School of Psychology and Speech Pathology

An Investigation into the Effects of Instructed Extinction on Physiological Responding and Conditional Stimulus Valence in Human Differential Fear Conditioning

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature: …….

Date: …….

Abstract

Treatments for anxiety disorders are efficacious but relapse rates are high. Developing interventions which will be successful in the long term has become a central focus of anxiety disorder research. Persisting negative valence of a feared stimulus has been correlated with higher relapse rates, but it is not yet clear whether negative stimulus valence responds to cognitive interventions. Instructed extinction is a laboratory analogue for cognitive therapy aimed at reducing the expectation that the feared aversive event will occur and involves informing participants before extinction that the unconditional stimulus (US) will no longer occur. The current thesis presents a series of five published papers examining whether conditional stimulus (CS) valence evaluations respond to instructed extinction in human fear conditioning and how different methodological aspects of instructed extinction affect physiological responding and CS valence.

The first paper presents a comprehensive review of studies using the instructed extinction manipulation in human fear conditioning and suggests that instructed extinction eliminates heightened physiological responses towards the CS, unless fear is conditioned to images of snakes and spiders or with a very painful US. The second paper reports a study examining the effect of instructed extinction on electrodermal responding, fear potentiated startle, and CS valence. The results suggest that instructed extinction eliminates differential electrodermal and fear potentiated startle responding at the beginning of extinction, but leaves differential CS valence intact. The third paper examines whether the reduction in physiological responding reported after instructed extinction occurs because the removal of the US electrode reduces the participants' arousal levels and renders the physiological indices less sensitive. A comparison between instructed extinction performed with the electrode attached and instructed extinction performed with the electrode removed provided no evidence that the removal/attachment of the US electrode influences instructed extinction. The fourth paper presents a comparison between scoring electrodermal responses in multiple latency windows during the CS or across the entire CS interval, on the data from an instructed extinction study. Multiple response scoring involves scoring two responses during the CS presentation – a first interval response which is more sensitive to orienting processes and a second interval response which is more sensitive to anticipatory processes. On the other hand, entire interval response scoring involves scoring the largest response occurring during the entire CS presentation and therefore might not be sensitive to the dissociation between orienting and anticipation that commonly occurs in the control group of instructed extinction

studies after the experimental procedure has been interrupted by the experimenter entering the participants' cubicle. As predicted, entire interval response scoring was not sensitive to this dissociation and did not capture the effects of instructed extinction, suggesting that multiple response scoring should be used to score electrodermal responses in instructed extinction studies. The fifth paper examines whether the elimination of differential physiological responding could occur in instructed extinction because removing the threat of the US completely during extinction reduces participants' overall arousal levels and renders the physiological indices less sensitive. This account was tested using an instructed reversal design, in which the CS- was paired with the US during reversal and the CS+ was presented alone. After instructed reversal, electrodermal responding to CS- increased, while, electrodermal responding to CS+ decreased, suggesting that the reduction in physiological responding to CS+ in instructed extinction is driven by the extinction instructions and not a reduction in overall arousal levels. Unexpectedly, CS+ valence increased after instructed extinction, while, CS- valence did not change.

Overall, the current thesis suggests that CS valence does not respond to instructed extinction, that the elimination of differential physiological responding after instructed extinction is not caused by a reduction in overall arousal levels, and that electrodermal responses from instructed extinction studies should be scored using multiple response scoring. The thesis presents an interesting dissociation between physiological responding and self-reported CS valence and the clinical applications and theoretical implications of this dissociation are explored. The effects of instructed reversal on CS valence during human differential fear conditioning is reported for the first time and the findings are discussed. The thesis concludes with a number of suggestions for future research in the domain of instructed extinction and in other forms of instructional designs.

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List of Publications Included as Part of the Thesis

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:10.1016/j.brat.2015.01.001

Luck, C. C., & Lipp, O. V. (2015). To remove or not to remove? Removal of the unconditional stimulus electrode does not mediate instructed extinction effects. *Psychophysiology, 52,* 1248-1256. doi:10.1111/psyp.12452

Luck, C. C., & Lipp, O. V. (2016). When orienting and anticipation dissociate — a case for scoring electrodermal responses in multiple latency windows in studies of human fear conditioning. *International Journal of Psychophysiology, 100,* 36-43. doi:10.1016/j.ijpsycho.2015.12.003

Luck, C. C., & Lipp, O. V. (2016). The influence of contingency reversal instructions on electrodermal responding and conditional stimulus valence evaluations during differential fear conditioning. *Learning and Motivation, 54,* 1-11. doi:10.1016/j.lmot.2016.05.001

Luck, C. C., & Lipp, O. V. (2016). Instructed extinction in human fear conditioning: History, recent developments, and future directions. *Australian Journal of Psychology, 68,* 209-227. doi:10.1111/ajpy.12135

Statement of Contribution of Others

The nature and extent of the intellectual input by the candidate and co-author has been validated and can be found in Appendix A.

……	…….....
Camilla Luck	Ottmar Lipp
(Candidate)	(Supervisor)

List of Additional Publications

Lipp, O. V., Cronin, S. L., Alhadad, S. S. J., & Luck, C. C. (2015). Enhanced sensitization to animal, interpersonal, and intergroup fear-relevant stimuli (but no evidence for selective one-trial fear learning). *Psychophysiology, 52*, 1520-1528. doi:10.1111/psyp.12513

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Chapter 1. Introduction

Fear is adaptive and functional – activation of the neural fear circuitry compels an organism to engage in defensive behavior (Quinn & Fanselow, 2006). In threatening situations this fear response is appropriate and facilitates survival. Problems occur, however, when the level of fear is inappropriate for the situation or when fear extends outside of threatening situations altogether. If these occurrences become frequent and impair an individual's functioning they are regarded as anxiety disorders (Quinn & Fanselow, 2006). Anxiety disorders are the most common psychological disturbance – without treatment, they are persistent, chronic, and self-perpetuating (Craske, Vansteenwegen, & Hermans, 2006). They have disabling effects on the individual (e.g. Olfson et al., 1997), increase the likelihood of other psychological disturbances (e.g, Hayward, Killen, Kraemer, & Taylor, 2000), and contribute to substance-use disorders (Swendsen et al., 1998). Although, efficacious treatments are available (Ougrin, 2011), unfortunately, one to two thirds of successfully treated patients will relapse within eight years (Craske, 1999). Developing treatments which are efficacious in both the short and long term has become the central goal of anxiety disorder research and basic research which examines fear acquisition, extinction, and relapse could hold the key to understanding how fear relapse can be reduced.

Fear Conditioning Paradigms

Fear relapse, also referred to as the return of fear, can be studied in healthy participants in the laboratory by making use of human fear conditioning paradigms. Conditioning paradigms provide a conceptual framework to study human fear learning and allow researchers to manipulate important variables while controlling for extraneous factors (Craske, Hermans, & Vansteenwegen, 2006). During fear conditioning, a conditional stimulus (CS; e.g. a neutral picture) is paired with an aversive unconditional stimulus (US; e.g. an electrotactile shock). With enough pairings, the CS becomes a signal for the US, eliciting physiological responses and developing negative valence (De Houwer, Thomas, & Baeyens, 2001; Lipp, 2006). In differential fear conditioning, which is typically considered the most reliable conditioning paradigm, one CS (CS+) is paired with the US, while another (CS-) is presented alone. Responding to CS+ is then compared to CS-, ensuring that changes in responding occur because of the CS-US relationship and not because of non-associative factors (Lipp, 2006).

The human differential fear conditioning paradigm has been used to model the acquisition, extinction, and relapse of human fear. During differential fear acquisition, the CS+ is paired with the US, while the CS- is presented alone (Lipp, 2006). Throughout acquisition, differential physiological responding and a differential perception of valence develops between CS+ and CS-, such that the CS+ elicits larger physiological responses and is rated as less pleasant than the CS- (De Houwer, Thomas, & Baeyens, 2001; Lipp, 2006). During extinction, both CS+ and CS- are presented alone and the differential physiological responding and valence evaluations between CS+ and CS- reduce (Lipp, 2006).

Factors Influencing the Return of Fear

Differential responding reduces throughout extinction because an inhibitory CS–noUS association is created (Bouton, 2002; 2004). The original excitatory CS–US association remains, at least partially, intact at the end of extinction, leading to the very common observation that conditional responding can reemerge after extinction in the absence of any additional CS–US pairings (Rachman, 1966; for a review see Vervliet, Craske, & Hermans, 2013). Three laboratory manipulations have been used to induce the return of fear – spontaneous recovery, renewal, and reinstatement. Spontaneous recovery occurs when the conditional response returns, or increases in strength, after a passage of time has occurred between extinction and re-test (Quirk, 2002). Renewal occurs when the conditional response returns after the CS is encountered in a context different to the extinction context (Bouton, 1993); and reinstatement occurs when the conditional response returns after unsignaled US presentations are administered (Rescorla & Heth, 1975).

A number of recent findings suggest that reducing the extent of negative valence the feared stimulus retains at the end of extinction (or treatment) could reduce the likelihood that relapse will occur. Higher levels of negative CS+ valence after extinction have been correlated with higher rates of conditional responding after a reinstatement manipulation (Dirkx, Hermans, Vansteenwegen, & Baeyens, 2004; Hermans et al., 2005; Zbozineck, Hermans, Prenouveau, Liao, & Craske, 2014). Similarly, when positive mood is induced via positive imagery training (a manipulation that involves listening to and imagining positive hypothetical scenarios), negative CS+ valence is reduced and participants show less reinstatement of self-reported fear and fear potentiated startle (Zbozinek, Holmes, & Craske, 2015).

Modeling Treatments in the Laboratory

Extinction training is an experimental analogue for exposure therapy and is very effective at reducing differential physiological responding between CS+ and CS- (Lipp, 2006). Extinction training also reduces differential valence evaluations but at a much slower rate and often the CS+ is still evaluated as unpleasant after extinction (Hofmann, De Houwer, Perugini, Baeyens, & Crombez, 2010). Clinically, this would suggest that after a client completes exposure therapy they would no longer experience heightened physiological arousal when they encounter their feared stimulus, but they may still evaluate this stimulus as unpleasant and this negative evaluation could later induce fear relapse. Fortunately, exposure therapy is not the only technique used to treat anxiety disorders. Cognitive therapy is included alongside exposure treatments, often with a focus on reducing the client's expectation that the feared aversive event will occur (Andrews, Crino, Lampe, Hunt, & Page, 1994).

One way of studying cognitive therapy in the laboratory is by using the instructed extinction manipulation (Cook & Harris, 1937). Instructed extinction involves informing one group of participants before the extinction phase that the US will no longer be presented, while a control group does not receive information about the CS– noUS contingency. Instructed extinction has been shown to robustly eliminate conditional physiological responding to fear irrelevant stimuli (see Chapter 2 for a detailed discussion on the effect of instructed extinction on fear conditioned to fear relevant stimuli), but it is not yet clear whether this manipulation also reduces differential CS valence.

Lipp and Edwards (2002) examined the effect of instructed extinction on conditional fear acquired to fear relevant (snakes and spiders) and fear irrelevant (flowers and mushrooms) stimuli. Instructed extinction eliminated differential electrodermal responding to images of flowers and mushrooms, but not to images of snakes and spiders (for a detailed discussion of this effect see Chapter 2). A measure of CS valence was assessed after extinction and both the instructed and control groups evaluated CS+ as less pleasant than CS-. One interpretation for this finding is that instructed extinction did not affect differential CS valence evaluations, but as CS valence was assessed post-experimentally it is also possible that differential CS valence did not fully extinguish or was renewed when the valence assessment was performed outside the extinction context.

To reliably assess the influence of instructed extinction on CS valence, the CS valence assessment should occur immediately before and after the instructed extinction manipulation. If valence is measured post-experimentally it is not clear whether the findings occur because of the instructions, the extinction training, relapse, or a combination of these factors. Lipp, Oughton, and LeLievre (2003; Experiment 2) used a trial-by-trial assessment of CS valence throughout acquisition and extinction, allowing them to assess the immediate influence of instructed extinction on CS valence and at the same time as electrodermal responding. Instructed extinction did not influence CS valence at the beginning of extinction, but surprisingly, did not reduce differential electrodermal responding either. Without clear effects of instructed extinction on electrodermal responding, it is hard to interpret its effects on CS valence as it is possible that the instructions were not believed or that the manipulation was not successful.

Thesis Rationale

Although the findings of Lipp and Edwards (2002) and Lipp et al. (2003; Experiment 2) suggest that CS valence does not respond to instructed extinction, the methodological limitations present in both studies prevent strong conclusions. As negative valence increases the risk of fear relapse it is important to examine whether CS valence responds to cognitive therapy aimed at reducing expectations that an aversive event will occur. The primary aim of the thesis is to comprehensively examine how CS valence responds to instructed extinction in human differential fear conditioning. The secondary aim of the thesis is to examine various methodological aspects of instructed extinction and how these methodological decisions affect physiological responding and CS valence evaluations.

Thesis Outline

These aims have been addressed across the following five publications:

1. The first paper of the thesis is entitled ‘Instructed extinction in human fear conditioning: History, recent developments, and future directions’. This paper provides a comprehensive review of the instructed extinction studies conducted within a human fear conditioning paradigm over the last 80 years. This article provides detailed descriptions of the various measures, paradigms, and stimuli used in instructed extinction research as well as an integration and discussion of the findings. For practical reasons this paper is situated first in the thesis, however, it was published last and references a number of the papers that make up the empirical part of the current thesis.

2. The second paper of the thesis is entitled ‘A potential pathway to the relapse of fear? Conditioned negative stimulus evaluation (but not physiological responding) resists instructed extinction’. Across two experiments, this paper examines the influence of instructed extinction on CS valence (measured on a trial-by-trial basis), electrodermal responding, and fear potentiated startle. A third experiment examines whether the results could occur because of demand characteristics.
3. The third paper of the thesis is entitled ‘To remove or not to remove? Removal of the unconditional stimulus electrode does not mediate instructed extinction effects’. This paper examines whether removing the US electrode during instructed extinction is responsible for the immediate reduction in physiological responding that is often reported.
4. The fourth paper of the thesis is entitled ‘When orienting and anticipation dissociate – a case for scoring electrodermal responses in multiple latency windows in studies of human fear conditioning.’ This paper compares the use of two common techniques for scoring electrodermal responses (multiple response scoring and entire interval response scoring) on data from an instructed extinction experiment and provides evidence that instructed extinction studies should be scored using the multiple response scoring technique.
5. The fifth paper of the thesis is entitled ‘The influence of contingency reversal instructions on electrodermal responding and conditional stimulus valence evaluations during differential fear conditioning’. This paper uses an instructed reversal design to examine whether physiological responding reduces during instructed extinction because removing the threat of the US reduces the participants’ overall arousal levels – rendering the physiological indices of fear learning less sensitive.

The findings are then summarized and integrated in the overall discussion section. This section examines how the findings fit into current theoretical frameworks and presents recommendations for research practice, clinical practice, and future research.

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Chapter 2. Paper 1 – Instructed Extinction in Human Fear Conditioning: History, Recent Developments, and Future Directions

Luck, C. C., & Lipp, O. V. (2016). Instructed extinction in human fear conditioning: History, recent developments, and future directions. *Australian Journal of Psychology*, *68*, 209-227. doi:10.1111/ajpy.12135

Instructed Extinction in Human Fear Conditioning: History, Recent Developments, and Future Directions

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Abstract

Instructed extinction is an experimental manipulation that involves informing participants after the acquisition of fear learning that the unconditional stimulus (US) will no longer be presented. It has been used as a laboratory analogue to assess the capacity of cognitive interventions to reduce experimentally induced fear. In this review, we examine and integrate research on instructed extinction and discuss its implications for clinical practice. Overall, the results suggest that instructed extinction reduces conditional fear responding and facilitates extinction learning, except when conditional stimulus valence is assessed as an index of fear or when fear is conditioned to images of animal fear-relevant stimuli (snakes and spiders) or with a very intense US. These exceptions highlight potential boundary conditions for the reliance on cognitive interventions when treating fear in clinical settings.

Key words: anxiety, cognitive interventions, fear conditioning, instructed extinction, return of fear

Fear can be a learned response – a neutral stimulus will elicit fear independently if it has been associated with an aversive stimulus. There are a number of pathways in which this fear association can be formed – including repeated pairings between the neutral and the aversive stimulus (experiential learning); observing another individual displaying fear to the neutral stimulus (observational learning); or being informed that the neutral stimulus is predictive of the aversive event (informational learning) (Rachman, 1968; Rachman, 1977). If contained, fear is adaptive as it facilitates defensive responding allowing the escape from, or avoidance of, dangerous situations, but if fear becomes exaggerated or is not appropriately regulated, it can develop into an anxiety disorder (Quinn & Fanselow, 2006). Anxiety disorders are emotionally and economically costly and will affect 25% of the population during their lifetime (Kessler, Koretz, Merikangas, & Wang, 2004).

Developing treatments that are efficacious in both the short and the long term has become a central focus of research on anxiety disorders. The short term success of gold-standard treatments is well documented (Bisson & Andrew, 2007; Ougrin, 2011; Sánchez-Meca, Rosa-Alcàzar, Marín-Martinez, & Gómez-Conesa, 2010), but one to two thirds of these successfully treated patients will relapse within eight years (Craske, 1999). This clinical observation is consistent with results of laboratory research showing that fear extinction does not erase the original fear memory but instead lays down a new context-specific extinction memory (Bouton, 2002). After extinction learning, the original fear memory often re-emerges resulting in the return of fear (Rachman, 1966; for a review see Vervliet, Hermans & Craske, 2013). Understanding why fear re-emerges and how this phenomenon can be reduced in the laboratory is crucial to developing long-lasting treatments.

Common anxiety treatments and their effects on fear and fear relapse can be modelled in the controlled laboratory environment (Craske, Hermans, & Vansteenwegen, 2006). Instructed extinction is a laboratory manipulation which involves using instructions to break the association between the neutral stimulus and the aversive stimulus (Luck & Lipp, 2015a). It is often considered a laboratory analogue for a cognitive intervention and has been used in a number of different contexts and under a number of different names over the last 60 years. In this review we will give a brief overview of the paradigms and measures involved in instructed extinction research before examining the research conducted with this manipulation within the human fear

conditioning paradigm. After the review of the literature, we will integrate the findings, discuss their significance for clinical practice, and offer possible directions for future research.

A Brief Introduction to Human Fear Conditioning

Classical fear conditioning can be used to model the development, treatment, and relapse of human fear (Craske et al., 2006). During classical fear acquisition, a neutral conditional stimulus (CS), e.g. a picture or tone, is repeatedly paired with an aversive unconditional stimulus (US), e.g. an electrotactile shock or loud noise. After repeated pairings, the CS becomes a signal for the US and elicits fear responding independently. During classical fear extinction, the CS is presented alone, and fear to the CS reduces. In the laboratory, the return of fear can be examined with three experimental manipulations. Spontaneous recovery, the return of fear after the mere passage of time, can be assessed by presenting the CS after a break in the experiment or after the participants have returned to the lab at a different time. Renewal, the return of fear after a context change, can be assessed by examining responding to the CS in a context that differs from the one used during extinction training; and reinstatement, the return of fear after presentation of the aversive stimulus, can be measured by presenting the CS after un-signalled presentations of the US (Bouton, 2002; Vervliet, Hermans, & Craske, 2013).

Acquisition, extinction, and the return of fear can be assessed within two variations of the fear conditioning paradigm – single cue and differential fear conditioning. In a single cue design, participants are presented with one CS paired with the US, and their responding is compared with a control group who receive random, or explicitly unpaired, presentations of the CS and the US. The single cue design has been criticised as it does not control for orienting and other non-associative processes that may affect responding to the CS. Moreover, selecting the appropriate control is difficult and if an explicitly unpaired stimulus sequence is used, it can result in inhibitory conditioning to the CS. A differential fear conditioning design embeds the control for non-associative factors into a within participants design by using two CSs, one paired with the US (CS+) and another presented alone (CS-) (Lipp, 2006).

A number of important factors that can influence conditioning vary across studies, including the CS duration, the interval between the CS and the US (interstimulus interval; ISI), and the reinforcement rate (for a detailed discussion see Lipp, 2006). In delay conditioning, CS offset coincides with, or is preceded by, the onset of the US, whereas in trace conditioning, there

is a time interval between CS offset and US onset. Delay conditioning is usually acquired faster and is more robust than trace conditioning (see for instance Lipp, Siddle & Dall, 2003). The choice of CS duration largely depends on the measure used to index conditioning. If autonomic responses are to be measured long CS durations (typically 6 or 8 seconds) are usually used to separate the unconditional response elicited by the US from conditional responding to the CS. Shorter CS durations are acceptable if the response system used to index conditioning is quick (i.e. eye blink conditioning or self-report measures). The ISI is the duration between the onset of the CS and the onset of the US and is dependent on both the CS duration and the interval between the CS offset and US onset. The reinforcement rate is the percentage of times that the CS is paired with the US during acquisition out of the total number of CS presentations.

Human fear learning can be assessed across three different response levels – physiologically, behaviourally, and verbally (Lang, 1985). The focus of human fear conditioning research has been on physiological and verbal indices and we will describe the common measures used in studies of instructed extinction in this section. Each measure used to index fear learning has advantages and limitations, and therefore, the effect of instructed extinction on human fear should be assessed across a number of different measures.

Electrodermal Responding

Electrodermal responding reflects variations in the conductivity of human skin to electrical currents due to changes in sympathetic nervous system activation of the eccrine sweat glands (Dawson, Schell, & Filion, 2007). It is the most frequently used measure in human fear conditioning and the most common index of instructed extinction. Electrodermal responding is sensitive to the psychological processes important during associative learning, such as orienting to, and the anticipation of, salient events. It is not selectively sensitive to fear learning, however, showing the same response pattern regardless of whether an aversive or a non-aversive US is used (Lipp and Vaitl, 1990). Electrodermal responding can be scored by distinguishing multiple response components during the CS-US interval or by scoring a single response during the entire interval. If a long CS duration is used, a first interval response will emerge within 1-4 seconds of CS onset and a second interval response will emerge within 4-7 seconds (6 s ISI) or 4-9 seconds (8 s ISI) of CS onset (Prokasy & Kumpfer, 1973). First interval responding is more sensitive to orienting elicited by CS onset and second interval responding is more sensitive to the

anticipation of the US (Öhman, 1983), however there is considerable covariation. The entire interval scoring technique scores the largest response occurring during the CS-US interval as a single index. Luck and Lipp (2016) compared multiple response scoring and entire interval scoring of data from an instructed extinction study and provided evidence that, because of a dissociation between orienting and anticipation, the instructed extinction effects which were detected using multiple response scoring were lost with entire interval scoring.

Heart Rate

Heart rate changes provide a cardiovascular index of conditioning, and heart rate responses to a CS, in anticipation of a US, often consist of an initial deceleration, a transient acceleration, and a subsequent deceleration. The initial deceleration reflects orienting to the CS, whereas the second and third components reflect the anticipation of the US. Conditioned heart rate responses seem to be sensitive to the affective valence of the US, with the accelerative heart rate response component believed to reflect anticipation of an aversive stimulus as it is most prominent in studies using intense USs or fear-relevant CSs (Lipp, 2006).

Blink Startle Responding

Blink startle responding is a skeletal nervous system measure of the brainstem startle reflex. It is not under cognitive control and is linearly modulated by valence, such that startle responding is inhibited if elicited during pleasant stimuli and potentiated if elicited during unpleasant stimuli (Lang, Bradley, & Cuthbert, 1990), but only if these stimuli are high in arousal (Cuthbert, Bradley, & Lang, 1996). Startle responding is considered a robust measure of fear learning and there are some reports that startle is potentiated only during anticipation of aversive USs (Hamm & Vaitl, 1996). Others have argued that conditioning with aversive and non-aversive USs can elicit the same pattern of startle response modulation (Lipp et al., 2003).

Conditional Stimulus Valence

The addition of verbal measures of CS valence to conditioning designs has become popular due to the difficulties assessing valence reliably with physiological indices. CS valence can be assessed before and after conditioning training, or throughout conditioning (online) with a continuous response indicator (Lipp, 2006). Pre/post measures cannot index real-time changes in valence and may be confounded by renewal effects as they are frequently recorded in a different

experimental context. In instructed extinction studies continuous assessments of CS valence are preferred as they can be obtained during the CS immediately after the instructed extinction manipulation, allowing for the assessment of instructed extinction effects before additional learning occurs (Luck & Lipp, 2015a).

Unconditional Stimulus Expectancy

US expectancy is measured to assess participants' anticipation of the US or awareness of the CS-US contingency. US expectancy is often assessed as a manipulation check after the completion of the experiment by asking participants to identify which stimulus had been associated with the US. Alternatively, US expectancy can be assessed as a dependent variable online throughout conditioning training (Lipp, 2006).

Instructed Extinction Manipulation

Instructed extinction is an experimental manipulation that assesses whether receiving instructions about the absence of the US is sufficient to reduce conditional responding. During instructed extinction, the experimenter interacts with participants after the last acquisition trial. In the instruction group, participants are informed that the US will no longer be presented, and the devices used to deliver the US (shock electrode or headphones) are often removed. Responding in the instruction group is then compared with a control group, who experience a similar interaction with the experimenter (i.e. to check the electrodes) but are not given information about the CS-US contingency. To allow for the identification, and possible exclusion, of participants who did not believe the instructions, the experimental group are typically asked whether they believed the instructions after the experiment.

Assessing instructed extinction effects relative to a control group who are exposed to the same level of interaction with the experimenter, but not instructed, controls for the effects of the manipulation on overall arousal and, potentially, conditional responding. The shock electrode is often removed to strengthen the manipulation and reduce the number of participants who do not believe the instructions. Some argue that this removal could reduce arousal levels and add a non-cognitive component to the manipulation. A direct comparison between instructed extinction with and without shock electrode removal, however has failed to substantiate this concern (Luck & Lipp, 2015b). Generally two types of instruction effects can be assessed. Instructed extinction can abolish differential conditional responding on the very first trial of extinction or it can

facilitate extinction learning. A reduction of conditional responding on the first trial of extinction in the instruction group, relative to the control group, can be attributed to the provision of information alone. Facilitation of extinction learning can be considered an interactive effect between explicit extinction training and the instructional manipulation.

Instructed Extinction with Non-Fear Relevant Conditional Stimuli

Cook and Harris (1937) were the first to hypothesise that a conditional electrodermal response could be removed by breaking the CS-US association with verbal instructions. Using a single-cue short-delay conditioning paradigm (3s ISI – US presented at CS offset; for further details of individual experiments see Table 1), participants were conditioned with a tone and an electrotactile shock throughout acquisition. After instructed extinction, electrodermal responding was considerably reduced in the instruction group in comparison with the non-instructed control group. Soon after, this initial observation was confirmed by Mowrer (1938) who reported that the conditional electrodermal response could be 'be switched on and off' by removing and reattaching the shock electrode or by using a buzzer system to indicate phases in which the US could be expected.

Notterman, Schoenfeld and Bersh (1952) extended this line of research by confirming that the conditional heart rate response was also subject to instructed extinction. During acquisition, participants were conditioned using a single-cue trace conditioning design (7s ISI – 6s trace interval). Instructed extinction did not influence conditional heart rate responses within the first 5 extinction trials, but extinction learning was facilitated in the instruction group during the last 5 extinction trials.

Sensitisation is a non-associative learning process in which the mere presentation of aversive stimuli can enhance electrodermal responding to neutral stimuli. Silverman (1960) argued that because the earlier instructed extinction studies did not include a pseudo-conditioning control group, it was not clear whether instructed extinction was influencing a conditional response or a sensitised response. To confirm this, he compared the effect of instructed extinction on conditional electrodermal responding after three different acquisition procedures – conditioning with a 2.5s ISI (0.5s trace interval), conditioning with a 8s ISI (6s trace interval), or a pseudo-conditioning (unpaired) control group. Instructed extinction reduced electrodermal responding in the 2.5s ISI and the control group, but not in the 8s ISI group. The

reduction of electrodermal responding in the 2.5s ISI group confirmed that instructed extinction could reduce a conditional response, but failure to find instructed extinction effects using a 8s ISI is surprising especially in light of the significant reduction detected in the unpaired control group. Silverman suggested that the long trace interval could be anxiety arousing and protects against instructed extinction effects, but such an interpretation is not consistent with the results of Notterman et al. (1952), who also used a 6s trace interval.

Lindley and Moyer (1961) examined the effects of instructed extinction on the conditioned finger withdrawal response (conditional movement of the finger after electrotactile shock to the finger) after minimal and extended acquisition training. Participants were conditioned using a single-cue short-trace (1s ISI – 0.5s trace interval) conditioning paradigm. Consistent with research on electrodermal responding and heart rate, instructed extinction reduced the conditioned finger withdrawal response. There was also some evidence that this reduction was larger in the participants who received minimal acquisition training.

Wickens, Allen and Hill (1963) investigated whether US intensity could moderate the effect instructed extinction on the conditional electrodermal response. Using a single-cue short-delay conditioning paradigm (0.5s ISI – US presented at CS offset), participants were conditioned with a weak or a strong electrotactile shock. Instructed extinction did not influence conditional responding on the first extinction trial but did facilitate the speed of extinction learning relative to the control group. No interactions between US intensity and instructed extinction were detected. This finding was confirmed by Grings and Lockhart (1963) who examined whether US intensity and amount of acquisition training would moderate the effect of instructed extinction on the conditional electrodermal response. Using a single-cue long-delay conditioning paradigm (5s ISI – US presented at CS offset), all participants viewed three CSs paired with a different US intensity (high, medium, low). Half of the participants received 9 CS-US pairings (3 of each CS), and the other half received 36 CS-US pairings (12 of each CS). Instructed extinction reduced electrodermal responding on the first extinction trial of each CS but was not influenced by US intensity or the number of CS-US pairing during acquisition.

Bridger and Mandel (1964) failed to find facilitation of extinction learning after instructed extinction in a long-delay differential conditioning design (6s ISI – US delivered 1s before CS offset) using a painful electrotactile shock US. They hypothesised that conditional

electrodermal responding established via CS-US pairings would not respond to instructed extinction, but conditional electrodermal responding established via a threat of shock phase would be eliminated by instructed extinction. During acquisition, both the conditioning and the threat group acquired differential responding, which did not differ on the last acquisition trial. After instructed extinction, differential responding was eliminated in the threat group but remained intact in the conditioning group. Bridger and Mandel suggest that instructed extinction will eliminate a conditional response that was established via instructions but not a conditional response that was established via direct CS-US pairings. This suggestion is not consistent with the majority of instructed extinction studies in the literature but could occur because of the intense US that was used.

More consistent with prior research, Bridger and Mandel (1965) report that instructed extinction facilitated the extinction of a conditional electrodermal response established with direct CS-US pairings. Using a short-delay differential conditioning design (0.5s ISI – US on CS+ offset), the reinforcement rate during acquisition training was varied between groups. One group received acquisition training with a partial reinforcement schedule (25%) and another with a continuous reinforcement schedule (100%). The reinforcement schedule did not moderate the instruction effects. All groups (controls and instructions) showed continued differential responding on the first extinction trial, but the magnitude of this differential response was reduced in the instruction groups and subsequent extinction learning was facilitated.

Mandel and Bridger (1967) examined the effect of instructed extinction after conditioning with three different acquisition procedures – a forward conditioning short-delay group (0.5s), a forward conditioning long-delay group (5s), and a backward conditioning group. During acquisition, all groups acquired differential responding between CS+ and CS-. During the first five extinction trials, differential responding was absent in the backward conditioning groups (control and instruction), but still present in all other groups. Differential responding was not present in any group during the last five extinction trials.

In the studies reported by Bridger and Mandel, differential electrodermal responding was consistently present in the instruction groups during the first extinction trial, and instructed extinction did not facilitate the speed of extinction learning in Bridger and Mandel (1965) or Mandel and Bridger (1967). These findings suggest that conditional electrodermal responding is

not always eliminated immediately by instructed extinction. Mandel and Bridger (1973) suggest that strong instruction effects are not present in their studies because they used a very painful shock as the US. Wickens et al. (1963) and Grings and Lockhart (1963) have reported that US intensity does not moderate instructed extinction effects; however, the maximum US intensity in these studies was set by the participant to be unpleasant but not painful. In contrast, participants in Bridger and Mandel's studies received a pre-set shock intensity that was perceived by all participants as very painful. Mandel and Bridger report that 10% of the participants refused to continue participation and that many indicated fear or anger about remaining in the experiment. They assert that the mildly uncomfortable shock used in most prior studies would not permit the acquisition of conditional responses, which are not merely reflections of cognitive expectancy.

Fuhrer and Baer (1980) aimed to examine whether resistance to instructed extinction could be obtained with a less noxious electrotactile shock and whether instructed extinction effects would differ between a 0.5s ISI and a 5s ISI (delay conditioning – US on CS+ offset). Throughout the experiment, a continuous measure of US expectancy was assessed alongside electrodermal responding. All participants were informed after acquisition that the US would no longer be presented and participants were then divided into 'believers' and 'non-believers' based on their US expectancy ratings. During the first extinction block (3 extinction trials), participants who reported not expecting the US continued to show differential responding between the CS+ and CS- in both ISI groups. A similar, but non-significant, differential pattern was detected in the participants who reported still expecting the US, and differential responding was eliminated in all groups after the first extinction block. Fuhrer and Baer (1980) interpret their findings as a demonstration of conditional responding, which is inconsistent with cognitive expectancies after conditioning with mildly unpleasant US, but this interpretation should be treated with caution. Rather than comparing instructed extinction with a non-instructed control group, Fuhrer and Baer instructed all participants and split them into groups based on their US expectancy ratings. Furthermore, participants who reported not expecting the US continued to show differential responding during the first block of extinction, but this responding is compared with no significant differential conditioning in participants who reported still expecting the electrotactile shock. The finding that differential responding was eliminated in all groups by the second extinction block is consistent with Wickens et al. (1963) and Notterman et al. (1952) and is

unlikely to be a demonstration of resistance to instructed extinction, similar to those displayed by Mandel and Bridger using a less noxious US.

