however the control group (M = 21.71 years, SD = 6.68 years) did not differ from the electrode on group, $p > .999$, or the electrode off group, $p = .224$. Six participants who were aged over 34 years (control = 2, electrode off = 4) were considered outliers using Tukey's outlier identification method (Hoaglin, Iglewicz, & Tukey, 1986; Hoaglin & Iglewicz, 1987). When they were excluded from the analyses no differences between the groups were detected, F(2, 71) = 0.96, $p = .390$, $\eta p^2 = .027$ (control: M = 19.91 years, SD = 2.29 years; electrode on: M = 20.17 years, SD = 2.53 years; electrode off: M = 21.25 years, SD = 5.00 years). The number of spontaneous electrodermal responses displayed during the three minute baseline period did not differ between the groups (control: M = 23.25 responses, SD = 15.51 responses; electrode on: M = 23.33 responses, SD = 11.48 responses; electrode off: M = 21.67 responses, SD = 12.99 responses), $F(2, 77) = 0.13$, $p = .882$, $\eta p^2 = .003$. A difference in the US intensity between the groups was detected, $F(2, 77) = 3.86$, $p = .025$, $\eta p^2 = .093$, such that the electrode off group (M = 36.04 V, SD = 7.46 V) set the US intensity higher than the control group (M = 30.46 V, SD = 7.06 V), $p = .028$. The US intensity in the electrode off group and the electrode on group (M = 31.97 V, SD = 7.20 V), $p = .130$, and the electrode on group and the control group, $p > .999$, did not differ. The perceived US unpleasantness did not differ between groups, $F(2, 76) = 0.44$, $p = .644$, $\eta p^2 = .012$ (control: M = -1.21, SD = 1.02; electrode on: M = -1.30, SD = 1.06; electrode off: M = -1.48, SD = 0.90). The electrodermal responses to the US differed between blocks, $F(3, 72) = 91.31$, $p < .001$, $\eta p^2 = .792$, such that responses were higher in block one in comparison with blocks two, $p < .001$, three, $p < .001$ and four, $p < .001$, block two compared with block four, $p < .001$, and block three compared with block four, $p < .001$. Unconditional electrodermal responding did not differ between the groups (group: $F(2, 74) = 0.42$, $p = .659$, $\eta p^2 = .011$; Block × Group: $F(6, 146) = 1.72$, $p = .120$, $\eta p^2 = .066$). Five participants (control: 2, electrode on: 1, electrode off: 2) could not correctly identify the experimental contingencies. When these participants were excluded a similar pattern of results emerged and therefore the results of the entire sample are reported. Nine participants (electrode on: 7, electrode off: 2) reported that they did not believe the instructions and the results concerned with the effects of the instructed extinction manipulation are reported including and excluding these participants.

Habituation

First Interval Electrodermal Responding. The first interval electrodermal responses recorded during habituation are presented in the left panel of Figure 1. A main effect of Block, $F(1, 74) = 61.11$, $p < .001$, $\eta p^2 = .452$, and a Block × Group interaction, $F(2, 74) = 3.82$, $p = .026$, $\eta p^2 = .094$, confirmed that electrodermal responding significantly declined from block one to block two in the control, $F(1, 74) = 36.47$, $p < .001$, $\eta p^2 = .330$, electrode on, $F(1, 74) = 28.01$, $p < .001$, $\eta p^2 = .275$, and electrode off groups, $F(1, 74) = 5.19$, $p = .026$, $\eta p^2 = .066$. The magnitude of this decline was smaller in the electrode off group resulting in the Block × Group interaction. The remaining main effects and interactions did not reach significance, largest (CS × Block), $F(1, 74) = 0.91$, $p = .342$, $\eta p^2 = .012$.

Acquisition

First Interval Responding. The first interval electrodermal responses recorded during acquisition are presented in the second panel of Figure 1. A main effect of CS, $F(1, 74) = 50.08$, $p < .001$, $\eta p^2 = .404$, and a main effect of block, $F(3, 72) = 10.12$, $p < .001$, $\eta p^2 = .297$, were qualified by a CS × Block interaction, $F(3, 72) = 13.41$, $p < .001$, $\eta p^2 = .359$. Responding between CS+ and CS- did not differ during block one, $F(1, 74) = 0.01$, $p = .918$, $\eta p^2 < .001$, but during blocks two, $F(1, 74) = 37.20$, $p < .001$, $\eta p^2 = .335$, three, $F(1, 74) = 62.50$, $p < .001$, $\eta p^2 = .458$, and four, $F(1, 74) = 37.44$, $p < .001$, $\eta p^2 = .336$, CS+ elicited larger responses than CS-. The remaining main effects and interactions did not attain significance, largest (Block × Group), $F(6, 146) = 0.82$, $p = .556$, $\eta p^2 = .033$.

Second Interval Responding. The second interval electrodermal responses recorded during acquisition are presented in the left panel of Figure 2. A main effect of CS, $F(1, 74) = 62.35$, $p < .001$, $\eta p^2 = .457$, and a main effect of block, $F(3, 72) = 3.64$, $p = .017$, $\eta p^2 = .132$, were qualified by a CS × Block interaction, $F(3, 72) = 13.67$, $p < .001$, $\eta p^2 = .363$. Responding between CS+ and CS- did not differ during block one, $F(1, 74) = 0.16$, $p = .689$, $\eta p^2 = .002$, but during blocks two, $F(1, 74) = 22.12$, $p < .001$, $\eta p^2 = .230$, three, $F(1, 74) = 41.00$, $p < .001$, $\eta p^2 = .357$, and four, $F(1, 74) = 64.08$, $p < .001$, $\eta p^2 = .464$, CS+ elicited larger responses than CS-. The remaining main effects and interactions did not attain significance, largest (Block × Group), $F(6, 146) = 1.46$, $p = .196$, $\eta p^2 = .057$.

