School of Psychology

Reducing Return of Fear: The Dual Role of the Unconditioned Stimulus in the Acquisition and Long-lasting Reduction of Fear in Humans

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Declaration of Originality

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

The research presented and reported in this thesis was conducted in accordance with the National Health and Medical Research Council National Statement on Ethical Conduct in Human Research (2007) – updated March 2014. Studies conducted as part of this thesis received human research ethics approval from the Curtin University Human Research Ethics Committee (EC00262), Approval Number #HRE2016-0384 and #HR23/2014/AR2.

Signature: …….

Date: …….

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List of Common Abbreviations

ANOVA	Analysis of variance
CS	Conditioned stimulus
CS-	Conditioned stimulus, not reinforced
CS+	Conditioned stimulus, reinforced
DASS	Depression, Anxiety, and Stress Scales
EDA	Electrodermal activity
FPS	Fear potentiated startle
Hz	Hertz
ITI	Inter-trial interval
IU	Intolerance of uncertainty
IUS	Intolerance of Uncertainty Scale
M	Mean
m	Minute
µS	Micro Siemens
PTSD	Post-traumatic stress disorder
s	Second
SAD	Social anxiety disorder
SCRs	Skin conductance responses
SD	Standard deviation
SE	Standard error
SNAQ	Snake Phobia Questionnaire
SPQ	Spider Phobia Questionnaire
US	Unconditioned stimulus
UR	Unconditioned response
VR	Valence ratings
V	Volt

Abstract

Past research has provided us with a good understanding of fear acquisition and fear reduction. Briefly, fears are learned through association of initially neutral cues with a naturally aversive outcome (unconditioned stimulus [US]). Following such pairings, the initially neutral stimulus becomes a conditioned stimulus (CS) that is capable of eliciting the conditioned fear response. The most commonly employed method of fear reduction is extinction training, consisting of repeated presentations of the CS in the absence of the US, until conditioned responding is eliminated. Extinction training is an effective method of fear reduction, but does not prevent fear recovery, because extinction training does not eliminate the acquired fear association, but creates a novel, inhibitory CS-no US association that competes for expression with the original CS-US association.

Based on a large body of evidence showing that conventional extinction training does not yield long-lasting reduction of fear, the focus of the present thesis was on fear reduction methods that involve presentations of the CS *and* the US, specifically on occasionally reinforced extinction training, manipulation of the memory reconsolidation process through extinction training that was delivered after a memory reactivation procedure involving the US, and extinction training administered in conjunction with US devaluation. Whilst all of these methods resemble conventional extinction training to some extent, they may capitalise on different underlying mechanisms, such as the modification of the original fear memory trace through the manipulation of the memory reconsolidation processes, and may, therefore, be better suited to the long-lasting reduction of fear. However, at present, these approaches remain poorly understood and require further investigation.

Study 1 was designed to examine spontaneous recovery, reinstatement, and reacquisition of fear subsequent to occasionally reinforced and conventional extinction training. Past human fear conditioning research indicates that *partially reinforced extinction training,* conducted with occasional presentations of CS-US trials, may reduce the reacquisition of extinguished fear. Appetitive conditioning research conducted with animals additionally suggests that the rate of reacquisition could be further reduced through *unpaired extinction training*, involving the occasional presentation of the US in the inter-trial interval (ITI). Building on this line of research, the present

study examined whether recovery of extinguished fear could be reduced through extinction training that involves the occasional presentations of the US, either paired with the CS or presented in the ITI. Healthy volunteers (N = 72; M age = 21.61 years, SD = 3.95) underwent differential fear conditioning to neutral CSs (pictures of birds and fish) before undergoing partially reinforced, unpaired, or conventional extinction training. Fear recovery, as indexed by differential skin conductance responses (SCRs) and CS valence ratings, was assessed 10 minutes after completion of extinction training through tests of spontaneous recovery, reinstatement, and reacquisition. Results showed spontaneous recovery of differential SCRs subsequent to conventional, but not partially reinforced or unpaired extinction training. The results further suggest that unpaired, but not partially reinforced extinction training may prevent reacquisition of differential SCRs. There was no benefit of US presentations on the reinstatement of SCRs or on the recovery of conditioned negative evaluations of CS valence. These results show that the recovery of extinguished fear can be reduced through extinction training that is conducted with occasional presentations of the US, although unpaired extinction may be more effective in the reduction of fear recovery than partially reinforced extinction.

In Study 2, it was examined whether recovery of extinguished fear could be reduced through extinction training that is delivered during the memory reconsolidation period, a period during which previously consolidated memories are active and malleable. Past research indicates that behavioural manipulations of the reconsolidation process may prevent the recovery of fear to fear-irrelevant, but not necessarily to fear-relevant CSs. The present study provided the first direct comparison of post-reconsolidation recovery of fear to fear-irrelevant and fear-relevant CSs, using a memory reactivation trial consisting of an unsignaled presentation of the US, delivered at half the physical intensity as that employed during fear acquisition (*US-reactivation*). Healthy volunteers (N = 56; M age = 24.39 years, SD = 7.71) underwent differential fear conditioning to fear-relevant (pictures of snakes and spiders) and fear-irrelevant (geometric shapes) CSs on Day 1. Extinction training, with or without prior memory reactivation, was delivered 24 hours later. Assessments of fear recovery, conducted on Day 3, showed spontaneous recovery and reinstatement of differential SCRs subsequent to conventional extinction training, but not after extinction training that was delivered 10 minutes after US-

reactivation. No group differences were observed in conditioned negative evaluations of CS valence. Contrasting past research, the present findings demonstrate that post-reconsolidation recovery of fear is not a function of fear-relevance. The findings further indicate that a memory reactivation trial consisting of the US is capable of reactivating and destabilising multiple, distinct fear memories, and thereby facilitates subsequent reduction of fear to both CS types. Follow-up tests conducted after a delay of 8 to 12 months indicated that fear reduction achieved through the US reactivation-extinction procedure was indeed long-lasting, whereby participants in the US-reactivation group were able to verbalise the trained CS-US associations, but did not exhibit spontaneous recovery or reinstatement of fear, as indexed by SCRs.

Study 3 was based on the previously discussed reconsolidation study and past *US devaluation* research and examined whether a procedure that resembles US reactivation-extinction, but is not delivered during the memory reconsolidation period could also yield long-lasting reduction of fear. In line with extant reconsolidation literature, it was also examined whether prediction errors generated by the unsignaled presentation of the USs during the US devaluation phase mediate the post-devaluation reduction of fear. Healthy volunteers (N = 96; M age = 24.33 years, SD = 7.12) underwent differential fear conditioning to fear-irrelevant and fear-relevant CSs on Day 1 and were subsequently randomised into one of three groups: control group, *instructed*, or *uninstructed* US devaluation. US devaluation was administered immediately after acquisition training and consisted of three unsignaled presentations of the US, at half the physical intensity as that employed during acquisition training. Verbal instruction were used to manipulate prediction errors during the US devaluation phase. Extinction training was delivered after a delay of 10 minutes. Assessment of differential SCRs and CS valence ratings, conducted 10 minutes and 24 hours after the US devaluation (or control) phase, showed reduced conditioned responding in all groups. These findings indicate that conditioned responding was not a function of US devaluation. However, the interpretation of findings was further complicated by group differences during fear acquisition. As such, no definitive conclusions can be made about the role of US devaluation, or prediction errors, in the long-lasting reduction of fear.

The broad pattern of findings can be interpreted as follows: In line with previous research, the present results demonstrate that conventional CS-only extinction training is effective in the within-session reduction of fear, but does not prevent fear recovery. Combining US presentations with conventional extinction training, whether in the form of occasionally reinforced extinction training or as a means of memory reactivation prior to the delivery of extinction training, resulted in superior reduction of fear, as reflected in SCRs. The secondary dependent measure employed in the present studies, consisting of CS valence ratings, was not sensitive to the experimental manipulations. However, this double dissociation between SCRs and CS valence ratings is not uncommon in fear conditioning research. The combined electrodermal results indicate that an aversive US, a key determinant of fear acquisition, is also important for the long-lasting reduction of fear.

The fear reduction methods investigated in the present studies, specifically occasionally reinforced extinction training and US reactivation-extinction, might represent suitable alternatives to current exposure-based practices for the treatment of naturally occurring fears, pending further pre-clinical and clinical research. In conclusion, the results of the studies conducted as part of this thesis contributed to the broader human fear conditioning literature by demonstrating that the well-documented recovery of fear subsequent to CS-only extinction training can be reduced, and even eliminated, through fear reduction methods that involve exposure to the CS *and* the US.

Publications Included as Part of the Hybrid Thesis

The studies presented in Chapter 3 and Chapter 4 of this thesis have been published in the Journal *Behaviour Research and Therapy.*

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Thompson, A., McEvoy, P. M., & Lipp, O. V. (2018). Enhancing extinction learning: Occasional presentations of the unconditioned stimulus during extinction eliminate spontaneous recovery, but not necessarily reacquisition of fear. *Behaviour Research and Therapy, 108,* 29-39. doi:10.1016/j.brat.2018.07.001

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Chapter 1: Introduction

Past research indicates that a key mechanism underlying the development and treatment of fears, phobias, and anxiety disorders is associative learning (Craske, Treanor, Conway, Zbozinek, & Vervliet, 2014; Grillon, 2008; Pittig, Treanor, LeBeau, & Craske, 2018). Associative learning theories (e.g., Pearce & Hall, 1980; Rescorla & Wagner, 1972) postulate that fears are learned through association of initially neutral cues with naturally aversive events (unconditioned stimuli [USs]). After repeated pairings, the previously neutral cue (conditioned stimulus [CS]) becomes a reliable predictor of the US and future CS presentations activate a mental representation of the US and elicit the conditioned fear response (Davey, 1992). The association that is formed between the CS and the US allows organisms to anticipate and prepare for future threat and, as such, is an important adaptive mechanism that facilitates survival (Öhman & Mineka, 2001). However, fears may also become maladaptive and contribute to the development and maintenance of anxiety disorders, for instance when individuals avoid places or situations that are associated with an increased likelihood of encountering the feared outcomes (Pittig et al., 2018).

The current global prevalence rate of anxiety disorders is estimated at 7.3%, with approximately 11.6% of the population experiencing an anxiety disorder in a given year (Baxter, Scott, Vos, & Whiteford, 2013; Craske & Stein, 2016). In Australia, one in seven adults aged 16 to 85 years (14.4%) will experience an anxiety disorder in a given year, while the lifetime prevalence rate is estimated to be between 20% and 32%, for men and women respectively (Australian Bureau of Statistics, 2008). These conditions are commonly treated with exposure-based therapies, which are based on the principles of extinction learning (Craske, Hermans, & Vervliet, 2018; Craske et al., 2014). Even though such treatments are efficacious, not all individuals respond to them, while others experience a return of fear after treatment (Craske & Mystkowski, 2006).

The return of extinguished fear is a well-documented phenomenon, which has been observed in the experimental and in the clinical setting alike (Bouton, 2002; Craske et al., 2018; Craske et al., 2014). It is estimated that 19% to 62% of individuals experience a return of fear after the successful conclusion of treatment of anxiety disorders (Craske & Mystkowski, 2006). Given this high rate of

relapse, there is a need for continued research into methods of long-lasting fear reduction, including methods that may persistently eliminate the return of fear, for instance by modifying the original fear memory trace (e.g., Liu et al., 2014). Based on a large body of evidence showing that conventional methods of fear reduction, involving the repeated presentations of the CS in the absence of the US, do not yield long-lasting reduction of fear (e.g., Bouton, 2002), the focus of the present thesis was on fear reduction methods involving the CS *and* the US, such as *occasionally reinforced extinction* training (Bouton, Woods, & Pineño, 2004).

Although the use of an aversive US for the purpose of fear reduction may seem counterintuitive, given its central role in fear acquisition, past research indicates that fear reduction methods involving exposure to the CS and US may be more effective in the long-lasting reduction of fear than methods focusing on exposure to the CS only (e.g., Bouton et al., 2004; Liu et al., 2014). Furthermore, such approaches may provide enhanced translational utility to the treatment of naturally occurring fears, as exposure-based techniques employed in the treatment of anxiety disorders are likely to involve real or imaginal exposure to the CS *and* the US (Scheveneels, Boddez, Vervliet, & Hermans, 2016; Vervliet, Craske, & Hermans, 2013). Hence, in the present experimental research project, three methods have been examined, being: (a) occasionally reinforced extinction training with paired and explicitly unpaired US presentations (Chapter 3; Bouton et al., 2004); (b) manipulation of the memory reconsolidation process through extinction training that was delivered after a memory reactivation procedure involving the US (Chapter 4; Liu et al., 2014); and (c) extinction training administered after US devaluation (Chapter 5; Schultz, Balderston, Geiger, & Helmstetter, 2013).

1.1. Fear Learning

1.1.1. Classical Conditioning

The study of fear in the experimental setting and the development of real-life fears is based on the principles of classical conditioning (Craske et al., 2018; Craske et al., 2014). Classical conditioning is the process through which an initially neutral stimulus becomes a CS by being repeatedly paired with the US – a stimulus that naturally elicits a physiological response (Pavlov, 1927). The process is also referred to as Pavlovian conditioning, named after the Russian physiologist

Ivan Pavlov, who discovered many of the basic principles of classical conditioning through his experiments in dogs. Pavlov observed that the dogs would not only salivate when they ate food, but also when they were presented with cues that predicted the arrival of food. The association between the CS and the US was established by presenting dogs with a ticking metronome (CS) immediately before food (US) was delivered. Following several CS-US pairings, the CS, when presented by itself, elicited salivation in the dogs (conditioned response). Similar to the association formed between the ticking metronome and the food, associative learning is also the process underlying fear acquisition (e.g., Lipp, 2006a).

During fear acquisition an initially neutral stimulus, such as an animal, becomes a CS through association with an aversive US, such as an animal bite. Following fear acquisition, the CS becomes a reliable predictor of the US and elicits the conditioned fear response (e.g., Davey, 1992). The earliest demonstration of fear conditioning in humans was conducted by Watson and Rayner (1920) through the 'Little Albert' experiment. Extensive pre-experimental observations revealed that the 9-month old boy showed no fear reactions when presented with a range of novel objects, such as a white rat, rabbit, dog, or even a burning newspaper. However, after the pairing of a white rat (CS) with a loud noise (US), the infant started reacting fearfully towards the white rat on subsequent trials. Thus, Watson and Rayner provided the first systematic investigation into fear learning in humans, demonstrating that fears can be acquired through association of a CS with a naturally aversive US. Present-day fear conditioning experiments follow the same principles of fear acquisition, albeit with stringent ethical requirements, mildly aversive USs, better control conditions, and enhanced statistical power.

1.1.2. Differential Fear Conditioning Paradigm

Fear acquisition in the experimental setting typically involves the use of a differential Pavlovian fear conditioning paradigm (Lipp, 2006a; Lonsdorf et al., 2017), whereby one CS (CS+) is paired with an aversive US, while another CS (CS-) is presented by itself. Future presentations of the CS+ result in anticipation of the US, which is reflected in increased differential responding to the CS+, relative to the CS-, on behavioural, verbal, and physiological indices of conditioned fear (Lipp, 2006a). A key strength of the differential fear conditioning paradigm is its ability to control for non-

associative processes that may affect conditioned responding, such as the orienting response or sensitisation, as any potential effects of non-associative processes on conditioned responding would be reflected in responding to the CS+ and the CS- (Lipp, 2006a; Lonsdorf et al., 2017; Öhman & Mineka, 2001).

Conditioned stimuli employed in human fear conditioning research fall into two broad classes of fear-relevance, being biologically fear-irrelevant CSs, such as geometric shapes (e.g., Schiller et al., 2010), and biologically fear-relevant CSs, such as snakes (Lipp & Edwards, 2002), spiders (Kindt & Soeter, 2013), or angry faces (Lucas, Luck, & Lipp, 2018). According to the preparedness theory (Seligman, 1971), humans have evolved to preferentially associate aversive outcomes with biologically fear-relevant stimuli, meaning stimuli that posed a threat to our ancestors' survival (Öhman & Mineka, 2001). There is evidence to suggest that the use of fear-relevant CSs results in more rapid fear acquisition and slower extinction of fear than the use of fear-irrelevant CSs (Öhman, 2009; Öhman, Eriksson, & Olofsson, 1975; Öhman & Mineka, 2001), although competing explanations exist (i.e., sensitisation; Lovibond, Siddle, & Bond, 1993) and a recent review found little support for the *resistance to extinction hypothesis* (Åhs et al., 2018). However, as real life fears and phobias are typically associated with fear-relevant stimuli (Mineka & Öhman, 2002; Öhman, 2009), both classes of CSs were employed in the present research project, to examine if fears conditioned to fear-relevant and fear-irrelevant CSs are differentially sensitive to experimental manipulations, such as behavioural manipulations of the memory reconsolidation process (Chapter 4).

The types of USs used in human fear conditioning research are negatively valanced and may encompass auditory stimuli, such as a human scream (Den, Graham, Newall, & Richardson, 2015; Kredlow, Orr, & Otto, 2018a) or loud (white) noise (Fernandez-Rey, Gonzalez-Gonzalez, & Redondo, 2018; Hosoba, Iwanaga, & Seiwa, 2001), electrotactile stimulation (Dunsmoor, Campese, Ceceli, LeDoux, & Phelps, 2015; Schiller et al., 2010), or film-clips (Arnaudova & Hagenaars, 2017; Cheung, Garber, & Bryant, 2015). For an overview of additional but less-commonly employed US types, see Lonsdorf et al. (2017). In the present project, the US consisted of an unpleasant, but not painful electrotactile stimulus. Electrotactile USs are the most frequently employed aversive stimuli in

human fear conditioning research and have been shown to produce reliable conditioning effects across several indices of conditioned responding (Lipp, 2006a).

1.1.3. Physiological, Behavioural, and Verbal Indices of Fear

Fear is an emotional response that is expressed on the verbal, behavioural, and physiological level (Lang, 1985). Similar to real-life fears, experimentally induced fear is reflected in increased physiological arousal, due to the activation of the sympathetic branch of the autonomic nervous system (Kreibig, 2010). As such, fear may be reflected in: Increased sweat gland activity, which in turn increases electrodermal activity (e.g., Boucsein, 2012; Culver, Stevens, Fanselow, & Craske, 2018; Liu et al., 2014; Schiller et al., 2010); increased respiration (for a review, see Kreibig, 2010); or changes in heart rate (e.g., Gruss & Keil, 2019; Orr et al., 2000). Exposure to threatening cues can also modulate reflex responses, such as the eye blink reflex, which is potentiated during fear conditioning (i.e., fear potentiated startle [FPS]; e.g., Kindt & Soeter, 2013; Lang & Bradley, 2010; Lang, Bradley, & Cuthbert, 1998; Mertens & De Houwer, 2016); for an overview of additional indices of fear, see Lonsdorf et al. (2017).

On a behavioural level, fear may be expressed in escape from or avoidance of threatening stimuli or situations (Krypotos, Vervliet, & Engelhard, 2018; Lang & Bradley, 2010; Lovibond, 2006; Pittig & Dehler, 2018; Pittig et al., 2018; Shin & Newman, 2018) or through enhanced attention to feared cues (e.g., Abend, Pine, Fox, & Bar-Haim, 2014; Lipp, 2006b). The subjective experience of fear may be expressed on the verbal level, through an increased negative evaluation of CS+ valence, relative to CS- valence (e.g., Luck & Lipp, 2015; Zbozinek, Hermans, Prenoveau, Liao, & Craske, 2015) and through increased subjective ratings of fear of the CS+ (e.g., Mertens et al., 2018; Mertens et al., 2016). Two additional verbal indices have been employed in the experimental setting, being the awareness of the CS-US contingency as well as US expectancy ratings, meaning the estimated probability of the CS being followed by the US (for a discussion of contingency awareness and expectancy, see Lovibond & Shanks, 2002). In the present project, skin conductance responses (SCRs) and CS valence ratings were employed as primary and secondary dependent measures of fear,

respectively. Additionally, a CS-US contingency awareness questionnaire was administered after acquisition training, to assess participants' ability to verbalise the CS-US relationship.

1.2. Fear Reduction

1.2.1. Conventional Extinction Training

Treatment of phobias and anxiety disorders is commonly conducted through exposure-based therapies, during which clients are repeatedly exposed to feared stimuli (Craske et al., 2018; Craske et al., 2014). The repeated exposure to feared cues (CSs) in the absence of the feared outcome (US) is synonymous with *extinction training* conducted in the experimental setting (Bouton, 2000; Craske et al., 2018). Conditioned responding, including conditioned fear, is extinguished (i.e., reduced) through the repeated non-reinforced presentation of the CSs during extinction training (Bouton, 2000), whereby the term 'non-reinforced' refers to the absence of the US. While such training reduces conditioned responding, the dominant view of extinction proposes that extinction training does not result in the unlearning or elimination of the original fear association (i.e., the CS-US association), but creates a new, inhibitory association (i.e., CS-no US) that co-exists with the fear association (Bouton, 2000). As such, future CS presentations may activate the CS-no US or CS-US association, whereby the latter would allow for a return of extinguished fear (Bouton, 2000), although competing explanations of extinction and fear recovery exist (for a review, see McConnell & Miller, 2014).

1.2.2. Recovery of Fear

The recovery of extinguished responding, including the recovery of fear, is well-documented in the conditioning literature (e.g., Bouton, 2002; McConnell & Miller, 2014) and is reflected in larger conditioned responding to the CS+ than to the CS- in a differential fear conditioning paradigm (e.g., Kindt & Soeter, 2013). Recovery of extinguished responding may occur in a new context, one which differs from the extinction context (renewal; Bouton & Bolles, 1979); after the passage of time (spontaneous recovery; Pavlov, 1927); or after the unsignaled presentation of the US (reinstatement; Rescorla & Heth, 1975). Another marker of fear recovery is the phenomenon of rapid reacquisition (Frey & Butler, 1977; Konorski & Szwejkowska, 1950), tested through the administration of additional CS-US pairings after extinction of the conditioned response. Findings from animal research

indicate that reacquisition after extinction may occur at a faster rate than *de novo* conditioning (Napier, Macrae, & Kehoe, 1992; but see Ricker & Bouton, 1996), suggesting that at least part of the original fear learning is preserved during extinction and may be retrieved through future cue encounters.

Taken together, research has identified several pathways that may lead to the recovery of extinguished fear, lending support for the proposition that extinction training involves the acquisition of an inhibitory CS-no US association, rather than the elimination of the CS-US association (e.g., Bouton, 2002). Considering that clients will encounter feared cues, and potentially also feared outcomes, in daily life, the likelihood of fear recovery after successful completion of exposure therapy appears high – this may seem discouraging from a clinical point of view. However, extant literature suggests that fear recovery could be reduced, or even prevented, through methods that are capable of enhancing extinction learning (Bouton et al., 2004; Chapter 3 [occasionally reinforced extinction]) or by modifying the original fear memory trace (Agren et al., 2012; Liu et al., 2014; Schiller et al., 2010; Chapter 4 [reconsolidation]). A third method, which does not rely on the administration of extinction training, but has nevertheless been shown to reduce the strength of the fear response, is the post-acquisition reduction of US aversiveness (Davey, 1989; Rescorla, 1973; Schultz et al., 2013; Chapter 5 [US devaluation]). These methods differ in their application and underlying mechanisms, however, their common denominator is the application of the US in the reduction of fear. Extant literature suggests that these methods may yield superior reduction of fear, compared to conventional CS-only extinction training (e.g., Liu et al., 2014).

1.2.3. The Role of the US in Fear Reduction

1.2.3.1. Occasionally Reinforced Extinction Training

Craske et al. (2014) suggested that extinction learning during exposure therapy could be enhanced through *occasionally reinforced extinction*, which refers to extinction training that is conducted with occasional presentations of the aversive US, either paired with the CS (referred to as *partially reinforced extinction* in this thesis) or presented in an unpaired manner, through occasional delivery of the US in the middle of the inter-trial interval (referred to as *unpaired extinction* in this

thesis; Bouton et al., 2004). Taking into consideration that CS-US pairings are administered during acquisition training to condition a fear response (e.g., Lipp, 2006a), it appears counterintuitive that additional post-acquisition CS-US pairings should result in fear extinction. Yet, Craske and colleagues proposed that occasionally reinforced *extinction training* may enhance *extinction learning* through prediction errors that are generated by the unexpected presentation of reinforced CSs, meaning CSs that are followed by the US, amongst non-reinforced CS presentations. Extrapolating from this hypothesis, occasionally reinforced extinction training may allow participants to learn that an occasional CS-US pairing is followed by several CS-only trials (see also Bouton et al., 2004). In this manner, participants may learn that the US does not occur as often as expected (partially reinforced extinction) or that the CS is no longer a reliable predictor of the US (unpaired extinction). Learning information about the likelihood of future CS-US pairings or the relationship between the CS and the US (occasionally paired or unpaired), may be more conducive to the long-lasting reduction of fear than learning that the US occurs in some contexts, but not in others (conventional extinction training).

Evidence in support of this proposition comes from the appetitive (Bouton et al., 2004) and aversive (Culver et al., 2018) conditioning literature. Appetitive conditioning experiments conducted with animals (Bouton et al., 2004) demonstrated that the speed of reacquisition of extinguished responding can be reduced through partially reinforced extinction training, relative to conventional extinction training. Bouton et al.'s findings also showed that reacquisition could be reduced even further through unpaired extinction training that involves the presentation of reinforcers (food) in the inter-trial interval (ITI). The reinforcement ratios used during extinction training in Bouton et al.'s study varied between experiments, whereby Experiment 1 utilised a US to CS ratio of 1:8 and 2:8, whereas Experiment 2 involved a gradual reduction of reinforcement across extinction training sessions, from a US:CS ratio of 1:8 to 1:24. As would be expected from training involving paired CS-US trials, the reduction of conditioned responding during extinction training was slower in the partially reinforced extinction group than in the conventional extinction group. Interestingly, the rate of extinction was not affected by the administration of unpaired reinforcers, which were delivered in

the ITI. Taken together, Bouton et al.'s findings indicate that extinction training conducted with occasional presentations of the US is superior in the reduction of reacquisition than conventional CS-only extinction training. Given the differences in the speed of extinction, the findings also suggest that the rate of within-session extinction of conditioned responding is not a good predictor of subsequent recovery of extinguished responding.