Lipp, Oughton, and LeLievre (2003; Experiment 2) examined the effect of instructed extinction on electrodermal responding and a continuous measure of CS valence using a differential long-delay conditioning paradigm (8s ISI – US followed CS+ immediately). During acquisition, differential first and second interval responses and differential valence evaluations were acquired between the CS+ and CS-. After instructed extinction, differential valence evaluations remained intact in both the control and the instruction group; however, no clear pattern of differential electrodermal responding was present in either the control or instruction group. Without a clear differential response in the control group, elimination of differential responding in the instruction group cannot be attributed to instructed extinction. The CS valence evaluations seemed to resist instructed extinction, however in the absence of clear instruction effects on electrodermal responding, the results of the CS valence measure should be interpreted with caution.

Sevenster, Beckers, and Kindt (2012) examined the effect of instructed extinction on electrodermal responding, blink startle, and online US expectancy throughout extinction training and after a reinstatement manipulation. In a differential long-delay (7.5s ISI – US presented 0.5s before CS+ offset) conditioning design, differential electrodermal responding, blink startle modulation, and US expectancy ratings were acquired throughout acquisition training in both the control and the instruction group. Following instructed extinction, differential US expectancy ratings and entire interval electrodermal responding was intact in the control group but eliminated in the instruction group. Differential startle modulation remained intact in both the control and the instruction groups on the first trial of extinction. Differential startle modulation was eliminated by the third extinction trial in the instructed group, while remaining intact across 11 extinction trials in the control group. Interestingly, differential US expectancy ratings re-emerged after a subsequent reinstatement manipulation in the control group but not the instruction group; however, no other between group differences emerged after reinstatement.

Across two experiments, Luck and Lipp (2015a) examined the effect of instructed extinction using a differential long-delay conditioning paradigm (6s ISI – US followed CS+ immediately), measuring electrodermal responding (Experiment 1), blink startle modulation

(Experiment 2), and online CS valence (Experiment 1 and 2). In Experiment 1, differential first and second interval electrodermal responding and differential valence evaluations were acquired throughout acquisition. Following instructed extinction, differential first and second interval electrodermal responding was eliminated in the instruction group by the first extinction block (2 trials). Differential first interval responding was eliminated in controls due to an increase in responding to CS-, but differential second interval responding was still intact. In contrast, differential CS valence evaluations were not affected by instructed extinction, with intact differential valence evaluations present in both groups and no effect of instruction across extinction. In Experiment 2, differential startle modulation and differential valence evaluations were acquired in both groups. Following instructed extinction, differential startle was eliminated in the instruction group by the first block but still intact in the control group. Differential valence ratings remained intact in both the control and the instruction group during the first extinction block, and valence evaluations did not differ between groups throughout extinction. In a third experiment, participants were asked to predict the outcome of an instructed extinction experiment after reading a detailed description of the procedure. Participants predicted that physiological responding would not change, and that CS+ valence would become more pleasant after instructed extinction. As these predictions were contrary to those observed in the experiments, the authors argue that the CS valence results are unlikely to reflect demand characteristics.

Luck and Lipp (2015b) examined whether the removal of the US electrode could be responsible for mediating instructed extinction effects by comparing an instruction (electrode attached) group, an instruction (electrode removed) group, and a non-instructed control group. Using a differential long-delay conditioning paradigm (6s ISI – US followed CS+ immediately), electrodermal responding and online CS valence was assessed. Throughout acquisition, differential first and second interval electrodermal responding and differential valence evaluations were acquired in all groups. Following instructed extinction, differential second interval electrodermal responding was intact in the control group, whereas differential first and second interval responding was eliminated in both instruction groups. Similar to Luck and Lipp (2015a), differential first interval responding was eliminated in the control group due to increased responding to the CS-. Differential valence evaluations were not affected by instructed

extinction, with intact differential valence present in all three groups at the beginning of extinction and no interaction with group throughout extinction training.

Summary

The research examining instructed extinction of fear conditioned to non-fear relevant stimuli has confirmed that it is effective at reducing conditioned fear across a number of different conditioning designs; this reduction, however, is not always evident on the first extinction trial. Conditional fear learning, assessed by electrodermal responding, heart rate, blink startle responding, and finger withdrawal, seems to be subject to instructed extinction. If self-reports of CS valence are measured, however, instructed extinction has been consistently shown not to have an effect. A number of potential moderators of the intervention have been explored, but many of these investigations have not yielded consistent results. Silverman (1960) suggests that instructed extinction may not affect fear after conditioning with a long-trace interval, but Notterman et al. (1952) used a long-trace interval and found a reduction of conditional responding. Lindley and Moyer (1961) found some evidence that instructed extinction effects were stronger after minimal acquisition training, but Grings and Lockhart (1963) found no evidence that the number of acquisition trials moderated instructed extinction effects. Bridger and Mandel (1965) report that instructed extinction effects do not differ after partial or continuous reinforcement training. Wickens et al. (1963) and Grings and Lockhart (1963) directly examined instructed extinction effects after acquisition training with different US intensities, and both report that US intensity did not moderate the effects. When a very intense US was used, however, Bridger and Mandel (1965) and Mandel and Bridger (1967) report that instructed extinction did not reduce conditional responding. Despite these minor inconsistencies, instructed extinction has been shown to be a robust and reliable manipulation that will facilitate extinction and, in some cases, eliminate conditional responding on the very first extinction trial, unless fear is indexed by CS valence evaluations and possibly after fear conditioning with a very intense US.

Instructed Extinction with Fear Relevant Conditional Stimuli

Seligman (1970) proposed that stimuli which posed a survival threat to ancestral humans were evolutionary prepared to associate with aversive events. Prepared associations were said to be rapidly acquired, resistant to extinction, and resistant to cognitive influence (for a review see:

Mallan, Lipp, & Cochrane, 2013). After this proposal, the instructed extinction manipulation became a way of assessing the proposed resistance to cognitive influence. To date, the instructed extinction manipulation has been used to examine three classes of fear-relevant stimuli – phylogenetic animal fear-relevant stimuli (snakes and spiders), social fear-relevant stimuli (angry faces and other race faces), and ontogenetic (modern) fear-relevant stimuli (guns). In this section we will review the instructed extinction studies which used these three classes of stimuli. Additional details of the experiments can be found in Table 2 (snakes and spiders) and Table 3 (social and ontogenetic stimuli).

Phylogenetic Animal Fear Relevant Conditional Stimuli (Snakes and Spiders)

Öhman, Erixon, and Löfberg (1975) examined whether fear conditioned to fear-relevant animals (snakes) would resist instructed extinction in comparison with fear conditioned to fear-irrelevant pictures (houses and faces). A single-cue long-delay conditioning design (8s ISI – US followed CS immediately) was used, measuring electrodermal responding and manipulating fear relevance between groups. Conditioning was present in both first and second interval electrodermal responding by the end of acquisition in all groups. After instructed extinction, second interval responding extinguished rapidly in all groups, but conditioning effects were still present in the first interval response of both fear-relevant groups (instruction and control). Conditioning effects, however, were absent in both fear-irrelevant groups (instruction and control), and therefore, resistance to instruction in the fear-irrelevant instruction group cannot be compared against a baseline instruction control group.

Hugdahl and Öhman (1977) replicated this finding using a differential long-delay (8s ISI – US on CS+ offset) conditioning design. Fear was conditioned to pictures of snakes and spiders (fear-relevant group) and pictures of circles and triangles (fear-irrelevant group). During acquisition, differential first and second interval electrodermal responding was acquired in all groups. Following instructed extinction, differential first interval responding was eliminated in the instructed fear-irrelevant group but still present in the non-instructed fear-irrelevant group. In contrast, differential first interval responding remained intact in both fear-relevant groups throughout extinction. Intact differential second interval responding was present in both fear-irrelevant groups throughout extinction, but in neither fear-relevant group.

Hugdahl (1978) examined whether fear conditioned to pictures of snakes and spiders would resist instructed extinction after a threat of shock acquisition phase. A differential long-delay conditioning design (8s ISI – US followed CS+ immediately) was used, comparing fear conditioned to images of snakes and spiders (fear-relevant) with fear conditioned to images of circles and triangles (fear-irrelevant). One group of participants received CS-US pairings during acquisition (conditioning group), whereas another group were told that the CS+ image would sometimes be followed by an electrotactile shock (threat group; the US was never presented). After acquisition, all participants were informed that the US would no longer be presented, and the shock electrode was removed. During acquisition, differential first and second interval responding was acquired in all groups. Regardless of the conditioning procedure used during acquisition, differential first interval responding was intact in both the conditioning and threat fear-relevant groups after instructed extinction. In contrast, differential first interval responding was abolished by instructions in the fear-irrelevant groups. There was a rapid decrease of differential second interval responding in the fear-irrelevant groups in comparison with the fear-relevant groups.

Cook, Hodes, and Lang (1986; Experiment 4) examined whether the tactile component of the shock was critical to the preparedness effects which had been observed by Öhman and his colleagues. Fear was conditioned to fear-relevant (snakes and spiders) and neutral pictures, with a US consisting of a loud noise and vibratory stimulus to the hand. Little detail about the experiment or analysis is included in the paper, but the authors report no differential effect of instructed extinction on fear-relevant and fear-irrelevant groups. Cook et al. (1986; Experiment 6) used a differential long-delay conditioning design (8s ISI – US followed CS+ immediately) to compare the effects of instructed extinction on conditional electrodermal and heart rate responding to fear-relevant (snakes and spiders) and fear-irrelevant (flowers and mushrooms) stimuli after conditioning with an electrotactile shock US or a loud noise US. Differential first interval electrodermal responding developed during acquisition in both the fear-relevant and fear-irrelevant groups. Instructed extinction reduced first interval electrodermal responding in all instruction groups, and differential responding remained only in the no instruction fear-relevant shock group. A similar pattern of results was obtained with heart rate responding, confirming that in this experiment, fear conditioned to snakes and spiders did not resist instructed extinction.

Soares and Öhman (1993) examined the effects of instructed extinction on electrodermal conditional responding to fear-relevant (snakes and spiders) or fear-irrelevant (flowers and mushrooms) stimuli that were presented either backwardly masked or unmasked during extinction. Participants were conditioned in a differential short-delay conditioning design (0.5s ISI – US followed CS+ immediately) and assigned to one of four groups – extinction with masked fear-relevant stimuli, masked fear-irrelevant stimuli, non-masked fear-relevant stimuli, or non-masked fear-irrelevant stimuli. Half of the participants within each of these groups were given extinction instructions, whereas the remaining half were not informed. During acquisition, responding to CS+ was larger than responding to CS- in all groups. When extinction was performed without the mask and without instruction, differential responding remained for both fear-relevant and fear-irrelevant stimuli. Instruction extinction, however, eliminated differential responding to neutral stimuli but left differential responding to both masked and unmasked fear-relevant stimuli intact (but reduced in magnitude).

Lipp and Edwards (2002) aimed to replicate reports that images of snakes and spiders resist instructed extinction and to assess whether instructed extinction influenced CS valence evaluations. Using a differential long-delay conditioning procedure (8s ISI – US presented at CS+ offset) participants were conditioned with fear-relevant (snakes and spiders) or fear-irrelevant (flowers and mushrooms) images. Participants rated the valence of the images on a 7-point Likert scale (-3 *unpleasant* to +3 *pleasant*) before and after conditioning and electrodermal responding was measured throughout the experiment. During acquisition, all groups acquired differential first and second interval responding. After instructed extinction, differential second interval responding was eliminated in the fear-irrelevant instruction group, but remained in the fear-irrelevant control group. Differential second interval responding remained in both the instructed and control fear-relevant groups. There was no evidence for a differential effect of instructed extinction on the first interval electrodermal responding; however, similar to Luck and Lipp (2015a; 2015b), this was likely due to an increase in responding to the CS- in the fear-irrelevant control group. Evidence for conditioning was obtained in the CS valence measure, but this did not interact with the instructional manipulation. This finding could suggest that instructed extinction did not affect the CS valence evaluations, but should be interpreted with care due to the limitations involved in using a post-extinction assessment of valence.

Luck and Lipp (under review-a; Experiment 1) aimed to replicate resistance to instructed extinction for fear conditioned to images of snakes and spiders using a within-participants design. The between-participants design has been criticised as the repeated exposure to fear-eliciting stimuli in the fear-relevant group could lead to between group differences in state anxiety, which could affect conditioning (Mertens, Raes, & De Houwer, 2016). Using a differential long-delay conditioning design (6s ISI – US presented at CS+ offset), participants viewed images of two fear-relevant (snake and spider) and two fear-irrelevant (bird and fish) animals. One picture from each fear relevance category was used as CS+ and the other as CS-. Differential first and second interval responding was acquired to both fear-relevant and fear-irrelevant images throughout acquisition. After instructed extinction, differential second, but not first, interval responding remained intact to fear-relevant images on the first extinction trial, whereas differential first and second interval responding to fear-irrelevant images was eliminated.

Social and Ontogenetic Fear Relevant Stimuli

Mallan, Sax, and Lipp (2009) assessed the influence of instructed extinction on blink startle modulation and first interval electrodermal responding after conditioning with racial in-group or out-group faces. A long-delay differential conditioning design (6s ISI – US presented at CS+ offset) was used, and Chinese male faces were used as the racial out-group within a group of Caucasian participants (most appropriate racial in- and out-groups in Australia). During acquisition, differential startle modulation and differential electrodermal responding was acquired in all groups. Following instructed extinction, the control group conditioned with out-group faces continued to show differential electrodermal and startle responding, but differential responding was extinguished in instructed participants conditioned with out-group faces. Differential responding was not present in participants conditioned with in-group faces throughout extinction, regardless of instruction group.

As part of a larger study, Olsson and Phelps (2004) examined the effect of instructed extinction on fear conditioned to angry faces after an instructed acquisition phase. Participants were informed that the CS+ would be paired with the electrotactile shock (US was never actually presented) and that the CS- would be presented alone. Differential responding was not present during acquisition; however, the acquisition analyses were focused on a subset of masked trials,

and it is unclear whether differential responding was present during the unmasked trials. After instructed extinction, differential responding was present between CS+ and CS- and was maintained during extinction. This finding suggests that fear conditioned to angry faces may resist instructed extinction, but this conclusion should be interpreted with care as differential responding was not present during acquisition, and the experiment was not designed to assess instruction effects as it was a small part of a larger study. Rowles, Lipp, and Mallan (2012) examined the effect of instructed extinction on fear conditioned to angry faces directly using a differential long-delay conditioning design (6s ISI – US presented at CS+ offset). During acquisition, one group of participants was conditioned with images of angry faces and another with images of happy faces. Both groups acquired differential first interval electrodermal responding, but after instructed extinction, only the angry control group showed differential responding, suggesting that fear conditioned to angry faces does not resist instructed extinction. A pre-post measure of CS valence showed evidence of conditioning, but this did not interact with the instructional manipulation.

Luck and Lipp (under review-a; Experiment 2) used a within-participants instructional design to examine whether fear conditioned to images of pointed guns would resist instructed extinction. Using a within-participants differential long-delay conditioning design (6s ISI – US presented at CS+ offset), participants viewed images of pointed guns (fear-relevant) and pointed hairdryers (fear-irrelevant). Throughout acquisition, differential first and second interval electrodermal responding was evident to images of guns and hairdryers; however, following instructed extinction, differential first and second interval electrodermal responding to both sets of images was eliminated.

Summary

The instructed extinction manipulation has been used in a number of studies to assess whether, as suggested by preparedness theory, fear conditioned to a range of fear-relevant CSs is encapsulated from cognition. There is substantial evidence that fear conditioned to images of snakes and spiders is not sensitive to instructed extinction. Of the eight studies designed to investigate this, six (Hugdahl, 1978; Hugdahl & Öhman, 1977; Lipp & Edwards, 2002; Luck & Lipp, under review-a; Öhman et al., 1975; Soares & Öhman, 1993) have reported that fear conditioned to snakes and spiders resists instructed extinction. There has been little evidence,

however, that fear conditioned to other classes of fear-relevant stimuli resists instructed extinction. Fear conditioned to other race faces (Mallan et al., 2009), angry faces (Rowles et al., 2012), and pointed guns (Luck & Lipp, under review-a) was reduced after instructed extinction.

Integration, Clinical Applications, and Future Directions

It is clear that instructed extinction has a long and rich history within human fear conditioning experiments. Instructed extinction experiments have used short and long CS durations, single cue and differential conditioning paradigms, different reinforcement rates and amounts, and a number of different conditional and unconditional stimuli. Despite this variation, the pattern of instructed extinction effects is remarkably consistent – instructed extinction reduces conditional fear as indexed by electrodermal responding, startle modulation, heart rate, conditioned finger withdrawal responding, and US expectancy ratings. This effect is not always present on the first trial of extinction, but with only a few exceptions, instructed extinction does facilitate the extinction of conditional fear.

The majority of studies have not assessed the effect of instructed extinction on the first trial of extinction, and in those studies that have, the results are mixed. Some authors report that conditional responding is eliminated prior to explicit extinction training, but others report that instructed extinction only facilitates extinction learning. As instructed extinction has been shown to eliminate conditional responding on the first extinction trial in a number of studies, it is possible that factors which vary across studies, such as the control of participant beliefs, could be influencing the results. Participants' belief in the instructions is a very powerful factor, and inclusion of participants who are sceptical about the validity of the instructions could mask instruction effects on the first trial of extinction (Luck & Lipp, 2015b; Mandel & Bridger, 1973).

Across the literature there have been three notable exceptions to the general pattern of instructed extinction results – instructed extinction does not affect CS valence; fear conditioned to snakes and spiders survives instructed extinction; and fear conditioned with a very painful electrotactile shock may resist instructed extinction. One potential explanation of these exceptions may be that emotional conditioning, prepared stimuli, and intensely aversive stimuli activate a subcortical fear-processing system which is more resistant to cognitive influence (Debiec & LeDoux, 2004; Öhman, 2005). More research is needed, however, to examine

whether there are more parsimonious explanations which could also account for these exceptions.

These 'exceptions' observed in the laboratory may have implications for clinical practice; however, there are limitations to the extent to which fear conditioned in the laboratory with an unpleasant US compares to the experiences of an individual suffering from, for instance, post-traumatic stress disorder. Nevertheless, differences in response to instruction observed across experiments may also manifest in clinical practice. The observation that fear conditioned with a very painful shock resists instructed extinction may suggest that fear responses seen in the clinic, which have been acquired based on intensely aversive real-life experiences, may be less responsive to cognitive intervention. Similarly, if fear conditioned to snakes and spiders, but not other animals, resists instruction in the laboratory, snake and spider phobias may require different approaches than those used for other small animal phobias. If there is a dissociation between the subjective dislike of feared situations and events and physiological responding after instructed extinction, then similar dissociations may be observed after successful treatment. Persisting negative valence predicts higher reinstatement rates after fear extinction (Dirkx, Hermans, Vansteenwegen, Baeyens, & Eelen, 2004; Hermans et al., 2005; Zbozinek, Hermans, Prenoveau, Liao, & Craske, 2015), and manipulations that reduce negative CS+ valence have been shown to reduce fear reinstatement (Zbozinek, Holmes, & Craske, 2015).

Instructed extinction is proposed as a laboratory analogue for cognitive interventions but falls short of capturing the complexity of cognitive interventions used in the clinical setting. Instructed extinction completely breaks the association between the feared stimulus and the aversive event, whereas cognitive therapy is used to bring the probability of negative outcomes more in line with reality. The robust decreases in physiological responding observed after instructed extinction may occur because of the certainty involved in the manipulation. Future research should examine the use of instructional manipulations that weaken the CS-US contingency, without breaking it completely. As a probability-based cognitive manipulation, instructed extinction does not capture a number of other aspects often targeted throughout cognitive therapy, such as reappraising the cost of the aversive event occurring and the client's ability to handle an aversive event if it was to occur. Negative valence, fear of snakes and spiders, and fears acquired based on very aversive events may still respond to these other aspects of cognitive therapy. In support of this idea, negative CS+ valence can be removed with a

cognitive intervention specifically targeting CS valence, rather than CS-US contingency (Luck & Lipp, under review-b). More research is required to disentangle the components involved in cognitive therapy, to examine the reliability of instructed extinction as an analogue for cognitive interventions, and to examine whether different types of cognitive interventions would be more effective at targeting negative valence and more robust fear responses.

Sevenster et al. (2012) is the only study to date to have assessed the effects of instructed extinction on the return of fear directly. In this study, instructed extinction did not influence the reinstatement of differential electrodermal responding or startle modulation but did reduce the return of differential US expectancy ratings. This initial finding is promising, but more follow-up research is needed to assess the effects of instructed extinction on the return of fear using renewal and spontaneous recovery procedures. Instructed extinction research in the laboratory has provided researchers with a number of interesting 'exceptions' which do require further study, but their implications should also be examined in clinical settings. Are cognitive interventions less effective for treatment of snake and spider phobias? Are they less effective when fear has been acquired in an intensely traumatic or negative situation? Is it possible to change the valence of the feared stimulus in the clinical setting, and does this reduce relapse? Instructed extinction research has come a long way since the first study was published in 1937, and now seems the time to translate some of its findings and implications to clinically based applied research.

Table 1. Instructed Extinction Research Using Non-Fear Relevant Conditional Stimuli

Study	Conditioning Design	CS	US	Conditioning and ISI	Acquisition	Extinction	Instruction Comparison	First Trial Effects	Facilitation?	Electrode Removal? (Instructed Groups)
Electrodermal Responding										
Cook and Harris (1937)	Single cue (no control)	3s light	Shock (duration not reported)	Delay – 3s (US on CS offset)	30 CS-US pairings (100% reinforcement)	Not specified	Between groups – control versus instruction	Not assessed	Yes	Not specified
Silverman (1960)	Single cue (unpaired control)	2s tone	6s shock	Trace – 2.5s and 8s ISI (0.5s and 6s interval between CS and US)	10 CS-US pairings (100% reinforcement)	15 CS alone trials	Between groups – instruction versus control (in each ISI condition)	Not assessed	2.5s ISI: Yes 8s ISI: No Unpaired: Yes	Not specified
Wickens, et al. (1963)	Single cue (unpaired control)	0.5s tone	0.1s shock	Delay – 0.5s ISI (US on CS offset)	10 CS-US trials (Strong US group: CS paired with a strong shock (with 10 weak shocks interspersed between trials); Weak US group: CS paired with a weak shock (with 10 strong shocks interspersed between trials);Control: 10 strong and 10 weak shocks (unpaired with the CS) (100% reinforcement)	5 CS alone trials	Between groups – instruction versus control (in each US intensity group)	No	Yes	Yes
Grings and Lockhart (1963)	Single cue (no control)	5s pictures (shapes)	Shock (duration not reported)	Delay – 5s ISI (US on CS offset)	Minimum reinforcement: 9 (3 of each CS) (100% reinforcement); Extended reinforcement: 36 (12 of each CS) (100% reinforcement)	3 CS alone trials (1 of each CS)	Between groups – instruction versus control (in each reinforcement condition)	Yes	Not assessed (only one of each CS trial)	Not specified
Bridger and Mandel (1964)	Differential conditioning	6s lights	0.5s shock (very painful)	Delay – 6s ISI (US delivered 1s before CS offset)	Shock group: 20 CS+ – US pairings (100% reinforcement); 20 CS- alone; Threat group: 20 CS+ alone trials (threatened);20 CS- alone trials	10 CS+ and 10 CS- trials (unreinforced)	Between groups – instruction versus control (in each of the shock and threat groups)	No	No	Yes

Bridger & Mandel (1965)	Differential conditioning	0.5s lights	0.5s shock (very painful)	Delay – 0.5s ISI (US on CS offset)	20 CS+ (5/20 reinforced in partial reinforcement group and 20/20 reinforced in continuous reinforcement group); 20 CS- alone trials.	30 CS+ and 30 CS- trials (unreinforced)	Between groups – instruction versus control (in each of the partial and continuous reinforcement groups)	Significant reduction (but continued differential responding)	Yes	Yes
Mandel and Bridger (1967)	Differential conditioning	Short ISI group: 0.5s lights. Long ISI group: 5s lights	0.5s shock (very painful)	Delay – 0.5s or 5s ISI (US on CS offset)	25 CS+ trials (15/25 reinforced); 25 CS- alone trials	10 CS+ and 10 CS- trials (unreinforced)	Between groups – instruction versus control (in each acquisition group)	No	Not possible to assess	Yes
Fuhrer and Baer (1980)	Differential conditioning	Short ISI group: 0.5s tones. Long ISI group: 8s tones	0.25s shock	Delay – 0.5s or 8s ISI (US on CS offset)	30 CS+ presentations (18 reinforced); 30 CS- presentations	10 CS+ and 10 CS- trials (unreinforced)	No control – all participants receive instructed extinction manipulation; Participants later split based on US expectancy scores.	No	Yes	Yes
Lipp et al. (2003)	Differential conditioning	8s pictures of vowels	.5s shock	Delay – 8s ISI (US on CS offset)	10 CS+ presentations (100% reinforcement); 10 CS- alone presentations	16 CS+ and 16 CS- presentations (unreinforced)	Between groups – control versus instruction	Not possible to assess	Not possible to assess	Yes
Sevenster et al. (2012)	Differential conditioning	8s pictures of shapes	2ms shock	Delay – 7.5s ISI (US presented 7.5s into 8s CS)	6 CS+ presentations (4 reinforced); 6 CS- alone presentations (Acquisition on Day 1)	16 presentations of CS+ and CS- (unreinforced)(extinction on day 2)	Between groups – control versus instruction	Yes	Yes	No
Luck and Lipp (2015a; Experiment 1)	Differential conditioning	6s pictures of neutral faces	0.2s shock	Delay – 6s ISI (US on CS offset)	8 CS+ presentations (100% reinforcement); 8 CS- alone trials	8 CS+ and 8 CS- presentations (unreinforced)	Between groups – control versus instruction	Yes [1]	Yes	Yes
Luck and Lipp (2015b)	Differential conditioning	6s pictures of neutral faces	0.2s shock	Delay – 6s ISI (US on CS offset)	8 CS+ presentations (100% reinforcement); 8 CS- alone trials	8 CS+ and 8 CS- presentations (unreinforced)	Between groups – control versus instruction (electrode attached) versus instruction (electrode removed)	Yes	Yes	Electrode attached: No; Electrode removed: Yes

Heart Rate										
Notterman et al. (1952)	Single cue (no control)	1s tone	6s shock	Trace –7s ISI (6s interval between CS and US)	18 CS presentations (11 reinforced – 61%)	11 CS (unreinforced); first extinction trial excluded	Between groups – control versus instruction	No	Yes	Not specified
Blink Startle Responding										
Sevenster et al. (2012)	Differential conditioning	8s pictures of shapes	2ms shock	Delay – 8s ISI (US presented 7.5s into 8s CS)	6 CS+ presentations (4 reinforced); 6 CS- alone presentations (acquisition on day 1)	16 presentations of CS+ and CS- (unreinforced) (extinction on day 2)	Between groups – control versus instruction	No	Yes	No
Luck and Lipp (2015a; Experiment 2)	Differential conditioning	6s pictures of neutral faces	0.2s shock	Delay – 6s ISI (US on CS offset)	8 CS+ presentations (100% reinforcement); 8 CS- alone trials.	12 CS+ and 12 CS- presentations (unreinforced)	Between groups – control versus instruction	Not possible to assess [2]	Yes	Yes
Finger Withdrawal										
Lindley and Moyer (1961)	Single cue (no control)	0.5s tone	0.2s shock	Trace – 1s ISI (.5s interval between CS and US)	Minimum reinforcement: until participant reached criterion of 4 conditioned responses in 5 consecutive trials (average 21 pairings); Extended reinforcement: 20 additional conditioning trials after reaching criterion	25 CS trials (unreinforced)	Between groups – control (no instructions/no pause) versus control (interrupted but no information given) versus instructed (informed to let the finger move automatically) versus instructed (informed to suppress finger movement)	Not assessed	Yes	Not specified (unlikely as shock embedded within experimental set-up)
US Expectancy										
Sevenster et al. (2012)	Differential conditioning	8s pictures of shapes	2ms shock	Delay – 8s ISI (US presented 7.5s into 8s CS)	6 CS+ presentations (4 reinforced); 6 CS- alone presentations (acquisition on day 1)	16 CS+ and 16 CS- (unreinforced) (extinction on day 2)	Between groups – control versus instruction	Yes	Yes	No
CS Valence										
Lipp et al. (2003)	Differential conditioning	8s pictures of vowels	.5s shock	Delay – 8s ISI (US on CS offset)	10 CS+ presentations (100% reinforcement); 10 CS- alone presentations	16 CS+ and 16 CS- trials (unreinforced)	Between groups – control versus instruction	No	No	Yes

Luck and Lipp (2015a; Experiment 1)	Differential conditioning	6s pictures of neutral faces	0.2s shock	Delay – 6s ISI (US on CS offset)	8 CS+ presentations (100% reinforcement); 8 CS- alone trials	8 CS+ and 8 CS- trials (unreinforced)	Between groups – control versus instruction	No	No	Yes
Luck and Lipp (2015a; Experiment 2)	Differential conditioning	6s pictures of neutral faces	0.2s shock	Delay – 6s ISI (US on CS offset)	8 CS+ presentations (100% reinforcement); 8 CS- alone trials	12 CS+ and 12 CS- (unreinforced)	Between groups – control versus instruction	No	No	Yes
Luck and Lipp (2015b)	Differential conditioning	6s pictures of neutral faces	0.2s shock	Delay – 6s ISI (US on CS offset)	8 CS+ presentations (100% reinforcement); 8 CS- alone trials	8 CS+ and 8 CS- trials (unreinforced)	Between groups – control versus instruction (electrode attached) versus instruction (electrode removed)	No	No	Electrode attached: No; Electrode removed: Yes

Notes:[1] Instruction effects were analysed in this study based on blocks – a reanalysis of the electrodermal responding data based on trials revealed that differential responding was not present on the first trial. [2] The first startle probe was in the second trial of extinction.

Table 2. Instructed Extinction Research using Phylogenetic Animal Fear Relevant Stimuli

Study	Conditioning Design	CS	US	Conditioning and ISI	Acquisition	Extinction	Instruction Comparison	First Trial Effects	Facilitation?	Electrode Removal?
Electrodermal Responding										
Öhman et al. (1975)	Single cue (no pseudo-conditioning control)	8s slides of snakes, houses, and faces	50ms shock	Delay – 8s ISI (US on CS offset)	10 presentations of snakes, houses, and faces (snakes paired with US for one group, houses paired with US for another, and faces paired with US for the third group)	10 snakes 10 houses 10 faces (unreinforced)	Between groups – instructed versus control	Not assessed	Fear irrelevant stimuli: Not possible to assess; Fear relevant stimuli: No	Yes
Hugdahl and Öhman (1977)	Differential conditioning	8s slides of a snake and spider (fear relevant) or a triangle and a circle (fear irrelevant)	Shock (duration not reported)	Delay – 8s ISI (US on CS offset)	10 CS+ (100% reinforcement); 10 CS- alone	14 CS+ and 14 CS- (unreinforced)	Between groups – instructed versus control (in each of the fear relevant and fear irrelevant groups)	Not assessed	Fear irrelevant stimuli: Yes; Fear relevant stimuli: No	Yes
Hugdahl (1978)	Differential conditioning	8s pictures of a snake and spider (fear relevant) or a triangle and a circle (fear irrelevant)	Shock (duration not reported)	Delay – 8s ISI (US on CS offset)	12 CS+ (100% reinforcement); 12 CS- alone	20 CS+ and 20 CS- (unreinforced)	All participants received instructed extinction manipulation	Not assessed	Fear irrelevant stimuli: Yes; Fear relevant stimuli: No	Yes
Cook et al. (1986; Experiment 4)	Differential conditioning	8s slides of snakes and spiders or neutral stimuli	Loud noise and vibrotactile sensation to arm (duration not reported)	Delay – 8s ISI (US on CS offset)	Not specified	Not specified	Between groups – instructed versus control (in each of the fear relevant and fear irrelevant groups)	No	No	Yes
Cook et al. (1986; Experiment 6)	Differential conditioning	8s slides of snakes and spiders (fear relevant) or flowers and mushrooms (fear irrelevant)	0.5s shock or 0.5s loud noise (between participants)	Delay – 8s ISI (US on CS offset)	8 CS+ (100% reinforcement); 8 CS- presented alone	20 CS+ and 20 CS- trials (unreinforced)	Between groups – instructed versus control (in each of the fear relevant shock and noise and fear irrelevant shock and noise groups)	No	No	Yes

Soares and Öhman (1993)	Differential conditioning	0.5s, 30ms, or 0.13s slides of snakes and spiders (fear relevant) or flowers and mushrooms (fear irrelevant)	0.5s shock	Acquisition: Delay – 0.5s ISI (US on CS offset); Extinction: Masked group: CS presented for 30ms followed by masking stimulus for 0.1s; Non-masked group: CS presented for 0.13s	12 CS+ (10 reinforced); 12 CS- (unreinforced)	16 CS+ and 16 CS- (unreinforced)	Between groups – instructed versus control (in each of the masking conditions)	Not assessed	Fear Relevant Stimuli: No; Fear irrelevant stimuli: Yes	Yes
Lipp and Edwards (2002)	Differential conditioning	8s images of snakes and spiders (fear relevant) or flowers and mushrooms (fear irrelevant)	0.2s shock	Delay – 8s ISI (US on CS offset)	10 CS+ (100% reinforcement); 10 CS- (unreinforced)	8 CS+ 8 CS- (unreinforced)	Between groups – instructed versus control (in each of the fear relevance categories)	Not assessed	Fear Relevant: No; Fear irrelevant: Yes	Yes
Luck and Lipp (under review-a; Experiment1)	Differential conditioning	6s images of fear relevant (snakes/spiders) and fear irrelevant (birds/fish)	0.2s shock and loud noise combination	Delay – 6s ISI (US on CS offset)	6 CS+ (100% reinforcement); 6 CS- alone (for both fear relevant and fear irrelevant stimuli)	6 CS+ and 6 CS- unreinforced trials (for both fear relevant and fear irrelevant stimuli)	Within-groups – all participants received extinction instructions	Fear Relevance: No; Fear irrelevant: Yes	Fear Relevance: No; Fear irrelevant: Yes	Yes
Heart Rate										
Cook et al. (1986-Experiment 6)	Differential conditioning	8s slides of snakes and spiders (fear relevant) or flowers and mushrooms (fear irrelevant)	0.5s shock or 0.5s loud noise (US varied between participants)	Delay – 8s ISI (US on CS offset)	8 CS+ (100% reinforcement); 8 CS- presented alone	20 CS+ and 20 CS- trials (unreinforced)	Between groups – instructed versus control (in each of the fear relevant shock and noise and fear irrelevant shock and noise groups)	No	No	Yes

Table 3. Instructed Extinction Research using Social and Ontogenetic Fear Relevant Stimuli

Study	Conditioning Design	CS	US	Conditioning and ISI	Acquisition	Extinction	Instruction Comparison	First Trial Effects	Facilitation?	Electrode Removal?
Electrodermal Responding										
Olsson and Phelps (2004)	Differential conditioning (threat of shock acquisition)	6s pictures of angry faces	Threat of shock (no actual presentations)	6s CS duration – no US presentations (in the masked group on masked trials the CS was presented for 33ms followed by the mask)	12 CS+ (unreinforced); 12 CS- (unreinforced)	10 CS+ and 10 CS- trials (unreinforced)	All participants in this part of the experiment received extinction instructions	No	No	Not Specified
Mallan et al. (2009)	Differential conditioning	6s pictures of Chinese faces or Caucasian faces (all males)	0.4s shock	Delay – 6s ISI (US on CS offset)	8 CS+ (100% reinforcement); 8 CS- presented alone	12 CS+ and 12 CS- trials (unreinforced)	Between groups – instructed versus control (in each of the fear relevance categories)	Not assessed	Yes	Yes
Rowles et al. (2012)	Differential conditioning	6s pictures of happy or angry faces (males)	0.2s shock	Delay – 6s ISI – (US on CS offset)	8 CS+ (100% reinforcement); 8 CS- presented alone	10 CS+ and 10 CS- trials (unreinforced)	Between groups – instructed versus control (in each of the fear relevance categories)	Not assessed	Yes	Yes
Luck and Lipp (under review-a; Experiment2)	Differential conditioning	6s pictures of fear relevant (guns) and fear irrelevant (hairdryers)	0.2s shock and loud noise combination	Delay – 6s ISI – (US on CS offset	6 CS+ (100% reinforcement); 6 CS- alone (for both fear relevant and fear irrelevant stimuli)	6 CS+ and 6 CS- alone trials (for both fear relevant and fear irrelevant stimuli)	Within-groups – all participants received extinction instructions.	Fear Relevance: Yes; Fear irrelevant: Yes	Fear Relevance: Yes; Fear irrelevant: Yes	Yes
Blink Startle										
Mallan et al. (2009)	Differential conditioning	6s pictures of Chinese faces or Caucasian faces (males)	0.4s shock	Delay – 6s ISI – (US on CS offset	8 CS+ (100% reinforcement); 8 CS- presented alone	12 CS+ and 12 CS- trials (unreinforced)	Between groups – instructed versus control (in each of the fear relevance categories)	Not assessed	Yes	Yes

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Chapter 3. Paper 2 – A Potential Pathway to the Relapse of Fear? Conditioned Negative Stimulus Evaluation (but not Physiological Responding) Resists Instructed Extinction

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A Potential Pathway to the Relapse of Fear?