Extinction

First Interval Responding. The first interval electrodermal responses recorded during extinction are presented in the third panel (all participants) and fourth panel (believers only) of Figure 1. Electrodermal responding to CS+ was marginally larger than electrodermal responding to CS-, $F(1, 74) = 3.84$, $p = .054$, $\eta p^2 = .049$. A main effect of block, $F(3, 72) = 5.93$, $p = .001$, $\eta p^2 = .198$, revealed that responding was larger in block one in comparison with block three, $p = .002$, and block four, $p = .002$. The remaining omnibus effects failed to reach significance, largest (Block × Group), $F(6, 146) = 1.52$, $p = .176$, $\eta p^2 = .059$. When the analyses were re-run removing the nine participants who did not believe the instructions, the main effect of CS did not attain marginal significance, $F(1, 65) = 2.73$, $p = .103$, $\eta p^2 = .040$ and the main effect of block remained, $F(3, 63) = 4.80$, $p = .004$, $\eta p^2 = .186$.

Second Interval Responding. The second interval electrodermal responses recorded during extinction are presented in the middle (all participants) and right panel (believers only) of Figure 2. A main effect of block, $F(3, 72) = 2.94$, $p = .039$, $\eta p^2 = .109$, revealed that responses in block one were larger than responses in block four, $p = .042$. A main effect of group, $F(2, 74) = 3.68$, $p = .030$, $\eta p^2 = .090$, and a CS × Group interaction, $F(2, 74) = 4.90$, $p = .010$, $\eta p^2 = .117$, were detected. In the control group, CS+ elicited larger electrodermal responses than CS-, $F(1, 74) = 8.65$, $p = .004$, $\eta p^2 = .105$, however, in the electrode on group, $F(1, 74) = 1.43$, $p = .236$, $\eta p^2 = .019$, and the electrode off group, $F(1, 74) = 0.14$, $p = .709$, $\eta p^2 = .002$, CS+ and CS- did not differ in responding. The remaining main effects and interactions did not attain significance, largest (Block × Group), $F(6, 146) = 1.19$, $p = .313$, $\eta p^2 = .047$. Analysis after removal of the participants who reported not believing the instructions yielded similar results (block: $F(3, 63) = 2.59$, $p = .061$, $\eta p^2 = .110$; group: $F(2, 65) = 4.69$, $p = .013$, $\eta p^2 = .126$; CS × Group: $F(2, 65) = 3.85$, $p = .026$, $\eta p^2 = .106$).

Instructed Extinction Manipulation – Trial Based Analysis

First Interval Responding. The first interval electrodermal responses recorded during the last trial of acquisition and the first trial of extinction are presented in Figure 3 (top panel). A main effect of CS, $F(1, 74) = 13.75$, $p < .001$, $\eta p^2 = .157$, a main effect of phase, $F(1, 74) = 8.87$, $p = .004$, $\eta p^2 = .107$, and a CS × Phase interaction, $F(1, 74) = 18.84$, $p < .001$, $\eta p^2 = .203$, were detected. Differential responding between CS+ and CS- was present on the last trial of acquisition, $F(1, 74) = 30.15$, $p < .001$, $\eta p^2 = .289$, but not on the first trial of extinction, $F(1, 74) = 0.01$, $p = .925$, $\eta p^2 < .001$. The remaining main effects and interactions did not attain significance, largest (Phase × Group), $F(2, 74) = 1.78$, $p = .176$, $\eta p^2 = .046$. The pattern of results did not change when the non-believers were removed (CS: $F(1, 65) = 14.35$, $p < .001$, $\eta p^2 = .181$; phase: $F(1, 65) = 11.76$, $p = .001$, $\eta p^2 = .153$; CS × Phase: $F(1, 65) = 19.40$, $p < .001$, $\eta p^2 = .230$).

Second Interval Responding. The second interval electrodermal responding recorded during the last trial of acquisition and the first trial of extinction is presented in the middle panel of Figure 3. A main effect of CS, $F(1, 74) = 22.86$, $p < .001$, $\eta p^2 = .236$, a main effect of phase, $F(1, 74) = 7.51$, $p = .008$, $\eta p^2 = .092$, a marginal main effect of group, $F(2, 74) = 3.00$, $p = .056$, $\eta p^2 = .075$, a CS × Phase interaction, $F(1, 74) = 23.19$, $p < .001$, $\eta p^2 = .239$, and a CS× Phase × Group interaction, $F(2, 74) = 3.44$, $p = .037$, $\eta p^2 = .085$, were detected. On the last trial of acquisition, responding to CS+ was larger than responding to CS- in all groups (control: $F(1, 74) = 9.23$, $p = .003$, $\eta p^2 = .111$; electrode on: $F(1, 74) = 25.03$, $p <.001$, $\eta p^2 = .253$; electrode off: $F(1, 74) = 11.54$, $p = .001$, $\eta p^2 = .135$). Following instructed extinction, differential responding between CS+ and CS- was present in the control group, $F(1, 74) = 4.20$, $p = .044$, $\eta p^2 = .054$, but not in the electrode on, $F(1, 74) = 1.53$, $p = .220$, $\eta p^2 = .020$, or electrode off groups, $F(1, 74) = 0.02$, $p = .887$, $\eta p^2 < .001$.

