Fear expression and return of fear following threat instruction with or without direct contingency experience

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Abstract

Prior research showed that mere instructions about the contingency between a Conditioned Stimulus (CS) and an Unconditioned Stimulus (US) can generate fear reactions to the CS. Little is known, however, about the extent to which actual CS-US contingency experience adds anything beyond the effect of contingency instructions. Our results extend previous studies on this topic in that it included fear potentiated startle as an additional dependent variable and examined return of fear following reinstatement. We observed that CS-US pairings can enhance fear reactions beyond the effect of contingency instructions. Moreover, for all measures of fear, instructions elicited immediate fear reactions that could not be completely overridden by subsequent situational safety information. Finally, return of fear following reinstatement for instructed CS+s was unaffected by actual experience. In sum, our results demonstrate the power of contingency instructions and reveal the additional impact of actual experience of CS-US pairings.

Keywords: fear; conditioning; instructions; skin conductance response; fear potentiated startle

Introduction

Adaptive behavior in changing environments critically relies on learning to predict potentially harmful events. However, fear learning can also be maladaptive and pathological when too pronounced or situational inappropriate. Fear conditioning, extinction and return of fear are used as laboratory analogues for the acquisition, exposure-based treatment and subsequent relapse in patients suffering from phobic fears (Beckers, Krypotos, Boddez, Effting, & Kindt, 2013; Mineka & Zinbarg, 2006). In fear conditioning, an initially neutral stimulus (conditioned stimulus, CS) is repeatedly paired with an aversive stimulus (unconditioned stimulus, US) and thereby the CS gains the capacity to elicit a fear response (conditioned response, CR). Repeated presentation of the CS without the US during extinction typically leads to a gradual weakening of the CR. However, the return of (conditioned) fear (ROF) can be facilitated by various conditions such as re-presentation of the US (reinstatement) (Bouton & Bolles, 1979; Haaker, Golkar, Hermans, & Lonsdorf, 2014; Rescorla & Heth, 1975).

The prediction of aversive events can be based on information acquired in different ways. With respect to the acquisition of fear, direct experience of CS-US pairings as well as observational and instructed fear have been identified as possible routes (e.g. Rachman, 1977). The role of actual CS-US contingency experience as laboratory model for the development of phobias has been a subject of debate because the etiology of fear and phobias can often be traced back to observational learning or verbal instructions (Field, 2006; Rachman, 1977). In addition, propositional theories of human associative learning highlight that observation, reasoning and verbal instructions are equally valid sources for learning as directly experiencing contingencies (De Houwer, 2009; Mitchell, De Houwer, & Lovibond, 2009; Olsson & Phelps, 2007). Therefore, studying instructions as a source of fear acquisition in the lab can be important to gain a better understanding of the etiology of fear and phobias and to spur theoretical development in our understanding of associative learning.

Numerous laboratory studies have demonstrated that verbal instructions are a potent means to generate or change fear reactions. For instance, verbal instructions and vicarious observations regarding CS-US contingencies are known to be sufficient to immediately establish (Cook & Harris, 1937; Grillon, Ameli, Woods, Merikangas, & Davis, 1991; Olsson & Phelps, 2004), alter (Lovibond, 2003; McNally, 1981) or extinguish fear reactions (Golkar, Selbing, Flygare, Ohman, & Olsson, 2013; Lipp & Edwards, 2002; Sevenster, Beckers, & Kindt, 2012a). Recently, it was also shown that extinction via instructions or observation attenuates the ROF through reinstatement (Golkar et al., 2013; Sevenster et al., 2012a).

To date, however, few studies have looked at the joint effects of the actual experience of and instructions about CS-US contingencies on fear expression and ROF. This could not only shed light on the unique contribution of both pathways to fear but also on their interaction. An additive effect of experience and instruction is expected on the basis of conditioning theories for phobic fear. For instance, Mineka and Zinbarg (2006) proposed that a trauma (e.g. being bitten by a dog) should lead to stronger fear reactions when it matches with previous beliefs on the trauma inducing stimulus (e.g. dangerous dog vs. non-dangerous dog). Similarly, it is known for decades that fear conditioning is more pronounced and extinction is attenuated when “biologically prepared” stimuli (e.g. snakes) are used as CSs (Öhman & Mineka, 2001). In line with this, it has been shown that the combination of threat information about an animal and an actual negative encounter indeed produced more fear in children than either threat information or a negative encounter alone (Field & Storksen-Coulson, 2007). Such an additive effect was, however, not observed in a similar study in adults (Ugland, Dyson, & Field, 2013). In these two studies however, participants were provided with general threat information rather than specific contingency instructions.

Moreover, possible differences in US expectancy between the threat and no-threat groups were not controlled for.

These issues were addressed in a recent study by Raes, De Houwer, De Schryver, Brass, and Kalisch (2014) that compared reactions to two CSs, both of which were instructed to be followed by an electro-tactile US during a second (“Test”) phase. In a cover story, participants were told that, in order to familiarize themselves with the procedure, the test phase would be preceded by a training phase that was identical to the test phase, except that the USs that would follow one of the CSs (CS instructed or CS-I) would be replaced by a placeholder (a drawing of a lightening bold). They were told that the placeholder was used simply to reduce the number of actual USs during the training phase. After these instructions, participants experienced the training phase in which the first CS (CS instructed + experienced or CSI+E) was followed by the US on some trials whereas the other CS (CS-I) was never followed by the actual US but only by the placeholder. During the later test phase, contrary to instructions, neither of the CSs was followed by the US, allowing for a test of conditioned responding under extinction. The results showed that the actual experience of CS-US contingency can enhance fear reactions beyond the effect of contingency instructions. In particular, Fear ratings during test were heightened for the CSI+E as compared to the CS-I while US expectancy ratings and skin conductance responses (SCR) did not differ significantly between both CSs. One possible explanation of this finding is that SCR and US expectancy may tap into more cognitive components of fear such as explicit CS-US contingency knowledge (Dawson, Schell, & Banis, 1986; Grings, 1973; Hamm & Weike, 2005; Reiss, 1980; Sevenster, Beckers, & Kindt, 2012b) whereas Fear ratings might reflect a more emotional component of fear (Hamm & Weike, 2005). From this perspective, instructions might primarily affect the cognitive components of fear whereas actual CS-US contingency experience might have an impact on the affective components.