Conditioned Negative Stimulus Evaluation (but not Physiological Responses) Resists Instructed Extinction

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Abstract

Relapse of fear after successful intervention is a major problem in clinical practice. However, little is known about how it is mediated. The current study investigated the effects of instructed extinction and removal of the shock electrode on electrodermal responding (Experiment 1), fear potentiated startle (Experiment 2), and a continuous self-report measure of conditional stimulus valence (Experiments 1 and 2) in human differential fear conditioning. Instructed extinction and removal of the shock electrode resulted in the immediate reduction of differential fear potentiated startle and second interval electrodermal responding, but did not affect self-reported conditional stimulus valence. A separate sample of participants (Experiment 3) who were provided with a detailed description of the experimental scenario predicted the inverse outcome, reduced differential stimulus evaluations and continued differential physiological responding, rendering it unlikely that the current results reflect on demand characteristics. These results suggest that the negative valence acquired during fear conditioning is less sensitive to cognitive interventions than are physiological indices of human fear learning and that valence reduction requires extended exposure training. Persisting negative valence after cognitive intervention may contribute to fear relapse after successful treatment.

Key words: fear conditioning, instructed extinction, electrodermal responses, fear potentiated startle, evaluative learning, fear relapse

Epidemiological data suggests that 25 percent of the population will develop an anxiety disorder at some stage in life (Kessler, Koretz, Merikangas, & Wang, 2004). It is thus reassuring that efficacious treatments are available for these conditions with exposure based and cognitive therapies emerging as the most commonly used interventions in clinical practice (Ougrin, 2011), both receiving consistent empirical support for a number of anxiety disorders (Bisson & Andrew, 2007; Ougrin, 2011; Sánchez-Meca, Rosa-Alcàzar, Marín-Martínez, & Gómez-Conesa, 2010). In spite of this considerable success, approximately one to two thirds of successfully treated patients will relapse within eight years (Craske, 1999). This figure highlights the need for continued research into the mechanisms underlying fear acquisition, reduction, and relapse – an understanding which is essential for the development of treatments with improved long term outcomes.

Fear is a basic emotion characterized by high levels of negative affect (displeasure) and physiological arousal (Lang, 1995). Classical fear conditioning models can provide a conceptual framework to study the development and treatment of human fear (Craske, Hermans, & Vansteenwegen, 2006). In the laboratory setting, a differential fear conditioning paradigm is often used, involving the presentation of two neutral conditional stimuli and an aversive unconditional stimulus (Lipp, 2006). During acquisition training, one conditional stimulus (CS+) is paired with the aversive unconditional stimulus (e.g. electrotactile stimulus), whilst the other is presented alone (CS-; Lipp, 2006). During fear acquisition, the CS+ becomes a valid predictor of the aversive unconditional stimulus, leading to the development of increased physiological responding and decreased valence ratings to the CS+ in comparison with the CS- (De Houwer, Thomas, & Baeyens, 2001; Lipp, 2006). Extinction training involves the repeated presentation of the CS+ without the unconditional stimulus and has been suggested as an experimental analogue to exposure based interventions (Kerkhof et al., 2009). Extinction training is very effective in eliminating differential physiological responding between CS+ and CS- and also reduces the negative valence acquired by the CS+, however, there is evidence that negative valence is more resistant to extinction than are the physiological indices of fear learning and thus requires extended extinction training (Lipp, Oughton, & LeLievre, 2003).

A very common finding in human fear learning is that after successful extinction of differential responding, conditioned responding can reoccur in a post-extinction test session, in the absence of any re-training or re-exposure to the feared stimulus (for a review see Vervliet,

Craske, & Hermans, 2013). This phenomenon is referred to as the return of fear (Rachman, 1966). To date, three mechanisms mediating the return of fear have been uncovered; spontaneous recovery: the return of fear following the mere passage of time, renewal: the return of fear following a context change, and reinstatement: the return of fear following unpredicted presentations of the unconditional stimulus (Bouton, 2002). It should be noted that as defined above (Lang, 1995), return of fear implies the recurrence of both physiological arousal and negative affect. However, under a less strict definition, the return of negative valence or physiological arousal alone could be interpreted as being a partial return of the fear response – an occurrence which could predispose the individual for full return of fear.

After observing that persisting negative valence towards the feared stimulus was correlated with higher reinstatement rates, Hermans et al. (2005) suggested that lingering negative valence could provide an additional pathway for the return of fear. Noting that negative stimuli preferentially associate with aversive outcomes (Hamm, Vaitl, & Lang, 1989) and that negative valence has been associated with escape and avoidance tendencies (Chen & Bargh, 1999), Kerkhof et al. (2009) developed this theory proposing, based on Lang's (1995) conceptualization of fear as a combination of high arousal and negative valence, that if negative valence persists after extinction, fear could return if the individual is put in a high arousal situation or state.

The human fear conditioning paradigm can also be used to examine the influence of cognition on the extinction of fear learning. Following, Mower's (1938) initial observation that electrodermal responding could be 'switched on and off' with signals informing the participants when an aversive unconditional stimulus was to be expected, researchers have used the instructed extinction paradigm as an experimental analogue for cognitive interventions to reduce fear. Instructed extinction involves informing one group of participants at the end of acquisition training that the aversive unconditional stimulus will no longer be presented, whilst a control group receives the same level of interaction with the experimenter, but is not informed. Frequently, the instruction that no further unconditional stimuli will be presented is accompanied by removal of the unconditional stimulus electrode (Hugdahl, 1978; Hugdahl & Öhman, 1977; see Sevenster, Beckers, & Kindt, 2012, for mere instruction effects). This manipulation has been shown to reduce the differential electrodermal responding acquired during fear conditioning unless the conditional stimuli used are pictures of snakes or spiders as fear conditioned to these

stimuli seems to be encapsulated from cognition (for a recent review see Mallan, Lipp, & Cochrane, 2013). However, electrodermal responding is not selectively sensitive to fear learning, showing the same pattern of responding regardless of whether the conditional stimulus is paired with an aversive or a non-aversive unconditional stimulus (Lipp & Vaitl, 1990). Fear potentiated startle is said to be a more selective index of conditioned fear (Hamm & Weike, 2005), but it is currently not clear whether instructed extinction also affects fear learning as indexed by fear potentiated startle, or the negative valence acquired during fear conditioning.

To date, two studies have assessed the effect of instructed extinction on conditioned fear as indexed by fear potentiated startle and have reached different conclusions. Whereas Mallan, Sax, and Lipp (2009) report that, like differential electrodermal responding, instructed extinction abolishes differential fear potentiated startle, Sevenster et al. (2012) report a dissociation between electrodermal responding and fear potentiated startle. In this study, instruction effects on differential electrodermal responses were immediate, i.e., evident on the very first trial of extinction training, whereas differential startle potentiation persisted for the first two trials of extinction. It should be noted, however, that relative to the non-instructed control group, extinction of fear as indexed by fear potentiated startle was accelerated considerably, as differential fear potentiated startle was absent after the first two extinction trials in the instructed group, but persisted across the first ten extinction trials in controls. Based on the latter finding it seems reasonable to conclude that conditioned fear as indexed by both physiological indices is subject to instructed extinction.

Whether instructed extinction affects the negative valence acquired by a CS+ during acquisition training is less clear. Lipp and Edwards (2002) and Rowles, Lipp, and Mallan (2012) included post-extinction assessments of conditional stimulus valence which seemed to be unaffected by instruction. Equivalent differential evaluation of CS+ and CS- was evident in all groups regardless of the nature of the conditional stimuli used or the instructions provided. However, as conditional stimulus valence was assessed after the completion of extinction training, it is not clear whether the differential conditional stimulus evaluations reflect on insensitivity to instruction or the renewal of fear due to a context change (Bouton, 2002; Vansteenwegen, Dirikx, Hermans, Vervliet, & Eelen, 2006). Lipp et al. (2003; Experiment 2) did not find an effect of instructed extinction on conditional stimulus valence using a continuous assessment during extinction training, however, these results need to be considered with care due

to fast extinction in the control group and no instruction effect on electrodermal responses.

The effect of instructed extinction on acquired conditional stimulus valence has also been examined in studies of evaluative conditioning which can inform studies of fear learning. In evaluative conditioning, pleasant and unpleasant pictures rather than aversive electrotactile stimuli are used as unconditional stimuli and conditional stimulus valence can be assessed immediately after instruction and during extinction training. Using such a paradigm, Lipp, Mallan, Libera and Tan (2010) failed to find an effect of instructed extinction on measures of conditional stimulus valence, immediate or delayed, although participants reported reduced expectancy of the unconditional stimuli immediately after instruction. Gast and De Houwer (2013) found valence measures to be sensitive to instructed extinction in their first, but not in their second experiment. However, the instructed extinction effect in Experiment 1 was not significant for participants who could correctly report the stimulus contingencies used during evaluative conditioning training. Taken together, results from evaluative conditioning seem to suggest no effect of instructed extinction on conditional stimulus valence, at least in participants who show evidence of learning during the initial training. It is unclear, however, whether these findings would transfer to fear conditioning that is acquired using a biologically significant aversive unconditional stimulus, such as electrotactile stimulus. Such an unconditional stimulus is likely to convey significantly higher levels of negative valence and emotional arousal than the presentation of an unpleasant picture.

To assess the effects of instructed extinction on electrodermal responses, fear potentiated startle, and conditional stimulus valence, two differential fear conditioning experiments were conducted using neutral faces as conditional stimuli and an aversive electrotactile stimulus as the unconditional stimulus. In Experiment 1, electrodermal responding and conditional stimulus valence were assessed and fear potentiated startle and conditional stimulus valence were assessed in Experiment 2. We examined electrodermal responding and fear potentiated startle in separate experiments to avoid contamination of electrodermal responses by the noise probes used to elicit startle responses and to replicate the results for conditional stimulus valence. Following the procedure used in the majority of prior instructed extinction studies, we removed the shock electrode as part of the instructional manipulation to ensure that the participants believed the instructions. Based on the review of the prior literature we predict that instructed extinction will reduce electrodermal responses and fear potentiated startle, whereas negative valence acquired

by the CS+ will persist.

Experiment 1

Method

Participants. Thirty-six (21 female) undergraduate students aged 17-52 years (*M* = 21.71) provided informed consent and volunteered participation in exchange for course credit. Participants were randomly assigned to one of two groups (instruction/removal, control). The pre-experimental ratings data of one participant was lost due to a recording error and evaluation data of three participants and the electrodermal responses of one participant were lost due problems with the recording device. These participants were included in the analyses of all remaining measures.

Apparatus/Stimuli. 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 were used as conditional stimuli. The pictures were 506 × 650 pixels in size, and were displayed for 6 seconds on a 17 inch color LCD screen. The two faces used as conditional stimuli during the experiment, the faces used as CS+/CS-, and whether the first trial of each phase was a CS+/CS- was counterbalanced across participants.

Conditional stimulus evaluations and physiological responding were recorded with a Biopac MP150 system, at a sampling frequency of 1000 Hz, using AcqKnowledge Version 3.9.1. Electrodermal responding was DC amplified at a gain of 5 μSiemens per volt and monitored using two 8mm Ag/AgCl electrodes filled with Mansfield R & D TD-246 electrode paste and attached using adhesive collars. Respiration was monitored with a chest gauge to control for respiration induced artefacts in electrodermal responding. Conditional stimulus valence was measured on a trial-by-trial basis using an evaluation joystick with the anchors, very unpleasant, neutral, and very pleasant. A Grass SD9 stimulator, pulsed at 50 Hz, was used to deliver a 200 ms electrotactile stimulus to the participants' preferred forearm via a concentric electrode. DMDX3.0.2.8 software (Forster & Forster, 2003) was used to record pleasantness ratings before and after conditioning training and to control stimulus presentation and timing.

Procedure. Participants were seated in front of the monitor in an experimental room, located adjacent to a control room. A respiration belt was fitted around their waist and two

electrodes were placed on the thenar and hypothenar prominences of their non-preferred hand. A shock electrode was attached with a bandage to their preferred forearm, and the participant completed a shock-work-up procedure to set the electrotactile stimulus to an intensity that was experienced as 'unpleasant, but not painful'. After the shock work-up procedure, the participants were subjected to a three minute baseline recording of their physiological responding whilst they relaxed and watched the blank computer screen.

After the baseline recording, the participants completed a pre-experimental rating task, in which participants were prompted to rate the faces on a pleasantness scale ranging from 1 to 9 (1 = unpleasant, 9 = pleasant). The conditional stimulus faces were displayed on the screen until a response was made. After the pre-ratings task, participants were informed that they would view pictures of faces, and that they were required to use the joy-stick to indicate whether they felt the face was pleasant, unpleasant, or neutral. To ensure that the valence ratings were not contaminated by the presence/absence of the unconditional stimulus, the participants were informed that they should rate each face as soon as it was presented on the screen. Valence ratings were made with the participants' preferred hand to ensure the movement of the joystick would not interfere with the skin conductance recording. After the task instructions the conditioning procedure was started. Conditioning consisted of habituation, acquisition, and extinction phases. During habituation, two faces, the CS+ and the CS-, were presented four times each. The habituation phase allows for the habituation of orienting responses to the conditional stimuli. The acquisition phase followed habituation immediately. During acquisition, the CS+ was presented eight times and unconditional stimulus onset coincided with the CS+ offset in a 100% reinforcement schedule, whereas the CS- was presented eight times alone. Extinction involved the presentation of both the CS+ and the CS- eight times each, but no electrotactile stimulus was presented during this phase. All conditional stimuli were presented for six seconds, and a blank rest screen was presented between trials for either 15, 18, or 21 seconds, randomly. Inter-trial interval duration was varied to avoid the participants predicting and anticipating the onset of the next CS.

For both the instruction/removal group and the control group, the experimenter entered the room at the end of acquisition. Participants in the instruction/removal group were informed that in the second part of the experiment the presentations of the electrotactile stimulus would cease and the shock electrode was removed. Participants in the control group were informed that

the shock electrode needed to be checked and it was removed and reattached. All participants were informed that the experiment would continue and that they should continue to evaluate the faces. After the last trial of extinction, the participants completed a post-experimental rating procedure that was identical to the pre-rating procedure. After this, the electrodes were removed and the participants were led into the control room to complete a post-experimental questionnaire. This required an assessment of contingency awareness in which the participants were shown four neutral faces and asked to indicate which two they had seen during the experiment and which face had been followed by the electrotactile stimulus. Participants were asked to rate how pleasant or unpleasant they found the electrotactile stimulus (-3 to +3 scale) and as a manipulation check the participants were asked to indicate whether they had believed the instruction that the electrotactile stimulus would not occur following the interruption (yes or no question; instruction/removal group only).

Scoring and Response Definition. To provide a measure of spontaneous electrodermal responding, any discernible response during the three minute baseline was counted (Dawson, Schell, & Filion, 2007). Respiration traces were inspected to identify cases when electrodermal responding might have been contaminated by deep breaths or excessive movement. No cases of excessive movement were identified and therefore no electrodermal responses were discarded. Electrodermal responses during conditioning were scored in three latency windows in accordance with Prokasy and Kumpfer's (1973) recommendations for scoring electrodermal responding in fear conditioning experiments. The First Interval Responding (FIR) was defined as responses starting within 1-4 seconds of the CS onset and Second Interval Responding (SIR) was defined as responses starting within 4-7 seconds of the CS onset. Responses to the unconditional stimulus were scored during acquisition, as responses starting within 7-10 seconds of the CS+ onset (Prokasy & Kumpfer, 1973). The use of multiple response windows (as opposed to single response) is recommended by Prokasy and Kumpfer (1973) as there is evidence that first interval responding reflects orienting to the conditional stimulus, second interval responding reflects the anticipation of the unconditional stimulus, and the unconditional response window reflects the response to the unconditional stimulus (Lockart, 1966; Stewart, Winokur, Stern, Guze, Pfeiffer, & Hornung, 1959). Moreover, there is evidence that different experimental manipulations will differentially affect first and second interval responding (Prokasy & Ebel, 1967).

During habituation, only FIRs were scored as they reflect orienting to the novel stimuli

(Öhman, 1983) and anticipation of the unconditional stimulus is not expected during this phase. The largest response starting within the latency window was scored and the magnitude of the response was calculated as the difference between response onset and peak (Prokasy & Kumpfer, 1973). 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 unconditional stimulus. In case of multiple responses, the largest response starting within the latency window was scored, regardless of whether the peak of the response was within the same latency window (Prokasy & Kumpfer, 1973). Electrodermal responding and valence ratings were averaged into blocks of two consecutive trials to reduce the influence of trial-by-trial variability. The conditional stimulus valence ratings were scored as the largest voltage deviation from a 1 second pre-stimulus baseline voltage that occurred within the 6 second CS presentation.

First and second interval electrodermal responding and conditional stimulus valence evaluations were subjected to separate 2 × 2 × n (Group [instruction/removal, control]) × CS [CS+, CS-] × Block [habituation = 2, acquisition = 4, extinction = 4]) factorial ANOVAs. As the influence of the instructional manipulation is expected in early extinction, additional 2 × 2 × 2 (Group [instruction/removal, control) × CS [CS+, CS-] × Block [1, 2]) factorial ANOVAs were performed on the electrodermal responding and conditional stimulus valence during early extinction. To examine whether differential responding was still present during the last block of extinction 2 × 2 factorial ANOVAs (Group [instruction/removal, control] × CS [CS+, CS-]) were performed on the electrodermal responding and conditional stimulus valence during the last block of extinction. Unconditional electrodermal responding during acquisition was subjected to a 2 × 4 (Group [instruction/removal, control] × Block [1, 2, 3, 4]) factorial ANOVA. Pre- and post-experimental ratings were subjected to a 2 × 2 × 2 (Group [instruction/removal, control] × CS (CS+, CS-) × Phase [pre- and post-experimental]) factorial ANOVA.

IBM SPSS Statistics 21 was used to conduct all analyses, and the significance level was set at .05. Multivariate F values (Phillai's Trace) and partial eta-squares are reported for all main effects and interactions. Follow-up analyses were conducted using the Bonferroni adjustment

provided by SPSS to protect against the accumulation of α error and adjusted *p* values are reported for these follow-up analyses.

Results

Preliminary Checks. Preliminary analyses revealed no difference between groups in age (instruction/removal: *M* = 21.17 years, *SD* = 4.30 years; control: *M* = 21.71 years, *SD* = 8.91 years), *t*(33) = 0.23, *p* = .820, the number of spontaneous electrodermal responses during the three minute baseline period (instruction/removal: *M* = 20.58 responses, *SD* = 13.26 responses; control: *M* = 21.47 responses, *SD* = 9.81 responses), t(34) = 0.23, *p* = .822, the unconditional stimulus intensity set by the participant (instruction/removal: *M* = 36.32 V, *SD* = 11.28 V; control: *M* = 35.00 V, *SD* = 9.35 V), t(34) = 0.38, *p* = .708, and the rated unconditional stimulus unpleasantness (instruction/removal: *M* = -1.61, *SD* = 1.40; control: *M* = -2.06, *SD* = 0.43), t(34) = 1.28, *p* = .209. The female to male ratio was larger in the control group (13:4) in comparison with the instruction/removal group (7:12), $\chi^2(1) = 5.71$, *p* = .017. Analysis of the unconditional electrodermal responses (responses to the electrotactile stimulus) during acquisition revealed a main effect of block, *F*(3,31) = 25.77, *p* < .001, $\eta p^2 = .714$. Electrodermal responding in block one was significantly higher than in blocks two, *p* < .001, three, *p* < .001, and four, *p* < .001. No analyses involving the factor group reached significance, confirming that the unconditional electrodermal responses did not differ between groups, largest (Block × Group interaction), *F*(3,31) = 0.92, *p* = .441, $\eta p^2 = .082$. Data from one participant in the instruction group who failed to correctly report the experimental contingencies were excluded where appropriate (acquisition, extinction and ratings). The pattern of results is very similar for the full and the reduced sample and both sets of statistics are reported when effects differ in significance. All participants in the instruction/removal group indicated that they believed the instructions.

Electrodermal responses. The first interval electrodermal responses for habituation are presented in the left panel of Figure 1. During habituation, first interval electrodermal responses declined from block one (*M* = 0.29, *SD* = 0.20), to block two (*M* = 0.20, *SD* = 0.20), as indicated by a main effect of block, *F*(1,33) = 10.19, *p* = .003, $\eta p^2 = .236$. All remaining main effects and interactions did not attain significance, largest (main effect of group), *F*(1,33) = 1.02, *p* = .321, $\eta p^2 = .030$.

The first and second interval electrodermal responses during acquisition are summarized

in Figure 1 (middle panel), and Figure 2 (left panel), respectively. During acquisition differential responding between the CS+ and the CS- emerged in both the first and the second interval responses for both groups. Analysis of the first interval responses, revealed a main effect of CS, $F(1,32) = 46.62$, $p < .001$, $\eta p^2 = .593$, a main effect of block, $F(3,30) = 4.41$, $p = .011$, $\eta p^2 = .306$, and a CS × Block interaction, $F(3,30) = 3.78$, $p = .021$, $\eta p^2 = .274$. This interaction confirmed that CS+ and CS- elicited similar levels of responding at block one, $F(1,32) = 1.13$, $p = .295$, $\eta p^2 = .034$, but that responding to CS+ was larger than responding to CS- in blocks two, $F(1,32) = 29.88$, $p < .001$, $\eta p^2 = .483$, three $F(1,32) = 30.86$, $p < .001$, $\eta p^2 = .491$, and four $F(1,32) = 12.98$, $p = .001$, $\eta p^2 = .289$. All other main effects and interactions did not attain significance, largest (main effect of group), $F(1,32) = 1.33$, $p = .257$, $\eta p^2 = .040$.

Analysis of the second interval responses revealed a main effect of CS, $F(1,32) = 16.57$, $p < .001$, $\eta p^2 = .341$, a main effect of group, $F(1,32) = 5.25$, $p = .029$, $\eta p^2 = .141$, a CS × Block interaction, $F(3,30) = 9.37$, $p < .001$, $\eta p^2 = .484$, and a CS × Block × Group interaction, $F(3,30) = 3.52$, $p = .027$, $\eta p^2 = .260$. Follow up analyses revealed that in the control group, responding to CS- was larger than responding to CS+ at block one, $F(1,32) = 8.05$, $p = .008$, $\eta p^2 = .201$. At block two, responses to CS+ and CS- did not differ, $F(1,32) = 0.18$, $p = .674$, $\eta p^2 = .006$, and at blocks three, $F(1,32) = 33.45$, $p < .001$, $\eta p^2 = .511$, and four $F(1,32) = 4.83$, $p = .035$, $\eta p^2 = .131$, CS+ elicited larger responses than CS-. In the instruction/removal group, no difference in responding between CS+ and CS- was detected at blocks one, $F(1,32) = 0.22$, $p = .645$, $\eta p^2 = .007$, or two, $F(1,32) = 1.67$, $p = .206$, $\eta p^2 = .049$, whilst larger responding was elicited by CS+ at blocks three, $F(1,32) = 6.82$, $p = .014$, $\eta p^2 = .176$, and four, $F(1,32) = 4.48$, $p = .042$, $\eta p^2 = .123$. All other main effects and interactions did not attain significance, largest (Block × Group interaction), $F(3,30) = 1.81$, $p = .167$, $\eta p^2 = .153$.

The first and second interval electrodermal responses recorded during extinction are summarized in the right panels of Figures 1 and 2. The differential responding between CS+ and CS-, acquired in first interval responses during acquisition, was not present during extinction in either group. The analyses revealed a main effect of block, $F(3,30) = 11.29$, $p < .001$, $\eta p^2 = .530$, a main effect of group, $F(1,32) = 10.66$, $p = .003$, $\eta p^2 = .250$, and a Block × Group interaction, $F(3,30) = 6.17$, $p = .002$, $\eta p^2 = .382$. Follow up analyses revealed that at blocks one, $F(1,32) = 16.78$, $p < .001$, $\eta p^2 = .344$, and two, $F(1,32) = 13.38$, $p = .001$, $\eta p^2 = .295$, responding was larger

in the control group in comparison with the instruction/removal group, whilst at blocks three, $F(1,32) = 2.95$, $p = .096$, $\eta p^2 = .082$, and four, $F(1,32) = 2.34$, $p = .136$, $\eta p^2 = .068$, the groups did not differ. All other main effects and interactions did not attain significance, largest (CS × Group interaction), $F(1,32) = 1.57$, $p = .220$, $\eta p^2 = .047$.

When the analyses were run only examining block one and two of extinction, a group difference was detected with the control group ($M = 0.25$, $SD = 0.20$) showing larger responding than the instruction/removal group ($M = 0.07$, $SD = 0.10$), as confirmed by a main effect of group, $F(1,32) = 17.09$, $p < .001$, $\eta p^2 = .348$. A main effect of block revealed that responding was larger in block one ($M = 0.19$, $SD = 0.18$) in comparison with block two ($M = 0.13$, $SD = 0.18$), $F(1,32) = 11.61$, $p = .002$, $\eta p^2 = .266$. The remaining main effects and interactions did not reach significance, largest (CS × Block), $F(1,32) = 1.56$, $p = .220$, $\eta p^2 = .047$. This group difference, along with visual inspection of Figure 1, suggested differential responding was eliminated at the beginning of extinction due to increased responding to CS- in the control group and decreased responding to CS+ in the instruction/removal group. To follow-up this observation a 2 × 2 × 2 (Group [instruction/removal, control) × CS [CS+, CS-] × Phase [last block of acquisition, first block of extinction] factorial ANOVA was performed, yielding a main effect of CS, $F(1,32) = 15.56$, $p < .001$, $\eta p^2 = .327$, a main effect of group, $F(1,32) = 8.02$, $p = .008$, $\eta p^2 = .200$ and a Phase × Group interaction, $F(1,32) = 8.64$, $p = .006$, $\eta p^2 = .213$. Follow-up analyses revealed that during the last block of acquisition there was no difference in responding between the instruction/removal and control groups, $F(1,32) = 1.43$, $p = .241$, $\eta p^2 = .043$, but that during the first block of extinction responding in the instruction/removal group was significantly reduced in comparison to the control group, $F(1,32) = 16.78$, $p < .001$, $\eta p^2 = .344$. Although suggested in Figure 1, the CS × Phase × Group interaction did not attain significance, $F(1,32) = 0.02$, $p = .897$, $\eta p^2 = .001$. The remaining main effects and interactions did not reach significance, largest (Phase × CS interaction), $F(1,32) = 3.45$, $p = .072$, $\eta p^2 = .097$. When responses in the last block of extinction were analyzed no main effects or interactions attained significance, largest (main effect of group), $F(1,32) = 2.34$, $p = .136$, $\eta p^2 = .068$, confirming that differential first interval electrodermal responding between the CS+ and the CS- had extinguished in both groups.

Inspection of the right panel of Figure 2 suggests that differential second interval electrodermal responding was present during early extinction in the control group, but not in the

instruction/removal group. Analyses of responses from the entire extinction phase revealed a main effect of block, $F(3,30) = 6.59$, $p = .001$, $\eta p^2 = .397$, a main effect of group, $F(1,32) = 7.76$, $p = .009$, $\eta p^2 = .195$, and a Block × Group interaction, $F(3,30) = 4.36$, $p = .012$, $\eta p^2 = .304$. Follow-up analyses confirmed that responding in the control group was larger than responding in the instruction/removal group during blocks one, $F(1,32) = 10.00$, $p = .003$, $\eta p^2 = .238$, and four, $F(1,32) = 4.40$, $p = .044$, $\eta p^2 = .121$. No differences in responding were detected between the groups during blocks two, $F(1,32) = 1.65$, $p = .208$, $\eta p^2 = .049$, and three, $F(1,32) = 1.18$, $p = .286$, $\eta p^2 = .035$. All other main effects and interactions failed to attain significance, largest (main effect of CS), $F(1,32) = 3.28$, $p = .080$, $\eta p^2 = .093$.

As the influence of the instructional manipulation on differential responding was expected in early extinction, the analyses were run including only blocks one and two. This revealed a main effect of group, $F(1,32) = 7.56$, $p = .010$, $\eta p^2 = .191$, a marginal Block × Group interaction, $F(1,32) = 4.09$, $p = .052$, $\eta p^2 = .113$, a marginal CS × Block interaction, $F(1,32) = 4.11$, $p = .051$ $\eta p^2 = .114$, and a CS × Block × Group interaction, $F(1,32) = 6.23$, $p = .018$, $\eta p^2 = .163$. Follow up analyses revealed that in the control group CS+ elicited larger responses than CS- at block one, $F(1,32) = 10.53$, $p = .003$, $\eta p^2 = .248$, but not at block two, $F(1,32) = 0.31$, $p = .583$, $\eta p^2 = .010$. Conversely, in the instruction/removal group, there was no differential responding at block one, $F(1,32) = 0.09$, $p = .767$, $\eta p^2 = .003$, or block two, $F(1,32) = 0.48$, $p = .493$, $\eta p^2 = .015$. The remaining main effects and interactions did not reach significance, largest (main effect of block), $F(1,32) = 3.63$, $p = .066$, $\eta p^2 = .102$.

When only the last block of extinction was included in the analyses a main effect of group was detected, $F(1,32) = 4.40$, $p = .044$, $\eta p^2 = .121$, which reflected smaller responding in the instruction/removal ($M = 0.05$, $SD = 0.09$) group in comparison with the control group ($M = 0.15$, $SD = 0.23$). The remaining main effects and interactions failed to attain significance confirming that differential second interval responding between CS+ and CS- was no longer present at the end of extinction, largest (main effect of CS), $F(1,32) = 0.09$, $p = .762$, $\eta p^2 = .003$.

Conditional Stimulus Valence Evaluations. The conditional stimulus valence evaluations obtained during habituation (left), acquisition (middle), and extinction (right), for both groups are summarized in Figure 3. Analysis of the valence evaluations recorded during habituation revealed a CS × Block × Group interaction, $F(1,31) = 4.55$, $p = .041$, $\eta p^2 = .128$. The

CS- was rated less pleasant in block one in comparison with block two in the instruction/removal group, $F(1,31) = 4.81$, $p = .036$, $\eta p^2 = .134$. All other comparisons failed to reach significance, largest (control CS+, block one in comparison with block two), $F(1,31) = 1.45$, $p = .237$, $\eta p^2 = .045$. The remaining main effects and interactions did not reach significant, largest (main effect of block), $F(1,31) = 1.11$, $p = .300$, $\eta p^2 = .035$.

At the beginning of acquisition, the pleasantness ratings of CS+ and CS- did not differ but as the experiment progressed CS+ was rated less pleasant than CS- in both groups. A main effect of CS, $F(1,30) = 12.59$, $p = .001$, $\eta p^2 = .296$, and a CS × Block interaction, $F(3,28) = 5.22$, $p = .005$, $\eta p^2 = .359$, confirmed these impressions. Follow up analyses revealed that CS+ and CS- were rated similarly at block one, $F(1,30) = 1.18$, $p = .286$, $\eta p^2 = .038$, but at blocks two, $F(1,30) = 13.07$, $p = .001$, $\eta p^2 = .303$, three, $F(1,30) = 12.57$, $p = .001$, $\eta p^2 = .295$, and four, $F(1,30) = 13.45$, $p = .001$, $\eta p^2 = .310$, CS+ was rated less pleasant than CS-. The remaining main effects and interactions did not reach significant, largest (CS × Block × Group interaction), $F(3,28) = 1.51$, $p = .233$, $\eta p^2 = .139$.