Bouton et al.'s (2004) findings were later replicated and extended to the reacquisition of operant responses in animals (Woods & Bouton, 2007) and to conditioned fear in humans (Culver et al., 2018). In a differential fear conditioning experiment, Culver et al. (2018) conditioned fear to neutral faces before extinguishing it through conventional or partially reinforced extinction training. Extinction training consisted of 24 CS+/- trials, of which six were reinforced (2:8 reinforcement ratio). Spontaneous recovery and reacquisition of fear were assessed one week later. The results showed a reduced rate of reacquisition of fear, as indexed by SCRs, subsequent to partially reinforced, but not conventional extinction training. In line with Bouton et al.'s (2004) findings, the occasional delivery of paired CS-US trials slowed the reduction of responding during extinction training, yet at the same time interfered with the reacquisition of fear during subsequent tests. However, in contrast to Bouton et al.'s research, conditioned responding in Culver et al.'s study failed to extinguish. Therefore, no inferences can be made about the effects of partially reinforced extinction training on the spontaneous recovery of fear, as, strictly speaking, fear that is not extinguished cannot "recover." Nevertheless, Culver et al. demonstrated that occasional presentations of reinforced trials during extinction training may slow the reacquisition of fear, although it is not known if such training could also reduce spontaneous recovery and reinstatement of extinguished fear.

In summary, Culver et al. (2018) provided evidence for the cross-species applicability of occasionally reinforced extinction training. Aspects that require further examination and were investigated in this thesis include: (a) application of the *unpaired* extinction procedure, involving *occasional* presentations of the US, to the extinction of fear in humans; (b) examination of spontaneous recovery, reinstatement, and reacquisition of extinguished fear subsequent to extinction training conducted with occasionally paired or unpaired US presentations; and (c) examination of

mechanisms mediating fear reduction through occasionally reinforced extinction training. Regarding the latter point, past research proposed several mechanisms that may mediate fear reduction in occasionally reinforced extinction training, including: Prediction errors, also referred to as *violations of expectancies* (Craske et al., 2014; Culver et al., 2018; Rescorla & Wagner, 1972; Vurbic & Bouton, 2014), US habituation (Rauhut, Thomas, & Ayres, 2001; but see Thomas, Longo, & Ayres, 2005), and sequential learning (Bouton et al., 2004; Capaldi, 1966, 1994).

1.2.3.1.1. Prediction Errors

The results reported by Bouton et al. (2004) and Culver et al. (2018) indicate that non-reinforced extinction training, which emphasises the within-session reduction of conditioned responding, may lead to greater levels of fear recovery than partially reinforced extinction training. A possible explanation for this effect is that partially reinforced extinction training enhances extinction learning by maximising prediction errors, generated through the omission of the US (in line with conventional extinction training) *and* through the unexpected presentation of reinforced CS+ trials (Craske et al., 2014; Weisman & Rodebaugh, 2018). Prediction errors represent a mismatch between actual and expected events (Fernández, Boccia, & Pedreira, 2016; Lee, 2009) and play a central role in the acquisition and extinction of conditioned responding (e.g., Holland & Schiffino, 2016; Pearce & Hall, 1980; Rescorla & Wagner, 1972; Vurbic & Bouton, 2014), as well as in the updating of consolidated memories (Exton-McGuinness, Lee, & Reichelt, 2015; Fernández et al., 2016; Lee, 2009; Sevenster, Beckers, & Kindt, 2012, 2013, 2014).

According to associative learning theories, such as the Rescorla-Wagner model (Rescorla & Wagner, 1972), fear acquisition and extinction learning are error-driven processes through which organisms learn which cues signal the delivery or omission of aversive events. During fear acquisition, learning is proposed to occur when there is a discrepancy between the outcomes predicted by the stimuli that are present on a given trial and the events that follow that trial. In other words, "organisms only learn when events violate their expectations" (Rescorla & Wagner, 1972, p. 75). The Rescorla-Wagner model is a US-driven model, which postulates that the acquisition of the CS-US association is mediated by prediction errors pertaining to the presence, absence, or value of the US

(for a discussion of prediction errors in other models of associative learning, see Fernández et al., 2016; Holland & Schiffino, 2016). When applied to fear conditioning, the Rescorla-Wagner model would postulate that fear learning is high when the associative strength of the CS is low, meaning when the CS is a poor predictor of the US and presentations of the US are 'surprising' (Kamin, 1969) or unexpected. Conversely, learning is proposed to cease when the CS becomes a reliable predictor of the US, meaning the presentation of the US does not generate prediction errors and the associative strength of the CS is at its maximum level – the *asymptote* of learning the US will support (Rescorla & Wagner, 1972). The same principles underlie extinction of conditioned responding, however, the prediction error that drives extinction learning is generated by the unexpected omission, rather than the unexpected presentation, of the US (Rescorla & Wagner, 1972).

In sum, the Rescorla-Wagner model suggests that learning occurs in the presence of prediction errors, but stops when there is nothing new to be learned. It should be noted, however, that additional factors may induce prediction errors and that such prediction errors are not readily accounted for by traditional models of associative learning (e.g., Pearce & Hall, 1980; Rescorla & Wagner, 1972), in particular when the prediction error pertains to the unsignaled presentation of the US, which occurs in the absence of the CS (e.g., Liu et al., 2014). The discussion of prediction errors is no longer limited to their role in associative learning models, but has been extended to encompass the updating of consolidated memories, including the reactivation and destabilisation of the (fear) memory trace (e.g., Fernández et al., 2016). Thus, the definition of prediction errors has evolved with the advancements made in reconsolidation research, having now a much broader conceptualisation than that initially proposed by associative learning models (e.g., Rescorla & Wagner, 1972).

According to recent findings (for reviews, see Exton-McGuinness et al., 2015; Fernández et al., 2016; Lee, 2009; Lee, Nader, & Schiller, 2017), prediction errors are not only created by the omission of the US (e.g., Rescorla & Wagner, 1972; Sevenster et al., 2013, 2014), but may also be generated by factors such as changes to the timing between CS and US onset (Díaz-Mataix, Ruiz Martinez, Schafe, LeDoux, & Doyère, 2013) or changes to the duration of the CS presentation (Agren et al., 2012), relative to acquisition. More importantly, however, prediction errors have been

shown to be a key determinant in the updating of consolidated memories, including fear memories (Fernández et al., 2016; Sevenster et al., 2012, 2013, 2014; see also Figure 1.1) and their role in the acquisition, extinction, and updating of conditioned responding has been supported through studies examining the neural and molecular mechanisms underlying prediction errors (Fernández et al., 2016; Holland & Schiffino, 2016; Schultz & Dickinson, 2000; Zhang, Haubrich, Bernabo, Finnie, & Nader, 2018). Given the large body of evidence supporting the role of prediction errors in learning, it is conceivable that extinction learning could be enhanced through a procedure that maximises prediction errors, such as the unsignaled presentation of the US or the occasional presentation of a CS-US trial amongst many CS-only trials (Bouton et al., 2004; but see Gershman, Jones, Norman, Monfils, & Niv, 2013; Shiban, Wittmann, Weissinger, & Mühlberger, 2015).

1.2.3.1.2. US Habituation

As occasionally reinforced extinction training involves further post-acquisition US presentations, another mechanism that may mediate fear reduction is US habituation (Rauhut et al., 2001). Habituation is a non-associative process that refers to the reduction in the strength of a response, such as a reflex response, to a stimulus that is presented repeatedly (Groves & Thompson, 1970). US habituation, as a procedure, typically involves repeated presentations of the US in the absence of the CS (but see Poulos, Furedy, & Heslegrave, 1979), resulting in a reduction of perceived US aversiveness (i.e., US devaluation; Davey, 1989; Rescorla, 1973; Storsve, McNally, & Richardson, 2012). As the magnitude of conditioned fear is influenced by the mental representation of the US, specifically its aversive properties, and US habituation reduces the perceived aversiveness of the US, the strength of the subsequent conditioned fear response would be reduced as well (Davey, 1992; Rescorla, 1973; Rescorla & Heth, 1975).

In this regard, Rauhut et al. (2001) reported that a series of extinction training sessions conducted with either four or 16 explicitly unpaired US presentations, reduced renewal and reacquisition of conditioned suppression in rats (an index of conditioned fear) to a greater extent than that observed after conventional extinction training. However, in addition to a reduction in fear recovery, Rauhut and colleagues also noted a reduction in the rate of acquisition of fear to a novel CS.

Consequently, it was concluded that unpaired US presentations during extinction training resulted in US habituation. However, a later replication of Rauhut et al.'s findings failed to corroborate the US habituation hypothesis (Thomas et al., 2005). Of note, the experimental design and extinction protocols in these studies diverged from Bouton et al.'s (2004) study, most notably through the use of an aversive conditioning preparation, in lieu of appetitive conditioning, and through the use of a US:CS ratio of 1:1 and 1:2, in lieu of a 1:8 and 2:8 ratio. It remains to be investigated whether a lower US:CS ratio, such as the one employed in Bouton et al.'s study, and the corresponding reduction of US presentations, would be sufficient to induce US habituation.

As a possible alternative explanation, it has been proposed that unpaired extinction training may weaken the CS-US association, and may do so to a greater extent than would be achieved through the mere omission of the US during CS-only extinction (Frey & Butler, 1977; Rescorla & Skucy, 1969; Rescorla & Wagner, 1972; Vervliet, Vansteenwegen, & Hermans, 2010). In a human fear conditioning experiment, Vervliet et al. (2010) reported that an unpaired extinction procedure consisting of eight CS and six US presentations, delivered in the ITI, prevented renewal of differential SCRs and US expectancy ratings, while conventional extinction training did not. Remarkably, Vervliet and colleagues did not observe the typical double dissociation between verbal (US expectancy) and physiological (e.g., SCRs or FPS) indices of conditioned responding reported in past research (e.g., Kindt & Soeter, 2013; Schultz et al., 2013). Previous studies reported spontaneous recovery, reinstatement, and rapid reacquisition of differential US expectancy ratings, even though physiological indices of conditioned responding indicated an absence of fear recovery (e.g., Kindt & Soeter, 2013). However, if we make the assumption that unpaired extinction training weakens the CS-US relationship, it is conceivable that the weakened fear association would be reflected in reduced US expectancy ratings. Therefore, the renewal of differential US expectancy in the conventional extinction group, but not in the unpaired extinction group (Vervliet et al., 2010), is in line with a weakened CS-US expectancy explanation. Nevertheless, as US habituation was not explicitly assessed in Vervliet et al.'s study, the exact mechanisms mediating the reduction of renewal are not known. As such, it is possible that fear reduction subsequent to unpaired extinction training is mediated by US

habituation, a weakened CS-US relationship, or, potentially, a combination of both (cf. Frey & Butler, 1977).

1.2.3.1.3. Bouton's Adaptation of Sequential Theory (Capaldi, 1966)

In contrast to the evidence reviewed in the preceding paragraph, but in line with the predominant view of extinction training as a type of new learning rather than 'unlearning' (Bouton, 2002; Vurbic & Bouton, 2014), Bouton et al. (2004) proposed that during occasionally reinforced extinction training an association is formed between reinforced trials and non-reinforced trials. Based on Capaldi's theory of sequential learning (Capaldi, 1966, 1994), Bouton et al. proposed that the key aspect learned during partially reinforced extinction training is that CS-US trials do not occur exclusively in the 'context' of other CS-US trials (i.e., in the acquisition context), but may also occur in the context of extinction trials (i.e., CS-only trials). Furthermore, the presentation of the US (or a CS-US trial) during extinction training is proposed to break the US's exclusive association with the acquisition context and, thereby, facilitate the generalisation of extinction learning to the test context, which in turn should reduce the recovery of conditioned responding (Vurbic & Bouton, 2014). It follows that such learning would decrease the rate of reacquisition, as a CS-US trial would signal the arrival of several CS-no US trials. Admittedly, while such an account of learning is readily applicable to partially reinforced extinction training, involving occasional presentations of CS-US trials amongst many CS-only trials, it is not readily applicable to unpaired extinction training, which does not involve any post-acquisition CS-US trials. Nevertheless, the presentation of unpaired USs during extinction training would still be expected to break the US's exclusive association with the acquisition context and may, therefore, facilitate the retrieval of extinction learning and reduce recovery of extinguished responding (Bouton et al., 2004). Additionally, according to Bouton et al.'s model, unpaired extinction training might reduce reinstatement of conditioned responding, due to the learned association between US-only and CS-only trials.

To summarise, previous examinations of conventional, partially reinforced, and unpaired extinction training indicated that extinction training that involves the presentation of the US may result in superior reduction of fear, relative to conventional CS-only extinction training (e.g., Bouton

et al., 2004; Rauhut et al., 2001). Extant literature further indicates that within-session extinction of conditioned responding is neither necessary (Culver et al., 2018) nor sufficient (Bouton et al., 2004) for the reduction of recovery from extinction phenomena, such as rapid reacquisition. Furthermore, given the diversity of methods employed in past reinforced extinction research (e.g., Bouton et al., 2004; Mickley et al., 2009; Vervliet et al., 2010), it is conceivable that different mechanisms mediate fear reduction in different types of reinforced extinction training, in particular since differences in the rate of reacquisition have been observed between unpaired and partially reinforced extinction (Bouton et al., 2004). The identification of underlying mechanisms and the utility of (occasionally) reinforced extinction in the reduction of fear recovery in humans, however, require further examination. Of particular interest for the treatment of naturally occurring fears is occasionally reinforced extinction (Craske et al., 2014), as exposure to aversive outcomes, whether real or imagined, may be distressing to the client. Therefore, a procedure that involves a relatively low number of US presentations may be easier to implement in the clinical setting and be better tolerated by clients than procedures that involve a comparatively large number of successive US presentations (e.g., Frey & Butler, 1977; Rauhut et al., 2001).

1.2.3.2. Consolidation and Reconsolidation of Fear

Conventional approaches to fear reduction, such as CS-only extinction training, focus on the inhibition of fear, but leave the CS-US association largely intact and, therefore, allow for a return of extinguished fear (e.g., Bouton, 2000; Craske et al., 2018). It follows that long-lasting reduction of fear could be achieved through the modification, or even elimination, of the original fear association (Agren et al., 2012; Schiller et al., 2010). Extant research proposes that this is indeed achievable, showing that consolidated memories can be modified in a manner that reduces, and even prevents, fear recovery (Kindt, Soeter, & Vervliet, 2009; Monfils, Cowansage, Klann, & LeDoux, 2009; Nader, Schafe, & LeDoux, 2000a; Schiller et al., 2010).

1.2.3.2.1. Consolidation of Fear Memories

Following acquisition training, new and vulnerable fear memories (i.e., the memory pertaining to the CS-US association) are stabilised and protected from disruption through the process

of *consolidation* (Dudai, 2004, 2012; Dudai, Karni, & Born, 2015; McGaugh, 2000). Consolidation transforms short-term memories into long-term memories through a multitude of neural and molecular processes that occur at the cellular and systemic level in the central nervous system (for recent reviews, see Runyan, Moore, & Dash, 2019; Visser, Lau-Zhu, Henson, & Holmes, 2018). Consolidation of new learning can occur rapidly and may only require a few minutes to hours to conclude, although consolidation at the systemic level, meaning the integration of the memory into a wider memory network, may take weeks to years to conclude (Visser et al., 2018). Stress hormones that are present during emotionally arousing events, in particular during events underlying fear learning (Pedraza et al., 2016), can further enhance consolidation, and thereby strengthen the memory for a particular experience (McGaugh, 2000).

During the initial period of vulnerability (i.e., within hours after training), consolidation of fear memories can be disrupted through the administration of pharmacological agents that target the respective underlying molecular processes (e.g., Lee, 2008; McGaugh, 2000; Nader, 2015; Schafe & LeDoux, 2000; Schafe, Nader, Blair, & LeDoux, 2001; Thomas, Saumier, Pitman, Tremblay, & Brunet, 2017), through the administration of electroconvulsive shocks (Duncan, 1949), or through interference created by new learning, such as the administration of extinction training immediately after fear acquisition (Myers, Ressler, & Davis, 2006; Norrholm et al., 2008; but see Merz, Hamacher-Dang, & Wolf, 2016; Schiller et al., 2008). Once consolidated, memories are immune to disruption, or at least this is what was assumed to be the case for approximately 100 years (McGaugh, 2000; Nader, Schafe, & LeDoux, 2000b), before advances in reconsolidation research challenged the tenets of the consolidation theory and demonstrated that consolidated memories can be altered.

1.2.3.2.2. Reconsolidation of Fear Memories

Misanin, Miller, and Lewis (1968) were first to demonstrate that consolidated fear memories in rats could be eliminated through the administration of an electroconvulsive shock. In accordance with present-day reconsolidation research (e.g., Kindt & Soeter, 2013), fear acquisition, manipulation of the memory reconsolidation process, and tests of fear recovery were separated in time by 24 hours, thereby allowing sufficient time for the consolidation of the fear memory trace on Day 1, as well as

for the conclusion of the memory reconsolidation period on Day 2 (e.g., Nader et al., 2000a; Visser et al., 2018). The key finding arising from Misanin et al.'s experiment was a reduction of the behavioural expression of fear in two groups of rats, one which received the electroconvulsive shock immediately after fear acquisition and one which received the electroconvulsive shock 24 hours later, subsequent to a memory reactivation trial consisting of a brief presentation of the CS. The fear response remained intact in rats that did not receive a memory reactivation trial prior to the delivery of the electroconvulsive shock on Day 2, indicating that the reduction of fear was not caused by the administration of the electroconvulsive shock per se, but by the administration of the electroconvulsive shock during a time period of memory lability. Thus, Misanin and colleagues demonstrated that previously consolidated fear memories were not immune to disruption, provided they were returned to an active state. This time-dependent window of lability is also known as the memory reconsolidation period (e.g., Nader et al., 2000a).

Reconsolidation refers to the process which restabilises previously consolidated memories following their reactivation and destabilisation (Nader & Hardt, 2009; Nader et al., 2000b). Interfering with reconsolidation through administration of pharmacological (e.g., Kindt et al., 2009) or behavioural interventions (e.g., Schiller et al., 2010) may modify the existing memory trace and persistently reduce fear recovery (Björkstrand et al., 2015; Liu et al., 2014; Schiller et al., 2010; see also Figure 1.1). While the name reconsolidation may imply that it constitutes a repetition of the consolidation process, extant literature suggests that consolidation and reconsolidation can be dissociated based on their neural and molecular signatures (Besnard, Caboche, & Laroche, 2012; Clem & Schiller, 2016; Lee, 2008; Lee, Everitt, & Thomas, 2004), although both processes also share common mechanisms (Cahill & Milton, 2019; Clem & Schiller, 2016).

Of note, because the exact mechanisms mediating fear reduction during the memory reconsolidation period are not fully understood (Beckers & Kindt, 2017), several terms exist to refer to the modification of the memory trace during the reconsolidation period, most commonly 'manipulation', 'disruption', 'interference', or 'updating' (e.g., Beckers & Kindt, 2017; Lee et al., 2017). These terms are used interchangeably, present thesis included, and will likely continue to be

used in this manner until we gain a better understanding of the mechanisms underlying fear reduction through the experimental manipulation of the reconsolidation process (see Beckers & Kindt, 2017 for a more detailed overview and justification of terminology used in reconsolidation research). Some of these terms imply that the original memory trace is updated with new information, in accordance with previous suggestions that reconsolidation serves to maintain memory relevance (Lee, 2009). For instance, research suggests that the *updating* or *manipulation* of the reconsolidation process can result in the reduction (Oyarzún et al., 2012; Schiller et al., 2010) or strengthening of fear (Lee, 2008; Tay, Flavell, Cassini, Wimber, & Lee, 2019), depending on the type of intervention or training delivered during the reconsolidation period (for a review, see Lee et al., 2017). In contrast, other terms imply that the post-reconsolidation reduction of fear is the result of reconsolidation *disruption* (Agren et al., 2012; Kindt et al., 2009), rather than updating of the original fear memory with new information, such as that acquired through extinction training (e.g., Monfils et al., 2009). Irrespective of the term used to refer to the experimental manipulation that is delivered during the reconsolidation period, it should be noted that the experimental manipulation of the reconsolidation process does not "erase" fear memories, as post-reconsolidation knowledge of the CS-US association typically remains intact (e.g., Kindt et al., 2009) despite the elimination of the emotional expression of fear, as reflected in reduced differential FPS (e.g., Kindt et al., 2009) or differential SCRs (e.g., Schiller et al., 2010).

Returning to early reconsolidation studies, findings reported by Misanin et al. (1968) sparked debate about the nature of memory and led to the proposition that the consolidation process does not represent a once-off stabilisation of memories, but that memories could be returned to an active and malleable state (Lewis, 1979). As such, Lewis' theory was in line with our current understanding of how memories are maintained and modified, specifically that consolidated memories can be reactivated and returned to an active and malleable state (Nader & Hardt, 2009; Visser et al., 2018). However, despite this promising early work, replication attempts of Misanin et al.'s (1968) findings yielded mixed outcomes in studies conducted with animals and humans (e.g., Rubin, 1976; Squire, Slater, & Chace, 1976; for a detailed overview of early research, see Beckers & Kindt, 2017; Schiller

& Phelps, 2011) and the study of reconsolidation was largely abandoned. Interest in reconsolidation research only re-emerged at the turn of the century, with the seminal work of Nader et al. (2000a).[1]

Nader et al. (2000a) demonstrated that the reconsolidation of an auditory fear memory in rats requires protein synthesis in the (lateral and basal) amygdala – the brain area responsible for the processing of the CS-US association (LeDoux, 2000, 2014). Inhibiting protein synthesis through a direct infusion of the protein synthesis inhibitor anisomycin into the amygdala reduced the rate of freezing (an index of fear) during tests of spontaneous recovery in rats that received a memory reactivation trial immediately before the administration of anisomycin. Conversely, the behavioural expression of fear remained intact in rats that received anisomycin without prior memory reactivation and in those that received the infusion 6 hours after the reactivation trial. Importantly, it was not the infusion of anisomycin per se that eliminated freezing, as the behavioural expression of fear was intact when tested 4 hours after the infusion (i.e., before the conclusion of the memory reconsolidation period), but not when tested after a delay of 24 hours. These results indicate that the reactivation of the auditory fear memory and the infusion of the amnestic agent reduced conditioned fear by interfering with the reconsolidation of the reactivated fear memory trace. Nader et al.'s findings also showed that reconsolidation is a time-dependent process which leaves memories vulnerable to disruption for up to 6 hours – this time window of lability has been also observed in humans (e.g., Schiller et al., 2010), although the exact time course of lability is not known (cf. Kindt & Soeter, 2018).

Nader et al.'s (2000a) findings were later replicated and extended to behavioural manipulations of reconsolidation, consisting of extinction training that was delivered during the reconsolidation period (Monfils et al., 2009). Monfils and colleagues observed reduced spontaneous recovery, renewal, reinstatement, and reacquisition of fear in rats that received extinction training 10 minutes or 1 hour subsequent to memory reactivation, but not in rats that received extinction training

[1] Please note that it is beyond the scope of this thesis to provide a comprehensive review of all early contributions made to the advancement of the field of reconsolidation (e.g., Przybyslawski & Sara, 1997; Sara, 2000). For excellent reviews of the history of reconsolidation research, please see Beckers and Kindt (2017); Nader (2013); Phelps and Schiller (2013); Schiller and Phelps (2011).

outside the reconsolidation period, being either 6 to 24 hours after memory reactivation or without prior memory reactivation. Hence, the results demonstrated that extinction training that is delivered during a time period when memories are thought to be active and malleable results in superior reduction of fear than that achieved through conventional extinction training, delivered outside the reconsolidation period.

In contrast to studies conducted with animals, reconsolidation research in humans advanced only in recent years, most notably through the seminal work of Kindt et al. (2009) and Schiller et al. (2010). Kindt et al. (2009) demonstrated that an oral administration of the beta-adrenergic antagonist *propranolol*, delivered before memory reactivation, can disrupt the reconsolidation of fears conditioned to fear-relevant CSs and prevent subsequent spontaneous recovery and reinstatement of fear, as indexed by decreased differential FPS. In contrast, differential FPS recovered in participants who received a placebo or propranolol without memory reactivation. Interestingly, the recovery of differential US expectancy ratings was not prevented through the disruption of the reconsolidation process, indicating that manipulation of reconsolidation does not eliminate the fear association (cf. Agren et al., 2012), but prevents the behavioural expression of fear.

Therefore, Kindt et al. (2009) demonstrated that it is possible for individuals to remember which cues signal the arrival of aversive outcomes, and yet not exhibit fear in the presence of these cues, as reflected in the absence of physiological responding that typically increases in anticipation of aversive outcomes (Lang & Bradley, 2010; Lang et al., 1998). From an evolutionary perspective, learning about potential threat is important for one's survival (Öhman & Mineka, 2001). Hence, retaining the declarative knowledge of the CS-US association serves an important adaptive purpose that allows individuals to prepare for potential threat, without experiencing debilitating fear or anxiety. From an ethical perspective, it is also reassuring that the disruption of the reconsolidation process does not erase fear memories and that impairments in the behavioural or emotional expression of fear are selective to the reactivated memories (e.g., Liu et al., 2014; Nader et al., 2000a; Schiller et al., 2010).