The follow-up analyses were re-run to confirm that both instruction groups differed from the control group but not from each other. This revealed that during the last trial of acquisition the groups did not differ in responding to CS+ or CS-, largest (responding to CS-, control vs. electrode off) $p = .189$, however on the first trial of extinction responding to CS+ was significantly larger in the control group in comparison with the electrode on group, $p = .018$ and the electrode off group, $p = .021$, but the electrode on and electrode off groups did not differ in responding to CS+, $p > .999$. The groups did not differ in responding to CS- on the first trial of extinction, largest (electrode on vs. electrode off) $p = .377$.

When the non-believers were excluded the CS × Phase × Group interaction attained marginal significance, $F(2, 65) = 2.52$, $p = .089$, $\eta p^2 = .072$. Follow-up analyses revealed the same pattern of responding, with continued differential responding at the beginning of extinction in the control group, $F(1, 65) = 4.35$, $p = .041$, $\eta p^2 = .063$, but not in the electrode on group, $F(1, 65) = 0.20$, $p = .653$, $\eta p^2 = .003$, or the electrode off group, $F(1, 65) = 0.18$, $p = .677$, $\eta p^2 = .003$. The remaining effects were similar (CS: $F(1, 65) = 26.00$, $p < .001$, $\eta p^2 = .286$; phase: $F(1, 65) = 9.99$, $p = .002$, $\eta p^2 = .133$; group: $F(2, 65) = 3.29$, $p = .044$, $\eta p^2 = .092$; CS × Phase: $F(1, 65) = 19.07$, $p < .001$, $\eta p^2 = .227$).

Tonic Electrodermal Level. An analysis of the tonic electrodermal level from the last trial of acquisition to the first trial of extinction revealed a main effect of CS, $F(1, 74) = 48.10$, $p < .001$, $\eta p^2 = .394$, and a Phase × CS interaction, $F(1, 74) = 22.41$, $p < .001$, $\eta p^2 = .232$. Before the last trial of acquisition, the tonic electrodermal level was higher before presentations of CS- ($M = 12.72$, $SD = 4.65$) than before presentations of CS+ ($M = 11.95$, $SD = 4.52$), $F(1, 74) = 61.73$, $p < .001$, $\eta p^2 = .455$, but before the first trial of extinction, there was no difference in the tonic electrodermal level before CS+ ($M = 12.16$, $SD = 4.95$) and CS- ($M = 12.20$, $SD = 4.76$), $F(1, 74) = 0.25$, $p = .616$, $\eta p^2 = .003$. The tonic electrodermal level is larger before CS- in acquisition due to the pseudo-random trial sequence. As a CS+/CS- is not presented for more than two consecutive trials, presentations of CS+ are more likely to precede presentation of CS- and therefore the tonic electrodermal level before CS- would be expected to be slightly higher as the previous trial was more likely to contain the electrotactile stimulus. This difference is absent on the first trial of extinction, as the electrotactile stimulus has not been presented for some time. The remaining main effects and interactions did not attain significance, largest (phase), $F(1, 74) = 1.18$, $p = .280$, $\eta p^2 = .016$. The pattern of results did not differ when the non-believers were removed (CS: $F(1, 65) = 48.37$, $p < .001$, $\eta p^2 = .427$, Phase × CS interaction: $F(1, 65) = 19.27$, $p < .001$, $\eta p^2 = .229$).

Conditional Stimulus Valence Evaluations

Habituation. The conditional stimulus valence evaluations recorded during habituation are presented in the left panel of Figure 4. No significant differences were detected during habituation, largest (block), $F(1, 75) = 2.25$, $p = .138$, $\eta p^2 = .029$.

Acquisition. The conditional stimulus valence evaluations recorded during acquisition are presented in the second panel of Figure 4. A main effect of CS, $F(1, 75) = 7.83$, $p = .007$, $\eta p^2 = .094$, a main effect of block, $F(3, 73) = 2.82$, $p = .045$, $\eta p^2 = .104$, and a CS × Block interaction, $F(3, 73) = 12.01$, $p < .001$, $\eta p^2 = .330$, were detected. Conditional stimulus valence evaluations of CS+ and CS- did not differ during blocks one, $F(1, 75) = 0.30$, $p = .586$, $\eta p^2 = .004$, or two, $F(1, 75) = 0.75$, $p = .389$, $\eta p^2 = .010$, but during blocks three, $F(1, 75) = 10.59$, $p = .002$, $\eta p^2 = .124$, and four, $F(1, 75) = 23.08$, $p < .001$, $\eta p^2 = .235$, CS+ was given lower valence ratings than CS-. All other main effects and interactions did not reach significance, largest (group), $F(2, 75) = 1.64$, $p = .202$, $\eta p^2 = .042$.