During extinction, both groups gave lower pleasantness ratings to CS+ ($M = -0.83$, $SD = 0.82$) in comparison with CS- ($M = -0.11$, $SD = 0.88$), and both conditional stimuli were rated as more pleasant as extinction progressed. The analyses confirmed these impressions revealing main effects of CS, $F(1,30) = 15.87$, $p < .001$, $\eta p^2 = .346$, and block, $F(3,28) = 5.75$, $p = .003$, $\eta p^2 = .381$. When compared with block one, the evaluations in block two, $p = .013$, and three, $p = .020$, were more pleasant. All other comparisons failed to reach significance, largest (block one in comparison with block four), $p = .088$. All other main effects and interactions did not attain significance, largest (CS × Bock), $F(3,28) = 2.02$, $p = .134$, $\eta p^2 = .178$.

As the influence of the instructional manipulation was expected in early extinction, the analyses were run excluding blocks three and four. This did not change the pattern of results, with analyses revealing a main effect of CS, $F(1,30) = 18.34$, $p < .001$, $\eta p^2 = .379$, and a main effect of block, $F(1,30) = 11.27$, $p = .002$, $\eta p^2 = .273$. The CS × Block × Group interaction was not significant, $F(1,30) = 0.06$, $p = .803$, $\eta p^2 = 002$, confirming that the instructional manipulation did not differentially affect the conditional stimulus valence evaluations. All remaining main effects and interactions did not attain significance, largest (Block × Group), $F(1,30) = 1.88$, $p = .181$, $\eta p^2 = .059$.

To examine whether differential valence ratings were still present at the end of extinction the analyses were re-run including only block four. This revealed a main effect of CS, $F(1,30) = 7.87$, $p = .009$, $\eta p^2 = .208$, which confirmed that across groups CS+ ($M = -0.64$, $SD = 0.91$) was rated as less pleasant than CS- ($M = -0.09$, $SD = 1.01$) during the last block of extinction. The remaining main effects and interactions did not reach significance, largest (main effect of group), $F(1,30) = 0.96$, $p = .335$, $\eta p^2 = .031$.

Pre- and Post-Experimental Pleasantness Ratings. The pleasantness ratings recorded before habituation and after extinction are displayed on the left side of Figure 4. Before habituation, CS+ and CS- received similar pleasantness ratings, however after extinction CS+ was rated less pleasant than CS-. This pattern emerged consistently for both groups. The analyses confirmed these impressions, revealing a Period × CS interaction, $F(1,31) = 10.44$, $p = .003$, $\eta p^2 = .252$. Follow-up analyses revealed that before the experiment, pleasantness ratings of CS+ and CS- did not differ, $F(1,31) = 0.93$, $p = .343$, $\eta p^2 = .029$, but after the experiment CS+ was rated less pleasant than CS-, $F(1,31) = 9.42$, $p = .004$, $\eta p^2 = .233$. All remaining effects did not reach significance, largest (main effect of CS), $F(1,31) = 3.44$, $p = .073$, $\eta p^2 = .100$.

Experiment 2

Method

Participants. Forty-four (26 female) undergraduate students volunteered participation in exchange for course credit. The participants' ages ranged from 16-59 ($M = 22.77$). All participants consented to the experiment and were fully informed. Participants were randomly assigned to one of two groups (instruction/removal, control). Recording error resulted in the loss of two participants' pre-experimental ratings data, four participants' post-experimental ratings data, and one participant's fear potentiated startle data. These participants were included in the analyses of all remaining measures.

Apparatus/Stimuli. Orbicularis oculi electromyography (EMG) was measured using two 4mm Ag/AgCl electrodes, one placed directly underneath the participants' left eye, and another below the corner of the left eye, approximately 1 cm to the left of the first electrode. A reference electrode was placed in the middle of the participants' forehead. All electrodes were fitted with adhesive collars and filled with a standard electrode gel, and impedances were checked to ensure they were lower than 10 kΩ. Orbicularis oculi EMG was recorded using AcqKnowledge Version

3.9.1 with a Biopac MP150 system, at a sampling frequency of 1000 Hz, and an amplification factor of 5000. Raw EMG was bandpass filtered with a low cut-off of 10 Hz and a high cut-off of 500 Hz. Startle blinks were elicited with a 105 dB bursts of white noise lasting 43 milliseconds with an instantaneous rise time, generated by a custom built noise generator and presented through Sennheiser headphones. Startle probes were presented 3.5 seconds or 4.5 seconds after the onset of the conditional stimulus and during the inter-trial intervals, 7 seconds after the conditional stimulus offset and 8 seconds before the onset of the next conditional stimulus. Before any stimulus presentations, three startle probes were presented to habituate startle responding, and to allow for a comparison of baseline startle magnitude between the groups. Two, four, and six startle probes were presented during CS+ and CS- in habituation, acquisition and extinction, respectively. Four probes were presented in the inter-trial interval of habituation, eight in acquisition, and twelve in extinction. During habituation, startle probes were placed in the second and fourth presentation of both the CS+ and the CS-. During acquisition startle probes were placed in the third, fourth, sixth and eighth presentation of the CS-; and the second, fourth, sixth, and eighth presentation of the CS+. During extinction startle probes were placed in the second, fourth, sixth, seventh, tenth and twelfth presentation of the CS-; and the second, fourth, fifth, eighth, tenth and twelfth presentation of the CS+.

Procedure. Eight additional trials (four CS+ and four CS-) were added during extinction, in order to allow sufficient time to examine changes in fear potentiated startle. Counterbalancing and the remainder of the procedure were conducted in the same manner as Experiment 1.

Scoring and Response Definition. Raw EMG was filtered offline (Band stop at 50 Hz followed by a bandpass filter, low cut-off of 30 Hz and a high cut-off of 500 Hz) rectified and smoothed (five point moving average). Blink startle magnitude was defined as the maximum of the rectified and smoothed response curve occurring within 120 milliseconds of the stimulus onset (Blumenthal et al., 2005). A trial was defined as missing if the baseline EMG recorded 50 milliseconds prior to probe onset was judged by visual inspection to be unstable, or if a spontaneous or voluntary blink immediately preceded the startle probe onset. A trial was defined as a non-response trial if no response onset could not be identified within 20-60 milliseconds of probe onset. Blink startle magnitudes elicited during the conditional stimuli were averaged into blocks of two consecutive trials, yielding one block for habituation, two blocks for acquisition and three blocks in extinction. Using all startles measured during conditioning as the reference

distribution, T-scores were calculated to reduce the impact of individual differences.

Startle magnitudes were subjected to separate 2 × 2 × n (Group [instruction/removal, control] × CS [CS+, CS-] × Block [habituation = 1, acquisition = 2, extinction = 3]) factorial ANOVAs. The remaining analyses were conducted in the same manner as for Experiment 1.

Results

Preliminary Checks. No differences between the groups were detected for age (instruction/removal: *M* = 22.77 years, *SD* = 9.82; control: *M* = 22.36 years, *SD* = 6.07), $t(42) = 0.17$, $p = .869$, gender (instruction/removal: 8 male:14 female; control: 10:12), $\chi^2(1) = 0.38$, $p = .540$, or the magnitude of the blink startle responses elicited during the baseline period (instruction/removal: *M* = 190, *SD* = 118; control: *M* = 190 , *SD* = 132), $t(40) = 0.06$, $p = .954$. The unconditional stimulus intensity level set (instruction/removal: *M* = 32.91 V, *SD* = 7.28 V; control *M* = 31.68 V, *SD* = 10.97 V), $t(42) = 0.44$, $p = .664$, and the rated unconditional stimulus unpleasantness level (instruction/removal: *M* = -1.82, *SD* = 0.66; control *M* = -1.86, *SD* = 0.71), $t(42) = 0.22$, $p = .828$, were similar in both groups.

Two participants in the control group, and one participant in the instruction/removal group failed to correctly identify which face had been paired with the unconditional stimulus. One participant in the instruction/removal group reported not believing the instructional manipulation. Data for these participants were removed from the analyses where appropriate (non-verbalizers from acquisition and extinction, non-believer from extinction, non-verbalizers and non-believer from ratings). The pattern of results is very similar for the reduced and the full sample and both sets of statistics have been reported only when effects differ in significance.

Fear Potentiated Startle. The magnitude of the blink startle responses recorded during habituation (left), acquisition (middle), and extinction (right) are summarized in Figure 5. In habituation, there were no differences in startle magnitude during CS+ and CS-, or between the groups, largest (CS × Group), $F(1,41) = 0.22$, $p = .641$, $\eta p^2 = .005$. During acquisition, fear potentiated startle magnitude was larger during presentations of CS+ (*M* = 55.42, *SD* = 7.64), in comparison with presentations of CS- (*M* = 50.66, *SD* = 7.05), and fear potentiated startle magnitude decreased from block one (*M* = 55.65, *SD* = 7.91), to block two (*M* = 50.43, *SD* = 6.77). The analyses confirmed these impressions yielding main effects of CS, $F(1,37) = 15.22$, $p < .001$, $\eta p^2 = .291$, and block, $F(1,37) = 18.81$, $p < .001$, $\eta p^2 = .337$. The remaining main effects

and interactions did not attain significance, largest (main effect of group), $F(1,37) = 2.60$, $p = .116$, $\eta p^2 = .066$.

During extinction, startle magnitude decreased with time, as confirmed by a main effect of block, $F(2,33) = 9.73$, $p < .001$, $\eta p^2 = .371$. Follow up analyses revealed that startle magnitude was larger during block one, in comparison with blocks two, $p = .001$, and three, $p = .001$, but that startle magnitude in bocks two and three did not differ, $p > .999$. A marginal Block × Group interaction, $F(2,33) = 3.22$, $p = .053$, $\eta p^2 = .163$, revealed that responding in the control group differed significantly between blocks one and two, $p < .001$, and blocks one and three, $p = .006$, whereas responding in the instruction/removal group only differed marginally between blocks one and three, $p = .052$. The remaining main effects and interactions did not attain significance, largest (main effect of CS), $F(1,34) = 2.85$, $p = .100$, $\eta p^2 = .077$.

As the influence of the instructional manipulation was expected in early extinction, the analyses were run excluding blocks two and three. This revealed a CS × Group interaction, $F(1,37) = 4.84$, $p = .034$, $\eta p^2 = .116$ (full sample: $F(1,41) = 3.90$, $p = .055$, $\eta p^2 = .087$). Follow up analyses revealed that, in the control group, startle magnitude was larger during CS+ than during CS-, $F(1,37) = 4.54$, $p = .040$, $\eta p^2 = .109$ (full Sample: $F(1,41) = 4.34$, $p = .043$, $\eta p^2 = .096$), but that no difference was present in the instruction/removal group, $F(1,37) = 0.94$, $p = .339$, $\eta p^2 = .025$ (full Sample: $F(1,41) = 0.48$, $p = .492$, $\eta p^2 = .012$). The remaining main effects and interactions did not attain significance, largest (main effect of group), $F(1,37) = 2.62$, $p = .114$, $\eta p^2 = .066$. Examining the last block of extinction revealed that across groups responding to CS+ ($M = 46.64$, $SD = 5.20$) was still marginally larger than responding to CS- ($M = 44.43$, $SD = 5.02$), as confirmed by a marginal main effect of CS, $F(1,36) = 3.32$, $p = .077$, $\eta p^2 = .084$. The remaining main effects and interactions did not reach significance, largest (main effect of group), $F(1,36) = 0.97$, $p = .332$, $\eta p^2 = .026$.

Conditional Stimulus Valence Evaluations. The conditional stimulus valence evaluations recorded during habituation (left), acquisition (middle), and extinction (right) are summarized in Figure 6. During habituation, no significant differences were detected between the groups or between the conditional stimuli, largest (Block × Group), $F(1,42) = 3.31$, $p = .076$, $\eta p^2 = .073$. During acquisition, a main effect of CS was detected, $F(1,39) = 8.23$, $p = .007$, $\eta p^2 = .174$, and a CS × Block interaction, $F(3,37) = 6.04$, $p = .002$, $\eta p^2 = .329$ (full sample: $F(3,40) =$

2.65, $p = .062$, $\eta p^2 = .166$). Follow-up analyses revealed that CS+ and CS- were given similar pleasantness evaluations at block one, $F(1,39) = 1.62$, $p = .211$, $\eta p^2 = .040$ (full sample: $F(1,42) = 2.67$, $p = .110$, $\eta p^2 = .060$). At block two, CS+ was given marginally less pleasant evaluations than CS-, $F(1,39) = 2.90$, $p = .097$, $\eta p^2 = .069$ (full sample: $F(1,42) = 4.63$, $p = .037$, $\eta p^2 = .099$), and in blocks three, $F(1,39) = 9.50$, $p = .004$, $\eta p^2 = .196$ (full sample: $F(1,42) = 11.31$, $p = .002$, $\eta p^2 = .212$), and four, $F(1,39) = 14.65$, $p < .001$, $\eta p^2 = .273$ (Full sample: $F(1,42) = 14.51$, $p < .001$, $\eta p^2 = .257$), CS+ was rated less pleasant than CS-. The remaining main effects and interactions did not attain significance, largest (main effect of block), $F(3,37) = 1.44$, $p = .247$, $\eta p^2 = .104$.

Analyses of the extinction phase, revealed a main effect of CS, $F(1,37) = 4.74$, $p = .036$, $\eta p^2 = .114$, confirming that CS+ ($M = -0.82$, $SD = 0.95$), continued to be rated as less pleasant than CS- ($M = -0.48$, $SD = 0.91$). A marginal main effect of block was detected, $F(5,33) = 2.33$, $p = .065$, $\eta p^2 = .261$ (full sample: $F(5,37) = 2.62$, $p = .040$, $\eta p^2 = .262$), revealing that evaluations in blocks one, $p = .094$, and two, $p = .055$, were marginally more pleasant than evaluations in block five, and that evaluations in block two were more pleasant than evaluations in block four, $p = .040$. The remaining comparisons failed to reach significance, largest (block one in comparison with block six), $p = .114$. The remaining main effects and interactions failed to reach significance, largest (CS × Block interaction), $F(5,33) = 1.90$, $p = .121$, $\eta p^2 = .224$.

To assess the influence of the instructional manipulation in early extinction, the analyses were re-run including only blocks one and two. This revealed a main effect of CS $F(1,38) = 12.15$, $p = .001$, $\eta p^2 = .242$, and a CS × Block interaction $F(1,38) = 5.98$, $p = .019$, $\eta p^2 = .136$. Follow up analyses revealed that during block one, CS+ was rated marginally less pleasant than during block two, $F(1,38) = 3.03$, $p = .090$, $\eta p^2 = .074$, whilst the pleasantness evaluations did not differ between blocks for CS-, $F(1,38) = 2.34$, $p = .135$, $\eta p^2 = .058$. The CS × Block × Group interaction was not significant, $F(1,38) = 0.01$, $p = .938$, $\eta p^2 < .001$, confirming that the instructional manipulation did not differentially affect the conditional stimulus valence evaluations. The remaining main effects and interactions did not reach significance, largest (Block × Group), $F(1,38) = 3.08$, $p = .087$, $\eta p^2 = .075$. When this marginal Block × Group interaction is followed-up no comparisons reach significance, largest (control group: block one in comparison with block two), $F(1,38) = 2.06$, $p = .159$, $\eta p^2 = .052$. When only the last block of extinction was included in the analyses no main effects or interactions attained significance,

largest (main effect of CS), $F(1,37) = 2.25$, $p = .142$, $\eta p^2 = .057$, confirming that differential ratings of the CS+ and the CS- had extinguished in both groups.

Pre- and Post-Experimental Pleasantness Ratings. The right panel of Figure 4 summarizes the pleasantness ratings recorded before habituation and after extinction. Analyses revealed a main effect for CS, $F(1,33) = 5.50$, $p = .025$, $\eta p^2 = .143$, and a marginal Period × CS interaction, $F(1,33) = 3.19$, $p = .083$, $\eta p^2 = .088$ (full sample: $F(1,37) = 4.69$, $p = .037$, $\eta p^2 = .112$). Follow-up analyses revealed that before the experiment, pleasantness ratings of CS+ and CS- did not differ, $F(1,33) = 0.34$, $p = .564$, $\eta p^2 = .010$ (full sample: $F(1,37) = 0.47$, $p = .499$, $\eta p^2 = .012$), but after the experiment CS+ was rated less pleasant than CS-, $F(1,33) = 5.91$, $p = .024$, $\eta p^2 = .145$ (full sample: $F(1,37) = 7.95$, $p = .008$, $\eta p^2 = .177$). The remaining main effects and interactions did not attain significance, largest (CS × Group), $F(1,33) = 1.50$, $p = .230$, $\eta p^2 = .043$.

Discussion

Experiments 1 and 2 aimed to assess the influence of instructed extinction and removal of the shock electrode on electrodermal responses, fear potentiated startle, and conditional stimulus valence during differential fear conditioning. In Experiment 1, instructed extinction and removal of the shock electrode resulted in the elimination of differential second interval electrodermal responding, but did not affect conditional stimulus valence evaluations. This pattern of results was replicated and extended in Experiment 2. Instructed extinction and removal of the shock electrode eliminated differential startle modulation at the beginning of extinction, whilst, differential valence evaluations were not affected.

The current findings suggest that instructed extinction and removal of the shock electrode results in the immediate decline of differential physiological responding, but does not affect indices of conditional stimulus valence. As modulation of the startle reflex is not under conscious control (Lang, Bradley, & Cuthbert, 1990), the results of the physiological measures used in Experiment 2 are unlikely to reflect demand characteristics. Conversely, subjective valence ratings are susceptible to the effects of demand characteristics (Mitchell, Anderson, & Lovibond, 2003), as they are under the participants' conscious control. To ensure that the findings of Experiments 1 and 2 reflect a true dissociation between physiological measures and conditional stimulus valence an explanation of the current results based on demand

characteristics should be excluded.

Experiment Three

Demand characteristics can influence the outcome of an experiment when the participants can correctly infer the experimental hypotheses and desire to respond according to them (Mitchell et al., 2003). The participants in Experiments 1 and 2 might have hypothesized that consistent differential responding throughout the experiment was expected and therefore continued to differentially rate the conditional stimuli throughout extinction. If so, the results obtained could reflect demand characteristics rather than a failure of instructed extinction to affect conditional stimulus valence.

Cacioppo, Marshall-Goodell, Tassinary, and Petty (1992) developed a method to assess demand characteristics explicitly in a separate sample of participants. To determine whether the participants might have been able to infer the experimental hypothesis, and respond accordingly, they asked participants to read a detailed description of a particular experiment and predict its outcome. They argued that a demand characteristic explanation would be implausible if the participants were not able to predict the results of the prior experiment. In Experiment 3, we utilized this methodology to examine whether the results of the Experiments 1 and 2 could reflect demand characteristics.

Method

Participants. Sixty-three (56 female; age range: 17-42; M = 20.54) undergraduate students who had not participated in Experiments 1 or 2 volunteered participation in exchange for course credit and provided informed consent.

Demand Questionnaire Measure. The demand characteristic questionnaire is shown in Appendix 1. The questionnaire consisted of a description of the acquisition and extinction phase of the instructed extinction experiment, as well as a series of questions requiring the participants to predict the results of the experiment. Heart rate was chosen as an example of a physiological response as it seemed more familiar than electrodermal responding or fear potentiated startle to a first year undergraduate sample.

Procedure. Participants were instructed to read the descriptions and questions carefully and to answer as if they were trying to predict the outcome of the study.

Questionnaire Scoring. The responses to each question were examined and coded by the first author as describing either an increase, a decrease, or no change. For example, a response like 'the pleasantness rating will drop' would be recorded as a 'decrease'; a response like 'the physiological responses will increase in response to the face paired with the shock' would be recorded as an 'increase'; and a responses like 'I don't think the pleasantness rating of the CS- will change' was recorded as a 'no change'. If the participant's response could not be categorized into one of the three response categories it was recorded as missing. For each question, the results were calculated as a percentage of people who predicted each outcome.

Results

Demand Questionnaire Responses. The predictions obtained from the demand characteristic questionnaire are displayed in Table 1. In the acquisition scenario, the most common pattern of results reported was that the CS+ would become more unpleasant, and elicit larger physiological responses throughout acquisition; whereas the CS- would become more pleasant, and result in reduced physiological responses throughout acquisition. In the instructed extinction scenario, the most common pattern of results reported was that on the first trial of extinction, the physiological responses to both the CS+ and the CS- would not change, whereas the evaluations of CS+ would increase in pleasantness, and the evaluations of CS- would stay the same.

Discussion

Experiment 3 aimed to assess whether the findings of Experiments 1 and 2 reflect on demand characteristics. The method used by Cacioppo et al. (1992) was implemented asking a separate sample of participants to predict the outcome of the experiment after reading a detailed description of the instructed extinction procedure. The majority of participants predicted that the physiological responding would not change, but that the ratings of the CS+ would become more pleasant on the first trial after the instructional manipulation. That is, they predicted a dissociation between the physiological indices of fear learning and conditional stimulus valence in the opposite direction to that observed in Experiments 1 and 2, suggesting that the results of Experiments 1 and 2 are unlikely to reflect on demand characteristics.

General Discussion

The current study examined the effect of instructed extinction and removal of the shock electrode on physiological indices of human fear learning and conditional stimulus valence. Instructed extinction resulted in the immediate elimination of differential second interval electrodermal responding (Experiment 1) and differential startle magnitude (Experiment 2) in the instruction/removal group, while differential responding remained intact at the beginning of extinction in the control group. In both experiments conditional stimulus valence ratings did not respond to instructed extinction as shown by continued differential ratings between CS+ and CS- in both groups at the beginning of extinction. This is to our knowledge the first study showing that instructed extinction has no effect on conditional stimulus valence in a differential fear conditioning paradigm, whilst simultaneously showing an effect on the physiological indices of human fear learning. This pattern of results replicates previous instructed extinction studies (Mallan et al. 2009; Rowles et al., 2012) and suggests that conditional stimulus valence is not responsive to instructed extinction in a fear conditioning paradigm.

Visual inspection of Figure 1 suggests that instructed extinction affected differential first interval electrodermal responding in both participant groups, although in a different manner. In the instruction/removal group responding to the CS+ seems to decrease from the last block of acquisition to the first block of extinction, whereas in the control group, responding to CS- seems to increase from the last block of acquisition to the first block of extinction. The expected Phase × CS × Group interaction, was not significant, however, at the beginning of extinction differential first interval electrodermal responding was not present in either group, with a group difference confirming larger overall responding in the control group. Rowles et al. (2012) reported a similar increase in first interval responding to the CS- in the control group during early extinction. It is likely that increased responding to the CS- in the control group reflects sensitization of the orienting reflex to CS- due to the interaction with the experimenter, an effect not seen in the instruction/removal group as they were provided with safety information. The expected Group × CS interaction was evident in second interval responding which is less affected by orienting and more selectively sensitive to unconditional stimulus anticipation. This differential pattern of results across response windows supports the notion of using separate latency windows when scoring electrodermal responding (Prokasy & Kumper, 1973).

The effect of verbal instruction and removal of the shock electrode on differential fear learning as indexed by fear potentiated startle was significant in the reduced sample from which

data of four participants had been removed, three who failed to report the experimental contingency and one who did not believe the instructions. Exclusion of participants who fail to provide evidence of learning in a differential fear conditioning paradigm or fail a manipulation check is common in human fear conditioning research and the fact that a similar pattern of results emerged when these participants were retained speaks to the robustness of the results. Analysis of the full sample yielded a marginally significant Group × CS interaction ($p = .055$), and follow-up analyses revealed that startle magnitude was larger during CS+ than during CS- in the control group, but not in the instruction/removal group. One may argue that measures of contingency awareness taken after extinction do not provide a true reflection of conditioning during acquisition and underestimate the learning present at that stage (Shanks & St John, 1994). However, given the inclusion of a continuous conditional stimulus valence assessment and the focus on the effects of instructions provided after acquisition, the post-extinction measure of contingency awareness seemed to most appropriate way to tap this information in the current procedure (Dawson & Reardon, 1973).

As conditional stimulus evaluations can be susceptible to demand characteristics, we explicitly assessed participants' predictions of the experimental results in Experiment 3. After reading a detailed description of the study, the majority of participants predicted that instructed extinction would affect the conditional stimulus evaluations, but not physiological responding. As this prediction is not consistent with the pattern of results observed in Experiments 1 and 2, it seems unlikely that these results reflect on demand characteristics. It is possible that the demand characteristics of the participants predicting the outcome of a study they read about may differ from those of participants who are actually in the experimental situation. However, it seems unlikely that the demand characteristics developed in the latter group would be opposite to those developed in the former. The demand questionnaire was scored by the first author who was aware of the experimental aim and this may have biased the scoring. To minimize any bias, response options were determined before scoring and responses that did not align clearly with these predetermined options were scored as missing.

The current findings suggest that the negative valence acquired during fear conditioning is not responsive to cognitive interventions, a finding with significant clinical importance as cognitive interventions are commonly used in treatments of anxiety disorders. If persisting negative valence does drive return of fear as proposed by Kerkhof et al. (2009) and suggested in

the data of Hermans et al. (2005) then the current findings highlight the importance of using extended extinction training to reduce negative valence of the feared stimulus. Conditional stimulus valence has been shown to resist extinction in comparison with physiological indices of human fear learning, however extended extinction training can be effective at reducing differential valence ratings (Lipp, Oughton & LeLievre, 2003). This is supported by the finding that significant differential valence evaluations between CS+ and CS- were still present at the end of extinction in Experiment 1, but not in Experiment 2 which utilized a larger number of extinction trials.

The current study highlights the importance of future research to identify ways in which conditional stimulus valence can be effectively reduced. Although the current study provides evidence that conditional stimulus valence is not sensitive to verbal instructions that target the stimulus contingencies, instructions that target the valence of conditional or unconditional stimuli may effectively reduce the negative valence acquired by the conditional stimulus. Future research should examine whether instructions aimed at increasing the valence of the CS+ without any reference to the unconditional stimulus can affect the valence of the CS+. Consistent with this idea, past research on evaluative conditioning has shown that changing the affective value of an unconditional stimulus will change the affective valence of a CS+ that was associated with it (US re-valuation; Baeyens, Eelen, Van den Bergh, & Crombez, 1992).

Like a number of previous studies, the current research combined verbal instruction with removal of the shock electrode to implement the instructed extinction manipulation. This was done to reduce the number of participants who did not believe the instructions but renders it impossible to attribute any change in conditional responding to the provision of verbal information alone. It speaks, however, to the robustness of the differential valence evaluations which were maintained even though presentation of further unconditional stimuli was impossible. Future research should examine whether the presence of the electrode influences the effect of the verbal manipulation as one could argue that it increases participants' arousal. We would predict that retaining the stimulus electrode will not alter the effect of instructed extinction on conditional stimulus valence, but may influence the physiological indices of fear learning.

Past research has shown that physiological indices of emotion are critically dependent on emotional arousal (Bradley, Codispoti, Cuthbert, & Lang, 2001; Lang, Greenwald, Bradley, &

Hamm, 1993). Electrodermal responses are enhanced to arousing emotional stimuli, regardless of valence and the affect startle effect, startle facilitation during unpleasant stimuli and inhibition during pleasant stimuli, is observed if the stimuli are arousing, but not if they are low in arousal (Cuthbert, Bradley, & Lang, 1996). Thus, it may be that verbal instruction and removal of the shock electrode reduced arousal sufficiently to eliminate differential physiological responses while leaving self-reported valence unaffected. It should be noted that no evidence in support of this explanation was found when analyzing the tonic level of electrodermal activity one second prior to conditional stimulus onset. Instructed extinction and removal of the shock electrode did not differentially affect this index of general arousal, however, it may be that the manipulation did affect stimulus specific arousal rather than general arousal levels.

The arousal explanation offered above can be assessed utilizing an instructed counter-conditioning procedure. Rather than advising participants that no more unconditional stimuli will be presented, counter-conditioning involves the instruction that from now on the unconditional stimulus will be presented after the CS-. This manipulation should maintain the general level of arousal as well as the arousal level associated with one of the conditional stimuli. Extrapolating from the current results, we would predict that after instructed counter-conditioning electrodermal responses and fear potentiated startle will be enhanced during the CS-, whereas the CS+ would retain its negative valence and counter-conditioning trials would be required to alter this.

Regardless of the outcome of the future studies described above, the current results have significant practical implications. They suggest that even in the analogue procedure implemented in the laboratory, physiological indices of fear learning respond well to cognitive interventions but that negative valence towards a feared stimulus is durable and may resist cognitive intervention. As suggested by Kerkhof et al. (2009) this residual negative valence may play a critical role in the return of fear after treatment. To elaborate – it may be that after successful treatment of an anxiety disorder, the negative conditional stimulus valence comes to the fore again once a client is placed in a high arousal situation or faced with isolated presentations of aversive stimuli. It may well be that persistent negative valence provides a pathway for the return of fear.

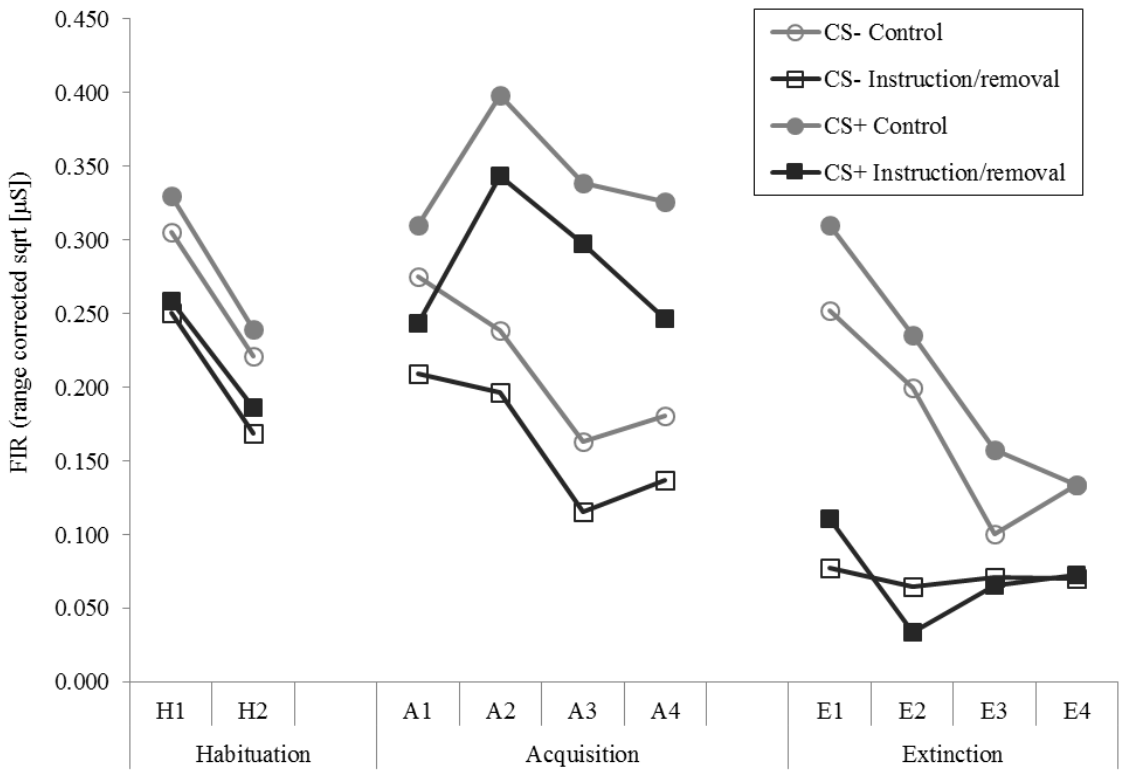

Figure 1. Mean electrodermal FIRs, for instruction/removal and control groups during habituation, acquisition, and extinction.

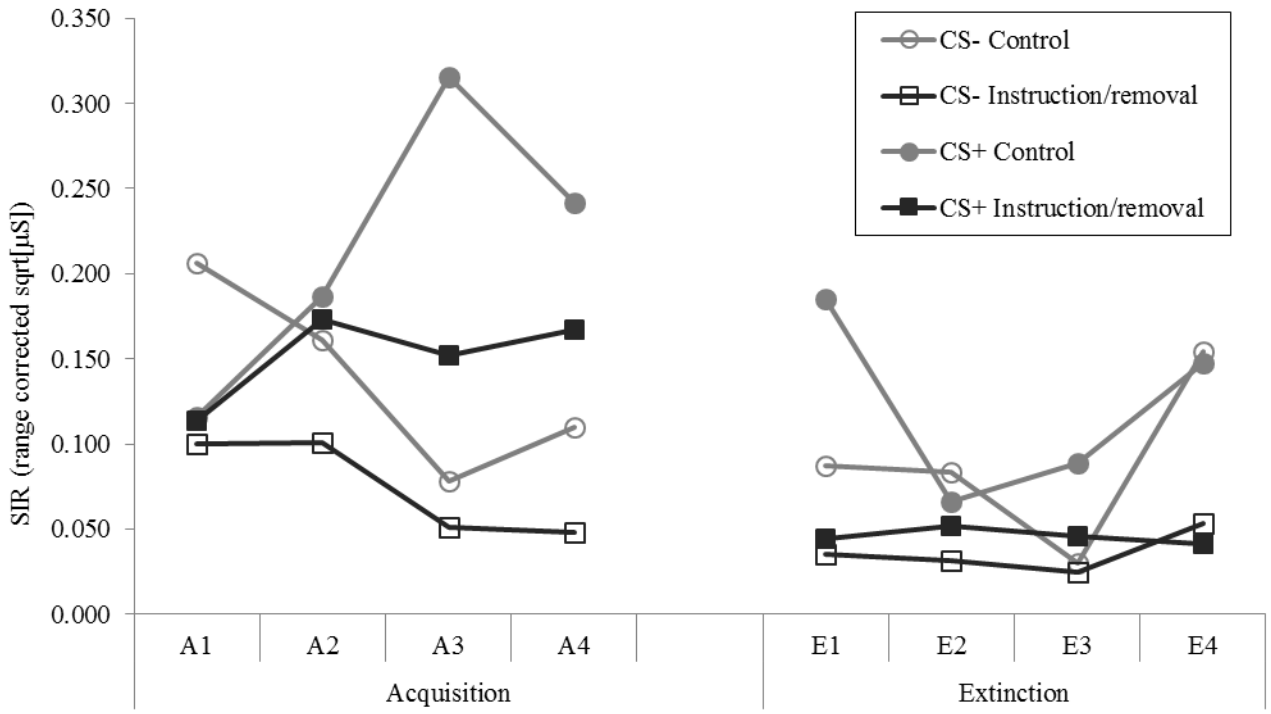

Figure 2. Mean electrodermal SIRs, for instruction/removal and control groups during acquisition, and extinction.