Kindt et al.'s (2009) findings have been corroborated through a series of studies conducted in the basic and applied setting (e.g., Brunet et al., 2011; Brunet et al., 2018; Kindt & Soeter, 2018; Kindt & van Emmerik, 2016; Soeter & Kindt, 2010, 2015a). However, as promising as these findings are, not all replication attempts have shown successful reduction of fear through the administration of propranolol after memory reactivation (e.g., Bos, Beckers, & Kindt, 2014; Chalkia, Weermeijer, Van Oudenhove, & Beckers, 2019; Schroyens, Beckers, & Kindt, 2017; Thome et al., 2016; Wood et al., 2015). Evidently, interference with reconsolidation, whether through pharmacological or behavioural means, requires further examination before reconsolidation-interfering interventions can be employed in the clinical setting. In this regard, behavioural manipulations of reconsolidation (Monfils et al., 2009) may pose a viable alternative to pharmacological manipulations, in particular since behavioural interventions do not carry any risks that may be associated with the use of pharmacological agents.

In the experimental setting, Schiller et al. (2010) were first to report successful behavioural manipulation of reconsolidation in humans, showing that spontaneous recovery of fear, conditioned to fear-irrelevant CSs, can be prevented if extinction training is delivered 10 minutes after memory reactivation. In contrast, spontaneous recovery of differential SCRs was observed after extinction training that was delivered without prior memory reactivation or outside the reconsolidation window (6 hours after memory reactivation). Reinstatement tests conducted one year later showed that fear was persistently eliminated. In a second experiment, Schiller and colleagues demonstrated that the effects of behavioural manipulations of reconsolidation were specific to the reactivated fear memory (i.e., the CSa-US association), as reflected in reduced reinstatement of differential SCRs to the reactivated CSa+, but not to the non-reactivated CSb+. Taken together, Schiller and colleagues translated findings from animal research (Monfils et al., 2009) to humans and showed that behavioural manipulations of the reconsolidation process can yield persistent reduction of fear in healthy volunteers and that these effects are specific to the reactivated CS.

Despite these encouraging findings, replication attempts of Schiller et al.'s (2010) results in human fear conditioning studies have yielded mixed results. Successful disruption of the reconsolidation process has been largely limited to studies employing fear-irrelevant CSs (Agren,

Björkstrand, & Fredrikson, 2017; Agren et al., 2012; Björkstrand et al., 2015; Fernandez-Rey et al., 2018; Grégoire & Greening, 2019; Hu et al., 2018; Johnson & Casey, 2015; Liu et al., 2014; Meir Drexler & Wolf, 2017; Oyarzún et al., 2012; Schiller, Kanen, LeDoux, Monfils, & Phelps, 2013; Steinfurth et al., 2014; but see Golkar, Bellander, Olsson, & Öhman, 2012 [Experiment 2]; Klucken et al., 2016). Notably, Agren et al. (2012) reported that delivery of extinction training during the reconsolidation period prevented reinstatement of differential SCRs and that this effect was mediated by the elimination of the fear memory trace in the human amygdala, as reflected in functional magnetic resonance imaging, conducted two days after the disruption of the reconsolidation process as well as during an 18-month follow-up (Björkstrand et al., 2015). It is rather remarkable that a behavioural intervention is capable of permanently altering brain activity and facilitating long-lasting reduction of fear. However, at present it is not known whether these effects are reliable, as replication attempts have yielded mixed results (Björkstrand et al., 2016, 2017; Klucken et al., 2016).

In contrast to studies conducted with fear-irrelevant CSs, behavioural manipulations of the reconsolidation process in fears conditioned to fear-relevant CSs proved less successful, showing a return of fear subsequent to extinction training that was delivered during the reconsolidation period (Fricchione et al., 2016; Golkar et al., 2012 [Experiment 1]; Kindt & Soeter, 2013; Meir Drexler et al., 2014; Soeter & Kindt, 2011 [Experiment 2]). Failed replication attempts led to the proposition of boundary conditions – factors that may interfere with the initiation or with the disruption of the reconsolidation process, such as the age or strength of the memory (for recent reviews, see Zhang et al., 2018; Zuccolo & Hunziker, 2019). It has been proposed that the use of fear-relevant CSs may yield “strong” fear memories that are not sensitive to behavioural manipulations of reconsolidation (Kindt & Soeter, 2013). However, seeing that the reconsolidation of such fears can be disrupted through the oral administration of propranolol (see Soeter & Kindt, 2011 for a comparison of pharmacological and behavioural manipulations), it appears that memory strength per se is not a likely boundary condition of reconsolidation.

Regarding the fear-relevance of the CS, there is evidence to suggest that biologically fear-relevant CSs, such as snakes, show superior conditioning which may resist extinction (Mineka &

Öhman, 2002; but see Åhs et al., 2018). Therefore, it is conceivable that fears conditioned to fear-relevant CSs may not be sensitive to extinction training that is delivered during the reconsolidation period. At the same time, past reconsolidation research has not directly compared effects of extinction training on the disruption of reconsolidation of fear memories conditioned to fear-irrelevant and fear-relevant CSs (Fricchione et al., 2016; Golkar et al., 2012; Kindt & Soeter, 2013). Cross-study comparisons are problematic due to methodological variations, such as the use of different reinforcement schedules during fear acquisition, differences in the types of CSs and USs employed, or variations in the use of measures of conditioned responding (for a review, see Kredlow et al., 2016). It should be pointed out that methodological variations are not specific to reconsolidation research, but a common factor that complicates comparison of findings in fear conditioning research (for a review, see Lonsdorf et al., 2017). In the absence of direct comparisons, no firm conclusions can be drawn about the effects of fear-relevance on the behavioural disruption of the memory reconsolidation process. Hence, this aspect has been examined in the reconsolidation study conducted as part of the present thesis.

1.2.3.2.3. Determinants of Successful Manipulation of Reconsolidation

In addition to the boundary conditions addressed in the preceding paragraph, as well as those reviewed elsewhere (Auber, Tedesco, Jones, Monfils, & Chiamulera, 2013; Faliagkas, Rao-Ruiz, & Kindt, 2018; Zhang et al., 2018; Zuccolo & Hunziker, 2019), a key determinant of successful disruption of reconsolidation is the procedure used to reactivate and destabilise the fear memory trace (for reviews, see Rodriguez-Ortiz & Bermúdez-Rattoni, 2017; Wideman, Jardine, & Winters, 2018). In this regard, both early (e.g., Misanin et al., 1968; Nader et al., 2000a) and more recent (De Oliveira Alvares et al., 2013; Monti et al., 2017; Pineyro, Monti, Alfei, Bueno, & Urcelay, 2014) research showed that presentations of a reminder, or reactivation, trial is necessary to return the consolidated fear memory trace to an active and labile state that allows for subsequent manipulation of the reconsolidation process. In the absence of a reactivation trial that destabilises the fear memory trace, neither behavioural (e.g., Liu et al., 2014; Schiller et al., 2010) nor pharmacological (e.g., Nader et al., 2000a; Soeter & Kindt, 2010) manipulations prevent the recovery of fear.

The majority of past research has employed a reactivation trial consisting of a non-reinforced presentation of the CS+ (e.g., Agren et al., 2012; Fernandez-Rey et al., 2018; Hu et al., 2018; Kindt & Soeter, 2013). CS-induced memory reactivation (referred to as *CS-reactivation* in this thesis) enables the selective reactivation and destabilisation of a specific fear memory, associated with the reactivated CS, while leaving other CS-US associations intact (Liu et al., 2014; Schiller et al., 2010). Although CS-reactivation has been successfully employed in past research (Agren et al., 2012; Oyarzún et al., 2012; Schiller et al., 2010), a non-reinforced CS-only trial resembles the first trial of extinction training and, depending on the learning history and experimental parameters, may lead to extinction learning rather than the destabilisation of the fear memory trace (for reviews, see Besnard et al., 2012; Exton-McGuinness et al., 2015). For a schematic representation of the determinants of memory reactivation, destabilisation, and interference with reconsolidation in human fear conditioning, see Figure 1.1.

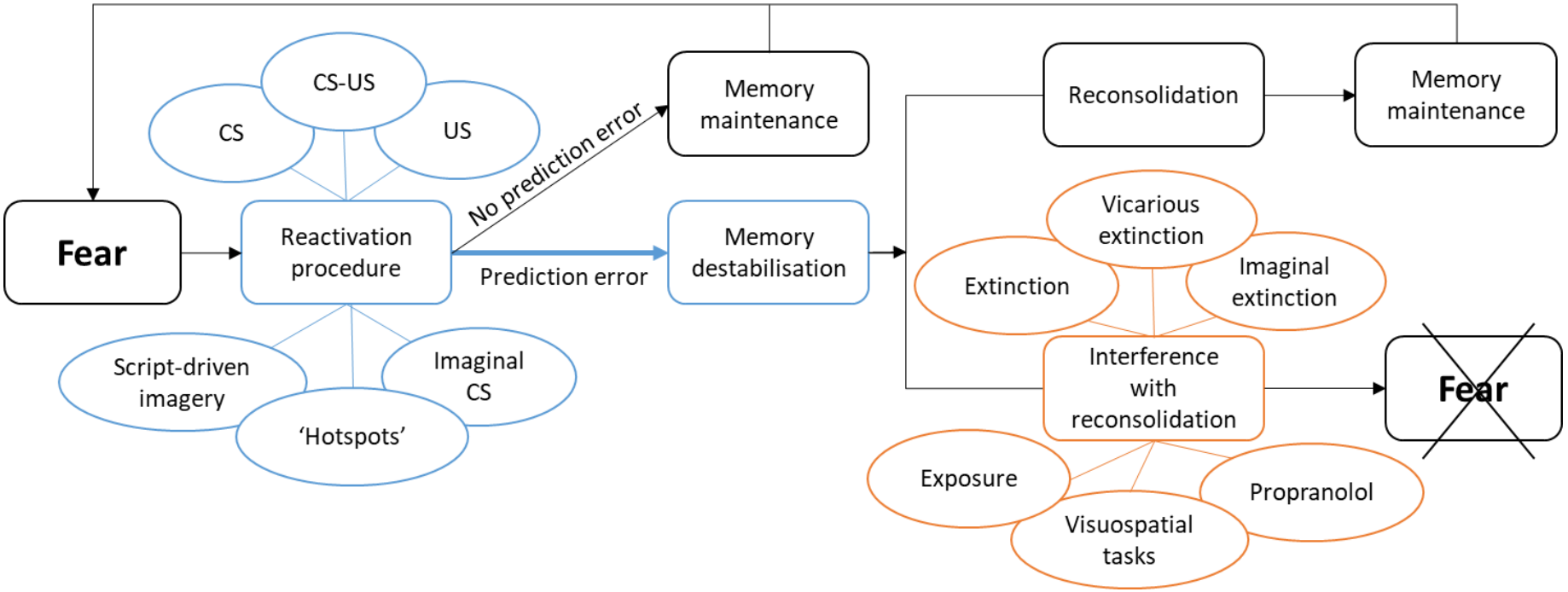


Figure 1.1. Interference with the reconsolidation of fear memories in human fear conditioning research. Reconsolidation is initiated through reactivation and destabilisation of the consolidated fear memory trace. A fear memory trace can be returned to its active and malleable state through a number of approaches, provided they generate a prediction error (Wideman et al., 2018), including: A brief presentation of the conditioned stimulus (CS; Schiller et al., 2010) or the unconditioned stimulus (US; Liu et al., 2014); a CS-US trial (Sevenster et al., 2013); script-driven imagery (Brunet et al., 2018); recalling distressing aspects of a trauma memory (i.e., hotspots; Kindt van Emmerik, 2016); or by imagining the CS (Grégoire & Greening, 2019). Following reactivation and destabilisation of the fear memory trace, the reconsolidation process restabilises the active and malleable memory trace and returns it to its original state, thereby preserving the fear memory (Exton-McGuinness et al., 2015). Interfering with memory reconsolidation prevents the restabilisation of the active memory trace, thereby allowing for the modification of the fear memory and reduction or elimination of the fear response. Successful interference with reconsolidation in human experiments has been demonstrated through the use of the beta-adrenergic receptor antagonist propranolol (Kindt et al., 2009); in vivo extinction training (Schiller et al., 2010); imaginal extinction training (Agren et al., 2017); vicarious extinction training (Golkar et al., 2017); exposure therapy (Telch et al., 2017); and visuospatial tasks (James et al., 2015).

1.2.3.2.3.1. Memory Destabilisation

Although memory destabilisation is a prerequisite for the initiation of memory reconsolidation (Pineyro et al., 2014), destabilisation and reconsolidation are distinct processes (Wideman et al., 2018). Destabilisation refers to the state of memory lability and vulnerability that is induced through memory reactivation, whereas reconsolidation is the process that restabilises the labile memory trace (Exton-McGuinness et al., 2015; Faliagkas et al., 2018; Lee et al., 2017; Wideman et al., 2018). Successful memory destabilisation is assessed indirectly, either through the successful disruption of the reconsolidation process, or lack thereof if the molecular processes underlying destabilisation are blocked prior to the presentation of the memory reactivation trial (for a review, see Wideman et al., 2018).

Extant literature suggests that memory destabilisation, and the corresponding initiation of the reconsolidation process, occurs in the presence of novel information that is relevant to prior learning (e.g., acquisition training), but creates a mismatch between expected and actual events (i.e., prediction error; Agustina López et al., 2016; Faliagkas et al., 2018; Fernández et al., 2016; Lee et al., 2017; Monti et al., 2017; Sevenster et al., 2012, 2013, 2014). The importance of prediction errors in memory destabilisation was demonstrated in a series of experiments conducted by Sevenster and colleagues (2012, 2013, 2014), who found that the reconsolidation process was not initiated through the mere presentation of retrieval cues, but required the presence of prediction errors.[2] Sevenster et al. (2013) reported that reactivation trials that lead to successful memory destabilisation may encompass reinforced or non-reinforced CS+ presentations, provided the reactivation trial is not fully predicted by prior learning history. Accordingly, non-reinforced CS+ presentations were found to initiate the reconsolidation process when asymptotic learning had occurred during acquisition training, whereas

[2] Of note, 'retrieval' in the context of reconsolidation research is a broad term that may refer to the recollection of previous learning, the behavioural expression of the memory, or memory reactivation (Lee et al., 2017). When the term retrieval is used in the present thesis, it is to refer to the recollection of memories, not to memory reactivation and destabilisation. As such, *reactivation-extinction* (Lee et al., 2017) will be used in lieu of the more commonly encountered term *retrieval-extinction*, to refer to behavioural manipulations of reconsolidation involving the use of extinction training.

in the absence of asymptotic learning, a reinforced CS+ trial was sufficient to destabilise fear memories.

In a subsequent experiment, Sevenster et al. (2014) further proposed that the destabilisation of fear memories may be contingent upon the number of prediction errors generated by the reactivation procedure, whereby a procedure that generated one prediction error appeared to initiate the reconsolidation process, whereas a reactivation procedure that generated 'multiple' prediction errors did not. The 'multiple prediction error' condition entailed four consecutive, non-reinforced CS presentations, procedurally resembling extinction training. Based on the 50% reinforcement schedule employed during acquisition training, it would be feasible to assume that the presentation of four non-reinforced CS trials provides greater opportunity for violations of US expectancy than would be afforded by one or two CS presentations used in the other groups. Even though repeated post-acquisition CS-only trials may generate prediction errors due to the unexpected omission of the US (Rescorla & Wagner, 1972), it is not necessarily the case that the magnitude of prediction error remains stable across repeated CS presentations. For instance, the Rescorla-Wagner model would stipulate that prediction errors decrease through repeated CS presentations, due to the organism learning that the CS is no longer a reliable predictor of the US (Rescorla & Wagner, 1972). The result of such learning is the extinction of conditioned responding (Rescorla & Wagner, 1972). Extinction and reconsolidation have been proposed to be mutually exclusive, meaning reconsolidation allows for memories to be updated, while extinction involves the acquisition of a new, inhibitory memory (Merlo, Milton, Goozée, Theobald, & Everitt, 2014), thereby preserving the original fear memory and allowing for a return of fear during future CS presentations (Bouton, 2002).

At present, our understanding of the necessary and sufficient conditions for the destabilisation of fear memories is still advancing. However, a large body of evidence suggests that prediction errors are indeed required for the initiation of the reconsolidation process (for reviews, see Exton-McGuinness et al., 2015; Fernández et al., 2016). While some findings indicate that there may be an optimal level of prediction error that determines whether CS-only reactivation trials result in extinction learning or in the destabilisation of fear memories (Merlo et al., 2014; Sevenster et al.,

2014), these findings are not readily reconciled with findings that indicate that an identical CS-reactivation procedure may facilitate the disruption of reconsolidation through pharmacological, but not through behavioural means (Soeter & Kindt, 2011), or with reports indicating that memory destabilisation can be achieved through reactivation procedures that appear to generate multiple prediction errors (Liu et al., 2014, discussed in the following paragraphs).

1.2.3.2.3.2. US-induced Memory Reactivation

It is conceivable that in contrast to pharmacological disruption of reconsolidation (e.g., through the oral administration of propranolol; Sevenster et al., 2013, 2014), the mere omission of the US during CS-reactivation may not be sufficient to generate the prediction error required to facilitate behavioural disruptions of reconsolidation (Kindt & Soeter, 2013; Soeter & Kindt, 2011), although it may do so if reactivation involves prolonged exposure to the CS (Agren et al., 2012; but see Hu et al., 2018) or when the outcomes of the reactivation trial are not fully predictable due to a low reinforcement schedule employed during acquisition training (e.g., 38%; Fernandez-Rey et al., 2018; Oyarzún et al., 2012; Schiller et al., 2010). The omission of the US, however, is not the only method of memory reactivation and destabilisation, despite its frequent use. Research indicates that reactivation procedures that entail the unsignaled presentation of the US (Dębiec, Díaz-Mataix, Bush, Doyère, & LeDoux, 2010; Huang et al., 2017; Liu et al., 2014; Luo et al., 2015; Zhu et al., 2018) or changes to the temporal relationship between the CS and the US (Agustina López et al., 2016; Alfei, Ferrer Monti, Molina, Bueno, & Urcelay, 2015; Díaz-Mataix et al., 2013) may also be used to destabilise fear memories. Additionally, US-driven memory reactivation procedures have been shown to destabilise multiple fear memories through a single, unsignaled presentation of the US, either delivered at its original intensity (Dębiec et al., 2010; Huang et al., 2017) or at reduced intensity, relative to that employed during acquisition training (referred to as *US-reactivation* in the present thesis; Liu et al., 2014).

In a series of experiments conducted with animals and humans, Liu et al. (2014) demonstrated that delivery of extinction training 10 minutes after US-reactivation prevented spontaneous recovery and reinstatement of fear to fear-irrelevant CSs 24 hours later. A six-month follow-up showed that

fear reduction was long-lasting, as reflected in the absence of reinstatement of differential SCRs. In line with previous studies using CS-reactivation (Schiller et al., 2010), Liu and colleagues also observed that fear reduction subsequent to reactivation-extinction was limited to CSs that were associated with the reactivated stimulus (see Figure 1.2 B).

Corroborating Schiller et al.'s (2010) earlier work, Liu and colleagues (2014) also demonstrated that successful reduction of fear recovery is contingent upon memory reactivation (Experiment 1) *and* the delivery of extinction training within the reconsolidation window, when memories are labile and open to modification (Experiment 1). The administration of the US at reduced intensity – an approach that resembles *US devaluation* (Chapter 5) – in the absence of immediate extinction training (i.e., when extinction was conducted 24 hours after US-reactivation) was not sufficient to prevent fear recovery (Liu et al., 2014). By incorporating the delayed extinction group and extinction-only group into Experiment 1, Liu and colleagues excluded alternative explanations for the persistent reduction of fear observed after US reactivation-extinction, namely extinction learning and US devaluation. Thus, irrespective of the type of memory reactivation procedure employed, whether CS- or US-driven reactivation, past research demonstrates that persistent reduction of fear recovery hinges on extinction training being delivered within the reconsolidation period – a brief period of memory lability, initiated through memory reactivation (Liu et al., 2014; Schiller et al., 2010).

As for the mechanisms underlying US-reactivation, examination of molecular processes in Liu et al.'s (2014) rat experiments showed that (a) changes in protein synthesis that are associated with the initiation of the reconsolidation process were not triggered by exposure to the CS or US only, but were contingent upon prior fear conditioning and subsequent reactivation of the fear memory; and (b) US-reactivation, relative to CS-reactivation, induced a greater modulation of molecular activity in brain areas implicated in fear learning in rats. This modulation of molecular activity was not related to US aversiveness. Overall, the combined findings from Liu et al.'s experiments conducted with animals and humans, in conjunction with previous reconsolidation research (e.g., Dębiec et al., 2010; Monfils et al., 2009; Nader et al., 2000a), have enhanced our understanding of mechanisms

underlying the reduced recovery of fear subsequent to manipulations of the memory reconsolidation process and, more importantly, allow us to distinguish between neural and molecular activity generated by reconsolidation update mechanisms and those triggered by other, unrelated processes, such as extinction training (e.g., Besnard et al., 2012).

Liu et al.'s findings further indicate that the key advantage of the US-reactivation procedure, relative to CS-reactivation, appears to be its ability to destabilise multiple fear memories that are related to the reactivated US (Figure 1.2; see also Huang et al., 2017). As such, CS-reactivation can be employed for the targeted reactivation and destabilisation of a single fear memory, or fear association (Figure 1.2 A), whereas US-reactivation can be used to destabilise multiple fear memories through a single reactivation trial (Liu et al., 2014; Figure 1.2 C, D). Subsequent administration of extinction training can interfere with the reconsolidation of the reactivated fear memories and prevent the return of fear to multiple CSs (Figure 1.2 C, D).

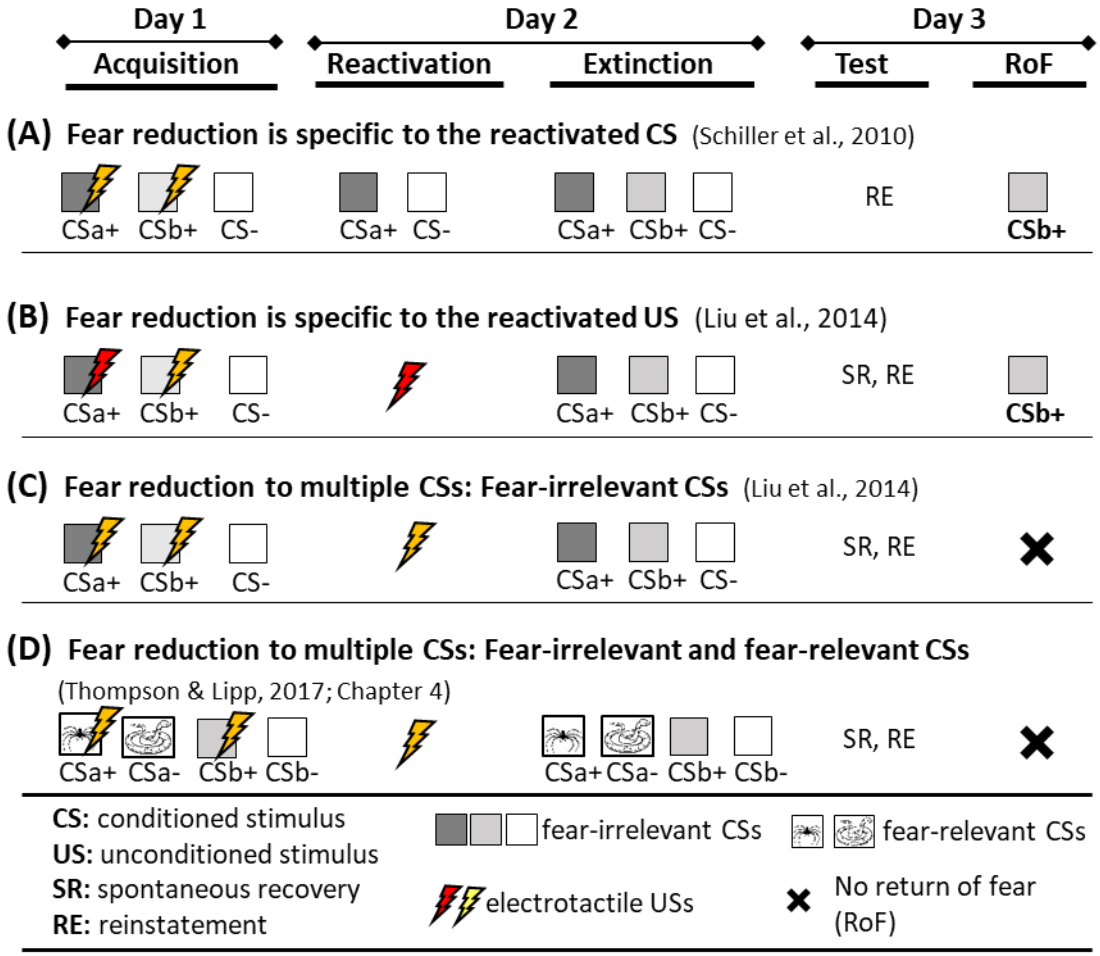


Figure 1.2. Return of fear subsequent to the reactivation-extinction paradigm conducted with CS- or US-induced memory reactivation. Fear reduction following reactivation-extinction is limited to CSs that are associated with the reactivated stimulus (**A, B**). CS-reactivation facilitates destabilisation of a specific fear memory, associated with the reactivated CS (**A**). US-reactivation facilitates destabilisation of multiple fear memories that are associated with the reactivated US (**C, D**). US-reactivation in the current investigation (Chapter 4) and past research (Liu et al., 2014) consisted of an unsignaled presentation of the US, delivered at half the physical intensity as that used during acquisition training. Please note that only select experiments are presented here, to highlight similarities and differences between CS- and US-reactivation procedures.