If this post-hoc explanation of the results of Raes et al. (2014) is correct, also other indices that tap primarily into affective components of fear should reveal an impact of actual CS-US pairings beyond the impact of contingency instructions. Fear potentiated startle (FPS) is a prime candidate for such an affective index of fear. The startle response is a defensive reflex, measured at the orbicularis oculi muscle that can be elicited by a sudden high intensity noise (Davis, 2006). The amplitude of the startle reflex is modulated by valence. It is potentiated in aversive emotional states (such as fear) and attenuated in positive emotional states (Lang, Bradley, & Cuthbert, 1990) and is often used to tap the affective component of fear learning (e.g., Hamm & Weike, 2005). In fact, it has been shown that FPS is less affected by verbal safety instructions (Sevenster et al., 2012a) and contingency awareness (Hamm & Weike, 2005; Sevenster, Beckers, & Kindt, 2014) during uninstructed conditioning than SCR. Thus, FPS represents a suitable measure to capture the emotional component of fear conditioning which we expect to be strongly affected by actual CS-US pairings. In addition, including FPS as an additional measure promises to be informative with respect to a striking finding of Raes et al. (2014), who report enhanced Fear and US expectancy ratings, but not SCRs, towards the CS-I (as compared to the CS-) already during training. Because participants were explicitly informed that the CS-I would only be followed by the US during a later test but not during the initial training, this finding shows that the effects of the threat instructions could not be completely overridden by subsequent situational safety instructions. As FPS has been shown to be especially insensitive to verbal safety instructions (Sevenster et al., 2012a), we predict that fear reactions to the CS-I during training will be specifically outspoken for FPS.

Finally, in a second extension of the design of Raes et al. (2014), we implemented a reinstatement procedure to complement the previous studies on the return of fear following reinstatement. The majority of studies on reinstatement in humans have used instructed acquisition (i.e., CS-US contingency instructions in combination with actual CS-US pairings) while extinction was with few exceptions uninstructed (Haaker et al., 2014). In these studies, instructed extinction as compared to uninstructed extinction leads to resistance to return of fear for SCRs and US expectancy ratings but not FPS (Sevenster et al., 2012a). Similarly, observational extinction (i.e., observing a third person being exposed to unreinforced post-acquisition CS trials) following regular fear conditioning also attenuated ROF following reinstatement (Golkar et al., 2013). While instructed and observational extinction seems to prevent the ROF, explicit tests of the effect of instructed vs. uninstructed fear *acquisition* are still awaited (Haaker et al., 2014). In an attempt to shed light on this question, our study for the first time directly compares the return of fear following reinstatement between two instructed CSs that differ in the presence or absence of a history of direct CS-US contingency experience. Given the assumption that both contingency instructions and CS-US pairings can contribute to the development of (pathological) fear, information about the impact of both pathways on ROF could shed new light on the long-term outcome of treatment of (pathological) fear.

Method

Participants

Forty-four right-handed volunteers were recruited through an online platform. Eight participants were excluded because of technical issues (N = 3), insufficient belief in the instructions (N = 4) or a failure to induce a fearful US (N = 1), leaving 36 participants for analyses (15 males, *mean age* = 26.89, *SD* = 4.87; *mean STAI-S score* = 32.67, *SD* = 5.67, range = 21 – 44). The sample size was based on the original study of Raes et al. (2014) (N = 32). The study was approved by the local ethics committee Hamburg (General Medical Council Hamburg) and volunteers were paid 20 Euro.

Materials

The Materials and Procedure used are largely identical to the previous experiment of Raes et al. (2014) and will thus be described briefly.

Experimental stimuli. Stimulus presentation was controlled with Presentation software (NeuroBehavioral Systems, Albany California, USA). Three blue snow fractals (200 by 200 pixels) in a white square presented in the center of a black background served as CSs (duration 8 s, see Figure 1) and a white fixation cross on a black background served as the ITI (duration 13, 15 or 17 s). The US was an electro-tactile stimulus administered to the back of the right hand with a 1 cm diameter surface electrode with a platinum pin (Specialty Developments, Bexley, UK). It consisted of three 2 ms rectangular pulses with an inter pulse interval of 40 ms. US administration was controlled via a Digitimer DS7A constant current stimulator (Hertfordshire, UK). In the training phase a picture of a lightning bolt (approximately 200 by 200 pixels) presented for 500 ms was used as the placeholder for the US.

Subjective ratings. US expectancy and Fear ratings referring to the most recent encounter for each CS were provided on 9-point Likert scales in blocks (i.e., 6 ratings per block). Before the rating block, participants were asked to think back to their last encounter with the stimuli and were reminded that the questions referred to the actual stimulation and not the picture of the lightning bolt. The Likert scales were accompanied by the caption “To what extent did you expect an electro-tactile stimulation while seeing this figure?” for US expectancy ratings and by “How much fear did you experience while looking at this figure?” for Fear ratings. Anchors for US expectancy ratings were (1) certainly not, (3) rather not, (5) uncertain, (7) rather certain and (9) certain. For Fear ratings anchors were (1) none at all, (3) very little, (5) uncertain, (7) to some extent and (9) very much. There were no time constraints for providing ratings. The sequence of trials was interrupted every nine trials for a rating block.

Manipulation checks. After the experiment, pleasantness and pain ratings were collected for both the acquisition US and the reinstatement USs on 9-point Likert scales. Pleasantness ratings were accompanied by the caption “How pleasant/unpleasant did you find the electrical (unexpected/unsignaled) stimulation?” and the anchors were: (1) very unpleasant, (5) uncertain, (9) very pleasant. Pain ratings were accompanied by the caption “How painful did you find the (unexpected/unsignaled) stimulation?” together with anchors: (1) totally not, (3) rather not, (5) uncertain, (7) rather much, (9) very much.

Questionnaires. Prior to the experiment, participants completed a German version of the State version of the State-Trait Anxiety Inventory (STAI-S; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983) and a general demographic questionnaire. After the experiment, participants completed an English custom-made questionnaire[1] about the credibility of the experimental instructions. In this questionnaire, participants had to indicate the clarity and believability of the instructions on a scale ranging from 0 (not at all) to 10 (very much so) and could additionally provide general remarks about the experiment.

--- Insert Figure 1 about here ---

Procedure

Start-up. Upon arrival at the laboratory, measurement and stimulation electrodes were attached. Participants then filled in the questionnaires and went through a work-up procedure to individually adjust US intensity to a level experienced as “unpleasant but not painful”. Ratings of the final intensity were verbally provided on a 10-point scale (*mean intensity* = 8.02 mA, *SD* = 6.80; *mean painfulness rating* = 8.53, *SD* = 0.71). Subsequently, participants were administered an initial announced electro-tactile stimulation to test their physiological reactions.

Instructions about the experimental procedure were provided as described before (Raes et al., 2014). Briefly, participants were informed about the two experimental phases (referred to as training and test phase). They were explicitly informed that in the training phase one stimulus (CSI+E) would be followed by the US while another stimulus (CS-I) would be followed by a picture of a lightning bolt as a placeholder for the US. As a cover story, the placeholder was said to be used to avoid the experience of a large number of USs before the actual test phase starts. A third fractal (CS-) was introduced as safe (never followed by the US, see Figure 1). Furthermore, participants were told that both the CSI+E and CS-I would be equally predictive of the US during the subsequent test phase. Explicit information about which two of these snow fractals may sometimes be followed by an US and which one would never be followed by the US (CS-) were provided. Assignment of the three fractals to the three CS types was counterbalanced across participants.