Extinction. The conditional stimulus valence evaluations recorded during extinction are presented in the third panel (all participants) and fourth panel (instruction believers only) of Figure 4. A main effect of CS confirmed that CS+ was rated as less pleasant than CS-, $F(1, 75) = 12.11$, $p = .001$, $\eta p^2 = .139$. A main effect of block, $F(3, 73) = 5.29$, $p = .002$, $\eta p^2 = .179$ revealed that evaluations were more negative in block one, compared with block three, $p = .002$, and four, $p = .003$, and block two compared with blocks three, $p = .014$, and four, $p = .012$. A marginal Block × Group interaction was detected, $F(6, 148) = 2.14$, $p = .052$, $\eta p^2 = .080$, however valence evaluations did not differ between groups in any of the extinction blocks, all *p's* > .242. This interaction reflected on slight differences between the groups in the overall valence across blocks. In the control group evaluations during block one were more negative than during blocks two, $p = .009$, three (marginal) $p = .051$, and four, $p = .031$. In the electrode on group, evaluations did not differ across blocks, all *p*'s > .999, and in the electrode off group, evaluations did not differ between blocks one and two, $p > .999$, whilst they were marginally more negative in block one compared with block three, $p = .064$, and four, $p = .054$, and more negative in block two compared with blocks three, $p = .008$, and four, $p = .004$. The remaining main effects and interactions did not reach significance, largest, (CS × Block), $F(3, 73) = 2.51$, $p = .065$, $\eta p^2 = .093$. When the non-believers were removed a similar pattern emerged (CS: $F(1, 66) = 10.32$, $p = .002$, $\eta p^2 = .135$; block: $F(3, 64) = 4.12$, $p = .010$, $\eta p^2 = .162$; CS × Block: $F(3, 64) = 3.25$, $p = .027$, $\eta p^2 = .132$; Block × Group: $F(6, 130) = 1.97$, $p = .075$, $\eta p^2 = .083$).

Instructed Extinction Manipulation. The conditional stimulus valence evaluations from the last trial of acquisition and the first trial of extinction are presented in the bottom panel of Figure 3. Analyses revealed a main effect of CS, $F(1, 75) = 21.76$, $p < .001$, $\eta p^2 = .225$, and a CS × Phase interaction, $F(1, 75) = 4.93$, $p = .029$, $\eta p^2 = .062$. The CS × Phase interaction revealed that although the CS+ and CS- were differentially rated during both phases, the CS+ was rated more pleasant on the first trial of extinction in comparison with the last trial of acquisition, $F(1, 75) = 5.27$, $p = .025$, $\eta p^2 = .066$, whereas, the valence evaluations of CS- did not differ between the last trial of acquisition and the first trial of extinction, $F(1, 75) = 0.50$, $p = .484$, $\eta p^2 = .007$. The CS × Phase × Group interaction, $F(2, 75) = 1.99$, $p = .144$, $\eta p^2 = .050$, did not attain significance confirming that instructed extinction did not affect the differential conditional stimulus evaluations. To further confirm this, follow-up analyses were performed, revealing continued differential evaluations of CS+ and CS- in all groups at the beginning of extinction, all *p's* < .043, and no differences between the groups at the beginning of extinction all *p's* > .999. The remaining main effects and interactions did not attain significance, largest (phase), $F(1, 75) = 1.95$, $p = .166$, $\eta p^2 = .025$. When the analyses were run excluding the non-believers a similar pattern emerged (CS: $F(1, 66) = 20.41$, $p < .001$, $\eta p^2 = .236$; CS × Phase: $F(1, 66) = 5.55$, $p = .021$, $\eta p^2 = .078$; CS × Phase × Group: $F(2, 66) = 1.87$, $p = .162$, $\eta p^2 = .054$).

Discussion

The current study assessed whether the effects of instructed extinction reported in prior studies of electrodermal fear conditioning can be attributed to the removal/attachment of the US electrode. We also aimed to provide a replication of Luck and Lipp's (2015) finding that CS valence does not respond to instructed extinction after fear conditioning. A differential fear conditioning paradigm was used comparing three groups – a control group who received no instructions, an instruction/electrode on group who were informed that the US would no longer be presented, but had the US electrode attached during extinction and an instruction/electrode off group who were informed that the US would not be presented and had the US electrode removed.

During acquisition, all groups acquired differential first and second interval electrodermal responding between CS+ and CS-. Following instructed extinction differential first interval responding was not present at the beginning of extinction in any group, while differential second interval electrodermal responding was present in the control group but absent in both instruction groups. The finding that the control group showed differential second interval electrodermal responding but not differential first interval responding at the beginning of extinction is not uncommon and has been reported in other instructed extinction studies (Luck & Lipp, 2015; Rowles, Lipp, & Mallan, 2012). This dissociation between electrodermal response indices likely reflects on differential effects of orienting and anticipation. First interval responding is very sensitive to orienting, whereas second interval responding is less affected by orienting. The interruption between acquisition and extinction is likely to have led to sensitization of the orienting reflex to the CS- in the control group. This effect was not seen in the instruction groups presumably because they were provided with safety information. Further evidence for this explanation is provided by the apparent re-emergence of differential first interval responding in the control group during the second block of extinction (see Figure 1). In the second interval responses, the instruction effects come out clearly, with an immediate reduction in differential electrodermal responding in both instruction groups, due to a reduction in responding to CS+. This is contrasted with evidence for differential responding at the beginning of extinction in the control group. The tonic electrodermal level, used as a general arousal index, provided no evidence that the arousal level reduced from acquisition to extinction in any group.