Taken together, Liu et al.'s (2014) findings demonstrate that the recovery of fear to multiple cues can be prevented through a single US-reactivation trial that is followed by extinction training 10 minutes later. Interestingly, the findings further indicate that fear reduction subsequent to US reactivation-extinction, but not CS reactivation-extinction, is not contingent upon extinction of all fear predictive cues (Experiment 3). Liu and colleagues observed that after fear acquisition to two CSs (CS1 and CS2), reactivation of CS1 and CS2 or reactivation of the US, followed by extinction of the CS1, yielded different outcomes during tests of spontaneous recovery and reinstatement, conducted after a delay of 24 hours. Whilst fear recovered to the non-extinguished CS2 subsequent to CS-

reactivation and CS1 extinction, fear did not recover to the CS1 or CS2 following US-reactivation and CS1 extinction.

These findings indicate that extinction of one CS only may disrupt the reconsolidation of multiple fear memories that are associated with the reactivated US. Considering that naturally occurring memories of traumatic or anxiety-arousing events are typically associated with multiple cues and each of these cues can elicit fear (e.g., Carpenter, Pinaire, & Hofmann, 2019; Schiller, 2014), the US reactivation-extinction procedure appears to be particularly suited to the treatment of anxiety and stress-related disorders. The procedure may eliminate the need for the identification of and exposure to all fear-predictive cues (CSs), thereby shortening treatment duration. This being said, as Liu et al. employed fear-irrelevant CSs only, an aspect that requires further examination is whether US-reactivation is capable of destabilising those type of fears that are typically associated with anxiety disorders, namely fears conditioned to fear-relevant CSs (Mineka & Öhman, 2002). This aspect has been examined in the reconsolidation study conducted as part of the present thesis (see Chapter 4).

Advancing research into manipulations of the memory reconsolidation process can improve our understanding of mechanisms underlying the maintenance and modification of fear memories and contribute to the development of more efficacious treatments for anxiety and stress-related disorders. US-reactivation, in particular, remains an under-utilised memory reactivation procedure in fear conditioning research. Due to its potential to destabilise multiple fear memories (Dębiec et al., 2010; Huang et al., 2017; Liu et al., 2014), including those conditioned to fear-relevant CSs, which proved difficult to destabilise in past research (e.g., Kindt & Soeter, 2013; but see Chapter 4), US-reactivation warrants further investigation in the basic and applied setting.

1.2.3.3. US Devaluation

The final US-driven method of fear reduction examined in this thesis was US devaluation (Davey, 1989, 1992), which refers to the post-acquisition reduction of the motivational value of the US (Davey, 1989; Storsve et al., 2012). Extant literature suggests that the strength of the conditioned response can be increased or decreased following initial acquisition training, by increasing (US

inflation; Rescorla, 1974) or decreasing (US devaluation; Rescorla, 1973) the value of the US. In fear conditioning research, US devaluation involves a reduction of the perceived aversiveness of the US, typically achieved through US habituation (Haesen & Vervliet, 2015) or through the repeated presentation of the US at a reduced physical intensity (Hosoba et al., 2001; Schultz et al., 2013).[3] Importantly, in contrast to the initial acquisition of the CS-US association, US devaluation is conducted in the absence of the CS and its effects on subsequent conditioned responding are mediated by non-associative processes, such as habituation mechanisms, rather than a decrease in associative strength (Davey, 1989; Haesen & Vervliet, 2015; Leer & Engelhard, 2015, but see Storsve, McNally, & Richardson, 2010 for a discussion of a dual role of associative and non-associative processes).

Considering the similarity between US devaluation and the US-reactivation procedure employed in reconsolidation research (Liu et al., 2014), the present US devaluation study was conducted to examine if persistent reduction of fear could be realised through a procedure that resembles US reactivation-extinction (Liu et al., 2014; Chapter 4), but is delivered immediately after acquisition training, rather than after a delay of 24 hours. The results of this study, therefore, contribute to our understanding of mechanisms underlying long-lasting fear reduction. Specifically, it was examined whether spontaneous recovery and reinstatement of fear to fear-relevant and fear-irrelevant CSs can be prevented without manipulating the memory reconsolidation process. In accordance with the reviewed reconsolidation research, it was also examined whether the effects of US devaluation are mediated by prediction errors. However, unlike the US-reactivation procedure employed in reconsolidation research (Liu et al., 2014), US devaluation in the present investigation encompassed the presentation of three, instead of one, unsignaled USs, at half the physical intensity as that employed during acquisition (Schultz et al., 2013).

In comparison to extinction training, US devaluation remains an under-utilised method of fear reduction. First employed in the 1970s in animal research, Rescorla (1973) observed that repeated

[3] The terms US habituation and US devaluation have been used interchangeably in some reports (Rescorla, 1973; Storsve, McNally, & Richardson, 2010), but not in others (Hosoba et al., 2001; Schultz et al., 2013). Here, the term US habituation will be used to highlight the use of a specific type of US devaluation procedure, involving presentations of the US at the same intensity as that employed during acquisition.

presentations of a loud noise US after acquisition reduced the conditioned fear response to the CS that had been paired with the US. Rescorla proposed that the repeated presentation of the US reduced its perceived aversiveness and, hence, subsequently reduced the magnitude of the conditioned fear response when the CS was presented again after the habituation procedure. Fear reduction, however, was only observed for a first-order, but not for a second-order conditioned response. Rescorla suggested that conditioned responding decreased in first-order conditioning (i.e., CS-US pairings) due to the stimulus-stimulus association (S-S) formed between the CS and the US. It follows that the conditioned response is a result of the CS activating a mental representation of the (devalued) US (e.g., Davey, 1992; Rescorla, 1973). In second-order conditioning (i.e., CS2-CS1 pairings), on the other hand, the CS was proposed to directly elicit the conditioned fear response (stimulus-response association [S-R]) without prior activation of the US memory and, therefore, was not sensitive to US devaluation (Rescorla, 1973).

The distinction between S-S and S-R associations is important when discussing US devaluation effects, because a reduction of US aversiveness would only be expected to be reflected in S-S associations, meaning when we assume that the presentation of the CS activates a mental representation of the devalued US, instead of directly eliciting the conditioned fear response (i.e., S-R association; Davey, 1992; Davey & McKenna, 1983; Rescorla, 1973). Of note, S-R associations are not limited to second-order conditioning (cf. Rescorla, 1973), but may also underlie first-order conditioning, as demonstrated in a series of fear conditioning experiments conducted with rats (Laborda & Miller, 2011); for a detailed discussion of S-S and S-R learning, see Holland (2008). Additionally, whether a conditioning protocol favours the formation of an S-S or S-R association may differ between animals and humans (Davey & McKenna, 1983). Past research indicates that fear learning in humans may involve the formation of an S-S association both in first- and second-order conditioning, due to the influence of cognitive processes that mediate conditioned responding (Davey, 1989; Davey & McKenna, 1983). In this regard, Davey and McKenna (1983) found that second-order conditioned fear was eliminated either through the devaluation of an aversive auditory US or through the use of verbal instructions pertaining to the absence of the US. Thus, it appears that US devaluation

may differentially affect conditioned responding in animals (e.g., Rescorla, 1973) and humans (e.g., Davey & McKenna, 1983).

The ability of humans to process verbal instructions and use other types of higher order cognitive functions presents researchers with a wider range of US devaluation methods, some of which do not require in vivo administration of the US (Reynolds, Field, & Askew, 2015). Examples of methods involving indirect experience with the US include imagery rescripting (Dibbets, Lemmens, & Voncken, 2018; Dibbets, Poort, & Arntz, 2012), eye movement desensitisation and reprocessing (Dibbets et al., 2018; Leer, Engelhard, Dibbets, & van den Hout, 2013), and observational learning (Reynolds et al., 2015). However, at present, methods involving direct experience with the US (Haesen & Vervliet, 2015; Hosoba et al., 2001; Schultz et al., 2013) appear to yield greater post-devaluation reduction of conditioned responding than methods involving indirect experience with the US (Dibbets et al., 2012).

Considering the broad range of US devaluation methods employed in past research and the differential effects US devaluation has on the renewal of conditioned responding in animals (Storsve et al., 2010, 2012) and humans (Davey & McKenna, 1983; Haesen & Vervliet, 2015), it is perhaps not surprising that US devaluation remains poorly understood. Findings from research conducted with animals, for instance, indicate that US devaluation reduces conditioned fear, but does not prevent its renewal or reinstatement (e.g., Storsve et al., 2010; Storsve et al., 2012). In contrast, research conducted with humans shows that a greater reduction of fear renewal can be achieved through US devaluation than through conventional extinction training (Haesen & Vervliet, 2015; Leer & Engelhard, 2015). However, at present it is not known whether the post-devaluation reduction in fear recovery is long-lasting, as delayed tests of conditioned responding, for instance conducted after a delay of 24 hours, are currently lacking. Hence, this aspect has been addressed in the US devaluation study conducted as part of this thesis (Chapter 5), through tests of spontaneous recovery and reinstatement, conducted 24 hours after delivery of US devaluation and/or extinction training.

Other aspects requiring further examination pertain to the optimal method of US devaluation, specifically whether the number of US presentations (70 US habituation trials: Poulos et al., 1979; vs.

3 US devaluation trials: Schultz et al., 2013) and the intensity of the US (i.e., reduced vs. not reduced) are important determinants of post-devaluation conditioned responding. Assuming that US intensity is reduced to decrease the perceived aversiveness of the US, questions arise about adequate control procedures to rule out alternative explanations, such as the effects of prediction errors generated by the reduction of US intensity and/or by the unsignaled presentation of the US (Fernández et al., 2016; Liu et al., 2014). There are additional questions that await further examination (see Chapter 5); however, the present investigation focused on two questions only: (a) whether the effects of US devaluation on conditioned responding are long-lasting and (b) whether they are mediated by prediction errors.

In light of the broad range of US devaluation methods used in past research, the choice of the procedure for the study conducted as part of this thesis was guided by two primary concerns, being (a) demonstrated effectiveness in the reduction of conditioned responding and (b) demands on participants. Hence, the procedure used by Schultz et al. (2013) was adapted for the present study, as it was shown that three presentations of the US, at half the intensity as that used during acquisition, were sufficient to reduce subsequent differential SCRs, and because a relatively brief US devaluation procedure (cf. Leer & Engelhard, 2015) reduces demands on participants. In contrast to Schultz et al., however, post-devaluation conditioned responding was assessed at two points in time, namely after a delay of 10 minutes and 24 hours, to test whether the effects of US devaluation on conditioned responding are long lasting (for further details pertaining to methods and results, see Chapter 5).

1.3. Summary, General Aims, and Rationale

In summary, fears are learned through association of initially neutral cues (CSs) with a naturally aversive outcome (US; Lipp, 2006a). Fear extinction, on the other hand, typically involves the repeated presentation of the CS in the absence of the US (Bouton, 2000). Despite successful within-session extinction of conditioned responding, recovery of extinguished fear is common, as extinction training does not eliminate the original fear association, but creates a new, inhibitory association (CS-no US) that co-exists with the original fear learning (i.e., the CS-US association) and competes for expression during post-extinction CS presentations (Bouton, 2002).

The return of extinguished fear is a well-documented phenomenon, which has been observed in the experimental and in the clinical setting (Bouton, 2002; Craske et al., 2018; Craske & Mystkowski, 2006; Craske et al., 2014). However, there is evidence to suggest that the recovery of fear could be reduced by modifying existing exposure-based practices (Craske et al., 2014). Hence, the key aim of this thesis was to examine three methods that may yield long-lasting reduction of fear, each of which involved presentations of the CS *and* the US (e.g., Liu et al., 2014).

In contrast to conventional CS-only extinction training, fear reduction methods that involve exposure to the CS and the US (e.g., Bouton et al., 2004) have received relatively little research attention, possibly because it appears rather counterintuitive to incorporate an aversive stimulus that is typically associated with fear acquisition (e.g., Davey, 1992) into protocols aimed at fear reduction. Indeed, past research shows that occasional presentations of the US during partially reinforced extinction training may slow the rate of extinction (Bouton et al., 2004) or prevent within-session extinction of fear (Culver et al., 2018) in animals and humans alike. Consequently, if rapid extinction of conditioned responding during extinction training (i.e., within-session extinction) is desired, training protocols that involve US presentations would be contraindicated. However, research also indicates that within-session extinction of conditioned responding is neither sufficient (Bouton et al., 2004; Brown, LeBeau, Chat, & Craske, 2017) nor necessary (Culver et al., 2018; Plendl & Wotjak, 2010) for the *between-session* reduction of conditioned responding. Hence, methods that focus on enhancing extinction learning (Culver et al., 2018) or the modification of the consolidated fear memory trace (Liu et al., 2014; Schiller et al., 2010) may be more effective in the long-lasting reduction of fear than methods focusing on the within-session extinction or habituation of conditioned fear (for reviews, see Craske, 2015; Craske et al., 2018; Craske et al., 2014). In this regard, presentations of the US either before (Liu et al., 2014) or during (Culver et al., 2018) the delivery of extinction training have been shown to yield superior reduction of fear recovery, relative to conventional extinction training that involves the repeated presentation of the CS in the absence of the US.

Past research has identified a number of US-driven approaches that may be suited to the long-lasting reduction of fear, including US habituation (Rescorla, 1973), US devaluation (Schultz et al., 2013), explicitly unpaired extinction (Vervliet et al., 2010), occasionally reinforced extinction (Culver et al., 2018), counterconditioning (Kang, Vervliet, Engelhard, van Dis, & Hagenaars, 2018; Thomas, Cutler, & Novak, 2012), or the manipulation of the fear memory reconsolidation process subsequent to memory reactivation procedures involving the unsignaled presentation of the US (Dębiec et al., 2010; Liu et al., 2014) or a CS-US trial (Agustina López et al., 2016; Alfei et al., 2015; Díaz-Mataix et al., 2013; Sevenster et al., 2013). These approaches differ in their application and underlying mechanisms, however, all of them are capable of reducing conditioned fear, whereby some methods have been shown to yield superior reduction of fear recovery, compared to conventional extinction training (e.g., Bouton et al., 2004; Liu et al., 2014; Vervliet et al., 2010). Importantly, the examination of a broader range of fear reduction methods may enhance our understanding of necessary and sufficient determinants of long-lasting fear reduction.

1.3.1. Study 1: Occasionally Reinforced Extinction

Study 1 (Chapter 3) builds on past research of occasionally reinforced extinction training (Bouton et al., 2004; Culver et al., 2018) and provides the first extension of Bouton et al.'s unpaired extinction procedure to fear extinction in a human differential fear conditioning paradigm, as well as providing the first direct comparison of the effects of conventional, partially reinforced, and unpaired extinction training on the recovery of extinguished fear in humans. Recovery of extinguished fear, indexed by differential SCRs and CS valence ratings, was assessed 10 minutes after the conclusion of extinction training, through tests of spontaneous recovery, reinstatement, and reacquisition.

Past research conducted with animals (Bouton et al., 2004) showed that partially reinforced and unpaired extinction training slowed the reacquisition of extinguished responding in an appetitive conditioning preparation. A replication and extension of partially reinforced extinction training to fear extinction in humans (Culver et al., 2018) corroborated Bouton et al.'s findings, showing greater interference with the reacquisition of fear subsequent to partially reinforced than conventional extinction training. A limitation of the study conducted by Culver and colleagues, however, was the

lack of fear extinction at the conclusion of extinction training. As such, no inferences could be made about the effects of partially reinforced extinction training on the spontaneous recovery of fear that was assessed one week later, because fear that is not extinguished cannot "recover." To address the possibility of reinforced trials to interfere with the extinction of conditioned responding (Bouton et al., 2004; Culver et al., 2018), the reinforcement schedule in the occasionally reinforced extinction study conducted as part of this thesis was reduced on the final block of extinction training from a US:CS ratio of 2:8 to 1:8. For a schematic representation of the experimental paradigm, including the reinforcement schedule employed during occasionally reinforced extinction training, see Chapter 3.

Based on the reviewed literature (Bouton et al., 2004; Craske et al., 2014; Culver et al., 2018), it was predicted that extinction learning would be enhanced through occasional presentations of the US during extinction training, which would be reflected in reduced fear recovery and reacquisition of fear, relative to non-reinforced extinction. In line with Bouton et al.'s findings, it was also predicted that reacquisition of extinguished fear would be slower after unpaired than partially reinforced extinction training.

1.3.2. Study 2: Reconsolidation

Study 2 (Chapter 4) builds on past reconsolidation research utilising extinction training to disrupt the reconsolidation process (Liu et al., 2014; Schiller et al., 2010) and provides the first application of the US reactivation-extinction procedure (Liu et al., 2014) to fear that was conditioned to fear-relevant CSs (pictures of snakes and spiders), as well as providing the first direct comparison of post-reconsolidation fear recovery to fear-relevant and fear-irrelevant CSs. Fear recovery was assessed through tests of spontaneous recovery and reinstatement, conducted after a delay of 24 hours and during a follow-up study, conducted 8 to 12 months after initial training (see addendum of Chapter 4 for results of follow-up study).

Past research demonstrated that extinction training that is delivered during the memory reconsolidation period, in contrast to conventional extinction training that is delivered without prior reactivation and destabilisation of the fear memory trace, can prevent the recovery of fear to fear-irrelevant CSs, such as geometric shapes (Liu et al., 2014; Schiller et al., 2010). Replication attempts

of the seminal work of Schiller et al., however, yielded mixed results (for reviews, see Beckers & Kindt, 2017; Kredlow et al., 2016). In particular the difficulties in replicating Schiller et al.'s findings with fear-relevant CSs led to speculations that *strong* fear memories, conditioned to fear-relevant CSs, may not be sensitive to behavioural manipulations of reconsolidation (e.g., Golkar et al., 2012; Kindt & Soeter, 2013). Nevertheless, a comparison of the effects of behavioural and pharmacological manipulations of reconsolidation in fears associated with fear-relevant CSs (Soeter & Kindt, 2011) indicated that it is not necessarily the strength of the fear memory that represents a boundary condition of reconsolidation, as fears conditioned to fear-relevant CSs were eliminated through the oral administration of propranolol, but not through extinction training that was delivered during the reconsolidation period. Extrapolating from these findings, it appears that additional factors may contribute to the successful disruption of the reconsolidation process, such as the prediction error generated by the memory reactivation procedure (for a review, see Wideman et al., 2018).

Two broad types of reactivation procedures have been employed in fear conditioning research: CS-reactivation (e.g., Kindt & Soeter, 2013) and US-reactivation (e.g., Liu et al., 2014). The prediction error generated by CS-reactivation is largely restricted to the unexpected omission of the US, relative to acquisition training. US-reactivation, on the other hand, may generate a stronger prediction error due to the unsignaled presentation of the US and the unexpected reduction of US intensity, relative to acquisition training (Liu et al., 2014). An additional advantage of US-reactivation is its ability to destabilise multiple fear memories, associated with the reactivated US, and thereby facilitate the disruption of their reconsolidation process through extinction training (Liu et al., 2014). However, an aspect that was not addressed by Liu et al. was the ability of US-reactivation to destabilise fears to fear-relevant CSs, which have proved resistant to behavioural manipulations of reconsolidation in past research (Golkar et al., 2012; Kindt & Soeter, 2013) and may, therefore, be resistant to destabilisation. This being said, as naturally occurring fears are typically associated with fear-relevant CSs (Mineka & Öhman, 2002), it is important to examine if such fears could be reduced through behavioural manipulations of reconsolidation.

Therefore, the aim of Study 2 (Chapter 4) was to apply the US reactivation-extinction procedure (Liu et al., 2014) to fears conditioned to fear-irrelevant and fear-relevant CSs and to examine whether post-reconsolidation recovery of fear differs as a function of CS fear-relevance. Fear recovery, indexed by differential SCRs and CS valence ratings, was assessed 24 hours after administration of the US reactivation-extinction procedure (or non-reminded extinction training in the control group). Follow-up tests of fear recovery were conducted 8 to 12 months after initial training. Based on the reviewed literature (Golkar et al., 2012; Kindt & Soeter, 2013), which has not found that behavioural interventions affect reconsolidation of fear conditioned to fear-relevant stimuli, it was hypothesised that there would be a larger level of post-reconsolidation fear recovery to fear-relevant than to fear-irrelevant CSs during tests of spontaneous recovery and reinstatement.

1.3.3. Study 3: US Devaluation

Study 3 (Chapter 5) was designed to examine whether fear recovery could be reduced through a US devaluation procedure that resembles US reactivation-extinction, but is not delivered during the memory reconsolidation period. In accordance with past reconsolidation research (Liu et al., 2014), it was also examined whether the effects of US devaluation on subsequent conditioned responding are mediated by prediction errors. Prediction errors are implicated in fear learning, fear reduction, and modification of consolidated fear memories (e.g., Fernández et al., 2016) and may, therefore, mediate or enhance the effects of US devaluation. In the present investigation, verbal instructions were used to manipulate the degree of prediction error generated by the unsignaled presentation of three USs, delivered at half the intensity as that employed during acquisition. The effects of US devaluation on conditioned responding were assessed after a delay of 10 minutes and 24 hours, through examination of differential SCRs and CS valence ratings during extinction training, spontaneous recovery, and reinstatement. For a schematic representation of the experimental paradigm and description of verbal instructions used during the US devaluation phase, see Chapter 5.

The present study builds on past US devaluation (Schultz et al., 2013) and reconsolidation research (Liu et al., 2014; Thompson & Lipp, 2017) with the aim to enhance our understanding of determinants of long-lasting fear reduction. Past US devaluation research indicates that the strength of

the fear response is not only determined by initial fear acquisition, but also by post-acquisition changes to the motivational value of the US (Davey, 1992; Davey & McKenna, 1983; Rescorla, 1973). It follows that a reduction of perceived US aversiveness may decrease the fear response during future CS presentations (Davey & McKenna, 1983). While there is evidence to suggest that US devaluation may reduce the renewal of fear (e.g., Haesen & Vervliet, 2015), it is not known if these effects are long-lasting or applicable to the reduction of spontaneous recovery and reinstatement.

Based on past reports of reduced post-devaluation differential SCRs at the start of extinction training (Schultz et al., 2013) and during tests of renewal (Haesen & Vervliet, 2015), it was predicted that differential SCRs would be lower in the US devaluation groups than in the control group, both 10 minutes after the US devaluation procedure as well as during tests of fear recovery, conducted after a delay of 24 hours. With regards to prediction errors, if the effects of US devaluation are mediated by prediction errors, post-devaluation differential SCRs would be lower in the uninstructed than in the instructed US devaluation group. Conversely, if prediction errors do not mediate US devaluation effects, there should be no differences in differential SCRs between the instructed and uninstructed US devaluation group. No directional predictions were made about the effects of US devaluation on conditioned CS valence ratings, due to the mixed findings reported in past research (Hosoba et al., 2001; Jensen-Fielding, Luck, & Lipp, 2017).

Chapter 2: Method

This chapter provides a general overview of the experimental paradigm, dependent measures, and other instruments employed in the studies conducted as part of this thesis. For study-specific methodological details, please see the respective empirical chapters (Chapters 3-5). In accordance with past human fear conditioning research, fear was conditioned through the use of a differential fear conditioning paradigm (e.g., Lipp, 2006a). Fear acquisition in the occasionally reinforced extinction study (Chapter 3) was conducted with fear-irrelevant CSs only, whereas fear conditioning in the reconsolidation (Chapter 4) and US devaluation (Chapter 5) studies was conducted with fear-relevant and fear-irrelevant CSs. During acquisition training, the CSs+ was paired with a mildly aversive electrotactile US on 100% of the CSs+ trials, while the respective CS- was presented by itself. A summary of key features of the experimental paradigms can be found in Figure 2.1. In all studies conducted as part of this thesis, recovery of extinguished fear was assessed through tests of spontaneous recovery and reinstatement; the occasionally reinforced extinction study also entailed tests of reacquisition. Tests of fear recovery were conducted between 10 minutes and 12 months after delivery of the experimental manipulation. The primary and secondary dependent measures consisted of differential SCRs and CS valence ratings, respectively.

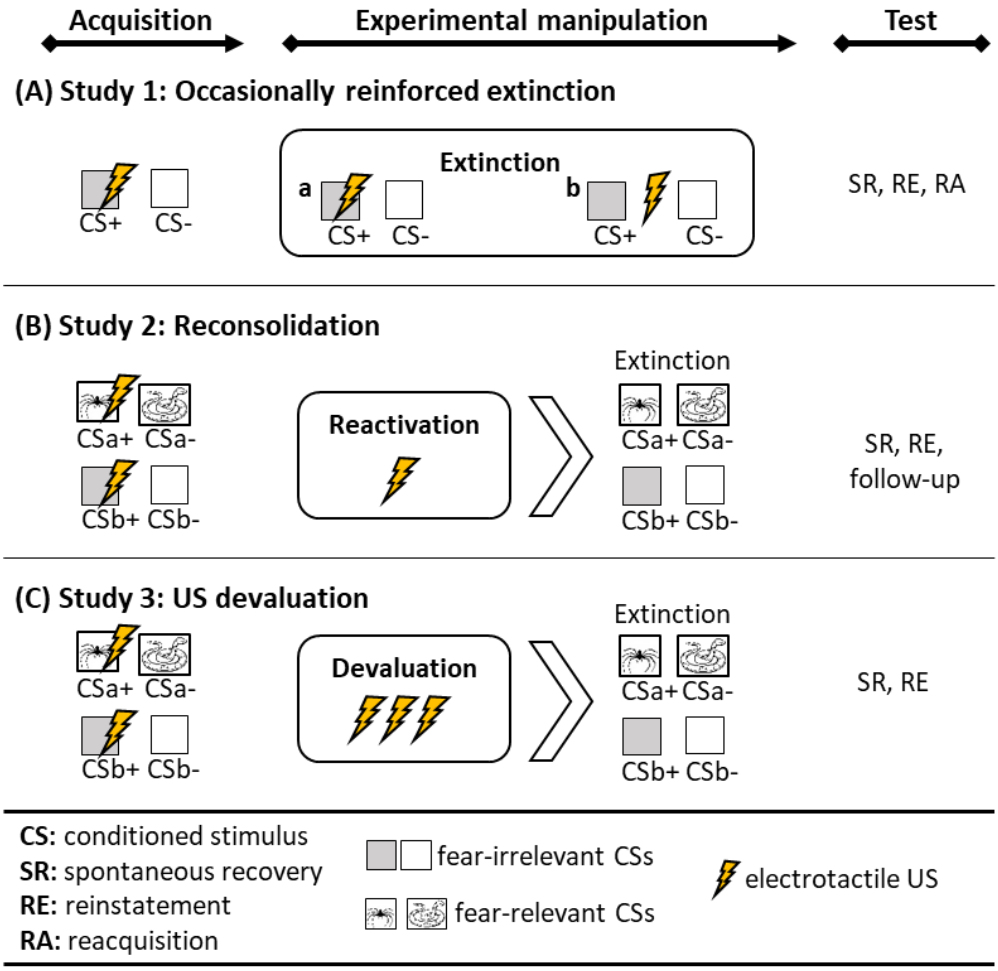


Figure 2.1. Schematic representation of experiments conducted as part of this thesis. The experimental manipulations consisted of occasionally reinforced extinction training (**A**), involving paired (**a**) or unpaired (**b**) US presentations; behavioural manipulation of the memory reconsolidation process (**B**); and US devaluation (**C**). In the reconsolidation study, follow-up tests of spontaneous recovery and reinstatement were conducted ≥ 8 months after initial training. The US intensity was halved for the purpose of the US-induced memory reactivation and US devaluation procedure, relative to US intensity used during acquisition.