Training phase. After a brief startle habituation with five startle probes (ISI of 3 s), the training phase started which consisted of 27 trials organized in three blocks of nine CSs (three per CS type). Stimulus presentation was randomized with the restriction of no more than two repetitions of the same CS type. The first presentation of both CSI+E and CS-I was always reinforced by the US or placeholder respectively, coinciding with CS offset. The second and third presentation was reinforced either for the CSI+E or CS-I in a counterbalanced fashion. Thus, in total two presentations of each CS type (CSI+E and CS-I) were reinforced.

Test phase. The test phase started with explicit instructions that both the CSI+E and the CS-I would be followed by the US from now while the CS- would remain unreinforced. In fact, this phase served as an extinction session as no US was administered following any CS. Apart from these instructions and US omission, stimulus timing and organization were identical to the training phase.

Reinstatement and reinstatement test. Following the last rating block of the test phase, three unannounced reinstatement USs were delivered (ISI of 5 s) to the participants while they saw a black background. The black background was identical to the background the fractals were superimposed on to maintain the experimental context (see Haaker et al., 2014 for a discussion of the role of the context in reinstatement). 17 s after the last reinstatement US, the first of nine (three of each CS type) additional unreinforced CS presentations started.

Psychophysiological recordings

Skin conductance responses (SCR). SCRs were measured using two disposable Ag/AgCl electrodes (2 cm diameter) attached to the distal and proximal hypothenar of the left hand. The signal was recorded using a BIOPAC MP-100 amplifier and Acqknowledge 3.9 software (BIOPAC Systems Inc, Goleta, California, USA). Data were manually scored offline using a custom-made program according to published recommendations (Boucsein et al., 2012): The first response initiating within a 0.9-4.0 s post stimulus onset (US or CS) and an amplitude >0.02 µS was considered. Reactions showing recording artifacts were treated as missing data points. Prior to analysis, skin conductance values were log-transformed to normalize the data and range-corrected to account for individual differences in skin conductivity.

Fear potentiated startle. Orbicularis oculi muscular activation was measured through two 5 mm Ag/AgCl electrodes attached to the lower eyelid of the right eye (Blumenthal et al., 2005). A ground electrode was placed on the forehead approximately two centimeters below the hairline. Startle responding was elicited using a 95 dB white noise burst presented binaurally through Sennheiser headphones (Wedemark, Germany). The raw signal was collected at 1000 Hz, amplified and filtered (28-500 Hz) with a BIOPAC MP-100 amplifier and recorded, rectified and integrated with Acqknowledge 3.9 software (BIOPAC Systems Inc, Goleta, California, USA).

During CS presentations in each block of the training and test phase, a startle probe was administered twice for each CS type - once after 5.5s and once after 6.5s. The first CS after the reinstatement USs was always startled to make sure that the rather transient effect of the reinstatement manipulation would be captured in the FPS data.

During the ITI, startle probes were administered in two thirds of the cases at either an early or a late time point while the remaining ITIs were not startled. For the 13 s ITI, the startle probe could be either administered after 5 or 6 s (early startle probe) or 8 and 9 s (late startle probe). For the 15 s ITI, these values were 5 and 6.5 s (early) and 9.5 and 11 s (late). Finally, for the 17 s ITI, these values were 5 and 7.5 s (early) and 11 and 13 s (late). Finally, for the reinstatement phase, one of the two versions was randomly selected.

Acquired data were scored offline with a custom-made program. Startle responses 20 - 120 ms post startle probe onset were scored (Blumenthal et al., 2005). Responses were treated as missing when confounded by recording artifacts or when spontaneous blinks occurred right before, during or right after the startle probe onset. Prior to analysis, FPS data were T-transformed. One participant was excluded from FPS analyses because he had a large proportion of unusable trials for this measure (85.39 %).

Statistical analyses

Before analysis, data from the physiological measures were averaged by three (SCR) or by two (FPS) trials per CS in order to reduce variance and to obtain an equal amount of data points as for the ratings (i.e., three per phase and one after reinstatement). The training and test phase were analyzed separately with mixed models ANOVAs with the within-subject factor CS type (SCR, US expectancy, Fear ratings: CSI+E, CS-I, CS-; FPS: CSI+E, CS-I, CS-, ITI). In addition, a second factor block (first, second or third) was added to the analysis of the test phase in order to assess extinction. Two additional ANOVA's were carried out to assess changes from the training to the test phase and from the test to the reinstatement phase respectively. First, the CSI+E/CS-I difference score for the training phase and the test phase was analyzed with a mixed model ANOVA with the factor phase (training, test). Second, responses from the last block of the test phase and the block after the reinstatement manipulation were compared with a phase (2) x CS type (for SCR: 3; for FPS: 4) mixed model ANOVA. For the reinstatement analysis, by trial results from the physiological measures were used because the reinstatement effect is transient (Haaker et al., 2014).

Greenhouse-Geisser corrections are reported when appropriate and the alpha level was set to .05.

Results

Manipulation checks

Four participants who rated believability as assessed by the custom-made questionnaire as 5 or less and one participant who consistently rated the US as pleasant and not painful in the post-experiment manipulation check ratings were excluded from the analyses (see the Materials and Participants sections). The remaining participants reported the instructions to be both clear (*mean* = 9.54, *SD* = 0.74) and believable (*mean* = 9.18, *SD* = 0.90). Furthermore, participants generally reported the US to be both rather unpleasant (*mean pleasantness rating* = 3.22, *SD* = 2.00) and moderately painful (*mean pain rating* = 6.33, *SD* = 1.17). Similar ratings were given for the reinstatement USs (*mean pleasantness rating* = 2.58, *SD* = 1.93; *mean pain rating* = 6.83, *SD* = 1.38).

Training phase

During the training phase, a significant main effect of CS type was observed for all measures, all p-values < .001 (see Table 1, Figure 2). Conditioned responses were stronger for CSI+E and CS-I than for CS-, showing fear expression on all measures, all p-values ≤ .005, with the exception of SCRs for which the CS-I only elicited trend-wise stronger responses than the CS-, $p = .075$. Furthermore, the CSI+E elicited significantly stronger responses than the CS-I in Fear ratings, US expectancy and SCRs, all *p*-values < .009, and trend-wise stronger response in FPS, *p* = .072 (see Table 1, Figure 2). Taken together, these results demonstrate enhanced cognitive and emotional responding during the training phase to the US-predictive CSI+E. Responses were, however, also enhanced to the CS-I despite instructions that this stimulus was explicitly safe during this but not a later experimental phase.