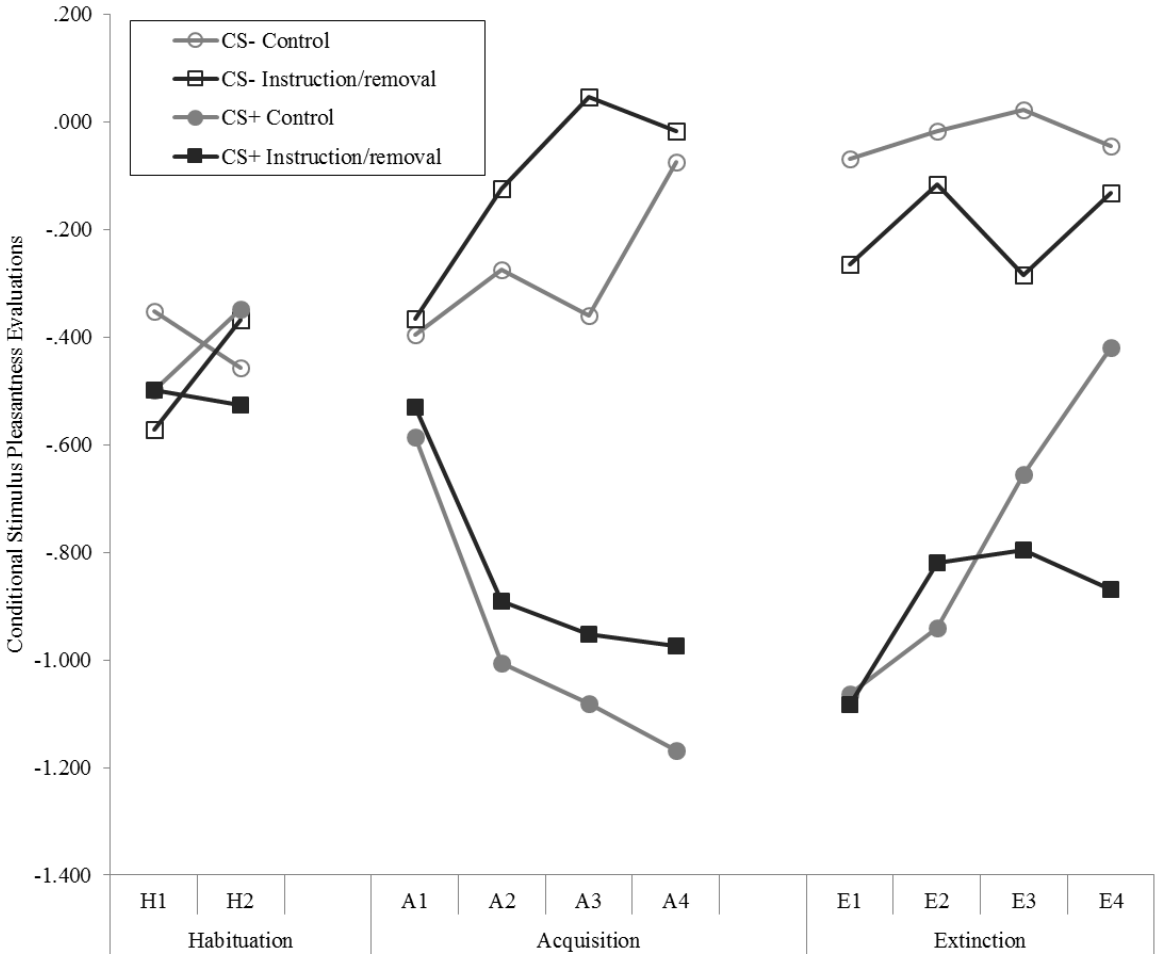

Figure 3. Conditional stimulus evaluations for instruction/removal and control groups during habituation, acquisition, and extinction.

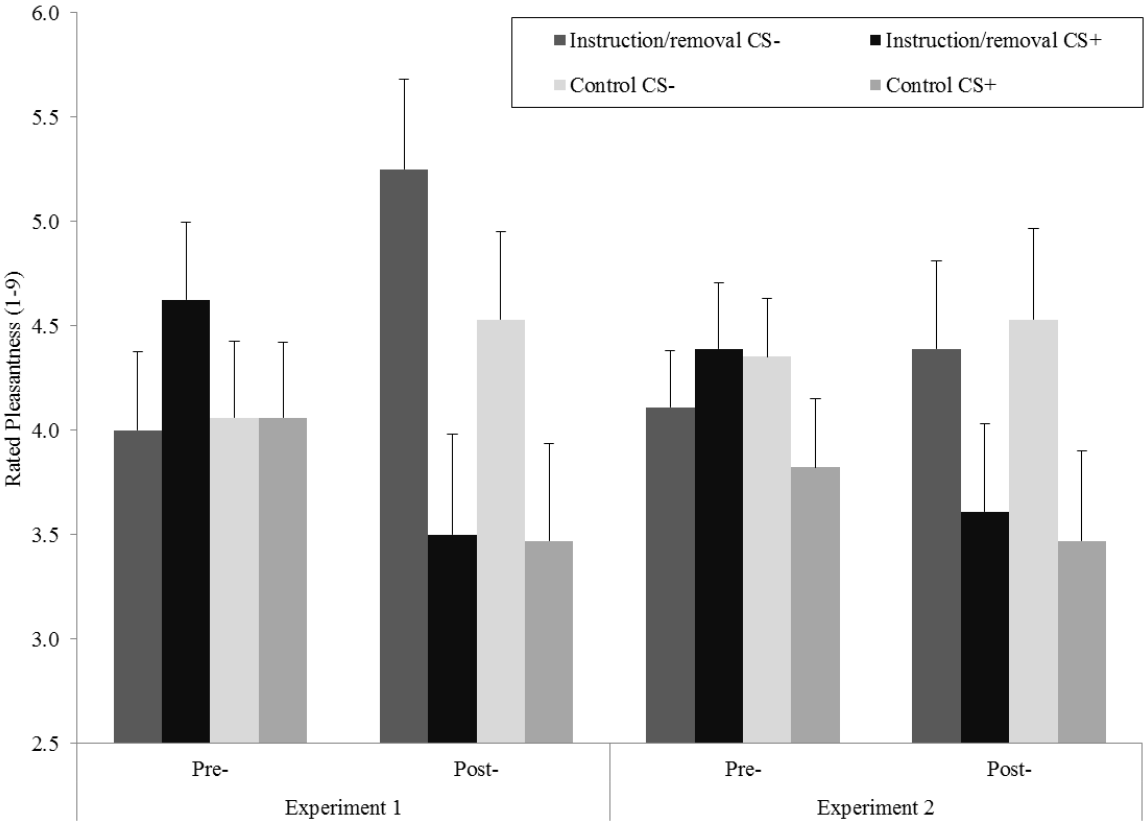

Figure 4. Pleasantness ratings collected pre- and post-experimentally for instruction/removal and control groups in Experiment 1 and 2 (error bars indicate standard errors of the mean).

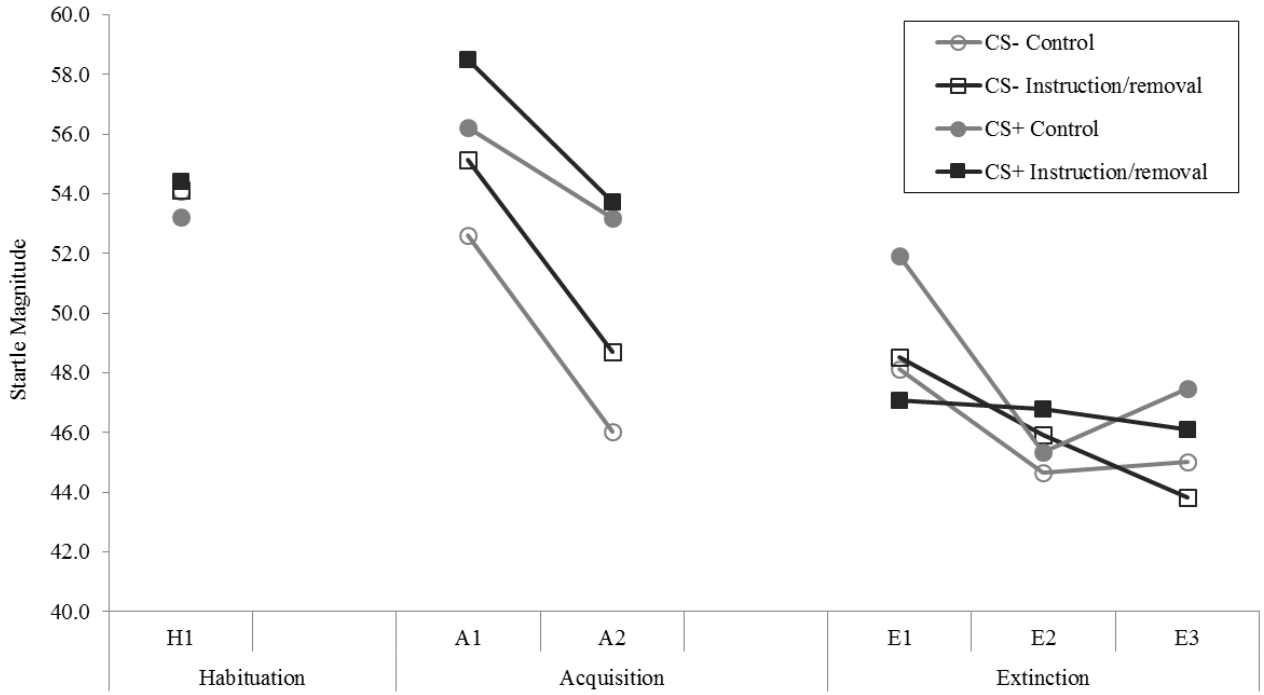

Figure 5. Startle magnitude elicited during habituation, acquisition, and extinction for instruction/removal and control groups.

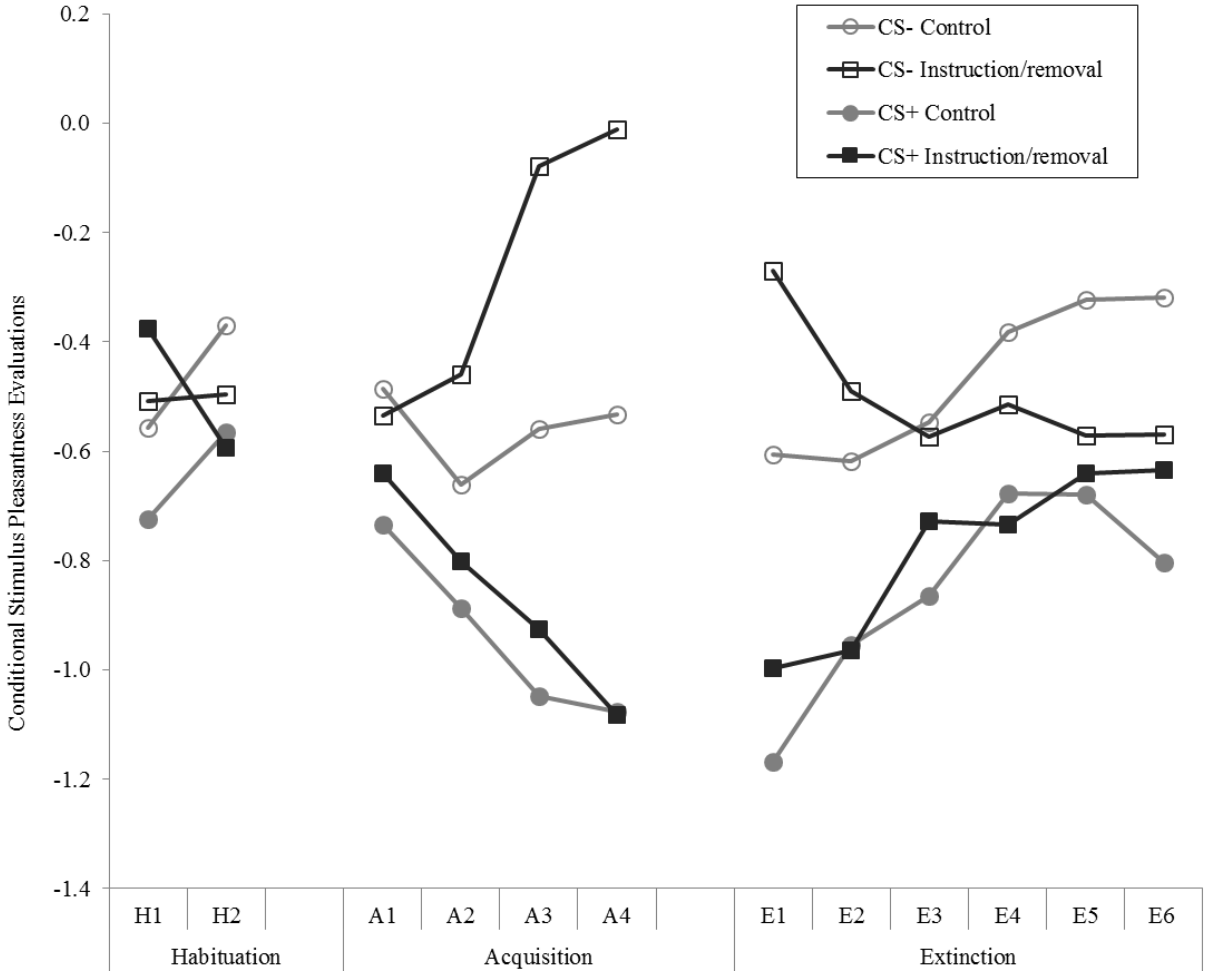

Figure 6. Conditional stimulus evaluations for instruction/removal and control groups during habituation, acquisition, and extinction.

Appendix 1. Demand Characteristics Questionnaire

Please read the following description carefully and answer the questions:

An experimenter is conducting a fear learning experiment looking at how associations are formed between different stimuli. The participant views repeated presentations of two different faces throughout the experiment. On each presentation one of them is followed by an unpleasant (but not painful) electric stimulus, and the other is presented alone. As a measure of fear, physiological responses (e.g. heart rate) to the faces are recorded throughout the experiment. The participant is also required to rate how they feel about the faces every time they are shown on the screen (i.e. whether they perceive the face as pleasant, unpleasant or neutral).

1. How do you think the **physiological responses** to the face *paired with the electric stimulus* will develop across the experiment*?*
2. How do you think the **physiological responses** to the *face presented alone* will develop across the experiment?
3. What do you think will happen to the participants' **pleasantness ratings** to the face *paired with the electric stimulus* throughout the experiment?
4. What do you think will happen to the participants' **pleasantness ratings** to the face *presented alone* throughout the experiment?

Halfway through the experiment, the experimenter informs the participant that the electric stimulus will no longer be presented, but that they will continue to view and rate the same two faces for the remainder of the experiment.

5. The first time the participants view the face that was previously *paired with the electric stimulus* after receiving the instructions, do you think their **physiological responses** will change?
6. The first time the participants view the face that was previously *presented alone* after receiving the instructions, do you think their **physiological responses** will change?
7. The first time the participants view the face that was previously *paired with the electric stimulus* after receiving the instructions, what do you think will happen to the **pleasantness rating** of the face?
8. The first time the participants view the face that was previously *presented alone* after receiving the instructions, what do you think will happen to the **pleasantness rating** of the face?

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Chapter 4. Paper 3 – To Remove or not to Remove? Removal of the Unconditional Stimulus Electrode does not Mediate Instructed Extinction Effects

Luck, C. C., & Lipp, O. V. (2015). To remove or not to remove? Removal of the unconditional stimulus electrode does not mediate instructed extinction effects. *Psychophysiology*, *52*, 1248-1256. doi:10.1111/psyp.12452

To remove or not to remove?

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

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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, while 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- (De Houwer, Thomas, & Baeyens, 2001; Lipp, 2006). 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, while 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 (2012) study, 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 reintroduction 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+ versus 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 a 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 that may affect differential physiological responding as physiological indices of positive and negative emotions are enhanced in response to high arousal stimuli (Cuthbert, Bradley, & Lang, 1996; Lang, Greenwald, Bradley, & Hamm, 1993). 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, while 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 pseudorandom 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 while 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 US 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 midway 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 any longer. Participants in the instruction/electrode off group were informed that they would not receive the electrotactile stimulus any longer 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 was defined as responses starting within 1-4 seconds of CS onset and second interval responding 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 US 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, 1987; Hoaglin, Iglewicz, & Tukey, 1986). 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, ηp^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 pseudorandom 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 *ps* > .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 *ps* > .999, and in the electrode off group, evaluations did not differ between blocks one and two, $p > .999$, while 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 *ps* < .043, and no differences between the groups at the beginning of extinction all *ps* > .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 reemergence 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 (Gast & De Houwer, 2013, Experiment 2; Lipp, Mallan, Libera, & Tan, 2010). 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 nonoccurrence 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.

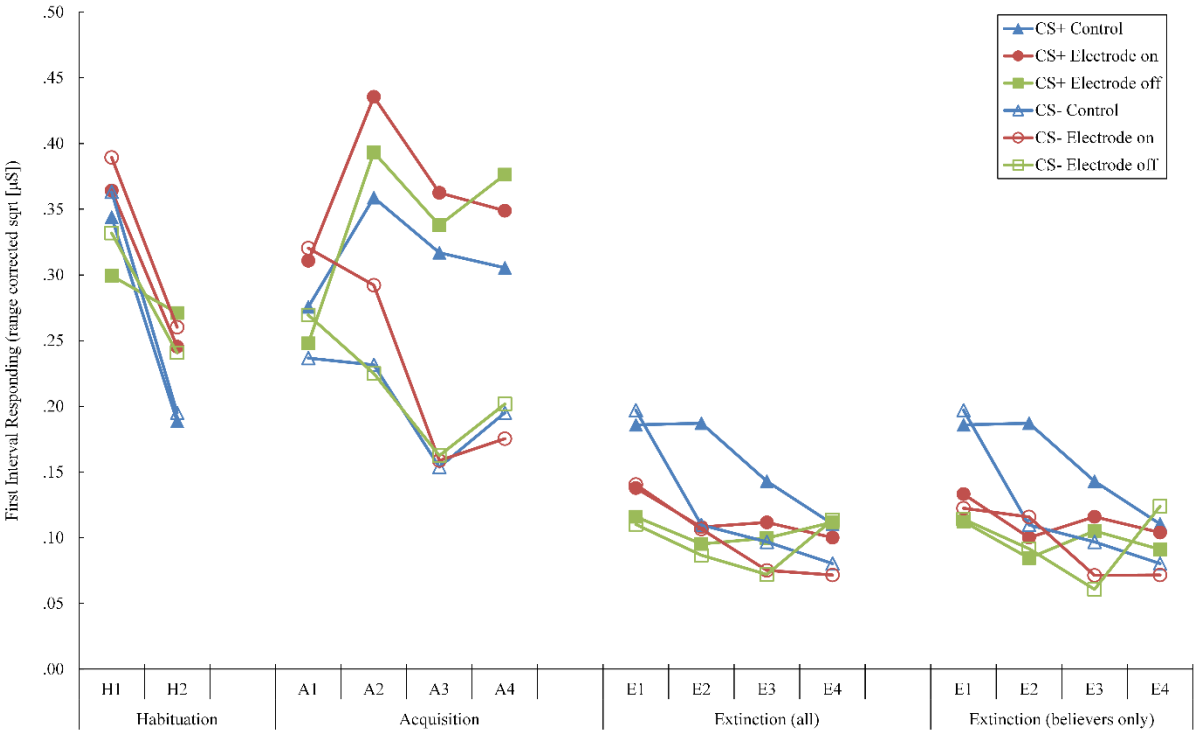

Figure 1. Mean first interval electrodermal responding during habituation, acquisition, and extinction. The fourth panel shows only responses from the participants who reported believing the instructions.

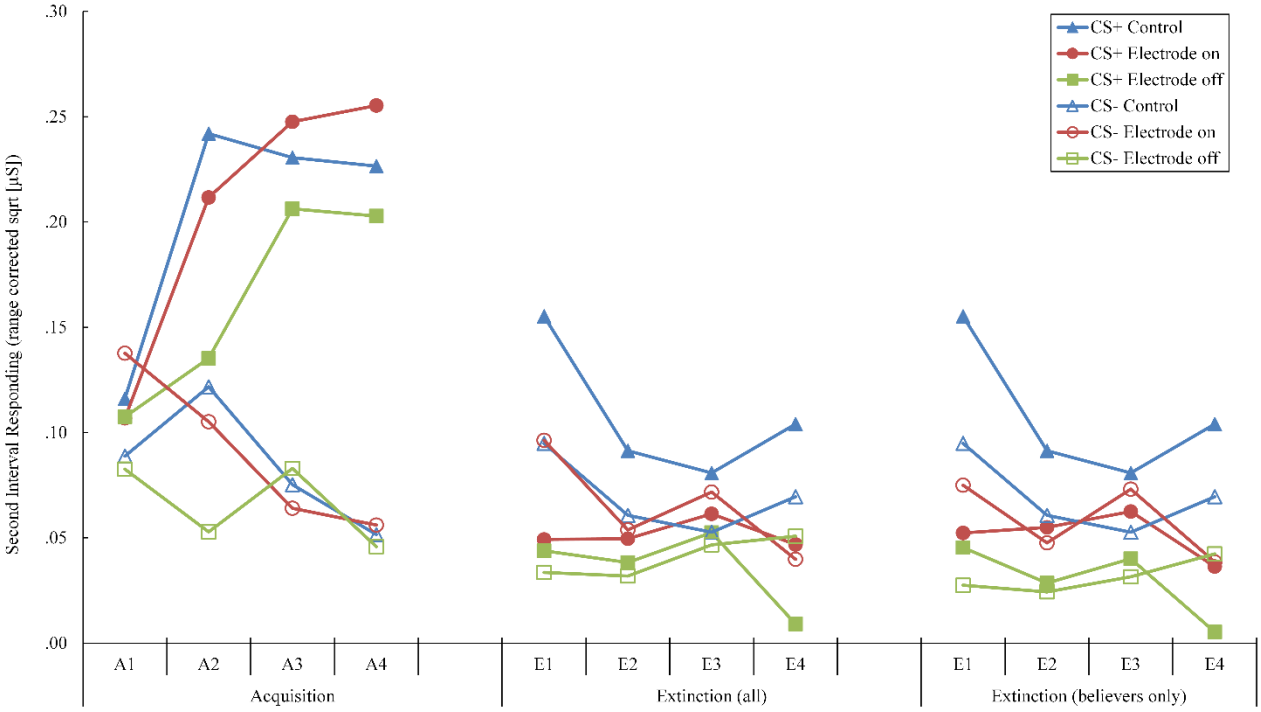

Figure 2. Mean second interval electrodermal responding during acquisition and extinction. The third panel shows only responses from the participants who reported believing the instructions.

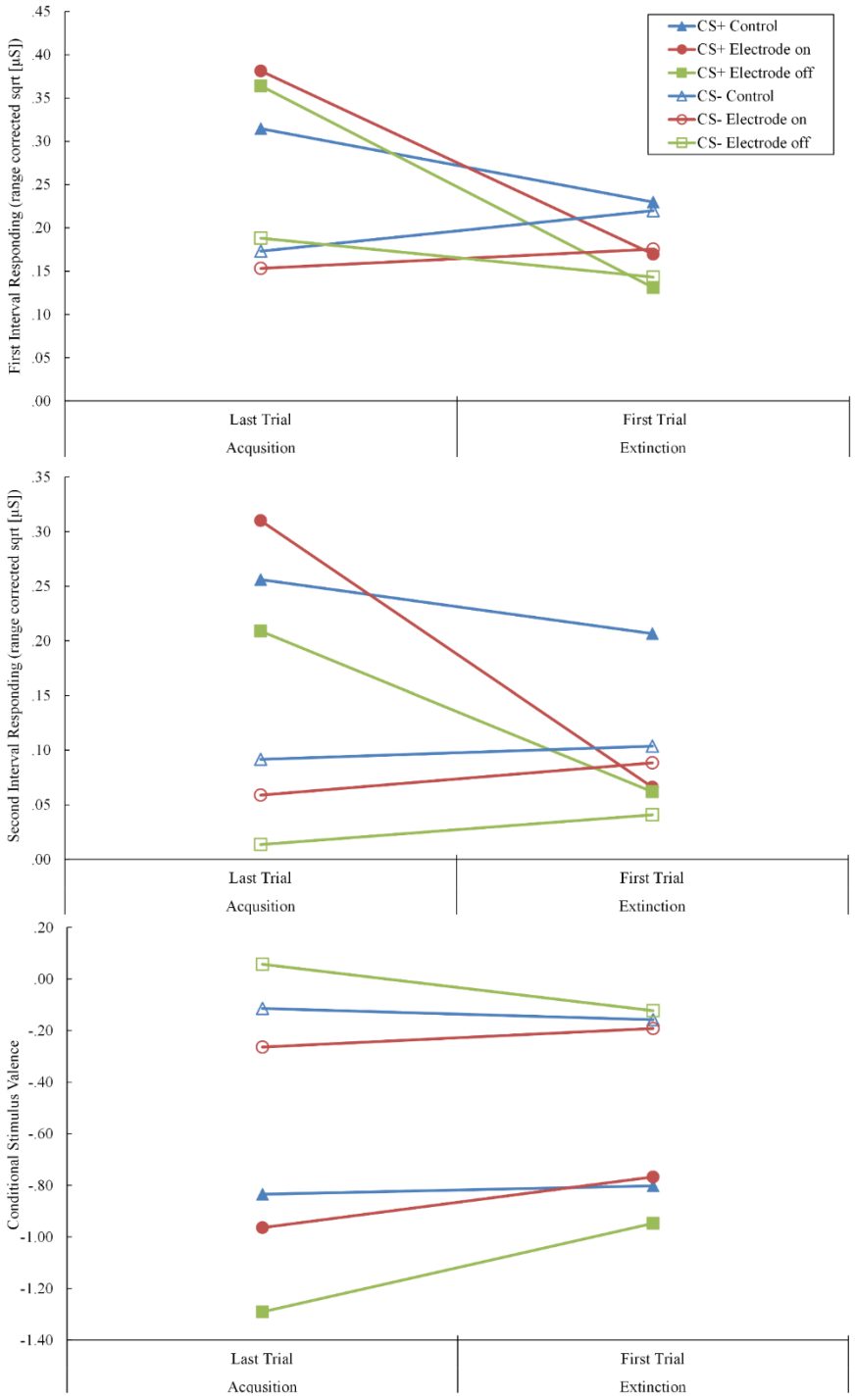

Figure 3. Comparison of first interval electrodermal responding (top), second interval electrodermal responding (middle), and conditional stimulus valence (bottom) from the last trial of acquisition to the first trial of extinction in participants who reported believing the instructions.

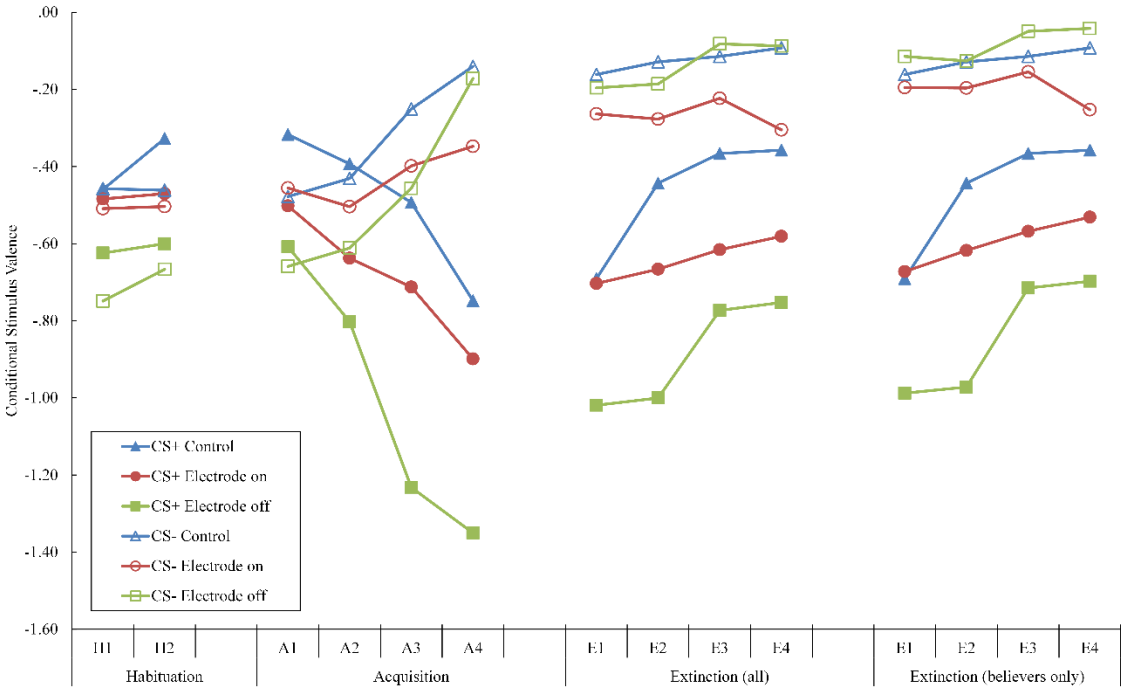

Figure 4. Conditional stimulus valence evaluations taken during habituation, acquisition, and extinction. The fourth panel shows only data from the participants who reported believing the instructions.

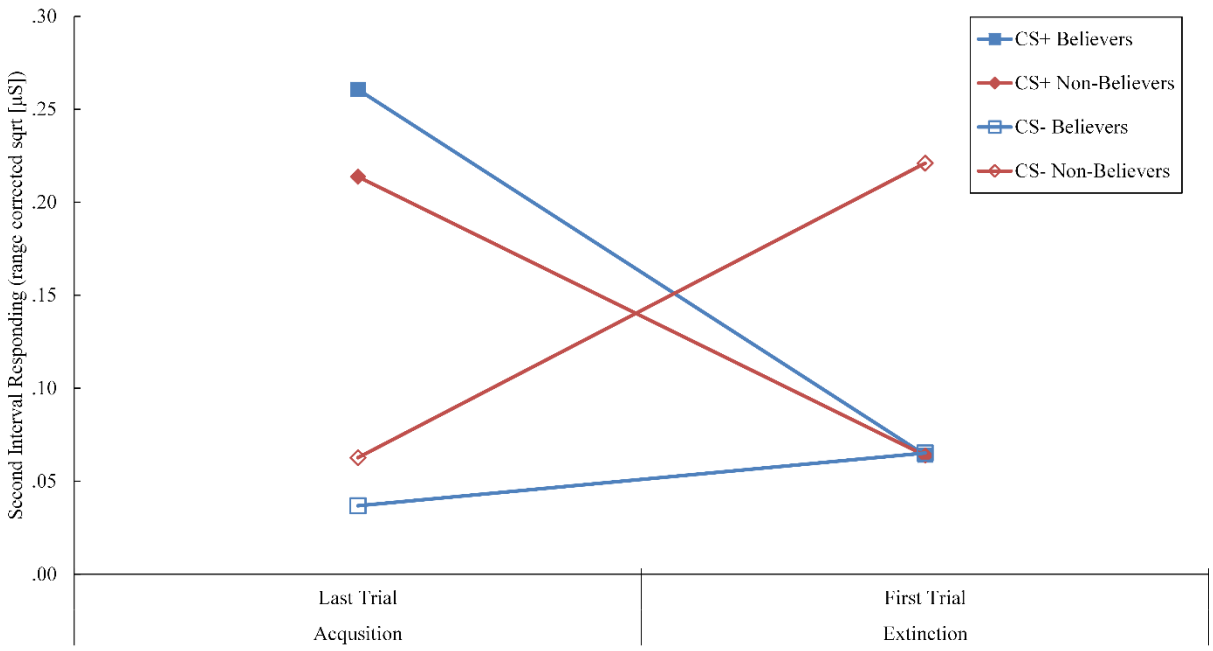

Figure 5. Mean second interval electrodermal responding in believers and non-believers of the instructions from the last trial of acquisition and the first trial of extinction.

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Chapter 5. Paper 4 – When Orienting and Anticipation Dissociate – A Case for Scoring Electrodermal Responses in Multiple Latency Windows in Studies of Human Fear Conditioning

Luck, C. C., & Lipp, O. V. (2016). When orienting and anticipation dissociate — a case for scoring electrodermal responses in multiple latency windows in studies of human fear conditioning. *International Journal of Psychophysiology, 100*, 36-43. doi:10.1016/j.ijpsycho.2015.12.003

When orienting and anticipation dissociate – a case for scoring electrodermal responses in multiple latency windows in studies of human fear conditioning

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Abstract

Electrodermal activity in studies of human fear conditioning is often scored by distinguishing two electrodermal responses occurring during the conditional stimulus-unconditional stimulus interval. These responses, known as first interval responding (FIR) and second interval responding (SIR), are reported to be differentially sensitive to the effects of orienting and anticipation. Recently, the FIR/SIR scoring convention has been questioned, with some arguing in favor of scoring a single response within the entire conditional stimulus-unconditional stimulus interval (entire interval responding, EIR). EIR can be advantageous in practical terms but may fail to capture experimental effects when manipulations produce dissociations between orienting and anticipation. As an illustration, we rescored the data reported by Luck and Lipp (2015b) using both FIR/SIR and EIR scoring techniques and provide evidence that the EIR scoring technique fails to detect the effects of instructed extinction, an experimental manipulation which produces a dissociation between orienting and anticipation. Thus, using a technique that scores electrodermal response indices of fear conditioning in multiple latency windows is recommended.

Key words: electrodermal responses, methodology, first interval responding, second interval responding, entire interval responding, conditioning, instructed extinction, differential fear conditioning.