2.1. Dependent Measures

2.1.1. Electrodermal Activity

In human fear conditioning studies, the most frequently employed physiological index of conditioned fear is electrodermal activity, specifically SCRs (Dawson, Schell, & Filion, 2007; Lonsdorf et al., 2017). SCRs reflect changes in skin conductance as a result of increased sweat gland activity, expressed in micro Siemens (µS; Dawson et al., 2007). Changes in skin conductance can be recorded through electrodes attached to palmar sites (Boucsein, 2012; Payne, Schell, & Dawson,

2016), such as fingers (e.g., Kindt & Soeter, 2013; Lipp & Edwards, 2002) or the thenar and hypothenar eminences of the non-dominant hand (e.g., Ho & Lipp, 2014), although non-palmar sites, such as feet (Schultz, Balderston, Geiger, & Helmstetter, 2013), are also suitable for the recording of electrodermal activity, despite being less responsive than palmar sites (Payne et al., 2016). Fear conditioning is reflected in larger differential SCRs to the CS+ than to the CS- (e.g., Lonsdorf et al., 2017), which occurs due to an increase in sweat gland activity in anticipation of the US (Boucsein, 2012; Lipp, 2006a).

An advantage of using SCRs in fear conditioning research is the non-invasive nature of the measure, which involves adhesion of two electrodes to the participant's hand, but places no additional demands on the participant, other than prior washing of hands and the requirement to refrain from excessive movement (Boucsein, 2012). Additionally, changes in skin conductance in response to the presentation of the CS+/- and the US (or its absence, during extinction training) can be measured continuously throughout each experimental test phase (e.g., Lonsdorf et al., 2017). However, as SCRs are not selectively sensitive to fear conditioning, but can be also elicited spontaneously or in response to positively valanced stimuli, scoring of SCRs is typically restricted to event-specific SCRs, meaning SCRs that are associated with the presentation of the CSs and US (Dawson et al., 2007). While SCRs are highly sensitive to psychological processes and conditioning manipulations (Dawson et al., 2007; Lipp, 2006a), they are also influenced by movement or breathing patterns (Boucsein, 2012; Lipp, 2006a). To minimise the influence of these factors on SCRs, participants in the studies conducted as part of this thesis were instructed to refrain from excessive movement. Additionally, respiration was recorded through the placement of a Biopac respiration belt around each participant's waist. A visual inspection of electrodermal data was conducted prior to the scoring of SCRs, to identify and eliminate SCRs that contained respiration-induced artefacts (Boucsein, 2012).

2.1.2. CS Valence Ratings

The secondary dependent measure of conditioned fear in the present research consisted of CS valence ratings. Conditioning effects in CS valence ratings are reflected in the decreased liking of the CSs+ that were paired with an aversive US (De Houwer, 2007; Hermans, Vansteenwegen, Crombez,

Baeyens, & Eelen, 2002). CS valence ratings can be recorded in an online or offline manner, meaning through ratings of CS+ and CS- valence on a trial by trial basis or at the conclusion of an experimental test phase (e.g., Hermans et al., 2002; Lipp & Edwards, 2002; Lipp, Oughton, & LeLievre, 2003; Luck & Lipp, 2015). Changes in valence can be assessed through the use of electronic or paper and pencil ratings scales (Lipp, 2006a). In a differential fear conditioning paradigm, fear acquisition, or more precisely the acquisition of unpleasantness (Lipp 2006), is reflected in increased differential negative evaluations of the CS+, relative to the CS- (e.g., Hermans et al., 2002).

In contrast to conditioned differential SCRs, conditioned negative valence may resist extinction (Lipp & Edwards, 2002; Luck & Lipp, 2015; but see Lipp et al., 2003). Failure to fully extinguish conditioned negative valence may be problematic for the treatment of fear, as residual negative valence is associated with the reinstatement of fear (Hermans et al., 2005; Zbozinek, Hermans, Prenoveau, Liao, & Craske, 2015). Hence, CS valence ratings were employed as a secondary dependent measure of conditioned responding in the present project.

The use of multiple measures of conditioned responding allows for a comparison of effects of experimental manipulations on different response systems, which reflect different dimensions of the fear response (Lang, 1985; Lang, Bradley, & Cuthbert, 1998), thereby enhancing our understanding of mechanisms underlying the development and reduction of fear. However, careful consideration must be given to the experimental parameters, as there is some evidence to suggest that the inclusion of multiple readout measures may affect fear conditioning and fear extinction, for instance by enhancing fear learning through the inclusion of startle probes or online US expectancy ratings (Lonsdorf et al., 2017; Sjouwerman, Niehaus, Kuhn, & Lonsdorf, 2016; Warren et al., 2014).

2.2. Materials and Methods

2.2.1. Participants

Healthy volunteers who were at least 18 years old and met inclusion criteria (i.e., no cardiovascular disease, seizure disorder, or pregnancy) were recruited for the present studies. Recruitment was conducted through the Curtin University research participation scheme. Participants were compensated through partial course credit or a modest financial compensation between 15 and

45 AUD per study. Sample size calculations for each study were determined based on sample sizes from past research (e.g., Luck & Lipp, 2015) and the need to counterbalance trial presentations across participants, meaning participants were recruited in multiples of eight. Ethical approval for the studies conducted as part of this dissertation was obtained from the Curtin University Human Research Ethics Committee.

2.2.2. Apparatus and Materials

2.2.2.1. Conditioned Stimuli

Two distinct classes of CSs were employed in the present project, consisting of fear-relevant and fear-irrelevant stimuli. Fear-irrelevant CSs encompassed colour images of geometric shapes, fish, and birds, whereas pictures of snakes and spiders served as fear-relevant CSs. As real life fears and phobias are typically associated with fear-relevant CSs, such as spiders (Mineka & Öhman, 2002; Öhman, 2009), both classes of stimuli were employed in the present project, to examine if fears conditioned to fear-relevant and fear-irrelevant CSs are differentially sensitive to experimental manipulations. The CSs were presented for 6 seconds (s) in the centre of a 17-inch colour LCD screen, over a black background; the assignment of pictures as CS+ or CS- and the trial order (whether the first trial of each phase was a CS+ or CS-) were counterbalanced across participants. Stimulus pictures, length of inter-trial intervals, and pseudo-randomised trial order varied across studies (for further details, see Chapters 3-5).

2.2.2.2. Unconditioned Stimulus

The US consisted of an electrotactile stimulus, which is the most frequently employed aversive stimulus in human fear conditioning research, as it produces reliable conditioning effects across several indices of conditioned responding (Lipp, 2006a). The US intensity was set by each participant to a level that was perceived as “unpleasant, but not painful.” US calibration was conducted at the start of the experiment and involved presentations of the electrotactile stimulation at progressively increasing intensity, starting at 0 Volts and terminating at an unpleasant, but not painful intensity. The perceived unpleasantness of the US was subsequently rated on a 9-point Likert scale, ranging from 1 (unpleasant) to 9 (pleasant).

The US was generated with a Grass SD9 stimulator (Grass Technologies, Middleton, WI) and was delivered to the wrist of the dominant hand via a concentric electrode. The shock was presented for 200 milliseconds (ms), was pulsed at 50 Hertz (Hz), and coincided with each CS+ offset during acquisition training; the CS- was never paired with the US. A 100% reinforcement schedule was employed to facilitate acquisition of fear – a prerequisite for the experimental manipulations and tests of fear recovery. The delivery of the US and CSs was controlled with DMDX 5.0.5 software (Forster & Forster, 2003).

2.2.2.3. Electrodermal Activity

Electrodermal activity was recorded through two self-adhesive, pre-gelled electrodes (Biopac Systems EL507), attached to the thenar and hypothenar eminences of the non-dominant hand. Electrodermal activity was DC amplified at a gain of 5 µS per volt and recorded with a Biopac MP150 system at a sampling frequency of 1000 Hz, using AcqKnowledge 4 (Biopac Systems, Goleta, CA). A Biopac respiration belt was fitted around the participant's waist to control for respiration-induced artefacts in SCRs.

2.2.2.3.1. Scoring and Response Definition

SCRs were scored offline in AcqKnowledge 4; responses that were confounded by movement- or respiration-induced artefacts were discarded (≤ 19 responses [≤ 0.14%] were discarded per study). In accordance with past research (Culver, Stevens, Fanselow, & Craske, 2018; Kindt & Soeter, 2013; Orr et al., 2000; Pineles, Orr, & Orr, 2009), SCRs elicited by the CSs were calculated by subtracting the mean skin conductance level during the 2 s baseline preceding CS onset from the largest skin conductance level occurring 1 to 6 s after CS onset; negative values were scored as zero and retained in the analyses. While other scoring methods exist (cf. Prokasy & Kumpfer, 1973), the advantage of the present method lies in its ability to capture the largest SCR that occurs in the 1 to 6 s response latency window following CS onset. Scoring the largest response in the entire response latency window, as opposed to scoring in the first (1-4 s) or second (4-7 s) response latency windows (Prokasy & Kumpfer, 1973), decreases the risk of underestimating the true extent of conditioned responding (Pineles et al., 2009) and, consequently, reduces the risk of underestimating the true extent

of fear recovery (for a comparison of scoring methods, see Luck & Lipp, 2016; Pineles et al., 2009). SCRs were subsequently range corrected, to control for individual differences in electrodermal activity (Lykken, 1972), and then square root transformed, to reduce the skew of the distribution (Dawson et al., 2007). The range correction was obtained by dividing each SCR by the largest SCR displayed by the participant. In line with previous research conducted in our laboratory (e.g., Lipp & Edwards, 2002), electrodermal responses were averaged into blocks of two consecutive trials for the purpose of data analysis, to reduce the influence of trial by trial variability (e.g., Lipp & Edwards, 2002).

2.2.2.4. Subjective Ratings

2.2.2.4.1. CS Valence Ratings

Offline CS valence ratings were obtained at the conclusion of the respective experimental test phase (e.g., after acquisition training), whereby participants were asked to rate the pleasantness of each CS on a 9-point Likert scale, ranging from 1 (unpleasant) to 9 (pleasant). The verbal instructions presented during the ratings task were as follows: "In this part of the experiment, you are asked to rate how pleasant/unpleasant the pictures are. Please use the scale below the picture to provide a rating from 1 (unpleasant) to 9 (pleasant)." While online valence ratings may be better suited to capturing trial-by-trial changes in CS valence, online ratings were not employed in the present research, to prevent potential interference with the recording of the primary dependent measure. To clarify, the concurrent recording of SCRs and CS valence ratings may create movement induced artefacts in SCRs due to the operation of a ratings scale (for a detailed discussion of advantages and disadvantages of the concurrent use of multiple readout measures in human fear conditioning research, see Lonsdorf et al., 2017; Sjouwerman et al., 2016; Warren et al., 2014). Ratings were obtained electronically through a custom-made Microsoft Access application, whereby stimuli were presented on the computer screen in randomised order and participants were instructed to rate stimulus valence on the scale located below the picture (Thompson, McEvoy, & Lipp, 2018).[4]

[4] In the reconsolidation study (Chapter 4), CS valence ratings were obtained through DMDX 5.0.5 software (Forster & Forster, 2003); the MS Access application was developed at the conclusion of this study and was used for the purpose of questionnaire and ratings data collection in subsequent studies.

2.2.2.4.2. CS-US Contingency Awareness

To assess whether participants were able to verbalise the CS-US contingency, a CS-US contingency awareness questionnaire was administered at the conclusion of acquisition training. The questionnaire contained images of the CSs and control stimuli that did not serve as CSs and required participants to indicate which pictures were followed by the electric shock. Participants in the reconsolidation study (Chapter 4) completed a paper and pencil version of this questionnaire, whereas participants in the occasionally reinforced extinction (Chapter 3) and US devaluation study (Chapter 5) were presented with an electronic version of the questionnaire. The electronic version resembled the paper and pencil questionnaire, with the exception that participants were asked to select Yes or No from a drop-down menu located below each image, to indicate whether the image was paired with the US. The paper and pencil version required participants to place a tick mark next to the pictures that had been paired with the US. As inability to verbalise the CS-US contingency may indicate failure to learn the fear association (Lipp, 2006a), data from participants who failed this test were excluded from statistical analyses (for further details, please see the respective empirical chapters).

2.2.2.5. Self-Report Questionnaires

Participant demographics and self-reported snake and spider fear, intolerance of uncertainty, depression, anxiety, and stress were recorded in the present project to ensure groups did not differ on variables known to affect conditioned responding (Lonsdorf & Merz, 2017). Self-report measures encompassed the Intolerance of Uncertainty Scale, short version (Carleton, Norton, & Asmundson, 2007); Depression, Anxiety, and Stress Scales, short version (Henry & Crawford, 2005; P. F. Lovibond & S. H. Lovibond, 1995); as well as the Spider and Snake Phobia Questionnaires (Klorman, Weerts, Hastings, Melamed, & Lang, 1974). The recorded participant demographics included gender, age, and ethnicity. Questionnaire data and participant demographics were collected electronically at the start of each experiment.

2.2.2.5.1. Depression, Anxiety, and Stress Scales (DASS-21)

The 21-item short version of the original 42-item DASS (P. F. Lovibond & S. H. Lovibond, 1995) was employed to assess levels of anxiety, depression, and stress participants experienced over

the past week. The DASS-21 is scored on a 4-point Likert scale, ranging from 0 (did not apply to me at all) to 3 (applied to me very much or most of the time), and comprises three subscales: The 7-item depression subscale (e.g., “I couldn’t seem to experience any positive feelings at all.”), which assesses symptoms such as hopelessness, self-deprecation, and anhedonia; the 7-item anxiety subscale (e.g., “I felt I was close to panic.”), assessing primarily somatic sensations of anxiety and subjective experience of anxious affect; and the 7-item stress subscale (e.g., “I found it hard to wind down.”), assessing tension-stress, including difficulty relaxing, nervous tension, irritability, and agitation (Lovibond, 1998; P. F. Lovibond & S. H. Lovibond, 1995; S. H. Lovibond & P. F. Lovibond, 1995). Raw subscale scores are multiplied by two, thereby yielding a possible total score of 0 to 42 for each subscale, in line with the full version of the DASS.

The DASS-21 was found to have excellent psychometric properties in community and clinical samples (Antony, Bieling, Cox, Enns, & Swinson, 1998; Crawford, Cayley, Lovibond, Wilson, & Hartley, 2011; Henry & Crawford, 2005; Tully, Zajac, & Venning, 2009). In adult community samples, the DASS-21 subscales demonstrated high internal consistencies (Cronbach's alpha ≥ .79; Crawford et al., 2011; Henry & Crawford, 2005) and good convergent and discriminant validity when compared with other validated measures of anxiety and depression, in line with the full version of the DASS (Crawford & Henry, 2003; Henry & Crawford, 2005). Internal consistencies in the present project were high, ranging from α = .78 (anxiety) to α = .87 (depression).

2.2.2.5.2. Intolerance of Uncertainty Scale-12 (IUS-12)

Intolerance of uncertainty (IU) was measured through the 12-item short version (Carleton et al., 2007) of the original 27-item IUS (Buhr & Dugas, 2002; Freeston, Rhéaume, Letarte, Dugas, & Ladouceur, 1994). The IUS-12 measures beliefs about and reactions to uncertainty, ambiguous situations, and the future and comprises two subscales: the 7-item Prospective IU (“Unforeseen events upset me greatly.”) and the 5-item Inhibitory IU scale (“I must get away from all uncertain situations.”). Prospective IU reflects cognitive appraisals of future uncertainty while Inhibitory IU is indicative of behavioural inhibition and avoidance (Carleton et al., 2007; McEvoy & Mahoney, 2012); the total IUS-12 score, which has been employed in the majority of past fear conditioning research

(e.g. Dunsmoor, Campese, Ceceli, LeDoux, & Phelps, 2015), reflects general IU (Carleton et al., 2007). Items are rated on a 5-point Likert scale, ranging from 1 (not at all characteristic of me) to 5 (entirely characteristic of me), yielding possible total scores of 12 to 60.

The IUS-12 was found to have good psychometric properties in undergraduate student and clinical samples (Carleton et al., 2007; Jacoby, Fabricant, Leonard, Riemann, & Abramowitz, 2013; Khawaja & Yu, 2010; McEvoy & Mahoney, 2011), although recent examinations of the underlying factor structure favour the use of the total score, reflecting a general IU factor, in lieu of sub-scale scores in undergraduate (Hale et al., 2016) and clinical samples (Shihata, McEvoy, & Mullan, 2018). Internal consistency in the present project was high for the total IUS-12 score ($\alpha \geq .88$).

2.2.2.5.3. Snake and Spider Fear Questionnaire

The degree of spider and snake fear in the US devaluation study (Chapter 5) was measured with the 31-item Spider Phobia Questionnaire (SPQ) and the 30-item Snake Phobia Questionnaire (SNAQ; Klorman et al., 1974). Both scales employ a true-false response format and measure fear and avoidance of spiders (e.g., "I avoid going to parks or on camping trips because there may be spiders about.") and snakes (e.g., "I dislike looking at pictures of snakes in a magazine."). The possible scores range from 0 to 30 (SNAQ) or 0 to 31 (SPQ), whereby higher scores are indicative of greater self-reported snake/spider fear. The SPQ and the SNAQ have been employed in past fear conditioning research (Bos, Beckers, & Kindt, 2014; Lipp & Waters, 2007; Mallan & Lipp, 2011; Pace-Schott, Verga, Bennett, & Spencer, 2012; Soeter & Kindt, 2015a) and demonstrated good psychometric properties in undergraduate samples (Klorman et al., 1974), evidencing good internal consistencies (SNAQ: .78 - .90; SPQ: .83 - .90) and 1-month test-retest reliabilities (≥ .60), but lower estimates of internal consistency of the SPQ (.43) have been reported in a small sample of undergraduate students (Muris & Merckelbach, 1996). Internal consistencies in the present investigation (US devaluation study) were high for the SPQ ($\alpha = .88$) and for the SNAQ ($\alpha = .92$). Please see the respective empirical chapters for the group means and standard deviations of individual difference measures and participant demographics.

Chapter 3: Study 1 – Occasionally Reinforced Extinction

Note: The following paper has been published in the Journal *Behaviour Research and Therapy.*

Thompson, A., McEvoy, P. M., & Lipp, O. V. (2018). Enhancing extinction learning: Occasional presentations of the unconditioned stimulus during extinction eliminate spontaneous recovery, but not necessarily reacquisition of fear. *Behaviour Research and Therapy, 108,* 29-39. doi:10.1016/j.brat.2018.07.001

Please note that no changes were made to the published article, other than the amendment of heading styles, to ensure consistency throughout this thesis (see Appendix A: Publication 1 for the print version of this article). As the spelling conventions used in the published article (American English) have not been changed, the spelling may deviate from that used in other parts of this thesis (Australian English).

3.3. Abstract

Background: Fears underlying anxiety disorders are commonly treated with exposure-based therapies, which are based on the principles of extinction learning. While these treatments are efficacious, fears may return after successful treatment. Past research suggested that post-extinction recovery of fear could be reduced through extinction training that involves occasional presentations of the aversive unconditioned stimulus (US), paired with the conditioned stimulus (CS). Here, we examined whether extinction training with occasionally paired or unpaired US presentations is superior in the reduction of fear recovery to non-reinforced extinction. **Method**: Following differential fear conditioning to neutral cues, participants (N=72; *M age*=21.61 years, SD=3.95) underwent either non-reinforced, partially reinforced, or unpaired extinction training. **Results**: Extinction involving paired or unpaired US presentations, but not non-reinforced extinction, eliminated spontaneous recovery of differential skin conductance responses (SCRs). Results further suggested that unpaired, but not paired, US presentations may guard against rapid reacquisition of differential SCRs. No benefits of US presentations during extinction were found on the reinstatement of SCRs or recovery of differential negative CS+ valence. **Conclusion**: Presenting USs during extinction training was more effective than non-reinforced extinction in the reduction of fear recovery, as indexed by SCRs, with unpaired extinction being more effective than partially reinforced extinction.

Key words: fear conditioning; occasionally reinforced extinction; partially reinforced extinction; unpaired extinction; reacquisition; spontaneous recovery; reinstatement; return of fear

3.4. Introduction

Past research has provided us with a good understanding of mechanisms underlying the development and reduction of fears, phobias, and anxiety disorders. Fears are acquired through association of neutral cues (conditioned stimuli, CSs), such as animals, with aversive outcomes (unconditioned stimuli [USs]; Davey, 1992), such as an animal bite. Through CS-US pairings we learn to predict which cues signal the arrival of aversive and potentially threatening events. While learning to fear cues which may pose a threat to our survival is an important adaptive mechanism that can protect us from harm and facilitate survival (Öhman & Mineka, 2001), fears may also become maladaptive and contribute to the development of anxiety and stress disorders, which can interfere with daily functioning (Foa & McLean, 2016). The current global prevalence rate of anxiety disorders is estimated at 7.3%, with approximately 11.6% of the population experiencing an anxiety disorder in a given year (Baxter, Scott, Vos, & Whiteford, 2013; Craske & Stein, 2016). Anxiety disorders are commonly treated with exposure-based therapies, which are based on the principles of extinction learning (Craske, Treanor, Conway, Zbozinek, & Vervliet, 2014). In its basic form, extinction training involves the repeated presentation of the CS, in the absence of the US, until a reduction of fear is achieved (Bouton, 2000).

While the efficacy of exposure therapies is well established, not all individuals respond to these treatments, while others experience a return of fear after successful treatment (Craske & Mystkowski, 2006; Weisman & Rodebaugh, 2018). Research suggests that extinguished fear may return, because extinction training does not result in the unlearning or persistent elimination of the original fear learning (i.e. the CS-US association), but creates a new, inhibitory association (CS-no US) that co-exists with the fear association (Bouton, 1993). As such, future CS presentations may activate the CS-no US or CS-US association, whereby the latter would allow for a return of fear (Bouton, 1993). Recovery from extinction phenomena are well-documented in the conditioning literature and include recovery of extinguished responding in a new context (renewal), after the passage of time (spontaneous recovery), after the unsignaled presentation of the US (reinstatement), or after additional post-extinction CS-US pairings (reacquisition; Bouton, 2002). Findings from animal research indicate that reacquisition after extinction may occur at a faster rate than de novo

conditioning (Napier, Macrae, & Kehoe, 1992; but see Ricker & Bouton, 1996), suggesting that the original fear learning is preserved during extinction and, thereby, may be retrieved through future cue encounters. Taken together, research has identified several pathways that may result in the return of fear following successful extinction training or the successful completion of exposure therapy.

When applied to an example of relapse in the clinical setting, for instance the return of social anxiety, which is characterized by fear of social situations in which individuals may be exposed to rejection, embarrassment, or negative evaluations by others (American Psychiatric Association, 2013), fear may recover when individuals are re-exposed to previously feared social situations (CS) or through exposure to additional CS-US pairings (reacquisition), such as receiving negative feedback (US) during a meeting at work (CS). Given the frequency with which feared cues and outcomes may be encountered in daily life, whether in a paired (CS-US) or unpaired manner (CS or US), the likelihood of fear recovery appears high - this may seem discouraging from a clinical point of view. However, recent evidence suggests that exposure therapy may be optimized in a way that would minimize recovery of extinguished fear, even in light of occasional post-extinction CS-US pairings.

A method of exposure therapy for reducing the return of fear proposed by Craske et al. (2014) involves *occasionally reinforced extinction*, meaning intentionally exposing clients to occasional presentations of the feared event (US) during exposure therapy. In the case of social anxiety, this may involve the delivery of rejection or *shame attacks* during exposure to social situations (Craske et al., 2014). While this idea appears counterintuitive as CS-US pairings are implicated in fear acquisition (Davey, 1992), extant literature suggests that occasional presentations of the US during extinction training may be superior to conventional, non-reinforced extinction in preventing recovery of extinguished responding (e.g. Bouton, Woods, & Pineño, 2004; Culver, Stevens, Fanselow, & Craske, 2018).