The two instruction groups did not differ in phasic or tonic electrodermal activity at any stage during extinction, suggesting that the presence of the US electrode itself did not affect electrodermal responding, whether differential or overall. Instead, the results suggest that the information given to the participants was responsible for the reduction in differential responding. Both instruction groups were provided with general safety information, ‘there will be no more presentations of the electrotactile stimulus’, and differential second interval electrodermal responding was eliminated on the first presentation of CS+ and CS- during extinction – before the participants were given any opportunity to learn the new stimulus contingencies. It would have been interesting to examine the difference in responding between participants who did and did not believe the instructions, but with only nine participants reporting not believing the instructions, statistical tests were not warranted in the current study. However, visual inspection of Figure 5 suggests that the non-believers show differential responding in a reversed direction, with responses to the CS- now exceeding responses to CS+. This pattern could suggest that they expected the electrotactile stimulus to follow the CS- instead, a finding which would be consistent with verbal reports given by a number of participants following the experiment. Exploring the pattern of responding in non-believers is an interesting avenue for future research and highlights the need to assess whether participants believe the instructions provided in instructed extinction studies.

The current study found no effect of instruction on the continuous measure of conditional stimulus valence, with all groups showing differential valence ratings between CS+ and CS- on the last trial of acquisition and the first trial of extinction. This provides a replication of the finding reported by Luck and Lipp (2015) and is in line with findings from the evaluative conditioning literature suggesting that in a picture-picture paradigm conditional stimulus valence resists instructed extinction (Lipp, Mallan, Libera, & Tan, 2010, Gast & De Houwer, 2013, Experiment 2). The current findings suggest that the dissociation between electrodermal responding and conditional stimulus valence is not simply caused by a drop in arousal decreasing the sensitivity of the physiological indices. More work is required, however to examine the boundaries of this dissociation and to determine the underlying mechanism. Rather than valence evaluations being impermeable to cognitive interventions, it could be that the target of an instructed extinction manipulation was not sufficient to reduce differential conditional stimulus valence, as the instructions targeted the anticipation of the US, but not the valence of the conditional stimuli. Future research could examine whether instructions targeting the valence of the conditional stimuli would be more effective in changing conditional stimulus valence evaluations. Future research could also examine the effects of instructed extinction in samples differing in levels of self-reported psychopathology.

The current study found that differential second interval electrodermal responding was eliminated due to a decrease in responding to the CS+ in both instruction groups. This seems to differ from the pattern reported by Sevenster et al. (2012). Visual inspection of the electrodermal data reported by Sevenster et al. (2012) suggests that responding to the CS+ did not change from the last trial of acquisition to the first trial of extinction, but that responding to the CS- actually increased. One possible explanation for this difference may be the presence of non-believers in Sevenster et al.'s sample. When the electrode was left attached we found that about 20% of the instruction group did not believe the instructions and there is some suggestion that these participants show a different pattern of responding.

In summary, we directly assessed the effects of removing the US electrode during an instructed extinction manipulation and have provided evidence that the removal of the US electrode does not explain the reduction in differential physiological responding seen as a result of instructed extinction. Instead, general safety information about US non-occurrence seems to drive this reduction in differential responding, providing evidence that changing the propositional structure of the CS-US relationship can change physiological responding on the first extinction trial. When deciding whether or not to remove the electrode as part of an instructed extinction manipulation, researchers should consider the specific requirements of their research, for instance whether the US will be presented after extinction training. Regardless of the aims of the research, however, a manipulation check to determine whether the participants believed the instructions should be included to examine whether believers and non-believers show a differential pattern of responding.

Figure Legends

Figure 1. Mean first interval electrodermal responding during habituation, acquisition, and extinction. The fourth panel shows only responses from the participants who reported believing the instructions.

Figure 2. Mean second interval electrodermal responding during habituation, acquisition, and extinction. The fourth panel shows only responses from the participants who reported believing the instructions.

Figure 3. Comparison of first interval electrodermal responding (top), second interval electrodermal responding (middle), and conditional stimulus valence (bottom) from the last trial of acquisition to the first trial of extinction in participants who reported believing the instructions.

Figure 4. Conditional stimulus valence evaluations taken during habituation, acquisition, and extinction. The fourth panel shows only data from the participants who reported believing the instructions.

Figure 5. Mean second interval electrodermal responding in believers and non-believers of the instructions from the last trial of acquisition and the first trial of extinction.

References

Boucsein, W., Fowles, D. C., Grimnes, S., Ben-Shakhar, G., Roth, W.T., Dawson, M.E., & Filion, D. L. (2012). Publication recommendations for electrodermal measures. *Psychophysiology, 49*, 1017-1034. doi:10.1111/j.1469-8986.2012.01384.x

M. G. Craske, D. Hermans & D. Vansteenwegen (Eds.), (2006). Fear and learning: From basic processes to clinical implications, 2006, APA Books: Washington.

Cuthbert, B.N., Bradley, M.M., & Lang, P.J. (1996). Probing picture perception: Activation and emotion. *Psychophysiology, 33*, 103-111. doi:10.1111/j.1469-8986.1996.tb02114.x

Dawson, M. E., Schell, A. M., & Filion, D. L. (2007). The electrodermal system. In J. T. Cacioppo, L.G. Tassinary & G.G. Bernston (Eds.), (2007). Handbook of Psychophysiology (pp. 159-181). Cambridge: Cambridge University Press.