Electrodermal activity has been a popular and widely reported autonomic index of conditional responding since the early studies of human fear conditioning. Since the 1960s, with the advent of using long conditional stimulus-unconditional stimulus intervals (CS-US interval) of six seconds or more, most researchers have agreed that separate response components can be observed during the CS-US interval, leading to the development of scoring techniques aimed at identifying and separating these components (Boucsein, 2012). The existence of multiple electrodermal responses is well accepted, but there is less agreement as to whether these responses reflect distinct psychological processes and whether information is lost if they are combined during scoring (Öhman, 1983; Pineles, Orr, & Orr, 2009).

Following calls to standardize the reporting of electrodermal activity in psychological research, Prokasy and Kumpfer (1973) reviewed the then extant literature on electrodermal activity as a measure of conditioning and argued in favor of distinguishing multiple responses during a CS-US interval of sufficient duration (usually 6 seconds or more). A first component (first interval response, FIR) was said to emerge within 1-4 s of CS onset and a second component (second interval response, SIR) shortly after this depending on the duration of the CS-US interval (within 4-7 s for a 6 s CS-US interval and 4-9 s for an 8 s CS-US interval). The FIR, was argued to be more sensitive to orienting elicited by CS onset whilst the SIR was said to be more sensitive to anticipation of the US (Öhman, 1983). A response to the US (third interval response, TIR) is scored within 1-4 s after the onset of the US. These scoring intervals are applied, regardless of whether the US onset occurs during the CS or coincides with the CS offset (delay conditioning) or whether there is a time gap between CS offset and the US onset (trace conditioning). Prokasy and Kumpfer maintained that both first and second interval responses were sensitive to associative learning, but that their separation was justified on the basis that experimental manipulations did not always affect both components in the same manner (Prokasy & Ebel, 1967), and that first and second interval responding were statistically independent (Prokasy & Ebel, 1967; Prokasy, Williams, Kumpfer, Lee & Jenson, 1973).

The use of separate latency windows when scoring electrodermal responses can be questioned on pragmatic and theoretical grounds. Scoring in multiple latency windows is time consuming and not easily automatized, and reporting results for two response components may be cumbersome and lengthen a report without adding additional information. Moreover, the separation of the response components can be difficult in the case of overlapping responses

rendering the scoring method subjective and potentially open to bias. On theoretical grounds, studies have frequently failed to support the notion that the two response components reflect dissociable psychological processes, yielding parallel results for FIR and SIR. Pineles et al. (2009) examined a selection of fear conditioning experiments which scored electrodermal responses in multiple latency windows and argued that, almost always, evidence for conditioning is found in both response components. They argued that separating response components may not be justified and provided evidence for this by rescoring the electrodermal responses obtained from a large study on differential fear conditioning (N = 287) using both a FIR/SIR component approach and an approach that scored a single response component, the entire interval response (EIR). The EIR was defined as the difference between skin conductance baseline (defined as the average skin conductance level 2 seconds before CS onset) and the peak skin conductance value observed anywhere within the CS-US interval of eight seconds (but before the onset of the unconditional response). The results were largely comparable across FIR, SIR, and EIR, however, although the FIR and EIR had similar effect sizes, SIR effect sizes were smaller. Indices of differential conditioning, difference scores between CS+ (CS paired with the US) and CS- (CS presented alone), between EIR and FIR were highly correlated, but correlations with SIR were not so robust.

There may be situations, however, in which experimental manipulations do produce meaningful dissociations between first and second interval responding, to which an EIR approach may be insensitive. One such case with significant empirical support is observed in studies of instructed extinction. During instructed extinction, one group of participants is informed after the completion of acquisition training that US presentations will cease, whilst the control group is interrupted in a similar manner but not informed about the changes to the CS-US contingency. Instructed extinction has been reliably shown to eliminate differential responding to CS+ and CS- at the very beginning of extinction. This conclusion, however, is often based solely on evidence from the SIR, as for the FIR instructed extinction effects are often masked by sensitization of the orienting reflex in the control group. Luck and Lipp (2015a, 2015b) and Rowles, Lipp, and Mallan (2012) report that differential SIR is immediately eliminated following instructed extinction in the instruction group, while differential SIR remains intact at the beginning of extinction in the control group. In contrast, differential FIR was eliminated in both groups at the beginning of extinction. Closer inspection suggests that in the instruction group

differential responding is eliminated due to a decrease in responding to CS+, but in the control group differential responding is eliminated due to an increase in responding to the CS-. This latter finding is interpreted to reflect sensitization of the orienting reflex caused by the interruption by the experimenter in the control group, an effect which is not seen in the instruction group as this group is provided with additional safety information.

Even though both differential FIR and SIR are eliminated after instructed extinction in the experimental group, it is crucial that evidence of intact differential responding be present in the control group to attribute the effect to the content of the instructions rather than to the fact that the experimental stimulus sequence was interrupted. Given the amplitude of the FIR tends to be larger than that of the SIR, we would predict that the EIR would reflect a response pattern similar to that seen for the FIR, and therefore would not allow for the detection of instructed extinction effects. In order to examine this possibility we applied the FIR/SIR and the entire interval scoring technique to the data reported by Luck and Lipp (2015b). This study compared two instruction groups (US electrode attached and US electrode removed) with a non-instructed control group, measuring electrodermal responding and conditional stimulus valence evaluations. As the focus of the current paper is on the electrodermal data, not the effect of instructed extinction, the reader is referred to Luck and Lipp (2015b) for details about the conditional stimulus valence measure, the effect of removal/attachment of the US electrode, and a more comprehensive discussion of instructed extinction.

Method

Participants

Seventy-eight (47 female) undergraduate students, aged between 17 and 50 years (M = 22.28 years), volunteered participation. The participants were compensated with course credit or monetary compensation and the procedures were approved by the Curtin University ethics review board. The participants were randomly assigned to either the control (n = 24), the instruction (electrode-on) group (n = 30), or the instruction (electrode-off) group (n = 24). The larger number of participants in the electrode-on group is due to the replacement of participants who failed to believe the instructions. One participant's electrodermal responses were lost due to problems with the recording device.

Apparatus/Stimuli

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 were used as the conditional stimuli (CS). The pictures were 506 × 650 pixels in size and were presented on a 24 inch color LCD screen for 6 s. Counter-balancing was conducted across participants, varying three factors – the faces used in the experiment, the face used as CS+/CS-, and the nature of the first trial (CS+/CS-). The trial sequence was arranged in a pseudo-random order, such that a CS+ or CS- was not presented on more than two consecutive trials. The unconditional stimulus (US) was a 200 ms electrotactile stimulus, pulsed at 50 Hz and delivered by a Grass SD9 Stimulator to the participant's preferred forearm.

Electrodermal activity was recorded with two 8 mm Ag/AgCl electrodes filled with an isotonic gel and DC amplified at a gain of 5 μSiemens per Volt. A Biopac MP150 system, using AcqKnowledge Version 3.9.1 at a sampling frequency of 1000 Hz was used to record the electrodermal responding and respiration data, and DMDX 4.0.3.0 software (Forster & Forster, 2003) was used to control the stimulus presentation and timing.

Procedure

After washing their hands and providing informed consent the participants were seated in front of a monitor in a separate cubicle of the laboratory. The electrodermal electrodes were attached to the thenar and hypothenar prominences of their non-dominant hand. The US electrode was attached to their dominant forearm and the participants underwent a shock work up procedure to set the intensity of the electrotactile stimulus to a level they experienced as subjectively unpleasant but not painful. After the work-up procedure, the participants were asked to relax and watch a blank computer screen while a three minute baseline of their electrodermal activity was recorded. After this baseline, participants were informed that they would view faces on the screen and that they should pay attention and evaluate the faces as pleasant or unpleasant. The conditioning sequence, which consisted of habituation, acquisition, and extinction phases was started. During habituation, both CS+ and CS- were presented a total of four times to allow for the habituation of orienting responses. Acquisition, which followed habituation immediately, involved eight presentations of the CS+ and the CS-, with the offset of the CS+ coinciding with the onset of the US in a 100% reinforcement schedule, whilst the CS- was presented alone. For

example, on a given trial either a 6 s CS- was presented alone or a 6 s CS+ was presented immediately followed by a 200 ms electrotactile stimulus. Then a blank rest screen was presented for either 11, 13 or 15 s before the onset of the next CS+ or CS-.

After the last trial of the acquisition, the experimenter entered the participants' room and informed them that the half-way point of the experiment had been reached and that the electrodes needed to be checked, before visually inspecting the electrodermal electrodes. For participants in the control group, the experimenter removed and reattached the shock electrode. For participants in the instruction/electrode-on group, the shock electrode was removed and reattached, and the participants were informed they would not receive the electrotactile stimulus anymore. For participants in the instruction/electrode-off group, the shock electrode was removed and the participants were informed they would no longer receive the electrotactile stimulus. After the interruption, all participants were informed that the experiment would continue and the extinction phase, consisting of eight unreinforced presentations of both the CS+ and the CS- was started. A blank rest screen, presented randomly for either 11, 13, or 15 seconds was used as the inter-trial intervals during the conditioning phases. Following extinction, the electrodes were removed and the participants were led into a separate room to complete a post-experimental questionnaire, in which they were asked to identify (from a set of four) which faces they had viewed during the experiment and which face had been followed by the electrotactile stimulus, as a measure of contingency awareness. As a manipulation check, participants were asked to indicate whether or not they had believed the instructions (yes or no question; instruction groups only).

Scoring and Response Definition

First and second interval scoring. As recommended by Prokasy and Kumpfer (1973), first interval responding (FIR) was defined as responses starting within 1-4 seconds of CS onset, second interval responding (SIR) was defined as responses starting within 4-7 seconds of CS onset. The largest response starting within the latency response window was scored and the magnitude was calculated as the difference between response onset and peak (Prokasy & Kumpfer, 1973). First and second interval responses were square root transformed to reduce the positive skew of the distribution (Dawson et al., 2007), and range corrected to reduce the effect of individual differences in response size (Boucsein et al., 2012; Dawson et al., 2007). The

largest response displayed by the participant, most often the response to the first or second presentation of the US, was used as the reference for the range correction. To avoid bias in the scoring, the scorer was blind to participant group and the nature of the CS trial (CS+ or CS-). To reduce the influence of trial by trial variability, FIR and SIR were averaged into blocks of two consecutive trials.

Entire interval scoring. The entire interval response (EIR) was scored as described in Pineles et al. (2009). The mean skin conductance level recorded during the two seconds immediately preceding the CS was subtracted from the highest skin conductance level recorded during the 6 s CS presentation. Subtraction of the baseline mean often resulted in a negative value for the EIR for which a zero response was substituted (40% of all responses). An additional measure of EIR was obtained by scoring the largest response starting within the 6 s CS presentation as the difference between response onset and response peak. This additional scoring methodology was included to ensure that any difference between the first and second interval scoring technique and the entire interval scoring technique was not due to differences in the way a 'response' was defined, i.e. highest skin conductance level in the CS-US interval - pre-CS baseline vs. actual identification of the largest response during the CS-US interval. The two EIR scoring methods yielded largely comparable results and therefore only responses based on Pineles et al. (2009) are reported, however the additional results are available on request. A square root transformation and range correction was conducted on the EIR in the same manner as for FIR and SIR and the EIR was averaged into blocks of two consecutive trials.

Statistical Analyses

FIR, SIR, and EIR 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. Bonferroni adjustments were used on all main and simple effect comparisons to protect against the accumulation of α error and adjusted *p* values have been reported for these follow-up

analyses. All analyses were conducted with IBM SPSS Statistics 22 with a significance level of .05, and Pillai's trace statistics have been reported.

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 control group ($M = 21.71$ years, $SD = 6.68$ years) did not differ from the electrode on group ($M = 20.17$ years, $SD = 2.53$ years), $p > .999$, or the electrode off group ($M = 25.50$ years, $SD = 10.93$ years), $p = .224$, however the electrode off group was older than the electrode on group, $p = .027$. Six participants 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 no age 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). As the pattern of results did not change when the analyses reported below were run excluding these participants, results for the entire sample are reported.

Habituation

First Interval Responding. The FIR recorded during habituation is 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, $F(2,74) = 3.82$, $p = .026$, $\eta p^2 = .094$, were detected. Responding significantly declined from block 1 to block 2 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, $F(1,74) = 5.19$, $p = .026$, $\eta p^2 = .066$, however 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), $F(1,74) = 0.91$, $p = .342$, $\eta p^2 = .012$.

Second Interval Responding. The SIR recorded during habituation is presented in the left panel of Figure 2. No main effects or interactions reached significance, largest (Block), $F(1,74) = 1.88$, $p = .175$, $\eta p^2 = .025$.

Entire Interval Responding. The EIR recorded during habituation is presented in the left panel of Figure 3. A main effect of block was detected, $F(1,74) = 52.53$, $p < .001$, $\eta p^2 = .415$, which confirmed that responding declined from block 1 to block 2. The remaining main effects and interactions did not reach significance, largest (CS), $F(1,74) = 1.76$, $p = .189$, $\eta p^2 = .023$.

Acquisition

First Interval Responding. The FIR recorded during acquisition is presented in the middle panel of Figure 1. Main effects of CS, $F(1,74) = 50.08$, $p < .001$, $\eta p^2 = .404$, and block, $F(3,72) = 10.12$, $p < .001$, $\eta p^2 = .297$, were qualified by a CS × Block interaction, $F(3,72) = 13.41$, $p < .001$, $\eta p^2 = .359$. During block 1, responding to CS+ and CS- did not differ $F(1,74) = 0.01$, $p = .918$, $\eta p^2 < .001$, however during blocks 2, $F(1,74) = 37.20$, $p < .001$, $\eta p^2 = .335$, 3, $F(1,74) = 62.50$, $p < .001$, $\eta p^2 = .458$, and 4, $F(1,74) = 37.44$, $p < .001$, $\eta p^2 = .336$, responding to CS+ was larger than responding to 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 SIR recorded during acquisition is presented in the middle panel of Figure 2. Main effects of CS, $F(1,74) = 62.35$, $p < .001$, $\eta p^2 = .457$, and block, $F(3,72) = 3.64$, $p = .017$, $\eta p^2 = .132$, were qualified by a CS × Block interaction, $F(3,72) = 13.67$, $p < .001$, $\eta p^2 = .363$. During block 1, responding did not differ between CS+ and CS-, $F(1,74) = 0.16$, $p = .689$, $\eta p^2 = .002$, but during blocks 2, $F(1,74) = 22.12$, $p < .001$, $\eta p^2 = .230$, 3, $F(1,74) = 41.00$, $p < .001$, $\eta p^2 = .357$, and 4, $F(1,74) = 64.08$, $p < .001$, $\eta p^2 = .464$, CS+ elicited larger responses than CS-. The remaining main effects and interactions did not attain significance, largest (Block × Group), $F(6,146) = 1.46$, $p = .196$, $\eta p^2 = .057$.

Entire Interval Responding. The EIR recorded during acquisition is presented in the middle panel of Figure 3. A main effect of CS, $F(1,74) = 80.61$, $p < .001$, $\eta p^2 = .521$, and a main effect of block, $F(3,72) = 8.97$, $p < .001$, $\eta p^2 = .272$, were qualified by a CS × Block interaction, $F(3,72) = 14.54$, $p < .001$, $\eta p^2 = .377$. During block 1, responding to CS+ and CS- did not differ, $F(1,74) = 0.15$, $p = .702$, $\eta p^2 = .002$, but during blocks 2, $F(1,74) = 41.63$, $p < .001$, $\eta p^2 = .360$, 3, $F(1,74) = 78.73$, $p < .001$, $\eta p^2 = .515$, and 4, $F(1,74) = 53.23$, $p < .001$, $\eta p^2 = .418$, CS+ elicited larger responses than CS-. The remaining main effects and interactions did not attain significance, largest (Block × Group), $F(6,146) = 1.61$, $p = .149$, $\eta p^2 = .062$.

Instructed Extinction Manipulation – Trial Based Analysis

First Interval Electrodermal Responding. The FIR recorded during the last trial of acquisition and the first trial of extinction is presented in the top section of Figure 4. A main effect of CS, $F(1,74) = 13.75$, $p < .001$, $\eta p^2 = .157$, and a main effect of phase, $F(1,74) = 8.87$, $p = .004$, $\eta p^2 = .107$, were qualified by a CS × Phase interaction, $F(1,74) = 18.84$, $p < .001$, $\eta p^2 = .203$. On the last trial of acquisition, responding was larger to CS+ in comparison with CS-, $F(1,74) = 30.15$, $p < .001$, $\eta p^2 = .289$, however, this differential responding was not present on the first trial of extinction, $F(1,74) = 0.01$, $p = .925$, $\eta p^2 < .001$. The critical CS × Phase × Group interaction did not reach significance, $F(2,74) = 0.51$, $p = .602$, $\eta p^2 = .014$, and follow-up analyses confirm that differential responding was not present in any group at the beginning of extinction, all *ps* > .642. The remaining main effects and interactions did not attain significance, largest (Phase × Group), $F(2,74) = 1.78$, $p = .176$, $\eta p^2 = .046$.

Second Interval Electrodermal Responding. The SIR recorded during the last trial of acquisition and the first trial of extinction is presented in the middle section of Figure 4. Main effects of CS, $F(1,74) = 22.86$, $p < .001$, $\eta p^2 = .236$, and phase, $F(1,74) = 7.51$, $p = .008$, $\eta p^2 = .092$, and a CS × Phase interaction, $F(1,74) = 23.19$, $p < .001$, $\eta p^2 = .239$, were qualified by a CS × Phase × Group interaction, $F(2,74) = 3.44$, $p = .037$, $\eta p^2 = .085$. 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 to 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 remaining main effects and interactions did not attain significance, largest (group), $F(2,74) = 3.00$, $p = .056$, $\eta p^2 = .075$.

Entire Interval Electrodermal Responding. The EIR recorded during the last trial of acquisition and the first trial of extinction is presented in the bottom section of Figure 4. Main effects of CS, $F(1,74) = 35.03$, $p < .001$, $\eta p^2 = .321$, and phase, $F(1,74) = 5.29$, $p = .024$, $\eta p^2 = .067$, were qualified by a CS × Phase interaction, $F(1,74) = 37.31$, $p < .001$, $\eta p^2 = .335$. On the last trial of acquisition, differential responding was present between CS+ and CS-, $F(1,74) = 73.06$, $p < .001$, $\eta p^2 = .497$, however, this differential responding was no longer present on the

first trial of extinction, $F(1,74) = 0.08$, $p = .777$, $\eta p^2 = .001$. The critical CS × Phase × Group interaction did not reach significance, $F(2,74) = 0.44$, $p = .645$, $\eta p^2 = .012$, and follow-up analyses confirm that differential responding was not present in any group at the beginning of extinction, all *ps* > .472. The remaining main effects and interactions did not attain significance, largest (Phase × Group), $F(2,74) = 1.63$, $p = .203$, $\eta p^2 = .042$.

Extinction

First Interval Electrodermal Responding. The FIR recorded during extinction is presented in the right panel of Figure 1. A marginal main effect of CS, $F(1,74) = 3.84$, $p = .054$, $\eta p^2 = .049$, revealed that electrodermal responding to CS+ was marginally larger than to CS-. A main effect of block, $F(3,72) = 5.93$, $p = .001$, $\eta p^2 = .198$, revealed that responding was larger in block 1 in comparison with block 3, $p = .002$, and block 4, $p = .002$. The remaining omnibus effects failed to reach significance, largest (Block × Group), $F(6,146) = 1.52$, $p = .176$, $\eta p^2 = .059$.

Second Interval Electrodermal Responding. The SIR recorded during extinction is presented in the right section of Figure 2. A main effect of block, $F(3,72) = 2.94$, $p = .039$, $\eta p^2 = .109$, revealed that responses in block 1 were larger than responses in block 4, $p = .042$. 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 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, $F(1,74) = 0.14$, $p = .709$, $\eta p^2 = .002$, responses to CS+ and CS- did not differ. The remaining main effects and interactions did not attain significance, largest (Block × Group), $F(6,146) = 1.19$, $p = .313$, $\eta p^2 = .047$.

Entire Interval Responding. The EIR recorded during extinction is presented in the right panel of Figure 3. A main effect of CS, revealed that responding to CS+ was larger than responding to CS-, $F(1,74) = 4.40$, $p = .039$, $\eta p^2 = .056$. A main effect of block, $F(3,72) = 2.82$, $p = .045$, $\eta p^2 = .105$, revealed that responding was larger in block 1 in comparison with block 4, $p = .032$, and block 4, $p = .002$. The remaining omnibus effects failed to reach significance, largest (Block × Group), $F(6,146) = 1.63$, $p = .143$, $\eta p^2 = .063$.

Discussion

The current paper aimed to investigate the sensitivity of three different electrodermal responses indices, first interval responding (FIR), second interval responding (SIR), and entire interval responding (EIR), to reflect the effects of an instructed extinction manipulation. Instructed extinction is known to produce robust, and meaningful, dissociations between FIR and SIR (see Luck & Lipp, 2015a; 2015b; Rowles, Lipp, & Mallan, 2012). We aimed to examine whether instructed extinction effects would be reflected in EIR by rescoring the data of Luck and Lipp (2015b).

Throughout the habituation phase, a main effect of block confirmed that both FIR and EIR showed evidence for habituation, however, no evidence for habituation was detected in SIR. This finding is consistent with the view that SIR is less sensitive to orienting, and supports the decision to only report FIR during the habituation phase. It should be noted that prior studies have reported changes in SIR during habituation (Pineles et al., 2009), but these changes were considerably smaller than those seen in FIR or EIR (effect sizes [η^2] of .01, .20 and .14, respectively) and may reflect on the larger sample size used in that study. During acquisition, evidence for conditioning was apparent in all electrodermal responses indices and as reported before, results of FIR, SIR, and EIR were comparable. The instructed extinction/control manipulation eliminated differential FIR and EIR in all groups when assessed either on the initial trial of extinction or across the entire extinction training. As described elsewhere (Luck & Lipp, 2015a, 2015b; Rowles, Lipp & Mallan, 2012), elimination of differential responding at the beginning of extinction as a result of the control manipulation is likely to reflect sensitization of the orienting reflex, resulting in increased responding to the CS-. Consistent with the proposal that SIR is less sensitive to the effects of orienting, the control group shows intact differential SIR at the beginning of extinction and across the entire extinction training. It is this intact differential SIR in the control group which allows the conclusion that the current results reflect on the content of the instructions provided rather than a general effect of interrupting the experimental procedure. The entire interval response is not sensitive to the apparent dissociation of orienting and anticipation, and cannot reflect the effects of instructed extinction as it was largely affected by the more prominent effects of orienting. Thus, the effects of instructed extinction would be lost if an EIR measure was used to reflect electrodermal responses in the current study.

To ensure that the current findings were not specific to a particular method of calculating the EIR, we also calculated EIR as the difference between response onset and response peak observed within the entire CS-US interval. As in the majority of differential conditioning designs (including that used by Pineles et al., 2009) a pseudorandom trial sequence is used in which a particular CS is not presented more often than twice consecutively, the presentation of a CS+ is more likely to precede the presentation of a CS- . During acquisition, the response elicited by the US will elevate the skin conductance baseline before the next trial leading to the well-established finding that CS- presentations have higher electrodermal baselines than CS+ presentations (see for instance, Luck & Lipp, 2015b). This baseline difference potentially underestimates the response to CS- which would artificially inflate the size of differential conditioning effects. Moreover, if a CS fails to elicit a response the slightly downward trajectory of the skin conductance trace should render the largest skin conductance value during the CS-US interval smaller than a pre-stimulus baseline yielding a nonsensical negative response value. In the current investigation, we found a similar pattern of results emerged for the EIR regardless of whether the response base was defined as the mean of a pre-CS baseline or the response onset within the CS-US interval. This is reassuring, but may reflect on the strong experimental manipulations (100% CS-US contingency) and large sample size in the current study.

The results of the current investigation support Prokasy and Kumpfer's (1973) recommendation that conditioning experiments should be designed and scored in such a way as to allow a distinction between first and second interval responding. We agree, and would expect, that in procedures where orienting and anticipation processes overlap, FIR, SIR, and EIR will yield largely comparable results, and that an entire interval scoring technique, which uses the skin conductance level at response onset as a reference, could accurately capture the experimental outcomes. Based on this it could be argued that the current examination is paradigm specific and not applicable to broader fear conditioning studies, however it is not always possible to predict a-priori when dissociations between different processes might occur and limiting the scoring to EIRs could lead to the loss of important information. Based on the current analysis, a strategy that scores electrodermal response indices of Pavlovian conditioning in distinct latency windows following the recommendations of Prokasy and Kumpfer (1973) seems advisable.

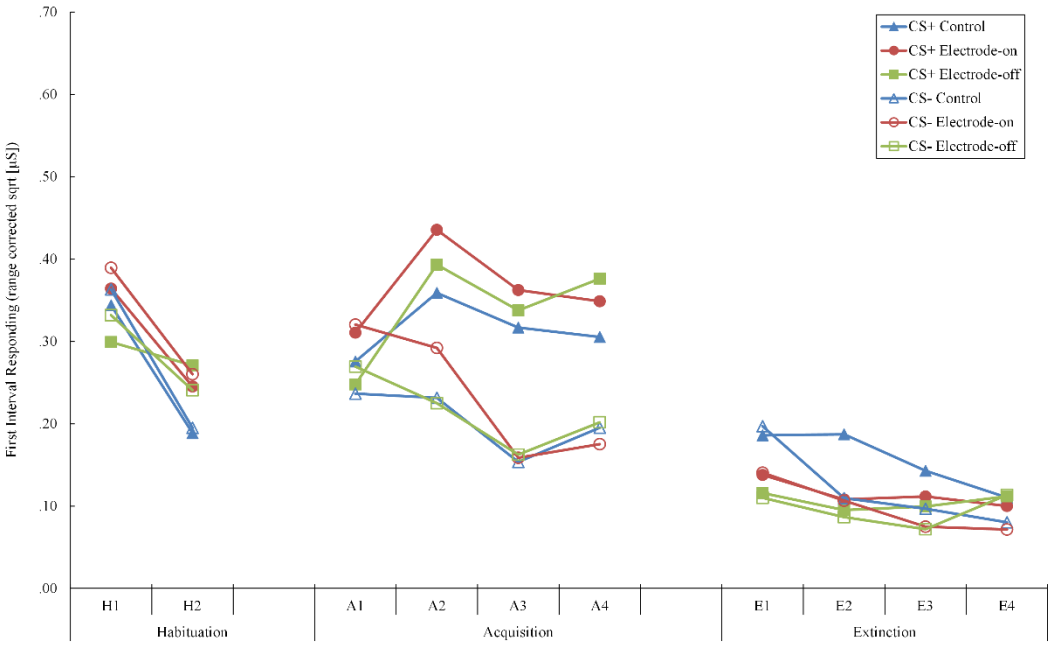

Figure 1. Mean first interval electrodermal responses during habituation, acquisition, and extinction phases.

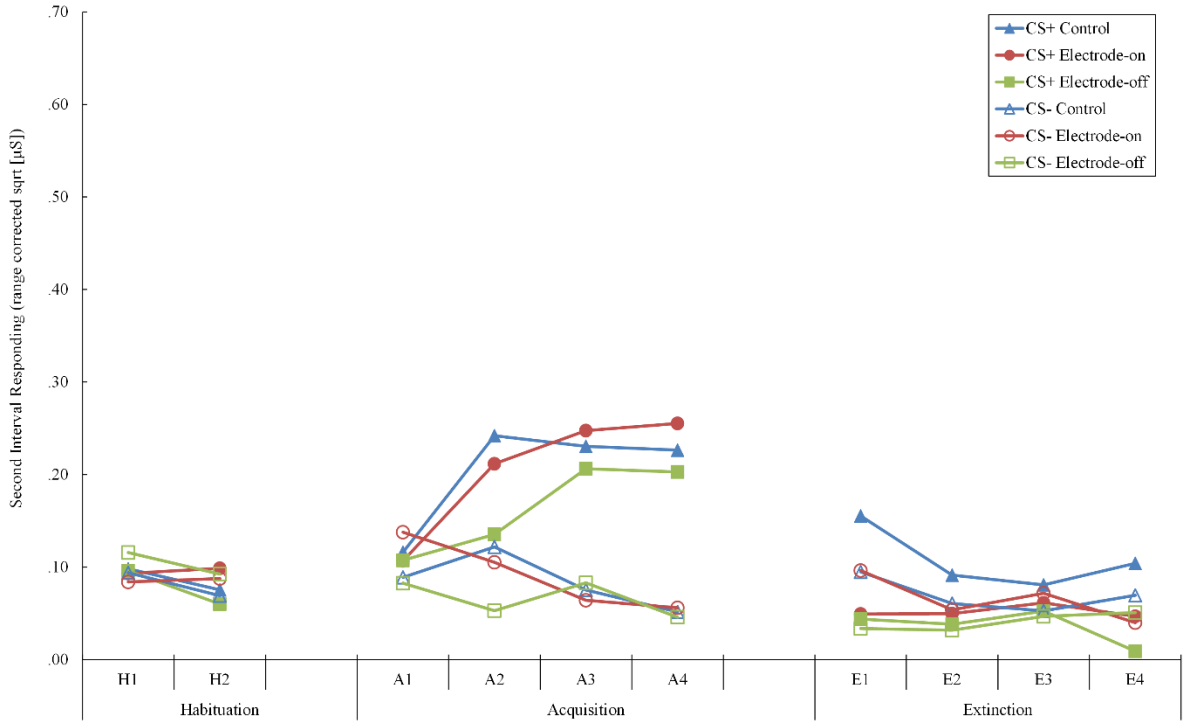

Figure 2. Mean second interval electrodermal responses during habituation, acquisition, and extinction phases.

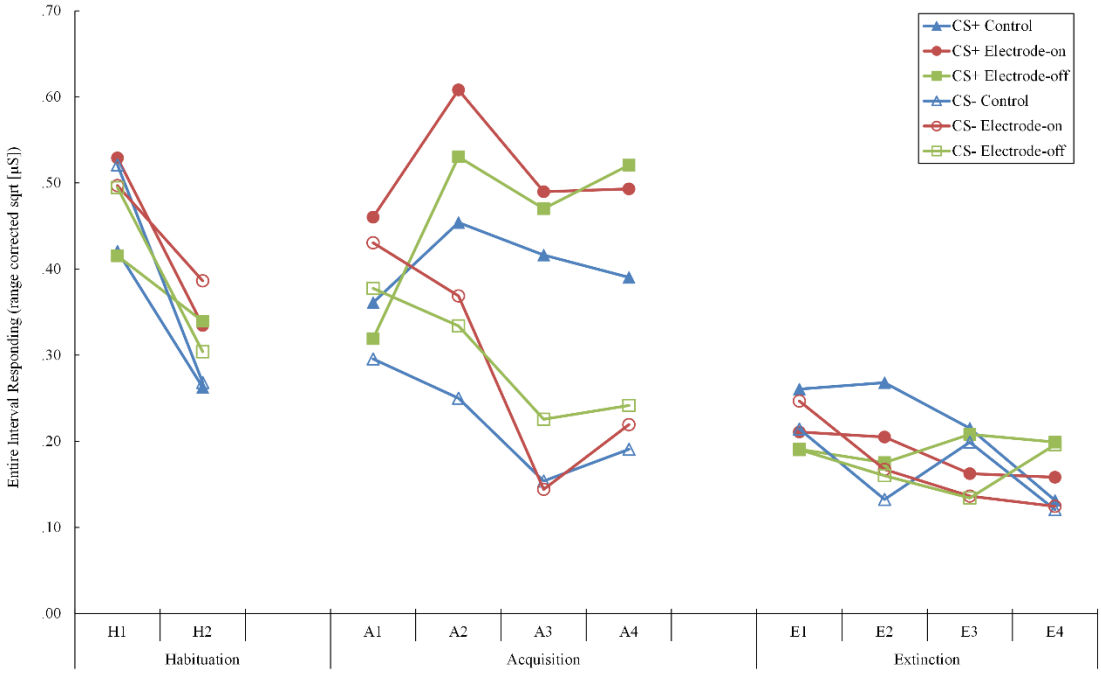

Figure 3. Mean entire interval electrodermal responses during habituation, acquisition, and extinction phases.

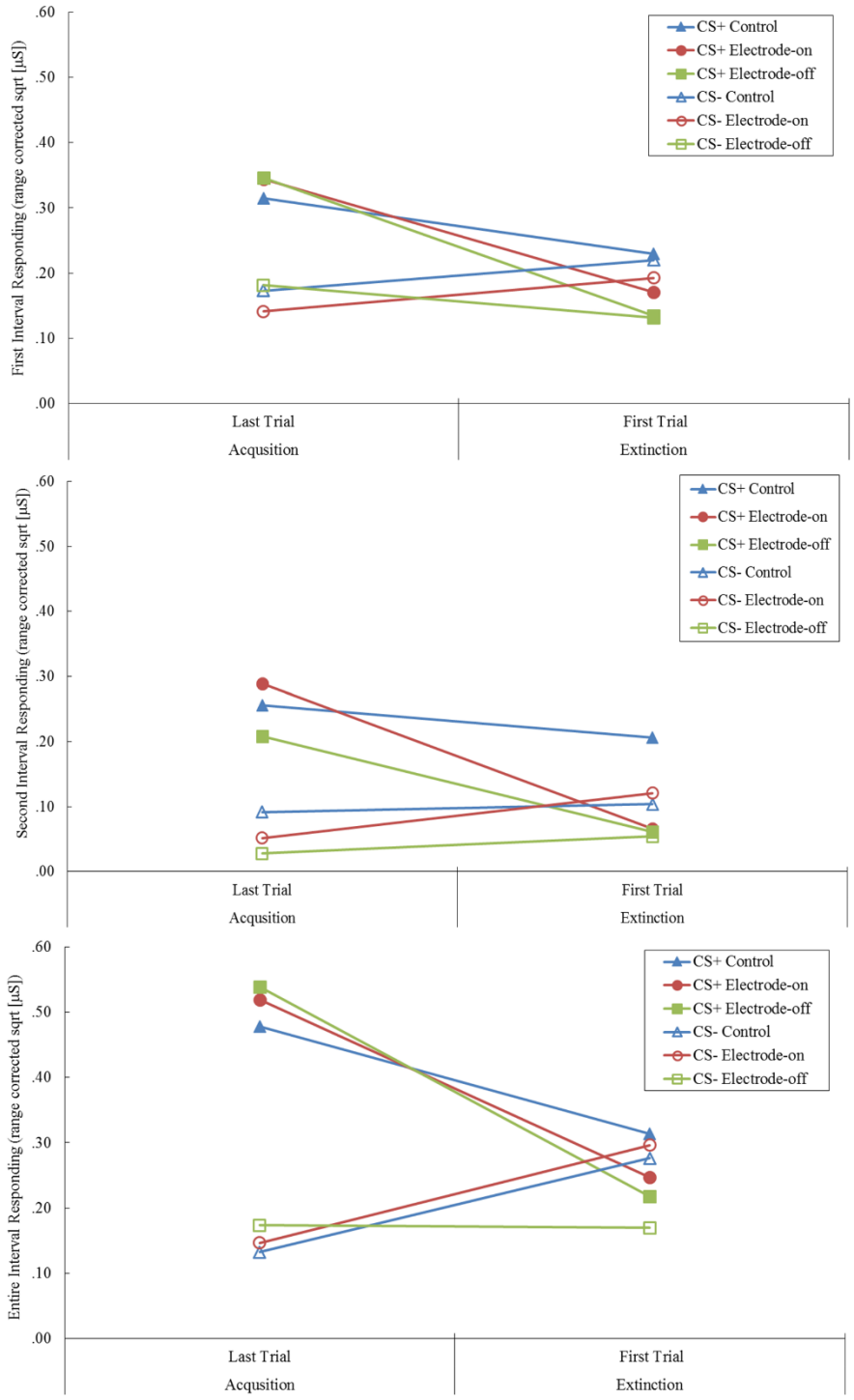

Figure 4. First interval (top), second interval (middle), and entire interval (bottom) electrodermal responses during the last trial of acquisition and the first trial of extinction.