Specifically, experiments conducted with animal subjects demonstrated that partially reinforced extinction training, involving occasional delivery of CS-US pairings, interfered with the reacquisition of extinguished responding in appetitive (Bouton et al., 2004) and operant conditioning preparations (Woods & Bouton, 2007). Of particular interest was the observation that partially reinforced extinction slowed the reduction of responding during extinction, as would be expected

from reinforced training, but protected against rapid reacquisition, relative to non-reinforced extinction. Additionally, compared to partially reinforced training, an unpaired extinction procedure, whereby reinforcers were not paired with the CS, but instead delivered in the inter-trial interval, further reduced the rate of reacquisition (Bouton et al., 2004 [Experiment 2]). Replication attempts with humans, however, have yielded mixed results in an appetitive conditioning study (van den Akker, Havermans, & Jansen, 2015), showing reduced reacquisition of US expectancies, but not self-rated *conditioned desires* for chocolate mousse, subsequent to partially reinforced and unpaired extinction training. That being said, the authors also reported group differences at baseline and differential effects of acquisition training on verbal (e.g. US expectancy) and physiological indices of conditioned responding (i.e. participants' rate of salivation in anticipation of food), making the overall interpretation of findings difficult.

An extension of Bouton et al.'s (2004) findings to human fear conditioning, on the other hand, has yielded more promising results, suggesting that partially reinforced extinction may successfully reduce the reacquisition of extinguished fear responses (Culver et al., 2018). Following differential fear conditioning to neutral cues, participants underwent either non-reinforced or partially reinforced extinction training. Similar to Bouton and colleagues' work, a 2:8 reinforcement schedule was used during extinction in the partially reinforced group, translating to six reinforced and 18 non-reinforced CS+ trials and 24 non-reinforced CS- trials. Tests of fear recovery showed that partially reinforced extinction training, relative to non-reinforced extinction, interfered with subsequent reacquisition of conditioned fear, as indexed by electrodermal responding. An aspect requiring further investigation, however, is the effect of partially reinforced extinction on the spontaneous recovery of extinguished fear. While Culver and colleagues observed reduced recovery of electrodermal responding to the CS+ after partially reinforced extinction, relative to non-reinforced extinction, these results must be interpreted with caution, as conditioned responding failed to extinguish during partially reinforced extinction training and, consequently, could not "recover." Nevertheless, the results of the reacquisition test provide evidence for cross-species applicability of partially reinforced extinction. The aim of the present study was to replicate and extend previous findings (Bouton et al., 2004; Culver et al., 2018) to the spontaneous recovery, reinstatement, and reacquisition of

extinguished conditioned responding in human fear conditioning, employing partially reinforced, unpaired, and non-reinforced extinction training. Furthermore, a direct comparison of occasionally paired and unpaired US presentations during extinction would also allow for examination of underlying mechanisms, which may differ across different types of reinforced extinction training (e.g. Bouton et al., 2004; Rauhut, Thomas, & Ayres, 2001; Rescorla & Skucy, 1969).

Several mechanisms have been proposed to account for the superior protection from fear recovery effects subsequent to reinforced and unpaired extinction training, compared to non-reinforced extinction, including: Weakening of the CS-US association through unpaired US presentations (Frey & Butler, 1977; Rescorla & Skucy, 1969; Vervliet, Vansteenwegen, & Hermans, 2010); US habituation (Rauhut et al., 2001; but see Thomas, Longo, & Ayres, 2005); sequential learning (Bouton et al., 2004; Capaldi, 1966, 1994); and enhanced extinction learning through violation of expectancies, also referred to as prediction errors (Craske et al., 2014; Culver et al., 2018; Rescorla & Wagner, 1972; Vurbic & Bouton, 2014).

Prediction errors are implicated in the acquisition and extinction of conditioned responding (e.g. Pearce & Hall, 1980; Rescorla & Wagner, 1972; Vurbic & Bouton, 2014), whereby learning is proposed to cease when the CS reliably predicts the delivery of the US (or its absence, in the case of extinction learning). Extinction learning may be enhanced through the occasional presentation of the US during extinction training, due to the violation of expectancies regarding the frequency of US presentations or changes to the CS-US relationship (e.g. Craske et al., 2014). For instance, the omission of the US at the onset of extinction provides an opportunity for new learning due to the mismatch between current information (CS-no US) and past learning (CS-US), while the presentation of occasionally paired and unpaired USs on later trials would sustain learning through the presentation of novel information that needs to be reconciled with prior learning. Hence, the occasional presentation of USs during extinction would allow participants to learn about the likelihood of future threat encounters, such as the frequency of US presentations, relative to CS-only trials, or the relationship between the CS and the US (i.e. occasionally paired or unpaired). Subsequent fear recovery could be reduced because participants learned that the CS predicts the absence of the US (unpaired extinction; Rescorla & Skucy, 1969; Vervliet et al., 2010) or that occasional CS-US trials

occur in the presence of many CS-no US trials (partially reinforced extinction). This proposition is also supported by Bouton et al.'s (2004) adaptation of sequential theory (Capaldi, 1966, 1994).

Bouton et al. (2004) proposed that the key aspect learned during partially reinforced extinction training is that CS-US trials do not occur exclusively in the "context" of other CS-US trials (i.e. acquisition), but may also occur in the context of extinction trials (i.e. a CS-US trial is followed by several CS-no US trials). Due to the association of reinforced trials with non-reinforced trials, reacquisition of extinguished responding may occur at a slower rate, as participants may expect a CS-US trial to be followed by further CS-no US trials. Bouton et al. further proposed that similar learning would occur during unpaired extinction, whereby unpaired US presentations would weaken the US's exclusive association with the acquisition context. Conversely, reacquisition subsequent to non-reinforced extinction is proposed to occur at a faster rate, as the omission of the US during training maintains the US's exclusive association with the acquisition context (i.e. CS-US trials occurring in the context of other CS-US trials). Hence, post-extinction presentations of reinforced trials would signal delivery of further reinforced trials and lead to rapid reacquisition. It should be noted that Bouton et al.'s model would also predict reduced reinstatement of fear, particularly in participants who received unpaired USs during extinction, but it is not readily applicable to spontaneous recovery, unless additional assumptions are made. Spontaneous recovery may depend, in part, on how easily the extinction memory can be retrieved, meaning the memory that a CS-only trial is more likely to signal further non-reinforced than reinforced trials. To summarize, there are several mechanisms that may account for reduction of fear recovery following reinforced extinction training, although, at present, they are still poorly understood and require further examination.

In the present study, we investigated whether occasional presentations of paired and unpaired USs during extinction training would result in superior reduction of spontaneous recovery, reinstatement, and reacquisition of fear, compared to non-reinforced extinction training. The fear association was established through differential Pavlovian conditioning (Culver et al., 2018; Lipp, 2006a), whereby one neutral cue (CS+) was continuously paired with an aversive electrotactile stimulus (US), while another cue (CS-) was presented by itself. Conditioned fear in differential paradigms is reflected in larger responding to the CS+, relative to the CS- (Lipp, 2006a). In line with

Culver et al. (2018), electrodermal responding and CS valence ratings were recorded as primary and secondary dependent measures of conditioned responding, respectively. Based on the reviewed literature (Bouton et al., 2004; Craske et al., 2014; Culver et al., 2018), we predicted that extinction learning would be enhanced through occasional presentations of the US during extinction training, which would be reflected in reduced fear recovery and reacquisition of fear, relative to non-reinforced extinction. In line with Bouton et al.'s findings, we also predicted that unpaired extinction would result in slower reacquisition of extinguished fear than partially reinforced extinction.

3.5. Materials and Methods

3.5.1. Participants

University students who met inclusion criteria (i.e. no cardiovascular disease, seizure disorder, or pregnancy) participated in exchange for partial course credit or a financial compensation of 30 AUD. After exclusion of one participant who failed to verbalize the CS-US contingency, data from 72 participants (44 females, 28 males; female-male ratio per group: 15:9 [non-reinforced and partially reinforced extinction group], 14:10 [unpaired extinction group]) were included in the analyses. The age range of participants was 18 to 38 years (M = 21.61 years, SD = 3.95). Ethical approval for this study was obtained from the Curtin University Human Research Ethics Committee.

3.5.2. Apparatus and Materials

3.5.2.1. Stimuli

In line with previous research (Culver et al., 2018), non-fear relevant stimuli have been employed in the present study. Conditioned stimuli (CS) included four color images of animals, two birds and two fish (sourced from the internet). Each participant was presented with a subset of two pictures, comprising one bird and one fish picture; stimulus sets were counterbalanced across participants. The pictures measured between 700 x 467 pixels and 700 x 541 pixels and were presented for 6 s, in the center of a 17-inch color LCD screen, over a black background, with an inter-trial interval of 13 to 17 s. To control for order effects, the assignment of bird and fish pictures as CS+ or CS- and the presentation order (whether the first trial of each phase was a CS+ or CS-) were counterbalanced across participants. Stimuli were presented in a pseudo-randomized order, whereby each CS was presented four times within blocks of eight trials, with the restriction that no more than

two consecutive CS+ or CS- trials were presented. The unconditioned stimulus (US) consisted of a mild electric shock, which was generated with a Grass SD9 stimulator (Grass Technologies, Middleton, WI) and was delivered to the wrist of the dominant hand via a concentric electrode. The shock was presented for 200 ms (pulsed at 50 Hz) and coincided with the CS+ offset (unless otherwise indicated); the CS- was never paired with the US. The delivery of the US and CSs was controlled with DMDX 5.0.5 software (Forster & Forster, 2003).

3.5.2.2. Electrodermal Activity (Skin Conductance Responses, SCRs)

Electrodermal activity was recorded through two self-adhesive isotonic gel electrodes (Biopac Systems EL507), attached to the thenar and hypothenar eminences of the non-dominant hand. Electrodermal activity was DC amplified at a gain of 5 micro Siemens (µS) per volt and recorded with a Biopac MP150 system at a sampling frequency of 1000 Hz, using AcqKnowledge 4 (Biopac Systems, Goleta, CA). A Biopac respiration belt was fitted around each participant's waist to control for respiration-induced artefacts in SCRs.

3.5.2.3. Subjective Evaluation of Stimulus Valence

Participants provided post-test CS and US valence ratings on a 9-point scale (from 1 [unpleasant] to 9 [pleasant]) at baseline, after acquisition, extinction, spontaneous recovery (CS ratings only), reinstatement test, and reacquisition. US valence ratings were not obtained after spontaneous recovery to prevent potential interference with subsequent reinstatement, as reinstatement requires the unexpected presentation of the US (Haaker, Golkar, Hermans, & Lonsdorf, 2014). Valence ratings were obtained electronically through a custom-made Microsoft Access application, whereby the stimuli were presented on the computer screen in randomized order and participants were instructed to rate stimulus valence on the scale located below the picture.

3.5.2.4. Self-report Questionnaires

To ensure groups did not differ on variables known to affect conditioned responding (Dunsmoor, Campese, Ceceli, LeDoux, & Phelps, 2015; Lonsdorf & Merz, 2017), participants were asked to complete the short version of the Intolerance of Uncertainty Scale (IUS-12; Carleton, Norton, & Asmundson, 2007). The 12-item IUS measures beliefs about and reactions to uncertainty, ambiguous situations, and the future (e.g. "Unforeseen events upset me greatly.") and comprises two

subscales: prospective IU (measures anxiety about future events) and inhibitory IU (indicative of behavioral inhibition or avoidance). The scale has good psychometric properties (Carleton et al., 2007) and demonstrated excellent internal consistency in the current sample (α = .90). For exploratory purposes, participants also completed the short version of the Depression, Anxiety, and Stress Scales (DASS-21; Henry & Crawford, 2005; Lovibond & Lovibond, 1995). The questionnaires were completed electronically at the start of the experiment.

3.5.2.5. Manipulation Checks

Following acquisition, participants were presented with a CS-US contingency questionnaire, containing the four stimulus pictures used in this study, and were asked to indicate which pictures had been paired with the US. As inability to verbalize the correct contingency may reflect a genuine failure to learn the CS-US relationship (Lipp, 2006a), data from one participant who failed this test were excluded from statistical analyses.

3.5.3. Procedure

A schematic representation of the experimental paradigm and the reinforcement schedule employed during extinction training is presented in Figure 1. Upon arrival in the laboratory, participants were informed about the experimental procedures and had the opportunity to ask questions, before providing information about current medication use and medical history. Participants were assigned to groups in the order they presented for testing, with the restriction that an approximately equal number of females and males were assigned to each group. Individuals who met inclusion criteria provided written consent, were seated in front of the computer screen, and were fitted with the skin conductance electrodes, respiration belt, and the shock electrode. After completing the self-report measures, participants were asked to relax and look at the blank computer screen while a 2-min baseline of their electrodermal activity was recorded. Subsequently, participants provided baseline CS valence ratings, set the US intensity to a level which was perceived as “unpleasant, but not painful,” and rated US valence.

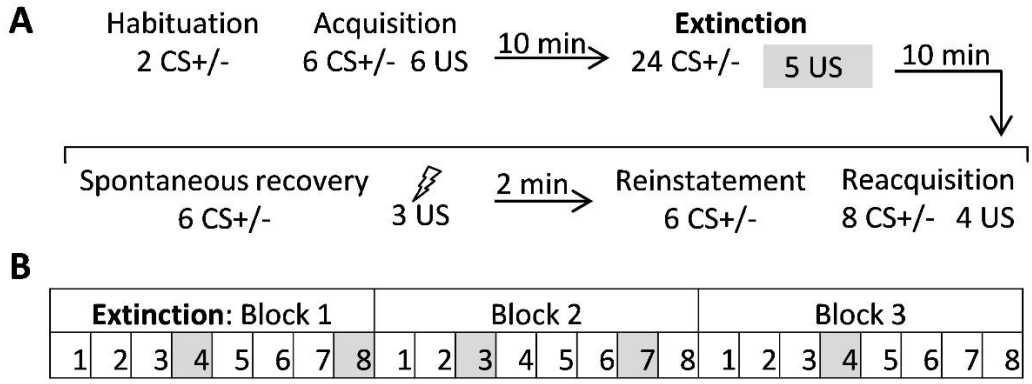


Figure 1. Schematic representation of the experimental paradigm (**A**) and reinforcement schedule during extinction training (**B**). Panel A illustrates the sequential order of experimental stages with the respective number of conditioned (CS) and unconditioned stimulus (US) presentations. Boxes in panel B denote delivery of the previously conditioned CS+ (CS- is not depicted here). The shaded boxes represent delivery of the US, which either coincided with the CS+ offset (partially reinforced extinction group) or was presented in the middle of the inter-trial interval (unpaired extinction group), either after CS+ offset or before CS+ onset.

3.5.3.1. Acquisition

Participants were asked to pay attention to the computer screen and to learn which CSs were followed by the US. Conditioning commenced with a habituation phase, consisting of two non-reinforced presentations of each CS, and was immediately followed by acquisition, which involved six presentations of the CS+ and the CS-. The US was presented on all CS+ trials. Thereafter, participants completed the CS-US contingency questionnaire and rated CS and US valence. The shock electrode was removed and a 10-min rest period was inserted during which participants were offered magazines to read. The break was included to allow electrodermal activity to return to a stable baseline before the start of extinction training.

3.5.3.2. Extinction

The shock electrode was reattached and participants were informed that they would be presented with several stimuli and asked to pay attention to the computer screen at all times. No information was provided about the types of stimuli to be presented, their frequency, duration, or contingencies. Extinction training consisted of 24 CS+/- presentations; the CSs+ were non-reinforced in the non-reinforced extinction (EXT) and unpaired extinction (UNP) group and partially reinforced (5 CS-US pairings; see Figure 1B) in the partially reinforced extinction (PRE) group. In the UNP

group, five US presentations were delivered in the middle of the inter-trial interval (ITI). Within each block of eight trials, the US was delivered on trial 4 and 8 (block 1), 3 and 7 (block 2), and on trial 4 (block 3). The US coincided with the CS+ offset in the PRE group. In the UNP group, the US was presented in the middle of the ITI, either after CS+ offset or in the ITI before CS+ onset, with the restriction that the US was never presented between two CSs+. As previous reports indicated that a 2:8 reinforcement ratio maintained differential responding at the end of extinction training (Culver et al., 2018), we decreased the reinforcement ratio on the last block of training to a 1:8 ratio. This decrease was in line with past research (Bouton et al., 2004) and served to facilitate loss of conditioned responding. Following extinction training, participants were asked to rate CS and US valence. Subsequently, the shock electrode was removed and a 10-min break was inserted (identical to the post-acquisition break).

3.5.3.3. Test of Fear Recovery

The shock electrode was reattached and participants were informed that they would be presented with several stimuli and were asked to pay attention to the computer screen at all times (instructions were identical to those presented at the start of extinction training). Assessment of spontaneous recovery involved six non-reinforced presentations of the CS+/-, which were followed by post-test CS valence ratings. Subsequently, participants received three unsignaled presentations of the US, with a duration of 200 ms and an ITI of 6 s. The computer screen remained switched on and displayed a black background (in line with previous training). After a 2-min delay, reinstatement of extinguished responding was tested through six non-reinforced presentations of the CS+/-. After rating CS and US valence, participants underwent partially reinforced reacquisition, comprising eight presentation of the CS+/-. The US coincided with the CS+ offset on 50% of the trials (1^{st}, 3^{rd}, 5^{th} and 6^{th} CS); the remaining trials were not reinforced. The physical intensity of the US during reinstatement and reacquisition was identical to that employed during acquisition. We employed a partial reinforcement schedule (in line with Bouton et al., 2004) to permit emergence of group differences (Lissek, Pine, & Grillon, 2006). The partial reinforcement schedule further served to enhance the ecological validity of the reacquisition test, as individuals would be more likely to

encounter occasional, than continuous, CS-US pairings in real life. At the conclusion of reacquisition, participants provided the final CS and US valence ratings.

3.5.4. Scoring and Response Definition

Electrodermal responses were scored offline in AcqKnowledge 4. Participants' baseline electrodermal activity was determined by counting all spontaneous responses that occurred during a 2-min rest period (Dawson, Schell, & Filion, 2007). A visual inspection of data was conducted to identify movement- or respiration-induced artefacts in SCRs. Eight SCRs (across groups) were discarded due to the presence of artefacts. In accordance with past research (Culver et al., 2018; Pineles, Orr, & Orr, 2009), SCRs elicited by the CSs were calculated by subtracting the mean skin conductance level during the 2 s baseline preceding CS onset from the largest skin conductance level occurring 1 to 6 s after CS onset. SCRs were range corrected to control for individual differences in electrodermal activity (Lykken, 1972) and then square root transformed to reduce the skew of the distribution (Dawson et al., 2007). The range correction was obtained by dividing each response by the largest response displayed by the participant. Electrodermal responses were averaged into blocks of two consecutive trials, to reduce the influence of trial by trial variability.

3.5.5. Statistical Analyses

Electrodermal responding during habituation was analyzed through a repeated measures analysis of variance (ANOVA) with group (EXT, PRE, UNP) as a between-groups factor and CS type (CS+, CS-) as a within-groups factor. Analysis of acquisition, extinction, and reacquisition data was conducted through mixed ANOVAs for repeated measures, with group as a between-groups factor and CS type (CS+ vs. CS-) and block/time as within-groups factors (acquisition: block 1-3; extinction: early vs. late phase; reacquisition: block 1-3). Extinction of conditioned responding was assessed with data from the early (block 1-2) and late phase (block 11-12) of extinction training, during which no USs were presented in any of the groups (the US presented at the end of trial 4/block 2 in the PRE and UNP group would affect responding on the subsequent trials, but not on trial 4). In line with past research (Dunsmoor et al., 2015), recovery of extinguished responding was assessed during the early phase (block 1) of spontaneous recovery and reinstatement tests, through separate repeated measures ANOVAs, with CS type (CS+ vs. CS-) as a within-groups factor and group as a

between-groups factor. CS valence ratings were analyzed through a series of mixed ANOVAs for repeated measures, with group as a between-groups factor and CS type (CS+ vs. CS-) and time as within-groups factors (acquisition: baseline vs. acquisition; extinction: acquisition vs. extinction; spontaneous recovery: extinction vs. spontaneous recovery; reinstatement: extinction vs. reinstatement). Reacquisition was assessed by means of a repeated measures ANOVA with group as a between-groups factor and CS type (CS+ vs. CS-) as a within-groups factor. Multivariate *F* values (Pillai's Trace) and partial eta squared values are reported for all main effects and interactions. Statistical significance was assessed at $\alpha = .05$; Bonferroni corrections were used for follow-up analyses to guard against the accumulation of a Type 1 error.

3.6. Results

3.6.1. Preliminary Analyses

The groups did not differ in age, selected US intensity, baseline CS or US valence ratings, baseline electrodermal activity, IUS-12 scores, or DASS-21 scores (Table 1). Selected US intensities ranged from 34 to 80 Volt, with a mean of 66.19 Volt (*SD* = 12.90).

Table 1

Means (M) and Standard Deviations (SD) for Age, US Intensity, Baseline Valence Ratings (VR), Baseline Electrodermal Activity (EDA), and Self-Report Questionnaires

	Non-reinforced extinction		Partially reinforced extinction		Unpaired extinction		
	M	*SD*	*M*	*SD*	*M*	*SD*	Test
Age	20.37	2.43	21.79	3.50	22.67	5.21	$F(2,69)=2.13$, $p=.127$
US intensity	63.38	13.39	68.17	11.59	67.04	13.67	$F(2,69)=0.90$, $p=.410$
Baseline VR							
CS+	7.25	1.51	6.71	2.05	7.38	1.35	$F(2,69)=1.09$, $p=.342$
CS-	6.96	1.57	6.88	2.15	7.54	1.56	$F(2,69)=1.00$, $p=.375$
US	3.88	1.92	3.83	1.88	3.42	1.74	$F(2,69)=0.45$, $p=.639$
Baseline EDA	12.42	9.60	11.50	8.89	10.13	8.35	$F(2,69)=0.40$, $p=.673$
IUS-12							
Prospective	18.46	5.12	19.62	5.45	18.96	5.49	$F(2,69)=0.29$, $p=.752$
Inhibitory	9.54	3.56	11.38	4.75	10.92	4.69	$F(2,69)=1.14$, $p=.324$
DASS-21							
Depression	8.67	10.05	7.92	7.99	9.00	7.55	$F(2,69)=0.10$, $p=.905$
Anxiety	6.17	5.04	7.50	8.18	7.75	7.65	$F(2,69)=0.35$, $p=.709$
Stress	10.75	7.73	13.75	9.57	14.25	9.35	$F(2,69)=1.08$, $p=.345$

Note. CS = conditioned stimulus, US = unconditioned stimulus, IUS-12 = Intolerance of Uncertainty Scale (short version), DASS-21 = Depression, Anxiety, and Stress Scales (short version). Baseline EDA refers to the number of spontaneous responses that occurred during a 2-min rest period. US intensity is reported in Volt.

3.6.2. Electrodermal Responding

3.6.2.1. Habituation

Electrodermal responding across groups is presented in Figure 2. Analysis of SCRs during habituation indicated that SCRs to CS+/- did not differ across groups during habituation, as reflected in the non-significant main effect of CS type, $F(1, 69) < 1$, and the CS type x group interaction, $F(2, 69) = 2.93$, $p = .060$, $\eta p^2 = .08$. Follow-up comparisons conducted for the trend towards significance in the interaction revealed larger SCRs to the CS- ($M = 0.57$, $SD = 0.05$) than to the CS+ ($M = 0.47$, $SD = 0.05$) in the EXT group (Figure 2A), $F(1, 69) = 4.10$, $p = .047$, $\eta p^2 = .06$, but not in the PRE or UNP group, both $F(1, 69) \leq 1.94$, $p \geq .168$, $\eta p^2 \leq .03$. As the interaction indicates that groups may have differed on their level of electrodermal responding to CS+/- at the start of acquisition, we conducted a separate 3 (group) x 2 (CS+, CS-) repeated measures ANOVA with data from the first trial of acquisition. The analysis revealed no significant group differences at the start of acquisition, as

reflected in the non-significant main effect of CS type, $F(1, 69) = 0.53$, $p = .471$, $\eta p^2 = .01$ and the non-significant CS type x group interaction, $F(2, 69) = 0.35$, $p = .709$, $\eta p^2 = .01$.