------ insert Figure 2 and Figure 3 about here ------

------ insert table 1-4 about here ------

Test phase

In the test phase, a significant main effect of CS type was observed for all measures, all *p*-values ≤ .001. Responses towards CSI+E and CS-I were significantly stronger than to the CS-, all *p*-values < .004 (see Table 2, Figure 2). In addition, CSI+E elicited significantly (US expectancy, Fear ratings, both *p*-values < .001) or trend-wise (FPS, *p* = .082)[2] stronger responses than CS-I, despite the fact that participants were told that both CSs would be equally predictive of the US during this experimental phase. For SCRs, however, there was no significant difference between CSI+E and CS-I, *F*(1,35) < 1. Thus, verbal instructions completely abolished differences between the merely instructed CS (CS-I) and the instructed and experienced CS (CSI+E) only in SCRs. For all other measures the effect of experience carried over from the training to the test phase which was reflected in a significantly or marginally maintained CSI+E/CS-I discrimination.

A significant main effect of block was also observed for all measures, all *p*-values < .001. Importantly, this main effect of block was qualified by an interaction between CS type and block for US expectancy, Fear ratings, and FPS, all *p*-values < .05. For US expectancy and Fear ratings, this interaction was due to decreasing responses for both the CSI+E and CS-I relative to the CS- (i.e., extinction). For FPS, the interaction was due to extinction of responding towards CSI+E but not towards CS-I (see Table 2 for contrasts).

Comparing training and test phase

As was noted by Raes et al. (2014), a change in the difference score between CS-I and CS- from training to test would show that there is not only an impact of threat information per se (i.e., that CS-I can be followed by the US), but also of the information about when the threat information is valid (i.e., that CS-I will be followed by the US only during test). We did indeed find that the CS-I/CS- difference was larger in the first block of the test phase than in the last block of the training phase for US expectancy, Fear ratings and SCR, all p-values < .03. For FPS, there was a weak trend in the same direction (p = .097, see Table 3 and Figure 3).

Reinstatement

For all measures, there was a main effect of time (pre or post reinstatement manipulation), showing that fear generally increased after reinstatement (generalized reinstatement), all p-values < .05 (see Table 4, Figure 2).

A significant time x CS interaction, p = .021, was observed only for Fear ratings (differential reinstatement, see Table 4). This interaction was due to increased Fear ratings for CS-I in comparison to the CS- after the reinstatement manipulation (p = .014), while response enhancement to the CSI+E following reinstatement did not differ from either response enhancement to the CS- or the CS-I (both *p's* >= .105, see Table 4).

Discussion

The present study investigated the effect of actual CS-US contingency experience beyond verbal instructions on different autonomous and declarative measures in an instructed fear expression paradigm. We thereby extended previous work (Raes et al., 2014) by including FPS as well as a reinstatement manipulation to the study. Thereby we were able to investigate for the first time reinstatement of fear to stimuli that differ in actual reinforcement experience but not in verbally assigned danger. Two CSs were explicitly told to predict US occurrence during the test phase of the experiment. Via a cover story participants were told that only one CS (CSI+E), but not the other (CS-I) would be followed by the US during an initial training phase. During the subsequent test phase however, contrary to instructions, none of the CSs were followed by the US.

We discuss three main findings: First, (a) CS-US contingency experience enhances fear reactions beyond the effect of verbal instructions in the test phase for subjective ratings (US expectancy, Fear) and (marginally) FPS reactions but not for SCRs and (b) discrimination between CS-I (instructed but never experienced) and the CS- became more pronounced from training to test for all dependent variables except for FPS, mirroring the provided information about CS-US contingencies. Second, verbal threat information can have profound effects that cannot be completely overridden by situational safety information ("better safe than sorry"). Finally, third, ROF does generally not differ for verbally transmitted fear with or without direct CS-US contingency experience. In the following, we will discuss these findings in depth.

First, results are in line with previous demonstrations that actual experience of CS-US contingencies enhances fear reactions beyond the effect of verbal instructions (Field & Storksen-Coulson, 2007; Raes et al., 2014). These and related results (e.g., Field & Storksen-Coulson, 2007) are in agreement with theories that highlight the role of conditioning in phobic fears which propose that a trauma should induce stronger effects when it matches previous beliefs (Mineka & Zinbarg, 2006). A previous study (Raes et al., 2014) observed such an additive effect of instruction and direct CS-US contingency experience only for Fear ratings but not any other dependent variable (SCRs, US expectancy). These findings could point to a difference between dependent measures that are thought to tap a more cognitive (US expectancy, SCRs) vs. emotional (Fear ratings) component of fear learning (e.g. Hamm & Weike, 2005; Sevenster et al., 2012a). The present study aimed at testing this hypothesis by including FPS as an additional dependent variable in the same paradigm. As in the previous study (Raes et al., 2014), no effect of direct CS-US contingency experience beyond the effect of contingency instruction (nonsignificant CSI+E/CS-I discrimination during test) was observed for SCRs. The significant CSI+E/CS-I discrimination observed during the training phase was completely abolished by instructions preceding the test phase and thus our results add to the interpretation of SCRs reflecting CS-US contingency knowledge (Hamm & Weike, 2005; Sevenster et al., 2012a). An effect of experience beyond instruction, as indicated by significant CSI+E/CS-I discrimination during the test phase was, however, evident for subjective ratings (both Fear and Expectancy ratings) and FPS (even though marginally significant). This maintained discrimination likely reflects remainders of the previous CSI+E-US contingency experience in the preceding training phase.

Based on this evidence, it seems unlikely that experience only adds an effect beyond instructions for measures that tap the emotional but not a rather cognitive component of fear or for measures that capture subjective ratings as opposed to psychophysiological reactions. In fact, interesting differences between both psychophysiological measures emerged. While FPS reactions during the test phase showed only marginal effects of both CS-US contingency experience during the preceding phase (marginal CSI+E/CS-I difference during test) and situational threat instruction provided before the test phase (marginal change in CS-I/CS- difference from training to test), SCRs were not influenced by direct CS-US contingency experience during the preceding phase (no CSI+E/CS-I difference during test) but were very sensitive to situational threat information (large change in CS-I/CS- difference from training to test). Thus, our results suggest that SCRs and FPS might be differentially sensitive to contingency instructions and direct experience respectively. This is in line with previous studies that demonstrated that FPS is less affected by verbal instructions and explicit contingency knowledge than SCR or subjective ratings (Sevenster et al., 2012a, 2012b). Alternatively, FPS might follow the initial threat information that both CSs will at a later time point be predictive of the US. This is reflected in a weak CSI+E/CS-I discrimination for FPS while both CSI+E and CS-I are potentiated against the CS- and the ITI. In sum, our data replicate previous findings that show an additive effect of experience and verbal threat information that, however, does not emerge in the same way in different dependent variables. Furthermore these inconsistencies between different dependent measures highlight the importance of multimodal assessment (e.g., different subjective measures and psychophysiological indicators of fear; see also: Mauss and Robinson, 2009).