De Houwer, J., Thomas, S., & Baeyens, F. (2001). Associative learning of likes and dislikes: A review of 25 years of research on human evaluative conditioning. *Psychological Bulletin*, *127*, 853–869. doi:10.1037//0033-2909.127.6.853

Forster, K., & Forster, J. (2003). DMDX: A Windows display program with millisecond accuracy. *Behavior Research Methods, Instruments, & Computers, 35*, 116-124. doi: 10.3758/BF03195503

Gast, A., & De Houwer, J. (2013). The influence of extinction and counterconditioning instructions on evaluative conditioning effects. *Learning and Motivation*, *44*(4), 312–325. doi:10.1016/j.lmot.2013.03.003

Grillon, C., & Ameli, R. (1998). Effects of threat of shock, shock electrode placement and darkness on startle. *International Journal of Psychophysiology, 28*, 223-231. doi: http://dx.doi.org/10.1016/S0167-8760(97)00072-X

Hoaglin, D. C., & Iglewicz, B. (1987). Fine-tuning some resistant rules for outlier labeling. *Journal of the American Statistical Association*, *82*, 1147-1149. doi:10.2307/2289392

Hoaglin, D. C., Iglewicz, B., & Tukey, J. W. (1986). Performance of some resistant rules for outlier labeling. *Journal of the American Statistical Association*, *81*, 991–999. doi:10.2307/2289073

Hugdahl, K. (1978). Electrodermal conditioning to potentially phobic stimuli: effects of instructed extinction. *Behaviour Research and Therapy*, *16*, 315–321. doi:10.1016/0005-7967(78)90001-3

Hugdahl, K., & Öhman, A. (1977). Effects of instruction on acquisition and extinction of electrodermal responses to fear-relevant stimuli. *Journal of Experimental Psychology. Human Learning and Memory*, *3*, 608–18. doi:10.1037/0278-7393.3.5.608

Lang, P. J., Greenwald, M. K., Bradley, M. M., & Hamm, A. O. (1993). Looking at pictures: Affective, facial, visceral, and behavioral reactions. *Psychophysiology, 30*, 261-273. doi: 10.1111/j.1469-8986.1993.tb03352.x

Lanzetta, J. T., & Orr, S. P. (1986). Excitatory strength of expressive faces: Effects of happy and fear expressions and context on the extinction of a conditioned fear response. *Journal of Personality and Social Psychology, 50*, 190-194. doi: 10.1037/0022-3514.50.1.190

Lipp, O. V. (2006). Human fear learning: Contemporary procedures and measurement. In M. G. Craske, D. Hermans & D. Vansteenwegen (Eds.), (2006). Fear and learning: From basic processes to clinical implications (pp. 37-52). Washington: APA Books.

Lipp, O. V., & Edwards, M. S. (2002). Effect of instructed extinction on verbal and autonomic indices of Pavlovian learning with fear-relevant and fear-irrelevant conditional stimuli. *Journal of Psychophysiology, 16*, 176-186. doi: 10.1027//0269-8803.16.3.176

Lipp, O. V., Mallan, K. M., Libera, M., & Tan, M. (2010). The effects of verbal instruction on affective and expectancy learning. *Behaviour Research and Therapy, 48*(3), 203-209. doi: http://dx.doi.org/10.1016/j.brat.2009.11.002

Lockhart, R. A. (1966). Comments regarding multiple response phenomena in long interstimulus interval conditioning. *Psychophysiology, 3*, 108-114. doi: 10.1111/j.1469-8986.1966.tb02687.x

Lovibond, P. F. (2004). Cognitive processes in extinction. *Learning and Memory, 11*, 495-500. doi: 10.1101/lm.79604

Luck, C. C., & Lipp, O. V. (2015). A potential pathway to the relapse of fear? Conditioned negative stimulus evaluation (but not physiological responding) resists instructed extinction. *Behaviour Research and Therapy, 66*, 18-31. doi: http://dx.doi.org/10.1016/j.brat.2015.01.001

Öhman, A. (1983). The orienting response during Pavlovian conditioning. In D. A. T. Siddle (Ed.), *Orienting and habituation: Perspectives in human research* (pp. 315-370). New York: Wiley.

Prokasy, W.F., & Kumpfer, K.L. (1973). Classical conditioning. In W. F. Prokasy & D. C. Raskin (Eds.), *Electrodermal activity in psychological research* (pp. 157-202). San

Diego: Academic Press.

Rowles, M. E., Lipp, O.V., & Mallan, K.M. (2012). On the resistance to extinction of fear conditioned to angry faces. *Psychophysiology, 49*, 375-380. doi:10.1111/j.1469-8986.2011.01308.x

Stewart, M.A., Winokur, G., Stern, J.A., Guze, S.B., Pfeiffer, E., & Hornung, F. (1959). Adaptation and conditioning of the galvanic skin response in psychiatric patients. *The British Journal of Psychiatry*, *105*, 1102-1111. doi:10.1192/bjp.105.441.1102

Sevenster, D., Beckers, T., & Kindt, M. (2012). Instructed extinction differentially affects the emotional and cognitive expression of associative fear memory. *Psychophysiology*, *49*, 1426–35. doi:10.1111/j.1469-8986.2012.01450.x

Tottenham, N., Tanaka, J.W., Leon, A.C., McCarry, T., Nurse, M., Hare, T. A., . . . Nelson, C. (2009). The NimStim set of facial expressions: Judgments from untrained research participants. *Psychiatry Research, 168*, 242–249. doi:10.1016/j.psychres.2008.05.006