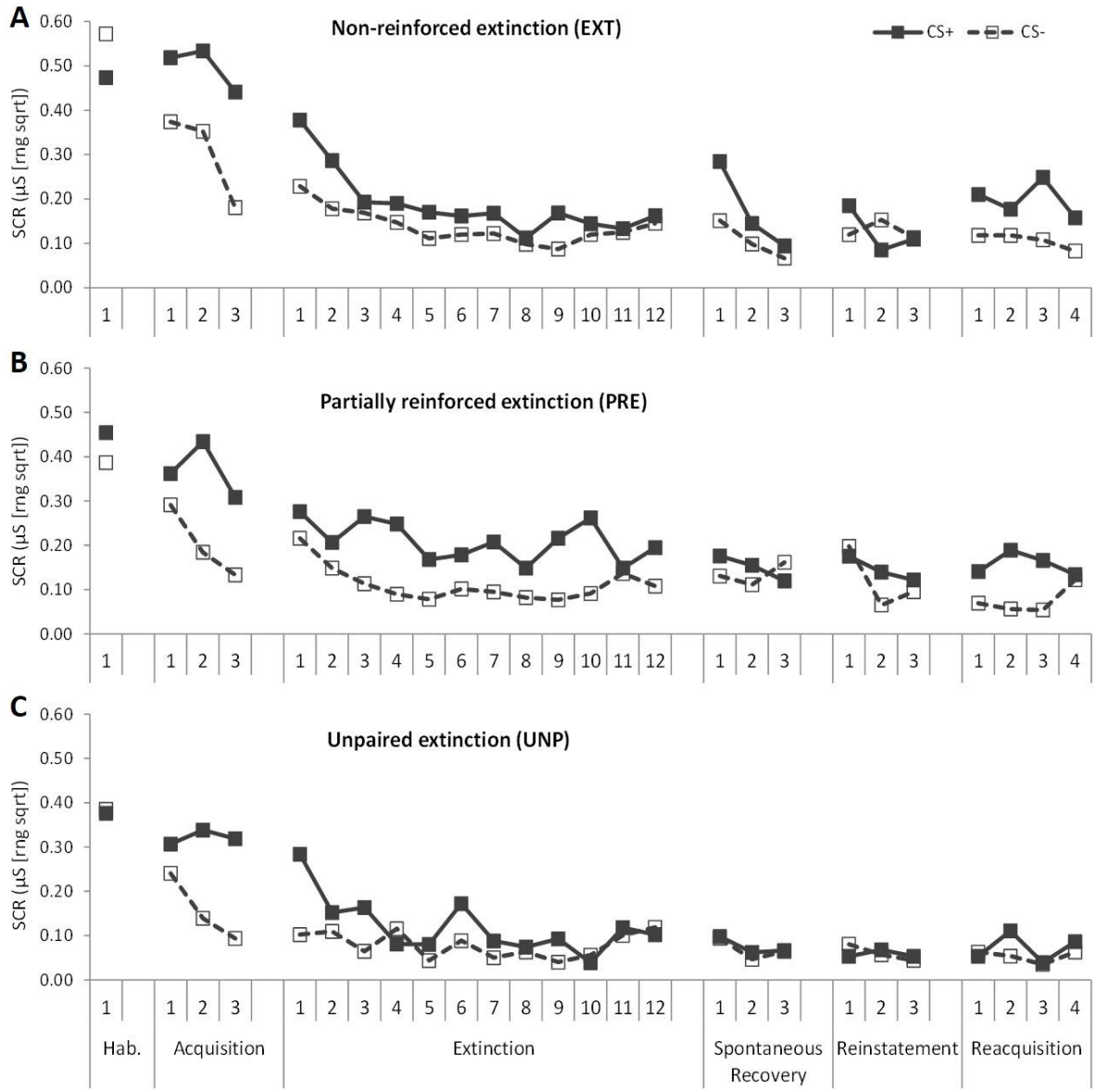


Figure 2. Mean skin conductance responses (SCRs) to reinforced (CS+) and non-reinforced (CS-) conditioned stimuli in the EXT (**A**), PRE (**B**), and UNP (**C**) group. SCRs are presented in blocks of two consecutive trials.

3.6.2.2. Acquisition

Differential SCRs were evident across groups during acquisition, as reflected in main effects of CS type, $F(1, 69) = 61.91$, $p < .001$, $\eta p^2 = .47$; block, $F(2, 68) = 12.18$, $p < .001$, $\eta p^2 = .26$, and a

CS type x block interaction, $F(2, 68) = 6.85$, $p = .002$, $\eta p^2 = .17$. The interaction reflects an increase in differential SCRs across blocks of acquisition, all $F(1, 69) \geq 13.16$, $p \leq .001$, $\eta p^2 \geq .16$. The remaining interactions did not attain significance, with the largest of the non-significant effects suggesting that acquisition of differential SCRs did not significantly differ across groups, (CS type x block x group), $F(4, 138) = 0.94$, $p = .443$, $\eta p^2 = .03$.

3.6.2.3. Extinction

A visual inspection of Figure 2 indicates that differential responding was larger during partially reinforced than during non-reinforced extinction. However, statistical analyses showed that differential responding during the reinforced stage of training did not interfere with the extinction of conditioned responding. Results of the ANOVA examining SCRs between the early (block 1-2) and late phase (block 11-12) of training revealed main effects of CS type, $F(1, 69) = 18.84$, $p < .001$, $\eta p^2 = .21$, and block, $F(3, 67) = 7.96$, $p < .001$, $\eta p^2 = .26$, which were qualified by a CS type x block interaction, $F(3, 67) = 4.69$, $p = .005$, $\eta p^2 = .17$. The interaction reflects differential electrodermal responding on block 1 and 2, both $F(1, 69) \geq 6.80$, $p \leq .011$, $\eta p^2 \geq .09$, but not on block 11 or 12, both $F(1, 69) \leq 1.27$, $p \geq .265$, $\eta p^2 \leq .02$, indicating that differential responding was extinguished in all groups. The remaining interactions did not attain significance, largest effect (CS type x block x group), $F(6, 136) = 1.17$, $p = .324$, $\eta p^2 = .05$. As Figure 2 indicates that differential responding may have been present on the last block of training in the PRE group, we subjected the mean SCRs of the CS+ and the CS- to a *t* test. In line with the between-groups comparisons, results confirmed that there were no significant differences between SCRs to the CS+ ($M = 0.20$, $SD = 0.22$) and the CS- ($M = 0.11$, $SD = 0.17$), $t(23) = 1.68$, $p = .106$, $d = 0.05$.

3.6.2.4. Spontaneous Recovery

Our primary prediction was that occasional US presentations during extinction training would result in less recovery of fear than non-reinforced extinction training. Inspection of spontaneous recovery data in Figure 2 suggests that differential responding recovered following non-reinforced extinction, but not after extinction conducted with occasionally paired or unpaired US presentations. This observation was confirmed by the results of statistical analyses, revealing a main effect of CS type, $F(1, 69) = 8.22$, $p = .005$, $\eta p^2 = .11$, which was qualified by a CS type x group interaction, $F(2,$

69) = 3.25, p = .045, ηp^2 = .09. The interaction reflects differential electrodermal responding in the EXT group, F(1, 69) = 13.19, p = .001, ηp^2 = .16, but not in the PRE group, F(1, 69) = 1.51, p = .223, ηp^2 = .02, or UNP group, F(1, 69) = 0.01, p = .917, ηp^2 < .01.

3.6.2.5. Reinstatement

A visual inspection of differential responding on block 1 of reinstatement (Figure 2) suggests that differential SCRs were reinstated in the EXT group, but not in the PRE or UNP group. However, these differences did not attain significance in the omnibus analysis. The results neither yielded a significant main effect of CS type, F(1, 69) = 0.04, p = .837, ηp^2 = .01, nor a CS type x group interaction, F(2, 69) = 1.48, p = .234, ηp^2 = .04, showing that differential SCRs did not differ across groups on the first block of reinstatement testing.

3.6.2.6. Reacquisition

Our first prediction was that occasional presentations of the US during extinction training would reduce reacquisition of extinguished responding, compared to non-reinforced extinction. To test this prediction, we conducted an ANOVA using data from block 1-3, which was the reinforced stage of reacquisition during which group differences would be expected to emerge (see also Figure 2). The results revealed a main effect of CS type, F(1, 69) = 17.29, p < .001, ηp^2 = .20, as well as a trend towards significance in the CS type x group interaction, F(2, 69) = 2.57, p = .084, ηp^2 = .07. The interaction reflects increased differential responding to the CS+, relative to the CS-, in the EXT group, F(1, 69) = 10.18, p = .002, ηp^2 = .13, as well as in the PRE group, F(1, 69) = 11.94, p = .001, ηp^2 = .15, but not in the UNP group, F(1, 69) = 0.31, p = .580, ηp^2 = .01. The main effect of block and remaining interactions did not attain significance, largest effect (block x group interaction), F(4, 138) = 1.09, p = .362, ηp^2 = .03.

To follow up on Bouton et al.'s (2004) findings, we also tested whether the rate of reacquisition would be slower after unpaired than partially reinforced extinction. The ANOVA conducted with data from the PRE and UNP group yielded a main effect of CS type, F(1, 46) = 9.87, p = .003, ηp^2 = .18, as well as a CS type x group interaction, F(1, 46) = 5.15, p = .028, ηp^2 = .10, reflecting larger differential SCRs in the PRE group (M = 0.11, SD = 0.17) than in the UNP group (M = 0.02, SD = 0.08), t(46) = 2.23, p = .031, d = 0.68. The main effect of block and remaining

interactions did not attain significance, largest effect (CS type x block interaction), $F(2, 45) = 1.30$, $p = .282$, $\eta p^2 = .06$. These results show that the rate of reacquisition differed between the PRE and UNP group; although, contrasting Bouton et al.'s findings, we did not observe reduced reacquisition, but an absence of reacquisition in the UNP group.

3.6.2.7. Examination of Underlying Mechanisms: US Habituation

The lack of reacquisition in the UNP group may indicate that the unpaired presentations of the US during extinction training resulted in US habituation. The reduced aversiveness of the US could have attenuated subsequent responding to the CS+ and slowed the rate of reacquisition (Rescorla, 1973). If US habituation occurred during extinction training in the UNP group, this would be reflected in smaller unconditioned responses (URs) in the UNP than in the PRE group.[1] The results of a 2 (group) x 5 (US presentations) ANOVA did not support the US habituation hypothesis. Results revealed a main effect of trial, $F(4, 43) = 6.76$, $p < .001$, $\eta p^2 = .39$, but no significant trial x group interaction, $F(4, 43) = 0.50$, $p = .736$, $\eta p^2 = .04$. The main effect reflects decreased URs across trials in both groups. However, the mean UR to the final US presentation in the PRE ($M = 0.74$, $SD = 0.34$) and UNP group ($M = 0.67$, $SD = 0.29$) resembled that on the last block of acquisition (PRE: $M = 0.58$, $SD = 0.25$; UNP: $M = 0.65$, $SD = 0.30$). Similarly, analysis of post-extinction US valence ratings showed that the US was rated as equally unpleasant in both groups (PRE: $M = 3.38$, $SD = 1.66$; UNP: $M = 3.96$, $SD = 1.73$), $t(46) = 1.19$, $p = .240$. The combined results, therefore, indicate that US habituation did not occur in either group.

3.6.3. CS Valence Ratings

3.6.3.1. Acquisition

Mean ratings of CS+ and CS- valence as well as negative evaluations of the CS+, relative to the CS-, are presented in Figure 3. Assessment of acquisition (baseline vs. post-acquisition ratings) showed increased negative evaluations of the CS+, relative to the CS-, in all groups. The repeated measures ANOVA yielded main effects of CS type, $F(1, 69) = 25.24$, $p < .001$, $\eta p^2 = .27$, and time,

[1] Respective unconditioned responses (URs) in the partially reinforced and unpaired extinction group were scored as the largest response starting in the 1 to 3 s window following US offset (Prokasy & Ebel, 1967). URs were range corrected and square root transformed prior to analysis.

$F(1, 69) = 46.98$, $p < .001$, $\eta p^2 = .41$, which were qualified by a CS type x time interaction, $F(1, 69) = 53.67$, $p < .001$, $\eta p^2 = .44$. The interaction reflects a significant difference between evaluations of the CS+ and the CS- in all groups, after acquisition, $F(1, 69) = 48.31$, $p < .001$, $\eta p^2 = .41$, but not at baseline, $F(1, 69) = 0.01$, $p = .944$, $\eta p^2 < .01$.

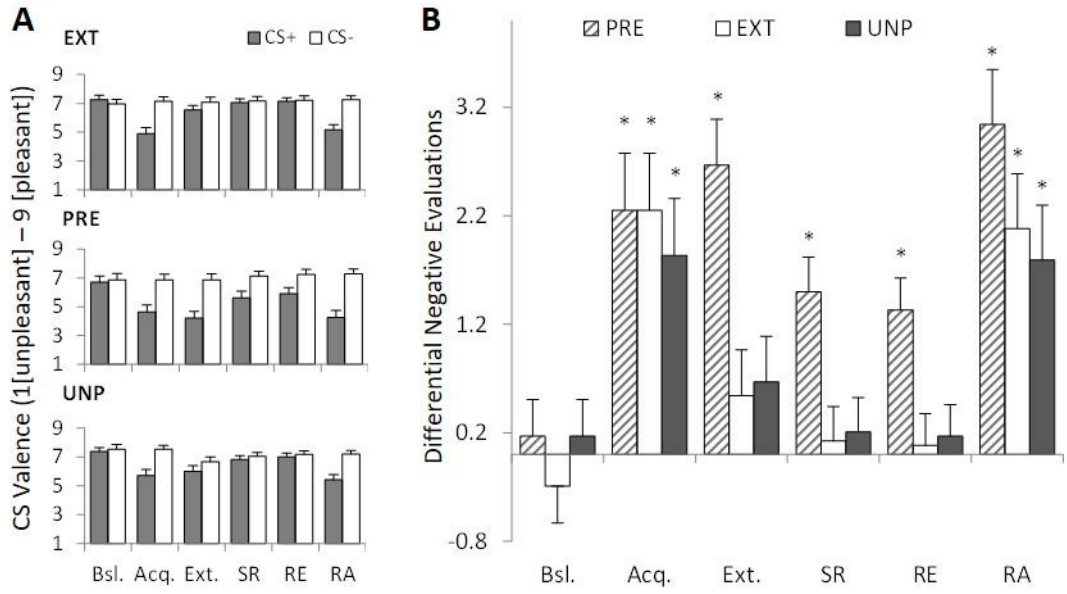


Figure 3. Mean conditioned stimulus (CS) valence ratings (**A**) and differential negative evaluations of the CS+, relative to the CS- (**B**) at baseline (Bsl.), after acquisition (Acq.), extinction (Ext.), spontaneous recovery (SR), reinstatement (RE), and reacquisition (RA). CS valence was rated from 1 (unpleasant) to 9 (pleasant). Error bars represent standard errors. The asterisk in panel B indicates significant negative evaluation of the CS+, relative to the CS-, at $p \leq .001$.

3.6.3.2. Extinction

Assessment of extinction of differential evaluations revealed a main effect of CS type, $F(1, 69) = 50.03$, $p < .001$, $\eta p^2 = .42$, as well as interactions of CS type x time, $F(1, 69) = 9.26$, $p = .003$, $\eta p^2 = .12$, and time x group, $F(2, 69) = 4.58$, $p = .014$, $\eta p^2 = .12$, which were qualified by a CS type x time x group interaction, $F(2, 69) = 5.61$, $p = .006$, $\eta p^2 = .14$. The three-way interaction reflects a significant decrease in negative evaluations from post-acquisition to post-extinction as well as elimination of differential evaluations in the EXT and UNP group, but not in the PRE group. Differential negative evaluations in the PRE group remained significant after extinction training, $F(1, 69) = 39.70$, $p < .001$, $\eta p^2 = .37$.

3.6.3.3. Tests of Fear Recovery

Assessment of spontaneous recovery and reinstatement data showed that this pattern was also reflected in ratings obtained after spontaneous recovery, CS type x group, $F(2, 69) = 8.39$, $p = .001$, $\eta p^2 = .20$, and after reinstatement, CS type x group, $F(2, 69) = 8.39$, $p = .001$, $\eta p^2 = .20$ (see also Figure 3B). No group differences were found in valence ratings obtained after reacquisition; negative evaluations of the CS+, relative to the CS-, were observed in all groups, as reflected in the significant main effect of CS type, $F(1, 69) = 62.76$, $p < .001$, $\eta p^2 = .48$, and non-significant CS type x group interaction, $F(2, 69) = 1.68$, $p = .193$, $\eta p^2 = .05$. In summary, analysis of valence ratings showed that, compared to electrodermal responding, post-test ratings of CS valence exhibited a different pattern of conditioned responding, whereby occasional CS-US pairings, but not unpaired US presentations, maintained differential evaluations after extinction, spontaneous recovery and reinstatement. Additionally, all groups rated the CS+ as significantly more unpleasant than the CS- after reacquisition, indicating that differential evaluations were reacquired.

3.7. Discussion

Our primary aim was to investigate if occasional presentations of the US during extinction training would provide enhanced protection from fear recovery than non-reinforced extinction. The overall pattern of results supported this hypothesis, although there were important differences between the effects of partially reinforced and unpaired extinction training. The results showed no differences in the acquisition and extinction of differential SCRs across groups. Group differences emerged during tests of fear recovery, whereby spontaneous recovery of extinguished differential SCRs was observed after non-reinforced extinction, but not after extinction conducted with occasional presentations of paired or unpaired USs. Analysis of SCRs during reinstatement failed to show significant group differences, indicating that the presentation of three unsignaled shocks did not reinstate differential SCRs in any group. The reacquisition test yielded somewhat unexpected results, indicating reacquisition of differential SCRs subsequent to partially reinforced and non-reinforced extinction, but not after unpaired extinction training.

In contrast to electrodermal data, post-test ratings of CS+/- valence did not reflect any benefits of occasional presentations of the US during extinction over non-reinforced extinction. While

acquisition of differential negative evaluations was in line with the electrodermal data, ratings obtained after the subsequent phases showed a different pattern of results. Negative evaluations of the CS+, relative to the CS-, were observed in the group that received partially reinforced extinction, but not in the groups that received non-reinforced or unpaired extinction, after extinction, spontaneous recovery, and reinstatement. Differential evaluations did not differ across groups after reacquisition, as the CS+ was rated as less pleasant than the CS- in all groups. The combined results demonstrate that partially reinforced and unpaired extinction training offer enhanced protection from fear recovery assessed by SCRs, relative to non-reinforced extinction. However, they also indicate that physiological and verbal indices of conditioned fear may be differentially sensitive to occasional presentations of the US during extinction, as reflected in the different pattern of results obtained from the analysis of electrodermal data and the analysis of valence ratings. Our results also indicate that occasional presentations of unpaired USs during extinction may be more effective in the long-lasting reduction of fear, as indexed by SCRs, than partially reinforced extinction, as unpaired extinction may prevent reacquisition of extinguished responding.

The overall pattern of results can be interpreted as being broadly consistent with past research to the extent that a) occasional presentations of the US during extinction resulted in superior attenuation of recovery from extinction effects than non-reinforced extinction (Bouton et al., 2004; Culver et al., 2018) and b) we observed a dissociation between physiological and verbal measures of fear (e.g. Culver et al., 2018; Luck & Lipp, 2015; Schultz, Balderston, Geiger, & Helmstetter, 2013; Thompson & Lipp, 2017). However, there were several important differences between our findings and those reported in past research. The results of the present study did not converge with previous reports (Bouton et al., 2004; Culver et al., 2018) that interference with reacquisition was greater following partially reinforced than non-reinforced extinction – reacquisition in our study did not differ between the control and partially reinforced group. While this finding was unexpected, in particular since partially reinforced extinction prevented spontaneous recovery of extinguished responding, the divergent results between this and Bouton et al.'s study may reflect on the use of an aversive conditioning preparation, as opposed to appetitive conditioning, as well as on the larger number of extinction trials employed by Bouton et al.

Repeated extinction sessions, conducted across multiple days, may have strengthened the animal subjects' memory of occasional CS-US trials occurring in the presence of many CS-no US trials, which, according to Bouton et al.'s (2004) adaptation of sequential theory (Capaldi, 1966, 1994), would slow the rate of reacquisition. It is possible that the 24 extinction trials employed in our study were not sufficient to create a robust extinction memory. On the other hand, Culver and colleagues (2018) employed the same number of extinction trials, albeit with six, instead of five, paired US presentations, and reported decreased reacquisition after reinforced than non-reinforced extinction. However, a closer examination of Culver et al.'s data suggests that differential SCRs were initially larger in the partially reinforced extinction group than in the control group (after the 1st and 2nd CS-US trial) and only decreased during the second half of reacquisition. The authors suggested that physiological responding decreased because participants acquired "physiological toughness," meaning an improved ability to cope with repeated exposure to aversive stimuli, as a result of partially reinforced extinction training. There was no evidence of decreased SCRs during reacquisition in the partially reinforced extinction group in our data, although this may reflect on the use of a partial reinforcement schedule during reacquisition. In contrast to continuous reinforcement (Culver et al., 2018), alternating CS-US with CS-no US trials may create more uncertainty about future threat. From an evolutionary perspective, even occasional threats may pose a risk to our survival. Hence, it may be of advantage to adopt a "better safe than sorry" approach in response to occasional threat encounters. In this sense, partially reinforced extinction training may protect against spontaneous recovery of fear, as a CS-only trial does not signal imminent danger, but the fear response may return when the likelihood of future threat increases, as would be the case after exposure to a CS-US trial. This proposition could be explored in future research through a direct comparison of reacquisition on a partial and continuous reinforcement schedule.

Analysis of electrodermal data during reacquisition further indicated that the mere reduction of the reinforcement schedule during extinction training, relative to that used during acquisition, may not be sufficient to prevent reacquisition of differential SCRs. This proposition was supported by the differential pattern of reacquisition results observed between the partially reinforced and the unpaired extinction group. Our results indicated that, in contrast to partially reinforced extinction, an unpaired

extinction procedure that involves the occasional presentations of the US in the inter-trial interval may not only reduce, but also prevent, reacquisition of differential SCRs. These results are in line with previous animal research conducted in the appetitive (Bouton et al., 2004) and aversive setting (Frey & Butler, 1977; Mickley et al., 2009; Rauhut et al., 2001; Thomas et al., 2005). Similarly, Vervliet et al. (2010) reported that extinction training consisting of eight non-reinforced CS trials and six unpaired electrotactile USs, presented during the inter-trial interval, reduced renewal of extinguished fear in humans. While the experimental methods differed across these studies, including the number of CS and US trials and the ratio of CS to US trials during extinction training, the outcomes of the present study and past research suggest that extinction training during which the US is retained, but no longer paired with the CS, is superior in the reduction of fear recovery to non-reinforced or partially reinforced extinction.

As a possible underlying mechanism, it has been proposed that unpaired presentations of the US weaken, or even eliminate, the CS-US association that was formed during acquisition (Frey & Butler, 1977; Rescorla & Skucy, 1969; Rescorla & Wagner, 1972; Vervliet et al., 2010). While the associative strength of the CS would also be reduced during non-reinforced extinction (Rescorla & Wagner, 1972), the presentation of unexpected unpaired USs would enhance extinction learning through increased prediction errors (e.g. Fernández, Boccia, & Pedreira, 2016; Todd, Vurbic, & Bouton, 2014). As the CS-US association is weakened, or eliminated, subsequent responding to the CS would be reduced.

A second mechanism proposed by Rauhut et al. (2001) involves US habituation. However, this proposition has not been supported through previous research (Thomas et al., 2005) or the results of the present study. Comparisons of unconditioned responses to the USs presented during extinction training showed no significant differences between the partially reinforced and unpaired extinction groups. Similarly, analysis of US valence ratings showed that the US was rated as equally unpleasant in both groups, making a US habituation explanation unlikely.

Finally, Bouton et al.'s (2004) model would not readily account for the results observed in our unpaired extinction group. While the model proposes that unpaired extinction weakens the US's exclusive association with the acquisition context, it would make the same prediction about partially

reinforced extinction. Therefore, it cannot account for the differences in responding during reacquisition between the paired and unpaired US group. Overall, the present results appear to be consistent with a weakened CS-US association explanation (Rescorla & Wagner, 1972; Vervliet et al., 2010). The reduced associative strength of the CS would explain the reduced recovery from extinction effects, but this hypothesis requires further examination, as extinction is generally recognized as a form of new learning, not unlearning (Bouton, 2002). Future fear conditioning research conducted with human participants may provide further insight into the mechanisms underlying extinction training with occasionally paired or unpaired USs through an examination of US expectancy ratings (Lovibond, 2006). Assessment of US expectancies on each trial of training may provide further information about what is learned during reinforced extinction training and how this learning may affect subsequent recovery of conditioned responding. It should be also noted that while the concurrent recording of multiple indices of conditioned responding may give us a better understanding of mechanisms underlying human fear learning (Lipp, 2006a), careful consideration must be given to the experimental parameters, to prevent response interference, such as movement-induced artefacts in SCRs due to the manual handling of a US expectancy scale.

As a limitation, we should note that the interpretation of reacquisition results must be treated with caution, as the respective group comparison only yielded a trend towards significance, indicating reacquisition of extinguished SCRs in the non-reinforced and partially reinforced extinction group, but not in the unpaired extinction group. Nevertheless, the results of spontaneous recovery showed that partially reinforced and unpaired extinction provided enhanced protection from fear recovery, as indexed by SCRs, compared to non-reinforced extinction training. Further, isolated comparisons of reacquisition data from the partially reinforced and unpaired extinction group supported previous observations (Bouton et al., 2004) that an unpaired extinction procedure results in stronger reduction of reacquisition of extinguished conditioned responding than partially reinforced extinction. Hence, the present extension of Bouton et al.'s findings provides further support for the utility of an extinction procedure involving occasional presentations of paired or unpaired USs in the reduction of fear recovery in humans and serves to inform future research.

It remains to be investigated why manipulations of extinction training showed no differential effects on the reinstatement of differential SCRs. It is possible that the large number of preceding CS-only trials, meaning CS trials during extinction training and those presented during tests of spontaneous recovery, reduced the effect of the reinstatement manipulation and, thus, prevented the observation of between group differences (for a review of reinstatement research, see Haaker et al., 2014). This proposition could be tested in future research, explicitly designed to assess the effect of occasional US presentations during extinction training on reinstatement. In this regard, tests of fear recovery, including reinstatement testing, could be conducted 24 hours after the conclusion of extinction training (e.g. Schiller et al., 2010; Thompson & Lipp, 2017). Additional areas of examination involve the applicability of the present extinction procedures to fears conditioned to fear-relevant CSs, such as snakes (Lipp, 2006b; Öhman & Mineka, 2001), as well as to pre-existing fears and phobias in clinical populations. Recruitment of clinical populations will enable future research to test the translational utility of reinforced extinction, including the applicability of reinforced extinction procedures to well-established (i.e. consolidated) fear associations (Dudai, 2012; Dudai, Karni, & Born, 2015).

Another aspect requiring further examination pertains to the differential effects of extinction training with occasional US presentations on physiological and verbal indices of conditioned fear. This dissociation is in accordance with previous fear conditioning research, suggesting that response systems which are governed largely by conscious, cognitive processes, such as ratings of CS valence or US expectancy, and physiological indices of conditioned responding are differentially sensitive to (manipulations of) extinction (Culver et al., 2018; Lipp & Edwards, 2002; Lipp, Oughton, & LeLievre, 2003; Schultz et al., 2013; Thompson & Lipp, 2017). However, the differences between ratings in the partially reinforced and unpaired extinction group at the end of extinction training may also point to different underlying mechanisms, as discussed previously.