Second, it is striking that the CS-I, which was said to be predictive of the US only in a later experimental phase, but to be explicitly safe during the initial training phase, elicited responses that were significantly enhanced as compared to the CS- in all dependent measures. This observation replicates the results of Raes et al. (2014) and extends them to FPS as an additional dependent measure. Together, this suggests that verbal threat information for a specific stimulus can have profound effects on both cognitive and autonomous measures that cannot be completely overridden by situational safety information (“better safe than sorry”). However, it needs to be acknowledged that in the current design we did not employ CSs that were purely verbally or Pavlovian conditioned. During the training phase, the CS-I was paired with the placeholder US which may have allowed for conditioning (Hofmann, De Houwer, Perugini, Baeyens, & Crombez, 2010; White & Davey, 1989). This may have led to an overestimation of the impact of verbal instructions during the practice phase and an underestimation of the impact of CS-US pairing experience during the test phase (see Raes et al., 2014 for an extensive discussion of this issue).

Third, after reinstatement, non-differential response enhancement (‘generalized reinstatement’) was observed for all dependent measures as often seen in human differential conditioning studies in reinstatement (Dirikx, Vansteenwegen, Eelen, & Hermans, 2009; for a review see Haaker et al., 2014) and other return of fear manipulations (Vervliet et al., 2013). In addition to this general reinstatement, that affects all CSs in a similar way, differential ROF to the CS-I as compared to the CS- was observed only in Fear ratings (‘differential reinstatement’). It is not uncommon in human studies that different dependent measures reflect a different quality (e.g., differential vs. generalized) of the reinstatement effect even in the same study (Haaker et al., 2014).

To date, the experimental and individual boundary of differential or generalized reinstatement as well as the mechanisms behind remain elusive (Dirikx et al., 2009; Haaker et al., 2014). The dissociation between these two qualities of reinstatement effects have only recently gained more attention. In rodent work on this topic nearly exclusively single-cue conditioning designs were used that do not allow for a discrimination between differential and generalized reinstatement effects, as there is only one conditioned stimulus. Differential conditioning protocols in turn allow for a dissociation between association-based and non-association based (e.g. sensitization) effect. However, it is important to note, that generalized reinstatement effects do not preclude genuine association-based mechanisms, as it may result from stimulus generalization and associative learning to the CS (discussed by Vervliet et al., 2013 in the context of renewal).

What is particularly striking with the present results is that the quantity and quality of reinstatement effects did not differ between both stimuli that were instructed to be predictive of the US, irrespective of whether this CS-US contingency was in fact experienced. These results might have important implications for clinical situations as they suggest that fears that are acquired via instructions have the same risk for relapse after treatment compared with fears that are acquired via the experience of aversive events. However, we cannot exclude that differences between our two CSs might arise under different conditions (Haaker et al., 2014). Nevertheless, our data illustrates how harmful and resistant verbally transmitted fears might be.

In line with the arguments put forward by Raes et al. (2014), we believe that our results put important constraints on theories of associative learning. Both experiments provide evidence that the actual experience of CS-US pairings can add to the effects of clear and believable contingency instructions. Associative learning models might explain this result by assuming that a CS-US association established on the basis of verbal instructions is further strengthened through subsequent CS-US pairings. Propositional models of associative learning, on the other hand, could argue that subsequent CS-US pairings add to the truth-value of propositions formed while receiving instructions. However, currently, both classes of models are underspecified with regard to the conditions under which actual experience can add to the effect of verbal instructions, which limits the possibility to interpret our data in favor of one model or the other (see Raes et al., 2014, for a more extensive discussion). Furthermore, models of associative learning will have to handle similarities and differences between different measures such as those observed in the current study. We have discussed dual-systems models in the introduction that highlight similarities between US expectancy ratings and SCRs in reflecting cognitive processing, which can be distinguished from measures that tap more into emotional processing such as FPS and Fear ratings (Hamm & Weike, 2005; Sevenster et al., 2012a). In the current study, however, CS-US pairings did not have the same effects on US expectancy and SCRs, which argues against the idea that both measures being affected by one common underlying (cognitive) factor. Dissociations between both measures have been reported before (e.g., Bechara et al., 1995; McAndrew, Jones, McLaren, & McLaren, 2012) while cognitive and emotional components of fear conditioning sometimes converge (e.g., Costa, Bradley, & Lang, 2014; Dawson, Rissling, Schell, & Wilcox, 2007), questioning the classification into cognitive versus emotional measures. Thus, carefully designed experiments employing a multimodal approach will be invaluable to further refine and develop models of associative learning.

Our study might be extended in several ways. First, neutral stimuli were used as CSs in this experiment. It cannot be excluded that results would be different if fear-relevant or “biologically prepared” stimuli were used as CSs (Hugdahl & Öhman, 1977; Hugdahl, 1978; Lipp & Edwards, 2002), in particular as theories relying on conditioning models of phobic fears propose that a trauma should induce stronger effects when it matches previous beliefs (Mineka & Zinbarg, 2006). Hence, effects of actual CS-US pairings might be particularly pronounced when fear-relevant stimuli are used as CSs. Furthermore, it would also be interesting to investigate conditions in which the trauma does not match the beliefs, for instance, when a stimulus was previously experienced or instructed to be safe or has been predictive of a positive event (e.g. a reward). Previous studies with observational learning suggest that such prior positive information could be protective for later acquisition of fear (Egliston & Rapee, 2007; Mineka & Cook, 1986). Second, it would be interesting in future studies to not pair the CS-I during the training phase with the placeholder US. Such a procedure would allow us to strengthen the conclusion that CRs to CS-I during training are due to the threat instructions rather than to the pairings between CS-I and the placeholder. Related to this, it would be interesting to include a pretesting phase in which CRs to the CSs are measured before any instructions have been given. Including this phase would provide a baseline for each participant and each CS for the effect of the threat instructions. Finally, the fact that participants directly experienced the electro-tactile stimulus might have influenced how participants reacted to our threat instructions. Other studies investigating effects of threat instructions have often not exposed the participants to the US before the experiment (Olsson

& Phelps, 2004; Soeter & Kindt, 2012). In future studies it would certainly be worthwhile to compare the effect of threat instructions between groups of participants that did or did not directly experience the US.

In sum, our data demonstrate that instructions represent a very powerful tool for the acquisition of fear and that verbal threat information can only partly be overridden by later situational safety information. We also demonstrate that direct experience can, at least for some dependent measures, have an effect beyond contingency instructions. Importantly, ROF as a model for clinical relapse, did not differ for fears that are acquired through instructions with or without compound CS-US experience. Taken together we provide evidence for the power and persistence of verbal threat information but also highlight the importance of considering different pathways to fear (direct experience, instructions) and stress the importance of multimodal assessment in experimental research.

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Tables

Table 1. Main effect of CS type for the training phase.