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Chapter 6. Paper 5 – The Influence of Contingency Reversal Instructions on Electrodermal Responding and Conditional Stimulus Valence Evaluations during Differential Fear Conditioning

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The influence of contingency reversal instructions on electrodermal responding and conditional stimulus valence evaluations during differential fear conditioning

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Abstract

In differential fear conditioning, the instruction that the conditional stimulus (CS) will no longer be followed by the unconditional stimulus (US; instructed extinction) reduces differential physiological responding (expectancy learning) but leaves differential CS valence evaluations (evaluative learning) intact. This dissociation suggests that expectancy, but not evaluative learning, responds to contingency instructions. Alternatively, as instructed extinction removes the threat of receiving the US, this dissociation could be caused by a drop in participants' arousal levels which could render the physiological indices of fear learning less sensitive. To test this alternative explanation, we examined the impact of an instructed reversal manipulation on electrodermal responding and CS valence evaluations. After instructed reversal, electrodermal responses to CS+ decreased and electrodermal responses to CS- increased, in the instruction, but not in the control group. In addition, there was some evidence for an instruction dependent change in CS valence, however, this finding seems limited to changes in CS+ valence and possible explanations for this finding are discussed. Overall, the study confirms that the dissociation detected in instructed extinction studies is unlikely to be caused by a drop in the participants' arousal levels.

Key words: fear conditioning, instructed reversal, instructed extinction, evaluative learning, expectancy learning, conditional stimulus valence, electrodermal responding

During classical fear conditioning, a neutral conditional stimulus (CS) is paired with an aversive unconditional stimulus (US). After repeated pairings, the CS generates an expectation that the US will occur (Lipp, 2006) and acquires negative valence (De Houwer, Thomas, & Baeyens, 2001). Dissociations between the predictive (expectancy) and the emotional (evaluative) components of human fear learning have been reported in response to instructed extinction (see Luck & Lipp, 2015a), generating debate about whether these components reflect different underlying mechanisms or operate under different boundary conditions.

Understanding the mechanisms underlying expectancy and evaluative learning is important from a number of viewpoints. Residual negative valence has been associated with higher relapse rates after fear extinction, and prior research suggests that CS valence may resist current fear and anxiety treatments (Hermans et al., 2005; Luck & Lipp, 2015a; Zbozinek, Hermans, Prenoveau, Liao, & Craske, 2015). From a theoretical perspective, there is some debate about whether Pavlovian conditioning can be considered the result of propositional processes alone or whether both propositional and associative processes co-occur during Pavlovian conditioning. According to single-process propositional theories, Pavlovian conditioning is the result of the formation and truth evaluation of non-automatic propositions regarding the CS-US relationship. Dual-process theories propose that automatic associations between CS and US representations also develop during CS-US pairings (see De Houwer, 2009 for a review and discussion of these theories). Some theories (see Baeyens, Eelen, Crombez, & Van den Bergh, 1992) propose that evaluative and expectancy learning are two different types of Pavlovian conditioning, both based on the formation of stimulus representations in memory. According to these theories, expectancy learning concerns the learning of predictive relationships in which the CS becomes a signal that the US will occur, whereas, evaluative learning concerns the learning of referential relationships, in which the CS becomes a stimulus which activates the mental representation of the US without generating an expectancy that the US will occur.

Dissociations between evaluative and expectancy learning in response to the same experimental manipulation could hold the key to understanding whether or not they have the same underlying mechanism. Expectancy and evaluative learning can be examined simultaneously using a differential fear conditioning paradigm. In this paradigm, one CS, the CS+, is repeatedly paired with the US, and another, the CS-, is presented alone. Electrodermal responding, a physiological index which is very sensitive to the CS-US contingency, and CS

valence evaluations are frequently collected as dependent measures, and both can be measured continuously throughout conditioning. Differential electrodermal responding and differential valence evaluations develop across training trials, such that CS+ elicits larger electrodermal responding and is rated as less pleasant than CS-. During extinction, CS+ and CS- are both presented alone and eventually the differential electrodermal responding and valence evaluations reduce and return to baseline levels. Using this paradigm, Luck and Lipp (2015a; 2015b) reported that instructed extinction, a manipulation which involves informing participants prior to the extinction phase that the US will no longer occur, results in the immediate elimination of differential electrodermal responding (and fear-potentiated startle), but leaves differential valence evaluations intact. These results can be interpreted to indicate that expectancy learning responds to the instructed CS+– noUS contingency immediately, but that evaluative learning continues to reflect the valence acquired during acquisition, requiring further Pavlovian training to reduce the negative CS+ valence. This interpretation is consistent with literature examining US expectancy and CS evaluation in picture-picture evaluative conditioning paradigms (Lipp, Mallan, Libera, & Tan, 2010). Alternatively, the elimination of differential physiological responding after instructed extinction could occur because participants' general arousal level is reduced after being informed that they will not receive US presentations anymore. Electrodermal responding is also sensitive to stimulus valence but only under conditions of high arousal (Bradley, Codispoti, Cuthbert, & Lang, 2001). As CS evaluations are not sensitive to the overall level of arousal, the dissociation between physiological and evaluative indices of fear learning could reflect the differential sensitivity of electrodermal responding and CS evaluations to changes in arousal.

An instructed reversal manipulation (Grings, Schell, & Carey, 1973) involves informing participants after acquisition training, that the contingencies will switch, such that CS+ will no longer be followed by the US, but that the US will now be presented after the CS-. This manipulation is unlikely to cause a drop in participants' overall arousal because of the ongoing threat of receiving the US and therefore provides a test of the arousal account described above. While instructed extinction involves examining safety instructions to the CS+, instructed reversal allows for the examination of both safety instructions to the CS+ and danger instructions to the CS-, providing a more comprehensive examination of the effects of instructions.

Effects of the instructional manipulation can be examined across the entire reversal phase or on the very first trial after the instruction was provided. Although differences between the instruction and control groups may be observed in both cases, the two assessments can indicate different processes. Instruction effects detected across the entire reversal phase could indicate that instructions facilitate learning of the new contingency (Instruction × Training interaction) and not necessarily a reversal change caused by the instructions alone. Differences on the first reversal trial, however, can be considered the effects of the instructional manipulation alone and provide for the strongest test of the instructed reversal manipulation. The nature of the first trial (CS+/CS-) presented after instruction should also be controlled because experiencing a contingency change on the first reversal trial (i.e. unreinforced CS+ or reinforced CS-) could lead participants to infer that the experimental contingencies have changed.

Using a differential fear conditioning paradigm, we examined whether electrodermal responding and trial-by-trial CS valence would respond to an instructed reversal manipulation. To be able to examine the effects of instructed reversal without any influence of additional learning (or inference), half of the participants received a CS+ as the first reversal trial and the others received a CS- as the first reversal trial. We hypothesized, based on the results of Luck and Lipp (2015a; 2015b), that electrodermal responding to CS+ would decrease and that electrodermal responding to CS- would increase on the first reversal trial in the instruction group but not in the control group. It was further hypothesized that CS valence would not be affected in either group.

Method

Participants

One hundred and forty-nine undergraduate students (95 female), aged between 17 – 43 years (M = 23.16) provided informed consent and volunteered participation in exchange for course credit or monetary compensation of AU$15. Participants were assigned to different CS order conditions[1] and then were randomly assigned to the control or instruction group. Twenty participants failed to correctly verbalize the experimental contingencies and were removed from

[1] Two experiments were conducted which were identical except for which CS was presented first during the reversal phase. To streamline the report, we have combined the experiments and added the factor CS order to the analyses.

the analyses. An additional 7 participants reported that they did not believe the reversal instructions and were removed from the reversal and instruction analyses. Five participants' electrodermal responses and two participants' conditional stimulus (CS) valence evaluations were lost due to problems with the recording device, and five participants did not provide complete before and after rating datasets. These participants have been included in the analyses of the remaining measures.

Apparatus/Stimuli

The CSs were 4 pictures of 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 presented on a 17-inch color LCD screen for 6 s. A pseudorandom trial sequence was used, such that a CS+/CS- was not presented more than twice consecutively. Counterbalancing was performed between participants, varying the nature of the first trial during acquisition (CS+/CS-), the face used as CS+/CS-, and the two faces used in the experiment. The unconditional stimulus (US) was a 200 ms electrotactile stimulus pulsed at 50 Hz and delivered by a Grass SD9 stimulator to the participants' preferred forearm. Physiological responding and CS evaluations were recorded with a Biopac MP150 system at a sampling frequency of 1000 Hz using Acqknowledge version 3.9.1. Electrodermal responding was DC amplified at a gain of 5 μSiemens per volt and CS evaluations were measured on a trial-by-trial basis using an evaluation joystick with the anchors 'very unpleasant', 'neutral', and 'very pleasant'. DMDX 3.0.2.8 software (Forster & Forster, 2003) was used to control the stimulus presentation and timing and to record the pleasantness ratings (Ratings A and B).

Procedure

Participants washed their hands, provided informed consent, and were seated in front of a monitor in a separate room adjacent to the control room. The respiratory effort transducer was fitted around their waist, and the electrodermal electrodes were attached to the thenar and hypothenar prominences of their non-dominant hand. The shock electrode was attached to their dominant forearm, and a shock-work up procedure was performed to set the US intensity to a level that was experienced as subjectively 'unpleasant, but not painful'. Participants were then asked to relax and watch the blank computer screen while a 3-min baseline of their electrodermal activity (EDA) was recorded. After the baseline recording, participants rated the CS faces on a 1

to 9 (1= unpleasant, 9=pleasant) Likert scale (ratings A) and were informed that they would see the faces displayed on the screen throughout the experiment. They were asked to use the evaluation joystick throughout the experiment to indicate how pleasant/unpleasant they found each face, and to make this evaluation as soon as the face was presented on the screen with their preferred hand – ensuring that the movement did not interfere with the electrodermal recording and that the presence/absence of the US, on a given trial, did not influence the evaluations.

After the participant confirmed that they understood what was required, the conditioning task, consisting of habituation, acquisition, and reversal phases, was started. During habituation, both CS+ and CS- were presented 4 times alone. During acquisition, the CS+ was presented 8 times, with the offset of the CS+ coinciding with the onset of the US in a 100% reinforcement schedule, while the CS- was presented 8 times alone. During habituation and acquisition, CS+ and CS- were presented in a pseudorandom sequence with the restrictions that the first 2 stimuli in a phase were a CS+ and a CS- and that no more than 2 consecutive stimuli were the same. After acquisition, the experimenter entered the participants' room and informed them that the mid-point of the experiment had been reached and that the electrodes needed to be checked, before appearing to visually inspect the electrodermal electrodes. Participants in the control group did not receive information about the CS-US contingency. Participants in the instruction group were informed that in the second part of the experiment the electrotactile stimulus would no longer be presented after the stimulus it had previously followed, but would switch to follow the other stimulus. Participants were asked to confirm they understood the instructions and told the experiment would continue. During the reversal phase, the CS+ (CS terminology from acquisition will be used consistently throughout both phases) was presented 8 times alone, and the CS- was presented 8 times with the offset of the CS- coinciding with the onset of the US in a 100% reinforcement schedule. The first 3 trials of the reversal phase differed depending on CS order group. Participants in the CS+ first group viewed 2 consecutive presentations of the CS+, followed by a CS- and then the counterbalanced pseudorandom trial sequence. Participants in the CS- first group viewed 2 consecutive presentations of the CS-, followed by a CS+ and then the counterbalanced pseudorandom trial sequence. Inter-trial intervals lasted 11s, 13s, or 15s from CS offset to CS onset and were randomly varied throughout the experiment. After the last reversal trial, participants completed another rating task (ratings B), which was identical to the one performed before conditioning, the electrodes were removed and the participant was led into

the control room for the post-experimental questionnaire. The questionnaire required participants to identify which faces were presented in the experiment and which face was followed by the electrotactile stimulus in the first and second part of the experiment. As a manipulation check, participants were asked to indicate whether they believed the instructions (instruction group only; yes or no question). Participants then rated the pleasantness of the electrotactile stimulus and the CS faces on a (-3 [*very unpleasant*] to +3 [*very pleasant*]) pleasantness scale (ratings C), before being debriefed and thanked.

Scoring and Response Definition

Electrodermal responding was scored in multiple latency windows as recommended by Prokasy and Kumpfer (1973) and Luck and Lipp (2016). First interval responding was defined as responses starting within 1-4 s of CS onset and second interval responding was defined as responses starting within 4-7 s of CS onset. The largest response starting within the latency window was scored and the response magnitude was calculated as the difference between response onset and peak (Prokasy & Kumpfer, 1973). The electrodermal responses were square root transformed to reduce the positive skew of the distribution (Dawson, Schell, & Filion, 2007) and then range corrected (using the largest response as a reference) to reduce the effect of individual differences in response size (Boucsein et al., 2012; Dawson et al., 2007). During habituation only first interval responses were scored as they reflect orienting to novel stimuli (Öhman, 1973). As a measure of spontaneous EDA, any discernible response displayed during the baseline period was counted (Dawson et al., 2007). The CS valence ratings provided with the response joystick were recorded by the Biopac MP150 system as voltage deviations. The joystick was spring loaded, such that after a response was made the joystick would return to the 'neutral' position. The valence ratings made during the 6 s CS presentation were scored as the largest voltage deviation from mean baseline voltage recorded 1 s prior to CS onset. To reduce the influence of trial by trial variability, electrodermal responding and CS valence evaluations were averaged into blocks of 2 consecutive trials[2]. All analyses were conducted with IBM SPSS Statistics 22 with a significance level of .05, and Pillai's trace statistics have been reported.

[2] As the influence of the instructional manipulation is expected during the first reversal trial the analyses concerned with the instruction effect are based on single trials.

Results

Preliminary Analyses

Two Pearson's chi-square tests were performed to ensure that the gender ratio did not differ in the instruction or CS order groups. To check for baseline differences between the groups a series of 2 (Group: instruction, control) × 2 (CS order: CS+ first, CS- first) univariate ANOVAs were performed on age, spontaneous EDA, US intensity, and US valence. The means and standard deviations for these variables are displayed in Table 1. The instruction groups, $\chi^2(1) = .240$, $p = .624$, and CS order groups, $\chi^2(1) = .362$, $p = .547$, did not differ in gender ratio. The CS- first group was older than the CS+ first group, $F(1,125) = 5.75$, $p = .018$, $\eta p^2 = .044$, and the CS+ first group set the US intensity marginally higher than the CS- first group, $F(1,125) = 3.28$, $p = .073$, $\eta p^2 = .026$. No other comparisons reached significance, all $Fs < 2.71$, $ps < .102$, $\eta p^2 s < .021$.

Habituation

The CS valence evaluations and first interval responding recorded during habituation (see left panels of Figures 1 and 2, respectively) were subjected to separate 2 (Group: instruction, control) × 2 (CS order: CS+ first, CS- first) × 2 (CS: CS+, CS-) × 2 (Block: 1, 2) mixed-model factorial ANOVAs.

Conditional Stimulus Valence. A CS × CS order interaction, $F(1,123) = 4.12$, $p = .045$, $\eta p^2 = .032$, revealed that participants in the CS- first group evaluated CS+ as less pleasant than CS-, $F(1,123) = 5.16$, $p = .025$, $\eta p^2 = .040$, whereas evaluations did not differ in the CS+ first group, $F(1,123) = 0.40$, $p = .530$, $\eta p^2 = .003$.

First Interval Responding. Responding decreased from block 1 to block 2, $F(1,121) = 61.50$, $p < .001$, $\eta p^2 = .337$, and responding was larger in the CS+ first group than in the CS- first group, $F(1,121) = 5.65$, $p = .019$, $\eta p^2 = .045$.

Acquisition

The CS valence evaluations, first interval responding, and second interval responding recorded during acquisition were subjected to separate 2 (Group: instruction, control) × 2 (CS order: CS+ first, CS- first) × 2 (CS: CS+, CS-) × 4 (Block: 1, 2, 3, 4) mixed model factorial

ANOVAs and are presented in Figures 1 (middle panels), 2 (middle panels), and 3 (left panels), respectively.

Conditional Stimulus Valence. A main effect of CS, $F(1,123) = 23.31$, $p < .001$, $\eta p^2 = .159$, and a CS × Block interaction, $F(3,121) = 14.53$, $p < .001$, $\eta p^2 = .265$, were moderated by a CS × Block × Group interaction, $F(3,121) = 3.48$, $p = .018$, $\eta p^2 = .079$. Differential valence was not present in either group during block 1 (*Fs* $(1,123) < 2.72$, *ps* $> .101$, ηp^2s $< .023$), however, during subsequent blocks CS+ was evaluated as less pleasant than CS- in both groups (all *Fs* $(1,123) < 4.90$, *ps* $> .028$, ηp^2s $< .037$). Although differential valence was present in both groups, valence evaluations to CS+ and CS- changed across blocks in the control groups, *Fs* $(3,121) > 5.58$, *ps* $< .002$, ηp^2s $> .121$, but not in the instruction groups, *Fs* $(3,121) < 2.21$, *ps* $> .090$, ηp^2s $> .053$.

First Interval Responding. Responses were larger in the CS+ first group than in the CS- first group, $F(1,121) = 4.94$, $p = .028$, $\eta p^2 = .039$. A main effect of CS, $F(1,121) = 60.38$, $p < .001$, $\eta p^2 = .333$, and a main effect of block, $F(3,119) = 11.28$, $p < .001$, $\eta p^2 = .221$, were moderated by a CS × Block interaction, $F(3,119) = 13.66$, $p < .001$, $\eta p^2 = .256$. Follow-up analyses revealed that responding to CS+ and CS- did not differ during block 1, $F(1,121) = 0.52$, $p = .470$, $\eta p^2 = .004$, but during subsequent blocks responding to CS+ was larger than to CS-, all *Fs* $(1,121) > 24.27$, *p*s $< .001$, ηp^2s $> .166$.

Second Interval Responding. A main effect of CS, $F(1,121) = 42.33$, $p < .001$, $\eta p^2 = .259$, was moderated by a CS × Block interaction, $F(3,119) = 9.07$, $p < .001$, $\eta p^2 = .186$. Follow-up analyses revealed that responding to CS+ and CS- did not differ during block 1, $F(1,121) = 0.46$, $p = .497$, $\eta p^2 = .004$, but responding to CS+ was larger than to CS- during subsequent blocks, all *Fs* $(1,121) > 4.67$, *ps* $< .034$, ηp^2s $> .036$.

Reversal

The CS valence evaluations, first interval responding, and second interval responding recorded during reversal were subjected to separate 2 (Group: instruction, control) × 2 (CS order: CS+ first, CS- first) × 2 (CS: CS+, CS-) × 4 (Block: 1, 2, 3, 4) mixed-model factorial ANOVAs and can be seen in the right panels of Figures 1, 2, and 3, respectively.

Conditional Stimulus Valence. A main effect of CS, $F(1,117) = 20.42$, $p < .001$, $\eta p^2 = .149$, was moderated by a CS × Group × CS order interaction, $F(1,117) = 3.99$, $p = .048$, $\eta p^2 = .033$. If a CS+ was presented first, the instruction group evaluated CS- as less pleasant than CS+, $F(1,117) = 9.18$, $p = .003$, $\eta p^2 = .073$, whereas evaluations did not differ in controls, $F(1,117) = 2.38$, $p = .126$, $\eta p^2 = .020$. If a CS- was presented first, the instruction group did not evaluate CS+ and CS- differently, $F(1,117) = 0.99$, $p = .321$, $\eta p^2 = .008$, but the control group evaluated CS- as less pleasant than CS+, $F(1,117) = 12.08$, $p = .001$, $\eta p^2 = .094$. A CS order × Block interaction, $F(3,115) = 3.46$, $p = .019$, $\eta p^2 = .083$, revealed when CS+ was presented first, overall evaluations did not differ across blocks, $F(3,115) = 0.87$, $p = .461$, $\eta p^2 = .022$, but when CS- was presented first, evaluations in block 1 were more pleasant than evaluations in subsequent blocks, all $ps < .037$, $F(3,115) = 4.31$, $p = .006$, $\eta p^2 = .101$. A CS × Block interaction, $F(3,115) = 17.60$, $p < .001$, $\eta p^2 = .315$, revealed that differential evaluations were not present during the first reversal block, $F(1,117) = 0.25$, $p = .616$, $\eta p^2 = .002$, but CS- was evaluated as less pleasant than CS+ during subsequent blocks, all $Fs(1,117) > 17.87$, $ps < .001$, $\eta p^2 s > .132$. The CS × Block × Group interaction approached significance, $F(3,115) = 2.64$, $p = .053$, $\eta p^2 = .064$, but follow-up analyses revealed the same pattern of differential valence in both groups.

First Interval Responding. Main effects of CS, $F(1,114) = 89.86$, $p < .001$, $\eta p^2 = .441$, and block, $F(3,112) = 10.94$, $p < .001$, $\eta p^2 = .227$, and a CS × Block interaction, $F(3,112) = 3.88$, $p = .011$, $\eta p^2 = .094$, were moderated by a CS × Block × Group interaction, $F(3,112) = 3.67$, $p = .014$, $\eta p^2 = .089$. In the control group, responding between CS+ and CS- did not differ during block 1, $F(1,114) = 0.13$, $p = .724$, $\eta p^2 = .001$, but during subsequent blocks responding to CS- was larger than responding to CS+, all $Fs\ (1,114) > 13.76$, $ps < .001$, $\eta p^2 s > .107$. In the instruction group, however, CS- elicited larger responding than CS+ during all blocks, block 1: $F(1,114) = 32.05$, $p < .001$, $\eta p^2 = .219$, subsequent blocks: all $Fs\ (1,114) > 14.06$, $ps < .001$, $\eta p^2 s > .109$. A CS × Group × CS order interaction, $F(1,114) = 6.39$, $p = .013$, $\eta p^2 = .053$, revealed that across reversal, responding to CS- was larger in the CS+ first instruction group in comparison with the CS+ first control group, $F(1,114) = 4.62$, $p = .034$, $\eta p^2 = .039$; no other differences between the groups reached significance, all $Fs\ (1,114) < 0.12$, $ps > .745$, $\eta p^2 s < .002$.

Second Interval Responding. A main effect of CS, $F(1,114) = 90.03$, $p < .001$, $\eta p^2 = .441$, was moderated by a CS × Block × Group interaction, $F(3,112) = 5.79$, $p = .001$, $\eta p^2 = .134$. In both groups, CS- elicited larger responding than CS+ during all 4 blocks, all $Fs\ (1,114) >$

3.97, *ps* < .049, ηp^2s > .033; however, during block 1, responding to the CS+ was larger in the control group than in the instruction group, $F(1,114) = 5.46$, $p = .021$, $\eta p^2 = .046$, and responding to the CS- was larger in the instruction group than in the control group, $F(1,114) = 4.69$, $p = .033$, $\eta p^2 = .039$. During block 2, responding to the CS+ was marginally larger in the instruction group than in the control group, $F(1,114) = 3.77$, $p = .055$, $\eta p^2 = .032$. The instruction and control group did not differ in responding to CS+ or CS- during any other stage of the reversal phase, all *Fs* (1,114) < 0.70, *ps* > .403, ηp^2s < .007.

First Trial Instruction Effects

In order to examine the effects of the instructions on responding to CS+ and CS- independent of any additional learning that may have occurred as a result of the initial reversal trial, a change score [first reversal trial – last acquisition trial] was calculated for evaluations of and electrodermal responses to CS+ in the CS+ first groups and CS- in the CS- first groups. To compare the magnitude of the instruction effects for CS+ (instructions should increase pleasantness and reduce electrodermal responses) and CS- (instructions should decrease pleasantness and increase electrodermal responses), the change scores in the CS- first group were inverted[3] and 2 (Group: instruction, control) × 2 (CS order: CS+ first, CS- first) between groups ANOVAs were performed and the 95% confidence intervals for the change scores were inspected. The (non-inverted) change scores for CS valence, first interval, and second interval responding are displayed in the left, middle, and right, panels of Figure 4, respectively.

Conditional Stimulus Valence. The 2 x 2 factorial ANOVA yielded no significant differences, largest $F(1,117) = 2.66$, $p = .105$, $\eta p^2 = .022$ (Group × CS order interaction) indicating that the change in stimulus evaluations did not differ across the 4 groups. The change score for CS+ valence in the instruction group, however, was significantly different from 0 as suggested by the 95% confidence interval [0.178, 0.837]. This was not the case in the other groups 95% CI [Instruction CS-: -0.501, 0.103; Control CS+: -0.278, 0.336; Control CS-: -0.514, 0.062].

[3] The signs for the CS- first group were inverted in order to remove the direction of the instruction effect (while still keeping individual variability). As some participant's instructions scores are positive others are negative taking the absolute values of the scores is not accurate as it does not take into account this variability. Inversing the score removes the direction while keeping the magnitude.

First Interval Responding. As can be seen in the middle panel of Figure 4, the change in first interval responding was larger in the instruction than in the control groups, $F(1,114) = 4.39$, $p = .038$, $\eta p^2 = .037$, and larger in the CS- first group than in the CS+ first group, $F(1,114) = 9.50$, $p = .003$, $\eta p^2 = .077$. Inspection of the 95% confidence intervals suggests that the increase in first interval responding to CS- in the instruction group was significant [0.154, 0.357], whereas there was no difference in the three other groups 95% [Instruction CS+: -0.140, 0.082; Control CS+: -0.089, 0.124; Control CS-: -0.017, 0.179].

Second Interval Responding. The change in electrodermal second interval responding was larger in the instruction than in the control groups, $F(1,114) = 8.33$, $p = .005$, $\eta p^2 = .068$. Second interval responses to CS+ decreased in the instruction, 95% CI [-0.230, -0.050], but not the control group, 95% CI [-0.092, 0.081], whereas second interval responses to CS- increased in the instruction group, 95% CI [0.037, 0.201], but not in the control group, 95% CI [-0.072, 0.087].

Pre/Post Pleasantness Ratings

Before analysis, the post-experimental pleasantness ratings (ratings C) were transformed from a 7 to a 9 point Likert scale. Pleasantness evaluations taken before habituation (ratings A), after reversal (ratings B), and post-experimentally were subjected to a 2 (Group: instruction, control) × 2 (CS order: CS+ first, CS- first) × 2 (CS: CS+, CS-) × 3 (Phase: ratings A, ratings B, ratings C) factorial ANOVA, see Figure 5. A main effect of phase, $F(2,120) = 7.38$, $p = .001$, $\eta p^2 = .109$, was moderated by a CS × Phase interaction, $F(2,120) = 11.27$, $p < .001$, $\eta p^2 = .158$. Ratings of CS+ and CS- did not differ before habituation, $F(1,121) = 0.11$, $p = .746$, $\eta p^2 = .001$, however after reversal, CS- was given lower pleasantness ratings than CS+, $F(1,121) = 15.07$, $p < .001$, $\eta p^2 = .111$. After the experiment, ratings of CS+ and CS- did not differ, $F(1,121) = 0.30$, $p = .585$, $\eta p^2 = .002$.

Discussion

In the current study, we examined the effect of reversal instructions on electrodermal responding and online conditional stimulus (CS) valence evaluations after differential fear conditioning. Prior studies of instructed extinction have reported that instructions eliminate differential physiological responding, while leaving differential CS valence evaluations intact (Luck and Lipp, 2015a; 2015b). This dissociation could indicate that different mechanisms

underlie expectancy learning and evaluative learning. Alternatively, it could occur because instructed extinction reduces arousal levels, rendering the physiological indices less sensitive to residual stimulus valence. An instructed reversal design permits the assessment of this proposition as the threat of receiving the unconditional stimulus (US), and therefore arousal, is maintained. Based on studies of instructed extinction we hypothesized that instructed reversal would reduce electrodermal responding to CS+, and increase electrodermal responding to CS-, in the instruction groups, but not the control groups. CS valence, however, was predicted to remain unchanged in both groups.

Throughout acquisition, differential first and second interval electrodermal responding was acquired, such that presentations of CS+ elicited larger responses than presentations of CS-. Differential valence evaluations were also acquired such that CS+ acquired negative valence relative to CS-. Reversal instructions affected electrodermal responses to CS+ and CS- as predicted. Analysis of the change in electrodermal responses from the last trial of acquisition to the first trial of reversal revealed that the instruction decreased electrodermal second interval responding to CS+ and increased electrodermal first and second interval responding to CS-. This change was evident on the very first trial of reversal, i.e., in the absence of any additional Pavlovian training. The finding that the instructed CS+ first group showed a decrease in electrodermal second interval responding to CS+, even though US presentations were expected on subsequent trials, indicates that the elimination of differential electrodermal responding after instructed extinction is not caused by a decrease in arousal levels.

While significant changes in second interval responding in response to instructed reversal were observed in both CS order groups, a change in first interval responding was significant only in the CS- first group. The absence of significant instruction effects in electrodermal first interval responding is not uncommon and has been reported in past studies of instructed extinction (see Luck & Lipp, 2015a; 2015b; Rowles, Lipp, & Mallan, 2012). It is likely that this is a side effect of the experimental manipulation as the interaction with the experimenter may increase orienting. The finding of differences between first and second interval responding in an instructed reversal design supports the argument that multiple response scoring is important, especially in instructional designs (see Luck & Lipp 2016 for more details and a FIR/SIR vs. EIR scoring comparison).

The overall analysis of the change from the last trial of acquisition to the first trial of reversal did not provide evidence for a significant change in CS valence evaluations; however, inspection of Figure 4 and the 95% CI suggests that CS+ valence in the instructed CS+ first group became more pleasant after the instruction. Although inspection of Figure 4 suggests that a similar change may have been evident for the instructed CS- first group, this change was not significant and occurred in both instructed and control participants. The pattern of results observed in the instructed CS+ first group may suggest that there are differences between the effects of instructed extinction and instructed reversal, with the latter able to affect both CS valence evaluations and electrodermal responses.

The differences between instructional designs could occur because, while instructed extinction only affects the valence of the CS+, reversal instructions target the valence of both CS+ and CS-. In the reversal design, not only does the absolute valence change (the CS+ is no longer paired with an aversive event), but also the relative valence (the CS+ is no longer the more negative of the two CSs). Differences between instructed extinction and instructed reversal could be explained by this CS- valence change if the participants make their evaluations in a relative fashion. It should be noted, however, that no such effect of instructed reversal was evident in the instructed CS- first condition or in Lipp et al's (2010) study of instruction effects on evaluative conditioning. Alternatively, a change in CS+ evaluation, but not CS- evaluation, may have been observed because the presentation of the CS+ alone during habituation allowed participants to form a CS+ –noUS representation which they could retrieve in response to the reversal instructions. No CS- –US pairings were presented before the reversal phase, and therefore participants would not have had the opportunity to form this representation. As electrodermal responding was immediately altered by the reversal instructions, it seems clear that relational propositions can be formed in response to instructions, but it is possible evaluative representations may not be able to form in a similar way based on instructions, but can be retrieved after instructions if a prior representation is available. This interpretation would be consistent with the failure of Lipp et al. (2010) to find an effect of instructed reversal on evaluative learning in a picture-picture paradigm as, unlike the current study, the picture-picture paradigm did not involve a habituation phase. It would not account for findings that instructed extinction failed to influence CS+ evaluations (Luck & Lipp, 2015a; 2015b) as these experiments

did include a habituation phase. As this interpretation is post-hoc it should be treated with caution until it has been empirically validated.

It is also possible that pre-existing valence differences in the CS- first group may have dampened the influence of the reversal instructions, leading to the observation that CS- valence did not respond to instruction. The CS- first group evaluated CS+ as less pleasant than CS- during habituation, and this intrinsic negativity may have reduced the impact of instructed reversal on CS- valence if participants evaluated the stimuli in a relative fashion. A counterbalanced trial sequence was used and any valence differences occurring before the experiment are likely to be chance effects. Despite this, if the CS+ was intrinsically a negative stimulus for the some participants they may have been more reluctant to evaluate CS- more negatively than CS+ after the reversal instructions. Inspection of the reversal phase data in Figure 1 supports these suggestions, as participants in the control CS- first group evaluated the CS- as more negative than the instruction CS- first group, even at the end of the reversal phase. It is not possible to exclude the possibility that these pre-existing valence differences could have dampened the effects of instructed reversal on CS- valence, and therefore more work seems to be required to clarify this inconsistency

In addition to online ratings of stimulus valence, participants also provided ratings of CS valence in Likert scales before and after Pavlovian training (Ratings A and B), and after completion of the experiment (Ratings C). The pleasantness evaluations taken immediately after reversal training (Ratings B) revealed the same pattern of results as present in the online ratings throughout reversal training, i.e., the CS- was rated as more negative than the CS+. Interestingly however, when participants were asked to rate the faces in a different context (Ratings C), participants did not evaluate CS+ and CS- differently. This finding is in line with reports that participants integrate stimulus valence across an entire experiment when providing post-experimental ratings in a context (defined in this instance by place and mode of measurement) that is different from that in which the most recent experimental contingency was experienced (Lipp & Purkis, 2006). More broadly, it highlights the importance of assessing the emotional response to an event in different contexts when assessing the effects of an intervention in experimental or applied settings.