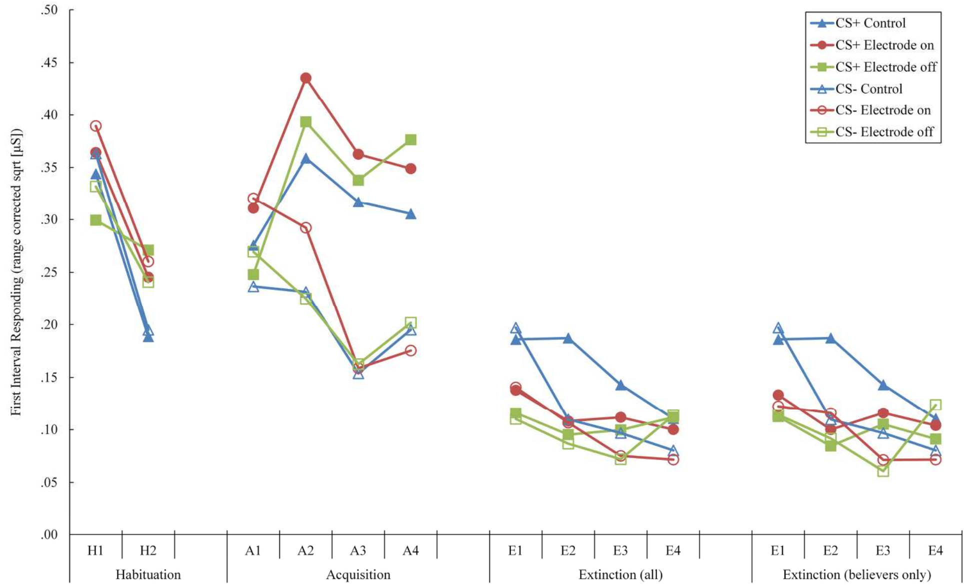

Figure 1. Mean first interval electrodermal responding during habituation, acquisition, and extinction. The fourth panel shows only responses from the participants who reported believing the instructions.
190x118mm (300 x 300 DPI)

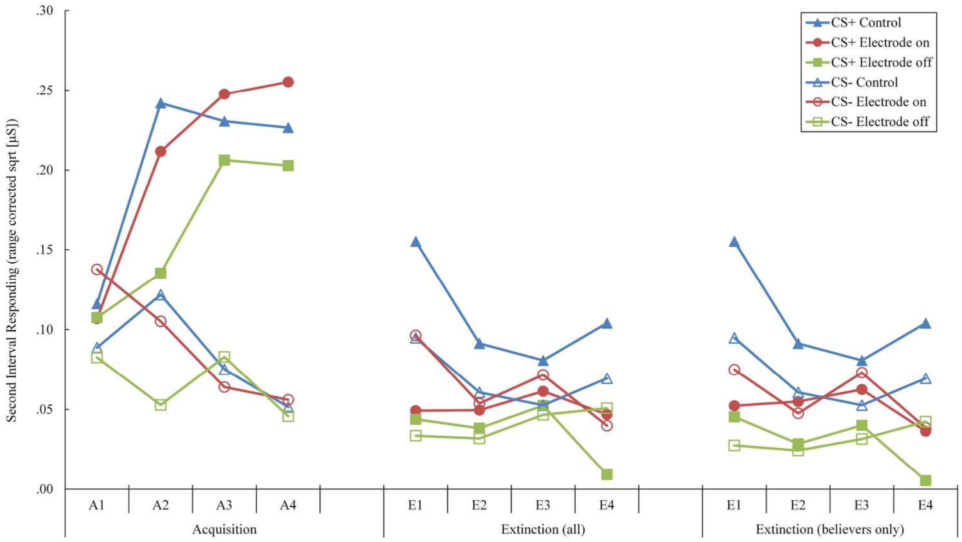

Figure 2. Mean second interval electrodermal responding during habituation, acquisition, and extinction. The fourth panel shows only responses from the participants who reported believing the instructions.
174x98mm (300 x 300 DPI)