DV	df	*F*	*Partial Eta²*	*p*-value	Contrasts
US expectancy	1.75, 61.23	65.98	.65	< .001	a1
Fear ratings	2, 70	63.01	.64	< .001	a2
SCR	2, 70	9.03	.21	< .001	a3
FPS	3, 102	17.40	.34	< .001	b

DV: dependent variable

[a]**All stimuli differ significantly or trend-wise ($p < .1$) from each other:**

1: CSI+E vs. CS-: $F(1, 35) = 208.70$, $p < .001$, *Partial Eta²* = .86; CS-I vs. CS-: $F(1, 35) = 32.80$, $p < .001$, *Partial Eta²* = .48; CSI+E vs. CS-I: $F(1, 35) = 22.82$, $p < .001$, *Partial Eta²* = .40

2: CSI+E vs. CS-: $F(1, 35) = 126.91$, $p < .001$, *Partial Eta²* = .78; CS-I vs. CS-: $F(1, 35) = 45.24$, $p < .001$, *Partial Eta²* = .56; CSI+E vs. CS-I: $F(1,35) = 19.06$, $p < .001$, *Partial Eta²* = .35

3: CSI+E vs. CS-: $F(1, 35) = 14.62$, $p = .001$, *Partial Eta²* = .30; CS-I vs. CS-: $F(1, 35) = 3.37$, $p = .075$, *Partial Eta²* = .09; CSI+E vs. CS-I: $F(1, 35) = 7.57$, $p = .009$, *Partial Eta²* = .18

[b]**No difference between CS- and ITI, all other contrast are significant or trend-wise ($p < .1$):**

CSI+E vs. CS-: $F(1, 34) = 28.08$, $p < .001$, *Partial Eta²* = .45; CS-I vs. CS-: $F(1, 34) = 11.62$, $p = .002$, *Partial Eta²* = .26; CSI+E vs. CS-I: $F(1, 34) = 3.44$, $p = .072$, *Partial Eta²* = .09; ITI vs. CS-: $F(1, 34) < 1$

Table 2. Main effects of CS type and block as well as CS type x block interaction for the test phase.

DV	df	*F*	*Partial Eta*^{2}	*p*-value	Contrasts
US expectancy					
CS type	1.33, 46.53	72.46	.67	< .001	a1
Block	1.36, 47.66	27.03	.44	< .001	b1
CS type x Block	4, 140	9.26	.21	< .001	c1
Fear ratings					
CS type	1.37, 47.82	70.63	.67	< .001	a2
Block	1.75, 61.18	27.38	.44	< .001	b2
CS type x Block	4, 140	15.74	.31	< .001	c2
SCR					
CS type	2, 70	8.78	.20	.001	d
Block	1.56, 54.49	32.70	.48	< .001	b3
CS type x Block	4, 140	1.59	.04	.188	
FPS					
CS type	2.24, 71.65	25.87	.45	< .001	e
Block	2, 64	29.85	.48	< .001	b4
CS type x Block	4.44, 142.02	2.45	.07	.043	f*

DV: dependent variable

[a]**All stimuli differ significantly from each other:**

1: CSI+E vs. CS-: $F(1, 35) = 104.17$, $p < .001$, *Partial Eta*^{2} = .75; CS-I vs. CS-: $F(1, 35) = 56.18$, $p < .001$, *Partial Eta*^{2} = .62; CSI+E vs. CS-I: $F(1, 35) = 18.94$, $p < .001$, *Partial Eta*^{2} = .35

2: CSI+E vs. CS-: $F(1, 35) = 95.55$, $p < .001$, *Partial Eta*^{2} = .73; CS-I vs. CS-: $F(1, 35) = 58.20$, $p < .001$, *Partial Eta*^{2} = .62; CSI+E vs. CS-I: $F(1, 35) = 18.77$, $p < .001$, *Partial Eta*^{2} = .35

[b]**For all measures, there is a significant linear decrease in responding over blocks:**

1: $F(1,35) = 32.82$, $p < .001$, *Partial Eta*^{2} = .48

2: $F(1,35) = 39.75$, $p < .001$, *Partial Eta*^{2} = .53

3: $F(1,35) = 38.94$, $p < .001$, *Partial Eta*^{2} = .53

4: $F(1,32) = 64.84$, $p < .001$, *Partial Eta*^{2} = .67

[c]**The difference between both CSI+E and CS-I and CS- decreases over blocks, linear contrasts with block:**

1: CSI+E vs. CS- * Block: $F(1, 35) = 20.40$, $p < .001$, *Partial Eta*^{2} = .37; CS-I vs. CS- * Block: $F(1, 35) = 18.34$, $p < .001$, *Partial Eta*^{2} = .34

2: CSI+E vs. CS- * Block: $F(1, 35) = 54.32$, $p < .001$, *Partial Eta*^{2} = .61; CS-I vs. CS- * Block: $F(1, 35) = 29.07$, $p < .001$, *Partial Eta*^{2} = .45

[d]**CSI+E and CS-I both differ from CS-, but not from one another:**

CSI+E vs. CS-: $F(1,35) = 9.37$, $p = .004$, *Partial Eta*^{2} = .21; CS-I vs. CS-: $F(1,35) = 16.66$, $p < .001$, *Partial Eta*^{2} = .32; CSI+E vs. CS-I: $F(1,35) < 1$

[e]**No difference between CS- and ITI, all other contrasts are significant or trend-wise ($p < .1$):**

CSI+E vs. CS-: $F(1, 32) = 40.70$, $p < .001$, *Partial Eta²* = .56; CS-I vs. CS-: $F(1, 32) = 21.27$, $p < .001$, *Partial Eta²* = .40; CSI+E vs. CS-I: $F(1, 32) = 3.23$, $p = .082$, *Partial Eta²* = .09; ITI vs. CS-: $F(1, 32) < 1$

[f]The difference between CSI+E and CS- decreases over blocks, but not the difference between CS-I and CS- or CSI+E and CS-I, linear contrasts with block:

CSI+E vs. CS- * Block: $F(1, 32) = 7.85$, $p = .009$, *Partial Eta²* = .20; CS-I vs. CS- * Block: $F(1, 32) = 2.20$, $p = .15$, *Partial Eta²* = .06; CS-I vs. CSI+E * block: $F(1, 32) = 1.45$, $p = .237$, *Partial Eta²* = .04

*The results are similar when ITI is included into the contrasts instead of CS-

Table 3. Main effect of experimental phase: Difference between CS-I and CS- in the last block of the training phase and the first block of the test phase.

DV	**Df**	***F***	***Partial Eta²***	***p*-value**
US expectancy	1, 35	6.04	.15	.019
Fear ratings	1, 35	8.96	.20	.005
SCR	1, 35	5.62	.14	.023
FPS	1, 31	2.93	.09	.097

DV: dependent variable

Table 4. Main effects of CS type and time as well as CS type x time interaction for the reinstatement analysis.