The current investigation confirms that the reduction of the physiological indices in response to instructed extinction does not occur because of a drop in arousal levels. Furthermore, the current study suggests that an instructional manipulation may also influence evaluative learning. Demonstrating that both expectancy and evaluative learning respond to the same manipulation provides some support for the propositional learning account, but strong theoretical conclusions cannot be drawn on the basis of the current data as the difference in valence changes between CS+ and CS- first groups needs further investigation. If CS+, but not CS-, evaluations respond to instructed reversal, the pattern of results would be more in line with dual process models. More research will be required to investigate whether changes in the evaluations of CS+ and CS- differ on the process level and to disentangle the mechanisms underlying evaluative learning.

Table 1. Means and Standard Deviations for the Variables Assessed in the Preliminary Analyses

	CS+ First		CS- First	
	Instruction	Control	Instruction	Control
Gender Ratio (male:female)	10:21	10:21	11:22	14:20
Age	21.19 (4.15)	22.65 (4.36)	24.18 (5.63)	23.47 (3.68)
Spontaneous EDA	21.50 (15.00)	17.03 (16.82)	16.13 (12.65)	17.74 (14.13)
US Level	3.25 (1.07)	3.36 (0.96)	3.08 (0.74)	2.95 (0.82)
US Valence	-1.94 (0.59)	-1.82 (0.78)	-1.61 (1.06)	-1.94 (0.55)

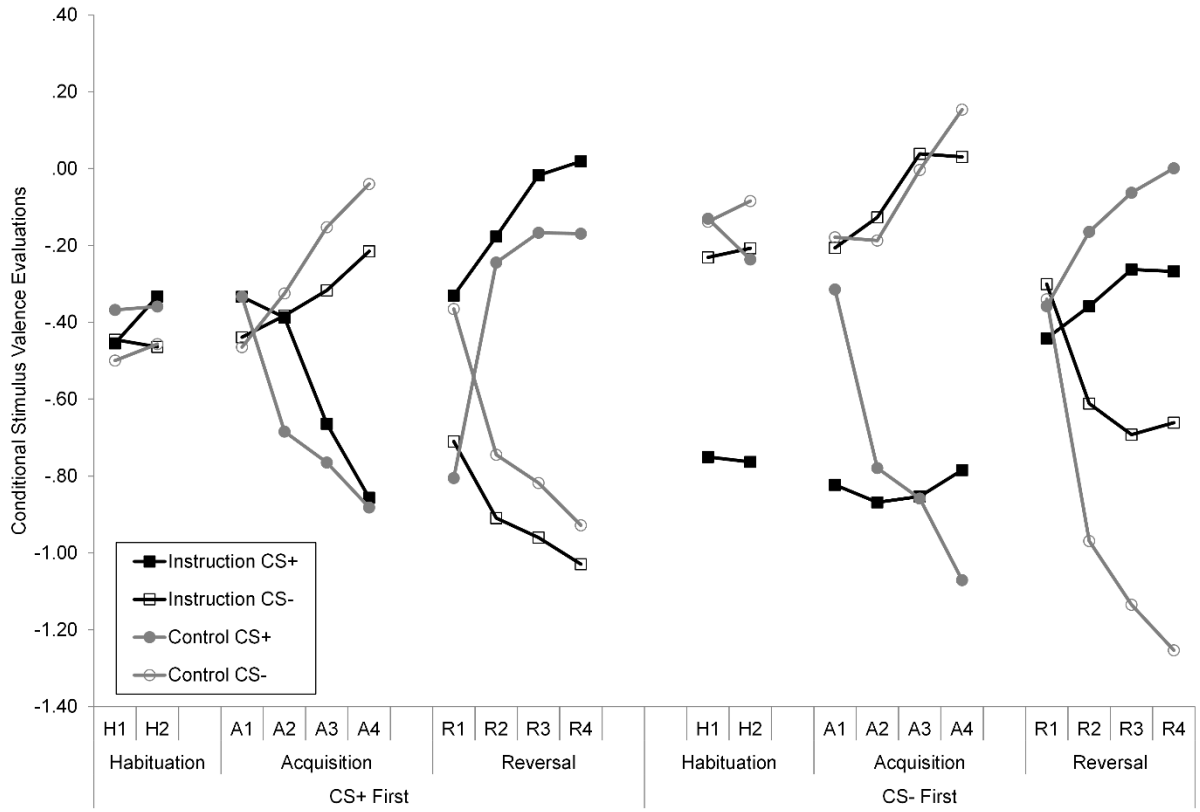

Figure 1. Conditional stimulus valence evaluations recorded throughout habituation, acquisition, and reversal

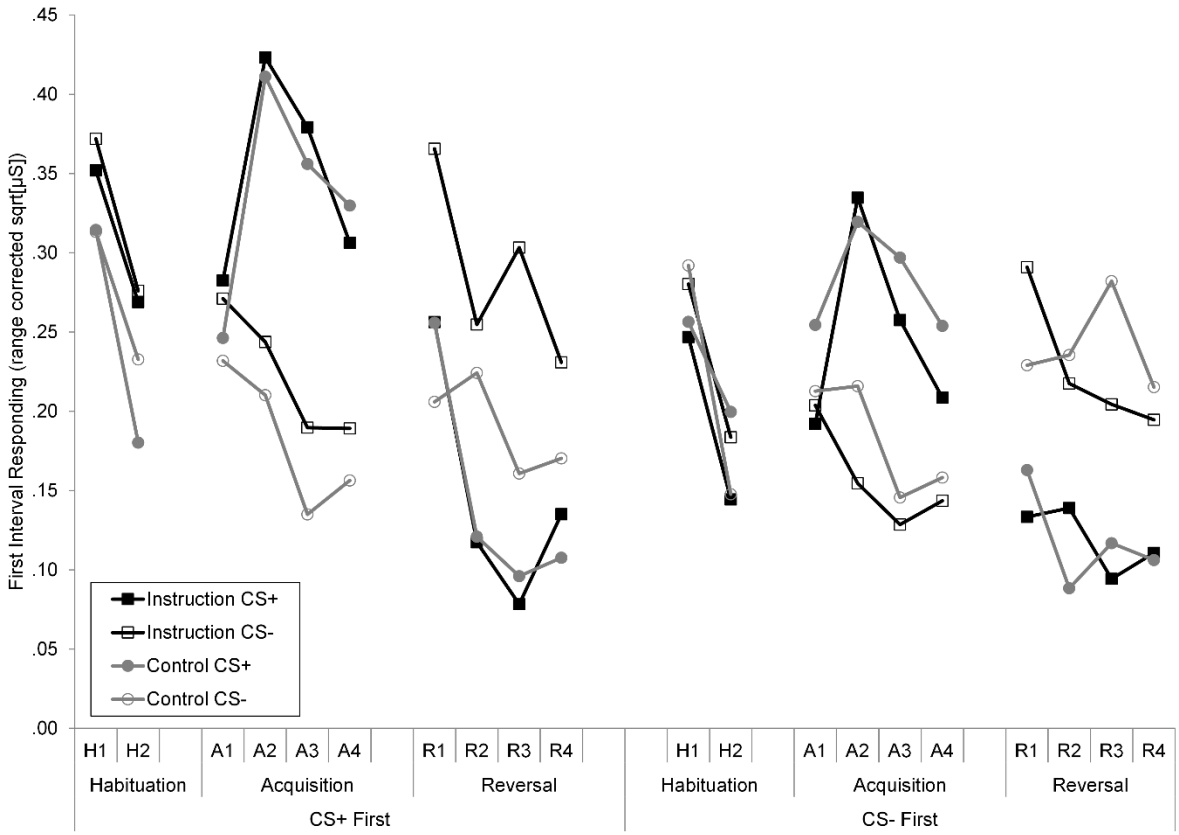

Figure 2. First interval electrodermal responding recorded throughout habituation, acquisition, and reversal.

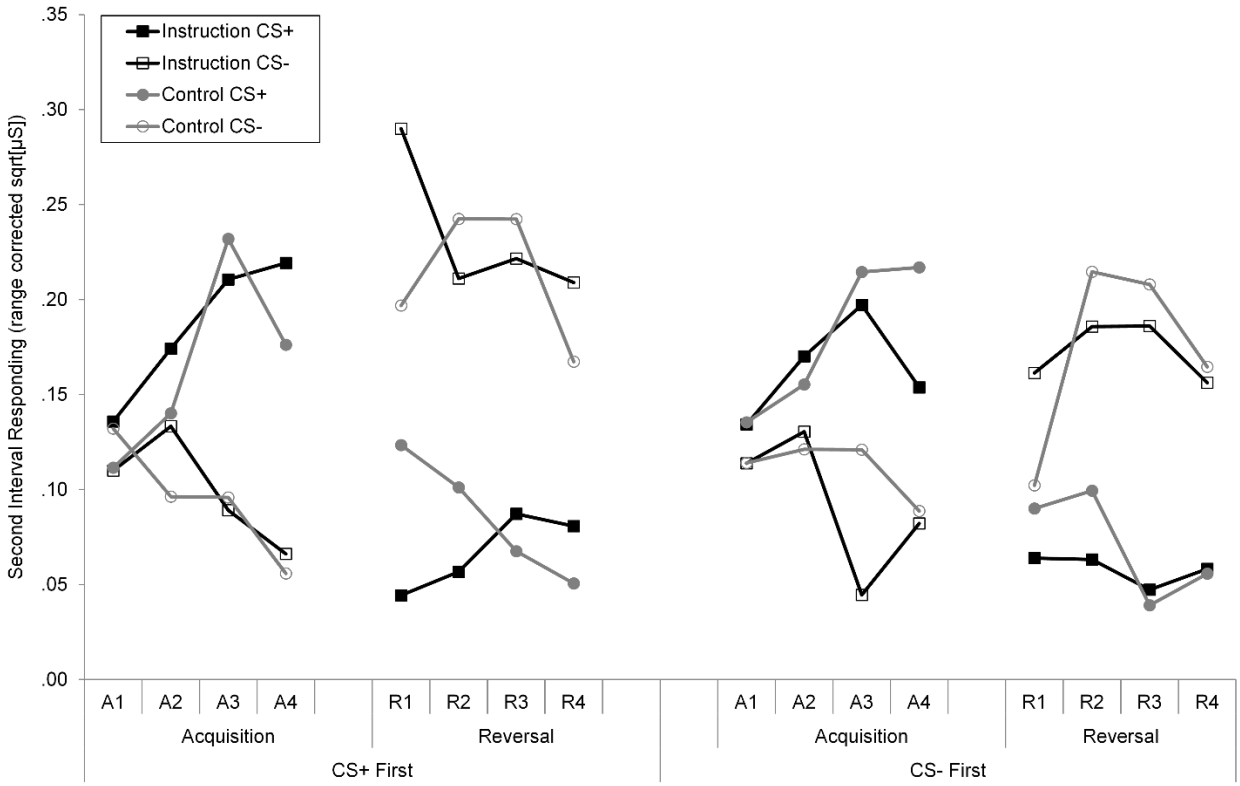

Figure 3. Second interval electrodermal responding recorded throughout acquisition and reversal.

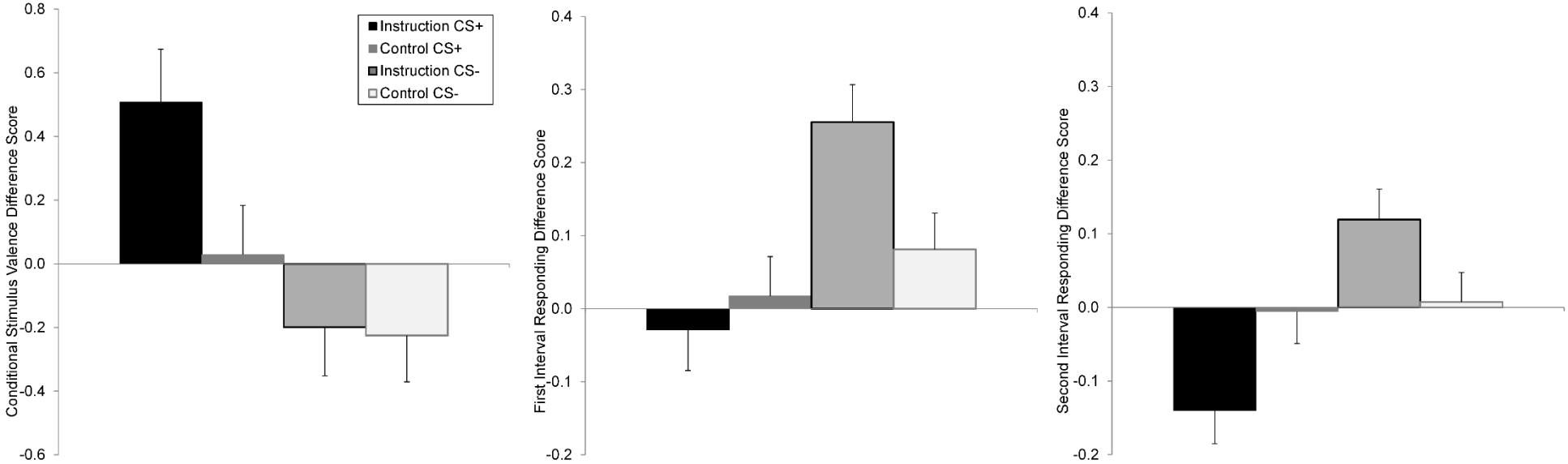

Figure 4. First trial difference scores (first reversal trial – last acquisition trial) for CS valence (left), first interval (middle), and second interval electrodermal responding (right). Positive values indicate that the stimulus is becoming more pleasant or that electrodermal responding is increasing. Negative values indicate that the stimulus is becoming less pleasant or that electrodermal responding is decreasing. (Error bars indicate standard errors of the mean).

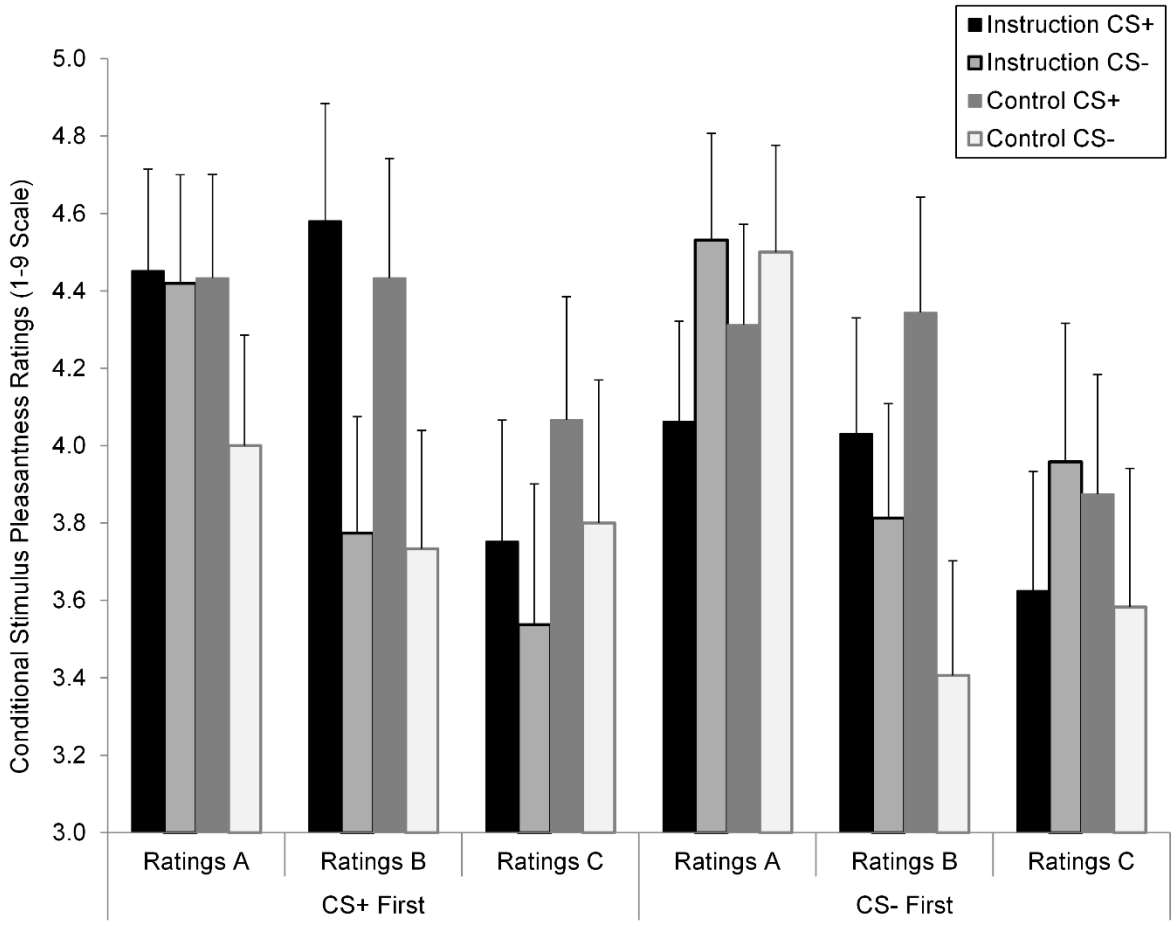

Figure 5. Conditional stimulus pleasantness ratings taken before conditioning (Ratings A), after reversal (Ratings B), and post-experimentally (Ratings C; Error bars indicate standard errors of the mean).

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Chapter 7. General Discussion

Treatments for anxiety disorders are efficacious in the short term – but relapse occurs in one to two thirds of successfully treated patients (Craske, 1999). Persisting negative evaluation of a previously feared stimulus has been shown to increase the risk of fear relapse (Dirkx, Hermans, Vansteenwegen, & Baeyens, 2004; Hermans et al., 2005; Zbozineck, Hermans, Prenouveau, Liao, & Craske, 2014). Negative valence is slow to extinguish (Hofmann, De Houwer, Perugini, Baeyens, & Crombez, 2010) and it is likely that after successful exposure therapy the previously feared stimulus will no longer elicit physiological signs of fear, but will still be evaluated as unpleasant. Cognitive therapy is often used alongside exposure therapy (Andrews, Crino, Lampe, Hunt, & Page, 1994), but more research is required to determine whether cognitive therapy can be used to reduce negative valence. The current thesis aimed to fill this gap by comprehensively examining one aspect of cognitive therapy – giving information about the feared aversive event occurring. The primary aim of the thesis was to examine whether conditional stimulus (CS) valence responds to instructed extinction in human differential fear conditioning. The secondary aim was to examine how different methodological aspects of instructed extinction could influence CS valence and physiological responding. These aims were addressed across four empirical papers.

The first empirical paper (Luck & Lipp, 2015a; Chapter 3) examined the influence of instructed extinction on electrodermal responding, fear potentiated startle, and CS valence evaluations. In Experiment 1, differential first and second interval electrodermal responding and differential valence evaluations were acquired throughout acquisition in the instruction and control groups. Following instructed extinction, differential first and second interval responding was eliminated in the instruction group, while, differential second interval responding remained intact in the control group. Unexpectedly, differential first interval responding was eliminated in the control group because of an increase in responding to CS-. The interaction with the experimenter in the control group, without the provision of safety information, likely caused an increase in orienting, eliminating differential first interval responding. We observed no effect of instructed extinction on CS valence evaluations at the beginning of extinction, or throughout the extinction phase, with both groups continuing to evaluate CS+ as less pleasant than CS- at the beginning of extinction.

In Experiment 2, these findings replicated using fear potentiated startle, a physiological index that is said to be more selectively sensitive to fear learning (Hamm &

Weike, 2005). Both groups acquired differential fear potentiated startle responses and differential valence evaluations throughout acquisition. Following instructed extinction, differential startle responses were eliminated in the instruction group, but remained intact in the control group. As in Experiment 1, instructed extinction did not influence CS valence evaluations at the beginning of extinction, or throughout the extinction phase. In Experiment 3, a separate sample of participants was recruited to determine the potential role of demand characteristics in explaining the results. The experimental scenario was described to the participants and they were asked to predict the outcome of the experiment. The majority of participants predicted that immediately following the instructions, CS+ valence would increase and physiological responding to CS+ would not change. As this pattern of results was opposite to that observed in Experiments 1 and 2, a demand characteristics explanation seems unlikely.

The second empirical paper (Luck and Lipp, 2015b; Chapter 4), examined whether the dissociation between physiological responding and CS valence might occur because the removal of the unconditional stimulus (US) electrode during instructed extinction reduces the participants' arousal levels. As physiological indices of fear learning are critically dependent on arousal (Bradley, Codispoti, Cuthbert, & Lang, 2001; Lang, Greenwald, Bradley, & Hamm, 1993), the immediate elimination of differential physiological responding after instructed extinction could be explained by a drop in the participants' arousal levels. To examine this possibility, the electrodermal responses and CS valence evaluations of three groups were compared after instructed extinction – an instruction (electrode attached), an instruction (electrode removed), and a non-instructed control group (electrode attached). Differential first and second interval electrodermal responding and differential valence evaluations emerged during acquisition in all groups. Following instructed extinction, differential first and second interval responding was eliminated in the instruction (electrode attached) and instruction (electrode removed) groups, while, differential second, but not first, interval responding remained intact in the control group. As in Luck and Lipp (2015a; Chapter 3), differential first interval responding was eliminated because of an increase in responding to CS- in the control group. As predicted, instructed extinction did not influence CS valence evaluations, on the first trial of extinction, or throughout the extinction phase – providing a replication of Luck and Lipp (2015a; Chapter 3). The observation that instructed extinction effects did not differ between the two instruction groups (electrode removed and attached) provides evidence that the elimination of differential physiological responding after

instructed extinction does not occur because of a drop in the participants' arousal levels and that removal of the US electrode is not a critical factor mediating instructed extinction effects.

The third empirical paper (Luck & Lipp, 2016a; Chapter 5) investigated the dissociation between first and second interval electrodermal responding reported in the control groups of Luck and Lipp (2015a & 2015b; Chapters 3 & 4). This most likely occurred because the instructed extinction manipulation caused a dissociation between orienting and anticipation. Scoring of electrodermal responses in multiple latency windows is particularly appropriate in these cases as first interval responding is more sensitive to orienting and second interval responding is more sensitive to US anticipation (Öhman, 1983). The entire interval scoring technique scores the electrodermal response as the maximum response occurring during the entire CS window (Pineles, Orr, & Orr, 2009). First interval responses typically have a larger amplitude than second interval responses and therefore the entire interval response is most likely to capture first interval responding. If only first interval responding was scored during instructed extinction, the meaningful dissociation between first and second interval responding would be lost and instructed extinction effects would not be detected. Luck and Lipp (2016a; Chapter 5) compared multiple response and entire interval scoring on the data reported in Luck and Lipp (2015b; Chapter 4). As predicted, entire interval scoring did not capture instructed extinction effects, with no difference between the instruction and control groups at the beginning of extinction, or throughout the extinction phase, in the entire interval response. These findings suggest that electrodermal responses in instructed extinction studies should be scored in multiple latency windows.

The fourth empirical paper (Luck & Lipp, 2016b; Chapter 6) examined a variation of the arousal hypothesis tested in Luck and Lipp (2015b; Chapter 4) by investigating whether removing the threat of the US itself could reduce the participants' overall arousal levels and render the physiological indices less sensitive. An instructed reversal design was used, in which the CS- was followed by the US during the reversal phase and the CS+ was presented alone. As this design maintains the continued threat of receiving the US, participants' overall arousal levels should not reduce. On the first trial of instructed reversal, first and second interval electrodermal responding to CS- increased; and second, but not first, interval electrodermal responding to CS+ decreased in the instruction groups. First and second interval electrodermal responding to CS+ and CS- did not change from the last trial of acquisition to the first trial of reversal in the control participants. Unexpectedly, participants

in the instruction group evaluated the CS+ as more pleasant on the first trial of the reversal phase, while evaluations of the CS- did not change. As expected, evaluations of both CS+ and CS- did not change from the last trial of acquisition to the first trial of reversal in the control participants.

Integration of the Current Findings into the Literature

The pattern of physiological responding reported after instructed extinction by Luck and Lipp (2015a & 2015b; Chapters 3 & 4) is consistent with the general pattern of findings in the literature for fear conditioned to fear irrelevant conditional stimuli. As reviewed in Luck and Lipp (2016c; Chapter 2), instructed extinction has been shown to eliminate conditional physiological responding, unless fear is conditioned to images of snakes and spiders or with a very painful electrotactile stimulus. The current thesis adds to this literature by excluding the possibility that the elimination of differential physiological responding occurs because of a reduction in the participants' general arousal levels. Instead, the results suggest that physiological responding primarily reflects the participants' expectations of the CS-US contingency – at least for fear conditioned with fear irrelevant stimuli. Electrodermal responding often converges closely with US expectancy, but fear potentiated startle is reported to be selectively sensitive to fear learning (Hamm & Vaitl, 1996, but also see Lipp, Siddle, & Dall, 2003). The observation that differential fear potentiated startle was eliminated by instructed extinction seems to contradict this claim, but it is possible that instructed extinction eliminates differential fear responding and only leaves differential valence intact. This possibility could be examined in future research by simultaneously assessing the impact of instructed extinction on fear potentiated startle and subjective fear ratings.

The dissociation between fear potentiated startle and CS valence evaluations reported in Luck and Lipp (2015a; Chapter 3) is also not in line with findings suggesting that fear potentiated startle is sensitive to stimulus valence under conditions of high arousal (Cuthbert, Bradley, & Lang, 1996). Luck and Lipp (2015a & 2016b; Chapters 4 & 6) provided evidence that the overall arousal levels do not drop after instructed extinction which should suggest that startle responding would still be sensitive to differential CS valence. At the beginning of extinction, however, while differential valence evaluations stayed intact, differential startle responding was eliminated. The results suggest that the ability of fear potentiated startle to capture stimulus valence is subject to additional unknown boundary conditions which require further investigation.

The current thesis provides strong evidence that CS valence does not respond to instructed extinction, extending the results reported by Lipp and Edwards (2002) and Lipp, Oughton, and LeLeviere (2003; Experiment 2). Luck and Lipp (2016b; Chapter 6) also provided the first examination of instructed reversal on CS valence evaluations. The pattern of CS valence after instructed reversal is not consistent with the pattern uncovered after instructed extinction, although the CS+ contingency is identical in the second phase of both procedures (CS+–noUS). There are, however, a number of other differences between extinction and reversal. In instructed extinction, the participants are given general safety instructions (no more US presentations) but in instructed reversal, the participants receive safety instructions about the CS+, but danger instructions about the CS-. It is possible that the CS+ is evaluated as more pleasant in the reversal design because the participants make their judgments relative to the CS-. In comparison with the CS-, which is now a danger signal, participants may view the CS+ as more pleasant. This could explain the differential effect of instructed extinction and instructed reversal on CS+ valence, but it cannot explain why evaluations of the CS- did not change after instructed reversal. If the CS+ was evaluated as more pleasant because the evaluative judgment was made relative to the 'unpleasant' CS-, then participants should have also evaluated the CS- as unpleasant after the instructions. In this experiment, however, the CS+ was evaluated as less pleasant than the CS- before acquisition in the CS- first group. It is possible that these pre-existing valence differences could have dampened the effects of instructed reversal on CS- valence. More research will be required to investigate this dissociation between CS+ and CS- valence after instructed reversal and to uncover the underlying mechanisms.

Implications for Theoretical Models of Evaluative and Expectancy Learning

The dissociation between physiological responding and CS valence after instructed extinction provides some support for dual process models of expectancy and evaluative learning. According to these models (see Baeyens, Eelen, Crombez, & Van den Bergh, 1992), evaluative and expectancy learning are two different forms of Pavlovian conditioning. While, expectancy learning concerns the learning of predictive relationships, in which the CS becomes a signal for the US; evaluative learning concerns the learning of referential relationships, in which the CS activates a mental representation of the US, without generating an expectancy that it will occur. The dissociation between physiological responding and CS valence after instructed extinction would be predicted by this dual-process framework. The extinction instructions target the relationship between the CS+ and the US and therefore

physiological responses (which seem to be most sensitive to the CS-US contingency in this paradigm) reduce after these instructions; but as the evaluative meaning of the CS is not dependent upon the expectation of the US, the extinction instructions leave CS valence evaluations intact.

Conversely, it is also possible that the mechanisms which underlie expectancy and evaluative learning are not distinct and the dissociation reported by Luck and Lipp (2015a; 2015b; Chapters 3 and 4) occurs, because the instructed extinction manipulation targeted the expectations of the US and not the evaluative meaning of the CS. Instructional manipulations which target the stimulus valence, rather than the CS-US contingency, could be more effective at changing CS valence evaluations. The finding that instructed reversal can influence CS valence provides some support for a single process propositional model (see De Houwer, 2009 for a review and discussion of these models), but the differential effect of the instructions on CS+ and CS- valence is not in line with this type of model. Strong theoretical conclusions should be withheld in this instance, however, because of the preexisting valence differences between CS+ and CS- in this study.

Methodology Recommendations for Instructed Extinction Research

The current thesis makes a number of methodology recommendations for future instructed extinction research. The findings of Luck and Lipp (2015b; Chapter 4) suggest that removal or attachment of the US electrode during instructed extinction is not important but that researchers should make their decision based on the specific requirements of their experiments. It is important, however, that researchers include a manipulation check to assess the participants' belief in the instructional manipulation as participants who do not believe the instructions can show different patterns of responding, such as increased responding to CS-. Luck and Lipp (2016a; Chapter 5) suggest that the electrodermal responses from instructed extinction studies should be scored in multiple latency windows to avoid overlooking important dissociations between orienting and anticipation, or missing the effect of the instructed extinction manipulation altogether. The current thesis also provides evidence that the timing of the assessment of instruction effects is critically important and that measures should be taken online and immediately after the manipulation. Post-experimental valence ratings in Luck and Lipp (2016b; Chapter 6) did not differ between the instruction and control groups even though CS+ valence did increase in the instruction group on the first trial of the reversal phase.

Implications for Clinical Practice

The current thesis suggests that cognitive interventions which target the expectation of the aversive event are very effective in reducing physiological responding but are not effective in reducing negative valence towards the feared stimulus. This is problematic as it could indicate that the current treatments are not effective at eliminating negative valence towards the feared stimulus. Instructed extinction is an analogue for cognitive therapy which targets the expectations of the feared aversive event, but it does not capture the full complexity of cognitive therapy used in the clinical situation (see Andrews et al., 1994). More research is required to examine whether other aspects of cognitive therapy, such as therapy targeting the valence of the feared stimulus directly would reduce negative stimulus evaluations.

Recommendations for Future Research

As suggested above, more research is required to explore the dissociation between CS valence and startle responding detected in Luck and Lipp (2015a; Chapter 3). One explanation for this dissociation is that stimulus specific arousal levels (rather than overall arousal levels) reduced after instructed extinction, rendering startle responses less sensitive to CS valence. This could be explored by simultaneously measuring self-reported arousal ratings and startle responses after instructed extinction. The dissociation between CS+ and CS- valence after instructed reversal reported in Luck and Lipp (2016b; Chapter 6) could be further explored by removing the habituation phase preceding the reversal experiment. Luck and Lipp (2016b; Chapter 6) suggest that a previous contingency representation may be required to change CS valence with an instructional manipulation targeting the CS-US relationship. If this phase is removed participants should not form a CS+–noUS representation and CS+ valence should not increase after reversal instructions.

More research is required to improve the clinical relevance of instructed extinction research and to examine different types of cognitive interventions in the laboratory. For instance, a study which uses a CS targeted instructional manipulation (rather than targeting the CS-US relationship) could be conducted to determine whether giving positive or negative information about the CS itself would change CS valence evaluations and physiological responding. Another possibility is to examine whether an instructed extinction manipulation which does not reduce the probability of the US occurring to zero would still be effective at eliminating differential physiological responding. As cognitive therapy focuses on bringing

expectations of the aversive event back in-line with reality, a laboratory manipulation which weakens the CS-US relationship, without eliminating it entirely, would be more applicable to the clinical situation.

More research is also required to examine whether instructed extinction influences how much fear relapse is observed after the experiment. Sevenster, Beckers, and Kindt (2012) report that instructed extinction did not affect the reinstatement of differential electrodermal responding or fear potentiated startle, but did reduce the reinstatement of differential US expectancy. This finding would suggest that instructed extinction can be used to reduce conditional expectancy ratings after reinstatement, but van den Akker, van den Broek, Havermans, and Jansen (2016) suggest that the return of conditional responding could actually be larger after instructed extinction because of the reduced 'surprise' or prediction error that occurs throughout extinction in the instruction group. More studies are needed to examine whether instructed extinction reduces or enhances conditional responding after reinstatement, renewal, and spontaneous recovery.

Concluding Remarks

The series of published papers which comprise the current thesis suggest that CS valence evaluations do not respond to instructed extinction. The thesis also excludes an arousal reduction account to explain the elimination in differential physiological responding which occurs after instructed extinction; and makes a number of methodology recommendations for future instructed extinction experiments. The observation that CS valence does not respond to instructed extinction suggests that cognitive therapy targeting the relationship between the feared stimulus and the aversive event will not be effective in eliminating negative stimulus valence. Fortunately however, a number of interesting avenues for future research are now open, such as exploring whether cognitive interventions targeting stimulus valence can be used to reduce negative valence evaluations. The instructed extinction manipulation is approaching its 80th anniversary and is still uncovering interesting findings which have implications for clinical practice and theoretical models – and probably will continue to do so for many more years to come.

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Appendix A. Co-Author Statement

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To Whom it May Concern,

I, Camilla Luck, was the major contributor to the conceptualisation and implementation of the following papers:

Luck, C. C., & Lipp, O. V. (2016). Instructed extinction in human fear conditioning: History, recent developments, and future directions. *Australian Journal of Psychology*. Advanced Online Copy. doi: 10.1111/ajpy.12135

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It was primarily my responsibility to review the literature, conduct the experiments, score the data, and conceptualise, draft, and proofread the above papers.

Camilla Luck

(Signed) .....

I, Ottmar Lipp, as the co-author, endorse that the level of contribution by the candidate indicated above is appropriate.

Ottmar Lipp

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