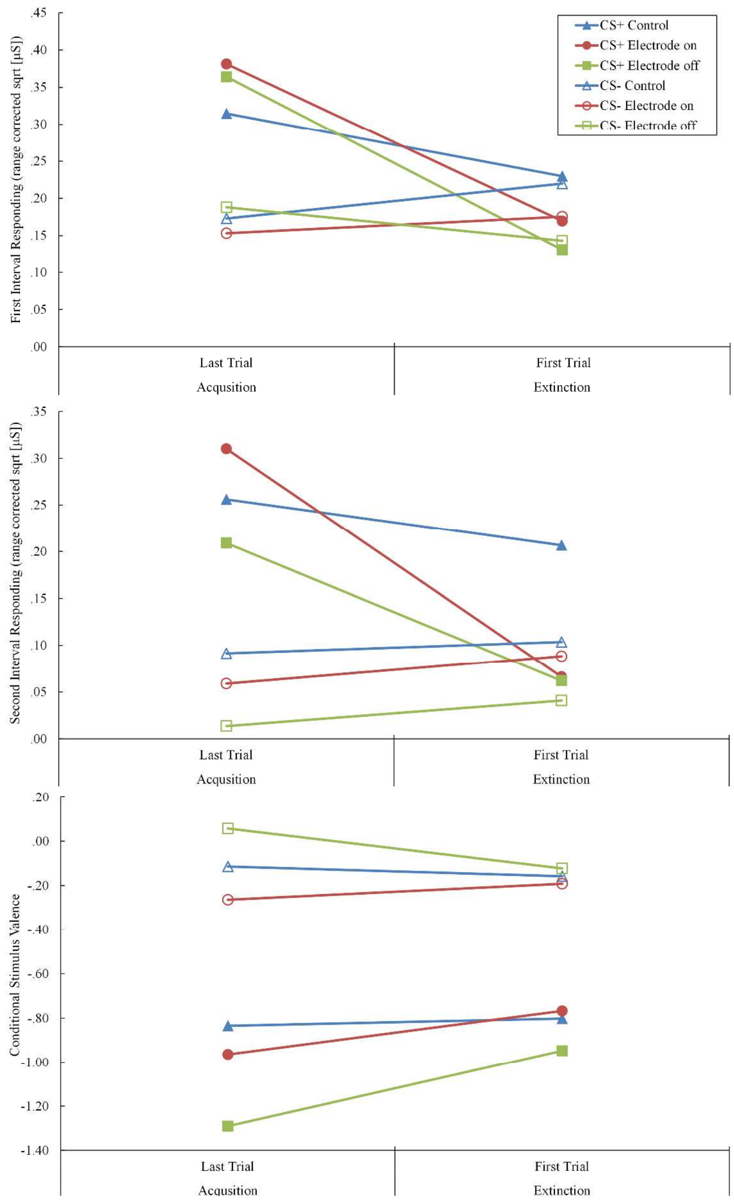

Figure 3. Comparison of first interval electrodermal responding (top), second interval electrodermal responding (middle), and conditional stimulus valence (bottom) from the last trial of acquisition to the first trial of extinction in participants who reported believing the instructions.
230x372mm (300 x 300 DPI)

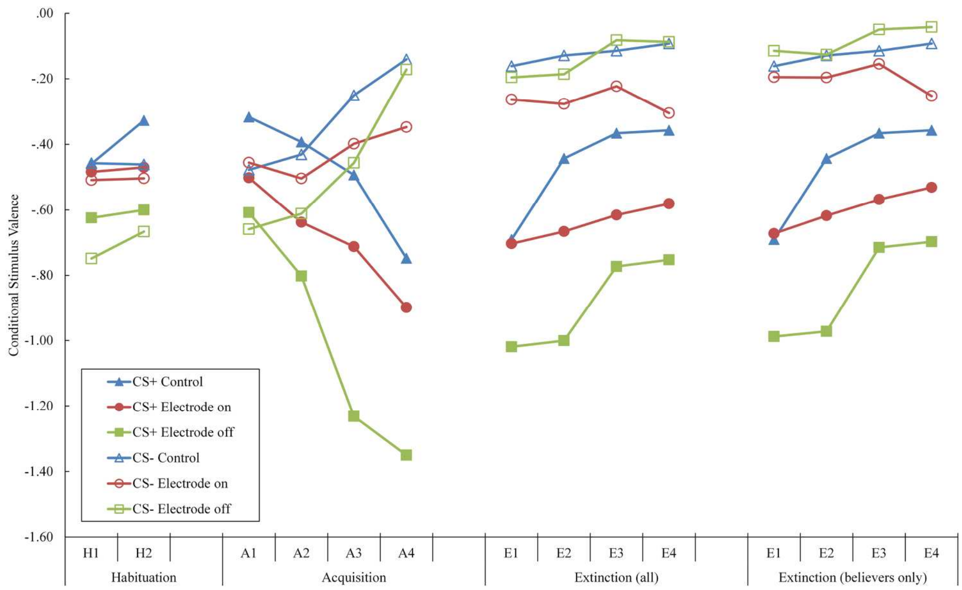

Figure 4. Conditional stimulus valence evaluations taken during habituation, acquisition, and extinction. The fourth panel shows only data from the participants who reported believing the instructions.
191x118mm (300 x 300 DPI)

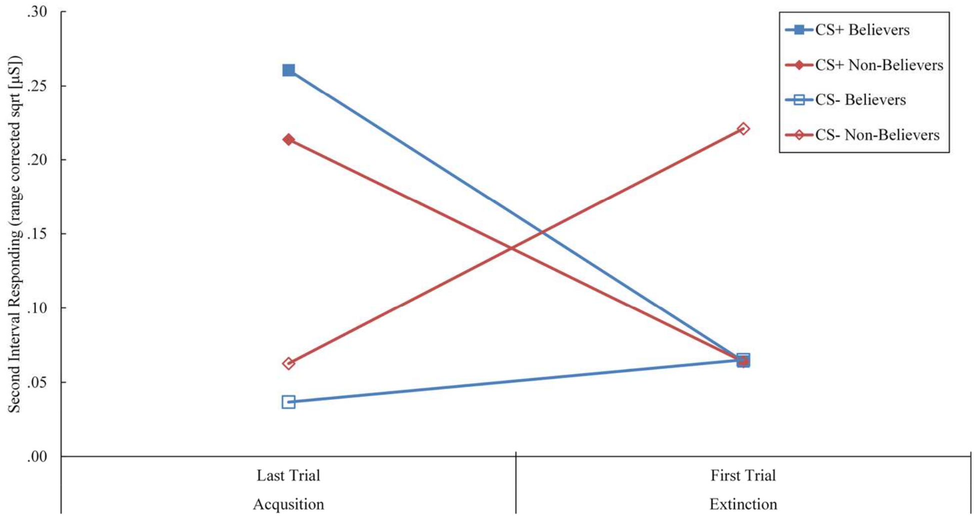

Figure 5. Mean second interval electrodermal responding in believers and non-believers of the instructions from the last trial of acquisition and the first trial of extinction.
140x74mm (300 x 300 DPI)