DV	df	*F*	*Partial Eta²*	*p*-value	Contrasts
US expectancy					
CS type	1.54, 54.00	55.91	.62	< .001	a1
Time	1, 35	8.03	.19	.008	b
CS type x Time	1.54, 53.75	1.33	.04	.269	
Fear ratings					
CS type	1.32, 46.25	54.48	.61	< .001	a2
Time	1, 35	21.33	.38	< .001	b
CS type x Time	2, 70	4.09	.11	.021	c
SCR					
CS type	2, 64	3.04	.09	.055	d
Time	1, 32	8.94	.22	.005	b
CS type x Time	1.58, 50.59	< 1	.01	.652	
FPS					
CS type	2, 40	2.73	.12	.077	e
Time	1, 20	5.51	.22	.029	b
CS type x Time	2, 40	< 1	.02	.671	*

DV: dependent variable

[a]**All stimuli differ significantly or trend-wise (p<0.1) from each other**

1: CSI+E vs. CS-: $F(1,35) = 86.63$, $p < .001$, *Partial* $Eta^2 = 0.71$; CS-I vs. CS-: $F(1,35) = 47.64$, $p < .001$, *Partial* $Eta^2 = 0.56$; CSI+E vs. CS-I: $F(1,35) = 6.70$, $p = .014$, *Partial* $Eta^2 = .16$

2: CSI+E vs. CS-: $F(1,35) = 66.08$, $p < .001$, *Partial* $Eta^2 = .65$; CS-I vs. CS-: $F(1,35) = 53.39$, $p < .001$, *Partial* $Eta^2 = .60$; CSI+E vs. CS-I: $F(1,35) = 3.71$, $p = .06$, *Partial* $Eta^2 = .10$

[b]**Post-reinstatement significantly stronger reactions than pre-reinstatement**

[c]**Response enhancement to CS-I differs significantly from CS- , but response enhancement to CSI+E and CS- and to CSI+E and CS-I does not differ:**

CSI+E vs. CS- * Time: $F(1, 35) = 2.78$, $p = .105$, *Partial* $Eta^2 = .07$; CS-I vs. CS- * Time: $F(1, 35) = 6.63$, $p = .014$, *Partial* $Eta^2 = .16$; CSI+E vs. CS-I * Time: $F(1, 35) = 1.76$, $p = .193$, *Partial* $Eta^2 = .05$

[d]**CSI+E differs significantly from CS- while all other stimuli do not differ**

CSI+E vs. CS-: $F(1,32) = 4.96$, $p = .033$, *Partial* $Eta^2 = .13$; CS-I vs. CS-: $F(1,32) < 1$; CSI+E vs. CS-I: $F(1,32) = 2.61$, $p = .12$, *Partial* $Eta^2 = .08$

[e]**CS-I differs significantly from CS- while all other stimuli do not differ**

CSI+E vs. CS-: $F(1,20) = 2.84$, $p = .11$, *Partial* $Eta^2=0.12$; CS-I vs. CS-: $F(1,20) = 5.13$, $p = .035$, *Partial* $Eta^2 = .60$; CSI+E vs. CS-I: $F(1,20) < 1$

*When taking the ITI into account (4 [CS type] x 2 [Time] analysis), there is still no CS type x Time interaction, $F(3,39) = 1.67$, $p = .19$, *Partial* $Eta^2 = .11$

Figures

Figure 1. Overview of the different CSs during the training phase. The CSI+E was paired with an electro-tactile stimulation (the Unconditioned Stimulus or US) and the CS-I was paired with a picture of a lightning bolt (the placeholder US).

Figure 2. Mean (A) US expectancy ratings, (B) Fear ratings, (C) fear potentiated startle responses and (D) skin conductance responses for CSI+E (instructed + experienced), CS-I (instructed), CS- and ITI across all experimental phases. Error bars represent SEM. Note that for the statistical analyses, physiological responses were averaged per two (FPS) or three (SCRs) trials for analyses concerning the training and the test phase.

Figure 3. Mean difference between CS-I and CS- in the training (last block) and the test phase (first block) for (A) US expectancy ratings, (B) Fear ratings, (C) fear potentiated startle responses and (D) skin conductance responses. Error bars represent SEM. Asterixes and hash indicate statistical significance (**$p < .01$, *$p < .05$, #$p < .1$).

Footnotes

[1]There was no specific reason why the credibility questionnaire was prepared in English. However, participants were recruited to be comfortable with an English speaking experimenter and none of the participants reported difficulties completing this questionnaire.

[2]When the analyses of the test phase is restricted to the first block only, which was the most sensitive block for effects of CS-US pairing experience in the study of Raes et al. (2014), fear reactions are significantly higher for CSI+E compared to CS-I on US expectancy, $F(1, 35)$ 10.85, $p = .002$, *Partial Eta²* = .24; Fear ratings $F(1, 35) = 21.09$, $p < .001$, *Partial Eta²* = .38; and FPS, $F(1, 33) = 6.20$, $p = .018$, *Partial Eta²* = 0.16; but not on SCR, $F(1, 35) < 1$.

Figure 1.

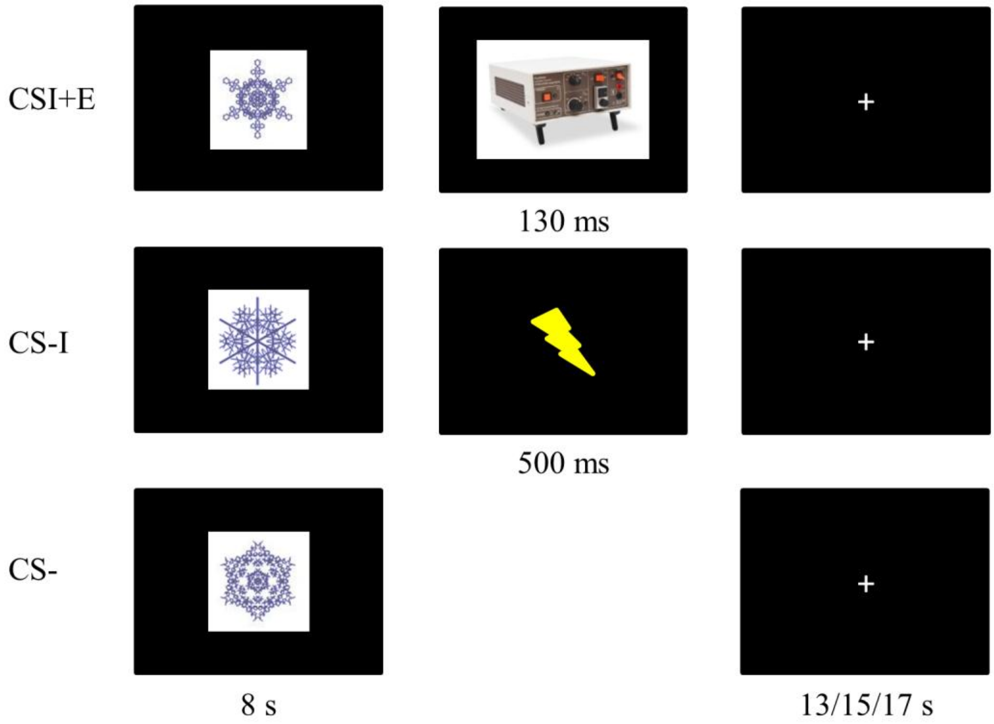
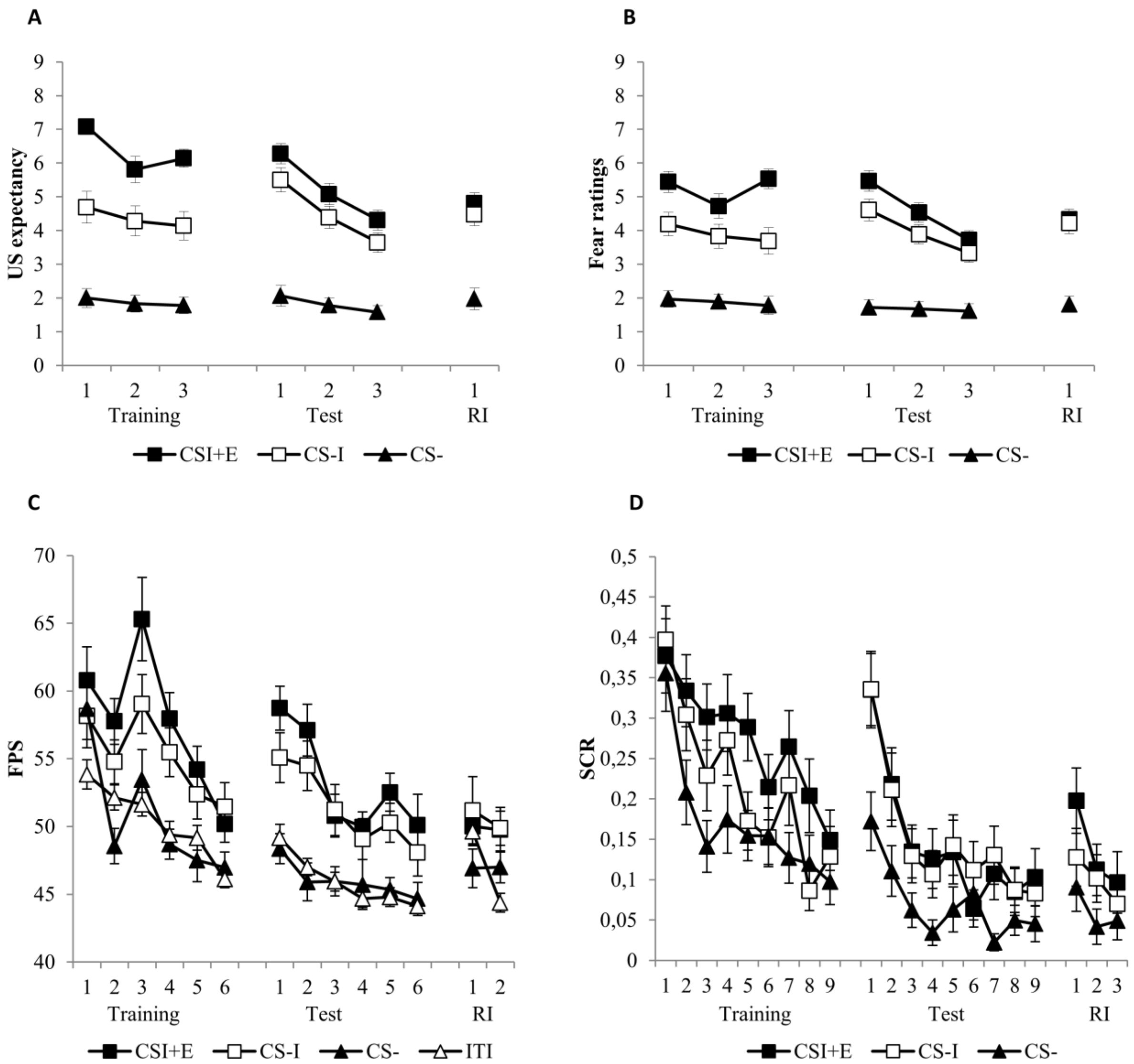


Figure 2.


A
US expectancy
Training
Test
RI
CSI+E
CS-I
CS-
B
Fear ratings
Training
Test
RI
CSI+E
CS-I
CS-
C
FPS
Training
Test
RI
CSI+E
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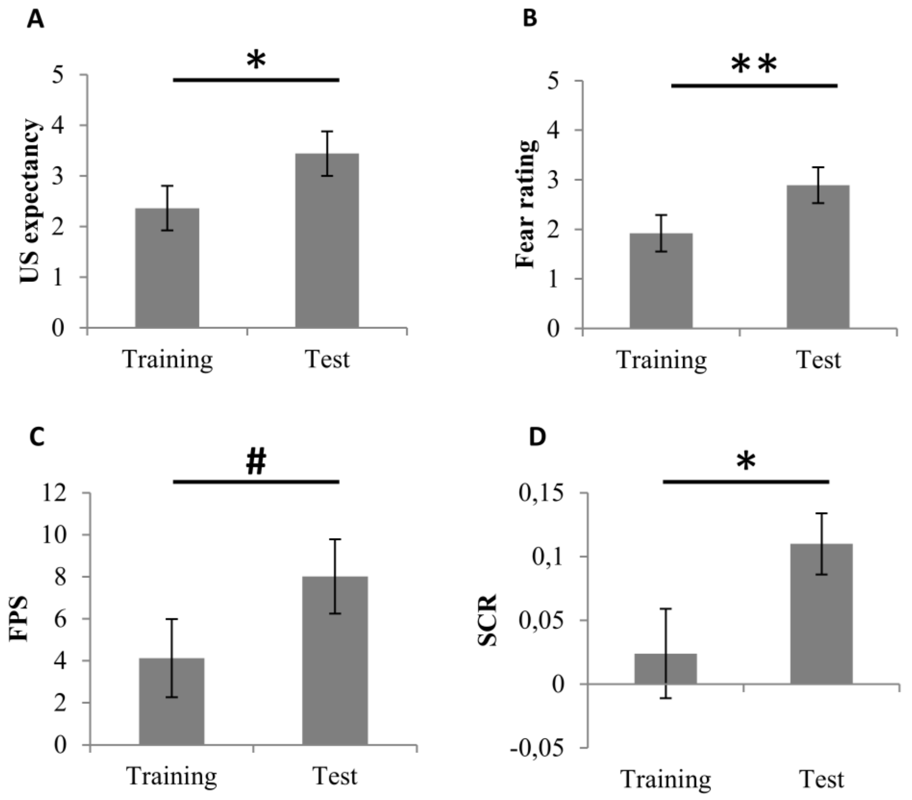
SCR
Training
Test
RI
CSI+E
CS-I
CS-

Figure 3.


A
*
US expectancy
5
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Training
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Fear rating
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10
8
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Training
Test
D
*
SCR
0,15
0,1
0,05
0
-0,05
Training
Test