In conclusion, the counterintuitive suggestion that presentations of aversive events during extinction training may enhance extinction learning and thus reduce subsequent recovery of the extinguished fear response (Craske et al., 2014) has been supported through the results of the present study. In this regard, our findings further indicate that unpaired extinction training may be more

effective in the reduction of recovery from extinction effects than partially reinforced extinction training, as unpaired extinction may guard against reacquisition of fear. When applied to the clinical setting, our results indicate that treatments focusing on the mere reduction of distress (in line with non-reinforced extinction) may be less effective in preventing fear recovery than treatments that provide clients with an opportunity to learn about the likelihood of future threat encounters (see also Craske et al., 2014; Weisman & Rodebaugh, 2018). Returning to our previous example of social anxiety disorder, during occasional exposure to a feared social situation and the feared outcome (CS-US pairing) clients may learn that the feared outcome occurs less often than expected. This learning may reduce the return of fear when the feared social situation (CS) is encountered between treatment sessions. For clients who are exposed to occasional CS-US pairings in daily life, such as negative feedback at work, it may be reassuring to know that such encounters may be beneficial for their overall treatment outcomes. In this sense, psychoeducation could be utilized to educate clients that occasional return of fear to cues in the environment is not an indicator of ineffective treatment, but an aspect that contributes to the reduction of maladaptive fears and, thereby, to the success of treatment.

We should add, however, that the translational utility of occasionally reinforced extinction requires further examination. It was not the authors' intention to suggest that a reinforced extinction procedure is readily applicable to the clinical setting, but to enhance our understanding of the mechanisms that affect extinction learning and to provide suggestions for future pre-clinical and clinical research (for a more detailed discussion of potential clinical applications, see: Craske, 2015; Craske et al., 2014; Krompinger, Van Kirk, Garner, Potluri, & Elias, 2018; Weisman & Rodebaugh, 2018). In this regard the procedure may lend itself more readily to the treatment of some, but not all, fears, phobias, or anxiety disorders. Conditions such as social anxiety disorder or specific phobias may benefit most from occasionally reinforced extinction, although the literature indicates the procedure may also be applicable to obsessive compulsive disorder (Krompinger et al., 2018). Clinical applications should also be guided by ethical considerations, in line with current exposure practices. As such, real life exposure to feared outcomes may be feasible from a pragmatic and ethical point of view in some, but not all, situations. Imaginal exposure may be considered in cases where

real life exposure is not feasible; however, the application of occasionally reinforced extinction during imaginal exposure requires further investigation.

Translating the unpaired extinction procedure to the clinical setting may be slightly more challenging, but not impossible if we consider that unpaired extinction does not involve the elimination of the feared outcome, but the presentation of the feared outcome (US) at unexpected times, separate from the situation (CS) that typically predicts the arrival of the US. It is conceivable that such training could be incorporated into imaginal exposure (e.g. Abramowitz & Arch, 2014), although more research is necessary to test this hypothesis.

Chapter 4: Study 2 – Reconsolidation

Note: The following paper has been published in the Journal *Behaviour Research and Therapy.*

Thompson, A., & Lipp, O. V. (2017). Extinction during reconsolidation eliminates recovery of fear conditioned to fear-irrelevant and fear-relevant stimuli. *Behaviour Research and Therapy, 92*, 1-10. doi:10.1016/j.brat.2017.01.017

Please note that no changes were made to the published article, other than the amendment of heading styles, to ensure consistency throughout this thesis (see Appendix B: Publication 2 for the print version of this article). As the spelling conventions used in the published article (American English) have not been changed, the spelling may deviate from that used in other parts of this thesis (Australian English).

4.1. Abstract

Extant literature suggests that extinction training delivered during the memory reconsolidation period is superior to traditional extinction training in the reduction of fear recovery, as it targets the original fear memory trace. At present it is debated whether different types of fear memories are differentially sensitive to behavioral manipulations of reconsolidation. Here, we examined post-reconsolidation recovery of fear as a function of conditioned stimulus (CS) fear-relevance, using the unconditioned stimulus (US) to reactivate and destabilize conditioned fear memories. Participants (*N*=56; 25 male; *M*=24.39 years, *SD*=7.71) in the US-reactivation and control group underwent differential fear conditioning to fear-relevant (spiders/snakes) and fear-irrelevant (geometric shapes) CSs on Day 1. On Day 2, participants received either reminded (US-reactivation) or non-reminded extinction training. Tests of fear recovery, conducted 24 hours later, revealed recovery of differential electrodermal responding to both classes of CSs in the control group, but not in the US-reactivation group. These findings indicate that the US reactivation-extinction procedure eliminated recovery of extinguished responding not only to fear-irrelevant, but also to fear-relevant CSs. Contrasting previous reports, our findings show that post-reconsolidation recovery of conditioned responding is not a function of CS fear-relevance and that persistent reduction of fear, conditioned to fear-relevant CSs, can be achieved through behavioral manipulations of reconsolidation.

Key words: reconsolidation; extinction; US-reactivation; prediction error; fear-relevance; return of fear

4.2. Introduction

The current focus of human memory reconsolidation research is on developing more efficient methods for long-lasting fear reduction. Research efforts in this area have increased since Schiller et al. (2010) demonstrated that fears, conditioned to fear-irrelevant stimuli (geometric shapes), can be permanently eliminated through safe and non-invasive behavioral interventions that target the memory reconsolidation process. These findings have since been replicated in other studies employing fear-irrelevant stimuli (Agren et al., 2012; Björkstrand et al., 2015; Johnson & Casey, 2015; Liu et al., 2014; Oyarzún et al., 2012; Schiller, Kanen, LeDoux, Monfils, & Phelps, 2013; Steinfurth et al., 2014), but see Golkar, Bellander, Olsson, and Öhman (2012 [experiment 2]) and Klucken et al. (2016). However, disruption of reconsolidation using behavioral interventions has not been demonstrated in studies employing fear-relevant stimuli (e.g. spiders; Fricchione et al., 2016; Golkar et al., 2012 [experiment 1]; Kindt & Soeter, 2013; Meir Drexler et al., 2014; Soeter & Kindt, 2011 [experiment 2]), leading to speculations that fear, conditioned to fear-relevant stimuli, may not be sensitive to behavioral manipulations of reconsolidation.

Reconsolidation is a time-dependent process that restabilizes reactivated memories (Nader, 2015). The purpose of reconsolidation is to update previously consolidated memories with novel information, in order to facilitate adaptation to the environment (Lee, 2009). Reconsolidation is initiated through reactivation and destabilization of the consolidated memory trace, by presenting cues associated with the original learning (Nader, 2013; Pineyro, Monti, Alfei, Bueno, & Urcelay, 2014). Once reactivated, memories become labile and are open to modification, before they reconsolidate and return to their inactive state (Nader, 2015; Nader, Schafe, & LeDoux, 2000a). Although the exact time course of memory reconsolidation is not known, it is believed that reconsolidation is completed within six hours of memory reactivation (Agren et al., 2012; Alberini, 2011; Nader et al., 2000a; Schiller et al., 2010). Interfering with reconsolidation during this period of lability through administration of pharmacological or behavioral interventions, such as extinction training, may modify the existing memory trace and persistently reduce the recovery of fear (Agren, 2014; Schiller et al., 2010). Conversely, when extinction training is administered without prior memory reactivation, fear may recover in a new context (renewal), after the passage of time (spontaneous recovery) or after

re-exposure to the aversive event (reinstatement), as extinction learning involves the acquisition of a new, inhibitory association and not the unlearning of the original fear response (Bouton, 2002).

Reconsolidation studies are typically conducted over the course of three consecutive days and involve differential Pavlovian fear conditioning (e.g. Schiller et al., 2010), whereby a neutral conditioned stimulus (CS+) is paired with an intrinsically aversive stimulus (unconditioned stimulus, US), while another CS is presented by itself (CS-). Future presentations of the CS+ result in anticipation of the US, which is reflected in increased differential responding to the CS+, relative to the CS-, on behavioral, verbal, and physiological indices of fear (Lipp, 2006a). During extinction (Day 2), typically delivered 10 minutes after administration of procedures which are thought to reactivate and destabilize fear memories, the CSs are presented without the US until differential responding is extinguished. Successful disruption of reconsolidation is inferred from the absence of differential responding during tests of fear recovery (e.g. Liu et al., 2014; Schiller et al., 2010).

As fear is expressed on the verbal, behavioral, and physiological level (Lang, 1985), several methods exist to measure conditioned fear. The most commonly employed measure in humans is electrodermal activity (skin conductance responses, SCRs) which increases during conditioning as a result of increased sweat gland activity (Boucsein, 2012; Dawson, Schell, & Filion, 2007). Fear learning is also reflected on verbal indices of conditioned responding, such as increased negative valence of the reinforced CS+ (De Houwer, Thomas, & Baeyens, 2001). Physiological and verbal indices of fear learning are said to be governed by dissociable implicit (non-conscious) and explicit (conscious) processes respectively (LaBar & Cabeza, 2006; D. H. Schultz, Balderston, Geiger, & Helmstetter, 2013; but see Sevenster, Beckers, & Kindt, 2012) and are differentially sensitive to manipulations of reconsolidation (e.g. Kindt & Soeter, 2013; Soeter & Kindt, 2010). By measuring multiple indices of conditioned responding, we can obtain a comprehensive understanding of processes underlying fear learning and fear reduction (Lipp, 2006a).

Extant literature indicates that post-reactivation extinction training is superior to extinction training alone in achieving lasting reduction of fear (Agren et al., 2012; Björkstrand et al., 2015; Liu et al., 2014; Schiller et al., 2010). However, at present, it is unknown whether all types of fear memories are sensitive to behavioral manipulations of reconsolidation. Relative to fear-irrelevant CSs,

phylogenetically fear-relevant CSs, such as snakes, show superior conditioning which resists extinction (Mineka & Öhman, 2002). Accordingly, it is possible that fears conditioned to different classes of stimuli may be differentially sensitive to behavioral manipulations of reconsolidation. However, conclusive evidence is lacking, as past research has either employed only fear-relevant or fear-irrelevant CSs (e.g. Fricchione et al., 2016; Golkar et al., 2012; Kindt & Soeter, 2013). Cross-study comparisons are problematic, due to methodological variations, such as the reinforcement rate employed during fear conditioning, number of acquisition trials, type and duration of the US or the memory reactivation procedure (for reviews see Auber, Tedesco, Jones, Monfils, & Chiamulera, 2013; Finnie & Nader, 2012; Kredlow, Unger, & Otto, 2016).

Differences across memory reactivation procedures deserve further consideration, as successful reactivation and destabilization of memories is a prerequisite for memory reconsolidation (Pineyro et al., 2014). The vast majority of past fear conditioning research (e.g. Golkar et al., 2012; Kindt & Soeter, 2013; Schiller et al., 2010) has employed an unreinforced presentation of the previously conditioned CS+ to reactivate fear memories ("CS-reactivation"). The success of this and other reactivation procedures is constrained by a number of boundary conditions. These include, but are not limited to, the age, strength and type of memory to be reactivated, type of reactivation procedure, and the 'prediction error' generated by the reactivating stimulus (for reviews of boundary conditions and prediction errors, please see Auber et al., 2013; Exton-McGuinness, Lee, & Reichelt, 2015; Fernández, Boccia, & Pedreira, 2016; Finnie & Nader, 2012; Lee, 2009).

The term 'prediction error' in reconsolidation research refers to a mismatch between past learning history, and actual events that are of relevance to prior learning and contain novel information that warrants updating or modification of memories (Exton-McGuinness et al., 2015; Fernández et al., 2016; Lee, 2009). An example of a manipulation that can generate a prediction error would be a change to the temporal CS-US relationship, by presenting the US 20 seconds earlier or later than expected, based on the trained CS-US interval (Díaz-Mataix, Ruiz Martinez, Schafe, LeDoux, & Doyère, 2013). An unreinforced presentation of the previously conditioned CS+ may also generate a prediction error, for instance when the duration of the CS presentation is increased, relative to training conditions (Agren et al., 2012). It has also been observed that CS-reactivation may trigger

memory reconsolidation when the consequences of the CS are not fully predictable, for instance following training on a partial reinforcement schedule (Oyarzún et al., 2012; Schiller et al., 2010), but see Golkar et al. (2012); Kindt and Soeter (2013). Overall, extinction training subsequent to CS-reactivation is more likely to disrupt the reconsolidation of fears conditioned to fear-irrelevant CSs (e.g. Schiller et al., 2010) than of those conditioned to fear-relevant CSs (Kindt & Soeter, 2013). There are several reasons as to why the CS-reactivation procedure may fail to destabilize these fear memories.

Briefly, it has been proposed that the strength of conditioned fears varies across training protocols. For instance, training with fear-relevant CSs is thought to result in strong fear associations which are resistant to extinction (Mineka & Öhman, 2002) and to behavioral manipulations of reconsolidation (Kindt & Soeter, 2013; Soeter & Kindt, 2011). It is also conceivable that the mere absence of the US during CS-reactivation does not create the prediction error necessary to facilitate behavioral manipulations of reconsolidation, even though the reactivation procedure is capable of supporting pharmacological manipulations (see Soeter & Kindt, 2011 for a comparison of these methods). It should be noted that there are a number of additional factors which may determine whether a manipulation results in memory destabilization. A comprehensive review of these factors is, however, beyond the scope of this paper. Readers interested in differences in prediction errors across memory types, training conditions, and computational models of associative learning may wish to consult Fernández et al. (2016); Holland and Schiffino (2016); or W. Schultz and Dickinson (2000).

Due to the mixed results from studies which used CS-reactivation, we employed a reactivation procedure that consisted of a single presentation of the US, at half the physical intensity used during acquisition ("US-reactivation"). This procedure has been successfully employed in past research (Liu et al., 2014), albeit only with fear-irrelevant CSs. Relative to CS-reactivation, the present reactivation procedure may be more likely to generate a prediction error, due to the mismatch between the actual and expected US intensity, as well as due to the unsignalled presentation of the US, in the absence of the CS. Based on previous reports, US-reactivation appears to be capable of reactivating and destabilizing multiple fear memories that are associated with the reactivating US (Liu et al., 2014). This procedure may be of relevance to the treatment of real-life fears, as these involve

multiple, often unknown, triggers (Schiller, 2014). It is conceivable that a single reactivation session could be sufficient to trigger the reconsolidation of multiple CS-US associations and facilitate the disruption of reconsolidation through extinction training, thereby preventing recovery of fear during future cue encounters (Dunbar & Taylor, 2017; Liu et al., 2014).

Here, we provided the first application of the US-reactivation procedure to fear, conditioned to fear-relevant stimuli, and investigated whether extinction training, delivered 10 minutes after reactivation, differentially affects the recovery of fear to fear-relevant and fear-irrelevant CSs. The study employed a mixed model design, whereby participants in the US-reactivation and control group were conditioned to fear-irrelevant and fear-relevant CSs (Olsson, Ebert, Banaji, & Phelps, 2005), but only participants in the US-reactivation group received a memory reactivation trial prior to extinction training. In line with previous research (Liu et al., 2014; Schiller et al., 2010), tests were conducted over the course of three consecutive days, involving differential Pavlovian conditioning (Day 1), reactivation-extinction/extinction-only (Day 2) and tests of fear recovery on Day 3. Electrodermal responding and CS valence ratings were recorded as primary and secondary dependent measures of conditioned fear, respectively. Based on the reviewed literature, which has not found that behavioral interventions affect reconsolidation of fear conditioned to fear-relevant stimuli, it was hypothesized that there would be a larger level of post-reconsolidation fear recovery for fear-relevant than for fear-irrelevant CSs during tests of spontaneous recovery and reinstatement.

4.3. Materials and Methods

4.3.1. Participants

University students who met inclusion criteria (i.e. no cardiovascular disease, seizure disorder, or pregnancy) participated in exchange for partial course credit or a financial compensation of 45 AUD. After exclusion of three participants who failed to verbalize the CS-US relationship on Day 1 (US-reactivation group: $n = 1$; control group: $n = 2$) and three participants from the US-reactivation group, who did not present for testing on Day 3, 28 participants each remained in the US-reactivation (15 male; $M = 23.54$ years, $SD = 7.05$) and control group (10 male; $M = 25.25$ years, $SD = 8.36$). Ethical approval for this study was obtained from the Curtin University Human Research Ethics Committee.

4.3.2. Stimuli and Measures

4.3.2.1. Stimuli

Fear-relevant CSs (CSa+/-) consisted of a spider (700 x 703 pixels) and snake (800 x 629 pixels) picture (Lipp, 2006b), while pictures of blue and yellow squares, measuring 700 x 700 pixels (Schiller et al., 2010), served as fear-irrelevant CSs (CSb+/-). The pictures were presented for 6 s, in the center of a 17-inch color LCD screen, over a black background, with an inter-trial interval of 10 to 14 s. To control for order effects, the assignment of snake and spider pictures as CSa+ or CSa-, the assignment of yellow and blue squares as CSb+ or CSb-, and whether the first trial of each phase was a CSa+/- or CSb+/- were counterbalanced across participants. Stimuli were presented in a pseudo-randomized order, whereby each CS was presented twice within blocks of eight trials. The US consisted of a mild electric shock, which was generated with a Grass SD9 stimulator (Grass Technologies, Middleton, WI) and delivered to the wrist of the dominant hand via a concentric electrode. The shock was presented for 200 ms (pulsed at 50 Hz) and coincided with the CSs+ offset; the CSs- were never paired with the US. In line with Agren et al. (2012), we employed a 100% reinforcement schedule to facilitate acquisition of fear on Day 1 – a prerequisite for subsequent manipulations of reconsolidation. The delivery of the US and CSs was controlled with DMDX 5.0.5 software (Forster & Forster, 2003).

4.3.2.2. Electrodermal Activity (Skin Conductance Responses, SCRs)

Electrodermal activity was recorded through two self-adhesive isotonic gel electrodes (Biopac Systems EL507), attached to the thenar and hypothenar eminences of the non-dominant hand. Electrodermal activity was DC amplified at a gain of 5 micro Siemens (µS) per volt and recorded with a Biopac MP150 system at a sampling frequency of 1000 Hz, using AcqKnowledge 4 (Biopac Systems, Goleta, CA). A Biopac respiration belt was fitted around the participants' waist to control for respiration-induced artefacts in SCRs. Electrodermal responses were scored offline in AcqKnowledge 4. Following a visual inspection of data, 10 SCRs (across groups) were discarded, due to the presence of respiration-induced artefacts. In accordance with past research (Kindt & Soeter, 2013; Pineles, Orr, & Orr, 2009), SCRs elicited by the CSs were calculated by subtracting the mean skin conductance level during the 2 s baseline preceding CS onset from the largest skin conductance

level occurring 1 to 6 s after CS onset. Responses below 0.02 µS were scored as zero and retained in the analysis (Kindt & Soeter, 2013). All SCRs were square root transformed and range corrected, to reduce the skew of the distribution as well as the influence of individual differences in electrodermal activity on conditioned responding (Lykken, 1972). The range correction was obtained by dividing each response by the largest response displayed by the participant. Electrodermal responses were averaged into blocks of two consecutive trials, to reduce the influence of trial by trial variability.

4.3.2.3. Valence Ratings

Participants rated CS valence on a 9-point scale (from 1 [unpleasant] to 9 [pleasant]) at baseline, after acquisition, spontaneous recovery, and reinstatement testing. Online ratings were not obtained, as these may interfere with the measurement of SCRs (Kindt & Soeter, 2013; Oyarzún et al., 2012). US valence was measured in an identical manner, following acquisition (US-reactivation and control group) and post-reactivation extinction training (US-reactivation group). DMDX 5.0.5 software was used to control stimulus presentation and to record CS ratings.

4.3.2.4. Manipulation Checks

Following acquisition, participants were presented with a CS-US contingency questionnaire, containing pictures of the CSs and two control stimuli, and were asked to indicate which stimuli had been paired with the US. As inability to verbalize the correct contingency may reflect a genuine failure to learn the CS-US relationship (Lipp, 2006a), data from participants who failed this test were excluded from statistical analyses. A second manipulation check was employed to assess whether participants in the US-reactivation group noticed the decrease in US intensity on Day 2: Participants were asked to rate US valence on a 9-point scale (from 1 [unpleasant] to 9 [pleasant]) and to indicate whether US intensity on Day 2 was lower, higher or the same as on Day 1.

4.3.3. Experimental Procedure

Unless otherwise indicated, participants were fitted with the skin conductance electrodes, respiration belt, and the shock electrode at the start of each session. Participants completed each stage of the experiment individually, while seated in front of a 17-inch color LCD screen. Please note that due to administrative limitations, data collection for the US-reactivation group was conducted six months before data collection in the control group. This separation in time has the potential to induce

confounds reflecting non-associative factors that affect responding. However, the within subject nature of the design limits the extent to which these can affect the results. Moreover, extensive preliminary testing failed to reveal any between group differences in overall responsiveness, fear learning or other factors that could affect the results.

4.3.3.1. Day 1: Acquisition

Participants were informed about the experimental procedures and had the opportunity to ask questions, before providing information about current medication use and medical history. Individuals who met the inclusion criteria provided written consent and set the US intensity to a level which was perceived as unpleasant, but not painful. After providing baseline CS valence ratings, participants were asked to pay attention to the computer screen and to learn which CSs were followed by the US. Conditioning commenced with a habituation phase, consisting of four presentations of each CS, and was immediately followed by acquisition, which involved eight presentations of CSa+/- and CSb+/-. Thereafter, participants completed the CS-US contingency questionnaire and provided US and CS valence ratings.

4.3.3.2. Day 2: (Post-reactivation) Extinction Training

Participants in the US-reactivation group were asked to remember what they had learned on Day 1, before receiving a memory reactivation trial, consisting of an unsignalled presentation of the US, at half the physical intensity employed on Day 1. The shock electrode was subsequently removed and participants were offered magazines to read during a 10-minute break. Participants in the control group commenced the session by reading magazines for 10 minutes and were not fitted with the shock electrode, to prevent potential reactivation of fear memories. Prior to extinction training, all participants were fitted with the shock electrode and were instructed to pay attention to the computer screen and remember what they had learned during previous stages of the experiment. Extinction training consisted of 10 unreinforced presentations of all CSs. Subsequently, participants in the US-reactivation group were asked to rate the valence and intensity of the US.

4.3.3.3. Day 3: Assessment of Differential Responding

Participants were asked to pay attention to the pictures on the computer screen; no further instructions were provided. Spontaneous recovery testing consisted of eight unreinforced

presentations of all CSs. CS valence ratings were obtained thereafter and were followed by three unsignalled presentations of the US for 200 ms each, at the intensity used during acquisition, with an inter-trial interval of 6 s. The computer screen was switched on and displayed a black background. After a 10-minute break (identical to Day 2), participants underwent reinstatement testing, consisting of eight unreinforced presentations of all CSs, followed by CS valence ratings.

4.3.4. Statistical Analyses

Electrodermal data from the US-reactivation and control group were analyzed through mixed analyses of variance (ANOVAs) for repeated measures, with group (US-reactivation vs. control) as between-subjects factor and fear-relevance (fear-relevant vs. fear-irrelevant), conditioning (CS+ vs. CS-) and block/time (habituation: block 1-2; acquisition: block 1-4; extinction: block 1-5; spontaneous recovery: block 5 extinction training vs. block 1 spontaneous recovery; reinstatement: block 4 spontaneous recovery vs. block 1 reinstatement) as within-subjects factors. CS valence ratings were analyzed in a similar manner, with group as between-subjects factor and fear-relevance (fear-relevant vs. fear-irrelevant), conditioning (CS+ vs. CS-) and time (baseline, acquisition, spontaneous recovery, reinstatement) as within-subjects factors. Multivariate *F* values (Pillai's Trace) and partial eta squared values were reported for all main effects and interactions. Statistical significance was assessed at $\alpha = .05$; Bonferroni corrections were used for follow-up analyses to guard against the accumulation of a Type 1 error.

4.4. Results

4.4.1. Preliminary Analyses

The groups did not differ in age, selected US intensity, baseline CS and US valence ratings or raw electrodermal responding during habituation (Table 1). A manipulation check confirmed that participants in the US-reactivation group registered the decrease in US intensity on Day 2, as the US was rated as less unpleasant on Day 2 ($M = 7.25$, $SD = 1.86$) than on Day 1 ($M = 3.50$, $SD = 1.75$), $t(27) = 7.50$, $p < .001$, $d = 2.08$, and was described as being of lower intensity than on Day 1.

Table 1

Means (M) and Standard Deviations (SD) for Age, Baseline Valence Ratings, US Intensity and Electrodermal Responding during Habituation in the US-Reactivation and Control Group

	US-reactivation		Control		
	M	*SD*	*M*	*SD*	*t*-Test
Age	23.54	7.05	25.25	8.36	$t(54) = 0.83$, $p = .410$
CS valence	5.41	0.90	5.60	1.39	$t(54) = 0.60$, $p = .552$
US valence	3.50	1.75	2.86	1.27	$t(54) = 1.57$, $p = .122$
US intensity	47.79	14.41	47.95	14.48	$t(54) = 0.04$, $p = .967$
Electrodermal responding	0.87	0.96	0.76	0.71	$t(54) = 0.51$, $p = .616$

Note. CS = conditioned stimulus, US = unconditioned stimulus. Electrodermal responding during habituation is reported in μSiemens; US intensity is reported in Volts.

4.4.2. Electrodermal Responding

Electrodermal responding in the US-reactivation and control group is presented in Figure 1. Analysis of electrodermal data during habituation yielded main effects of fear-relevance, $F(1,54) = 5.86$, $p = .019$, $\eta p^2 = .10$, and block, $F(1,54) = 55.12$, $p < .001$, $\eta p^2 = .51$, as well as fear-relevance x block, $F(1,54) = 8.87$, $p = .004$, $\eta p^2 = .14$, fear-relevance x conditioning x group, $F(1,54) = 6.82$, $p = .012$, $\eta p^2 = .11$, and fear-relevance x conditioning x block x group interactions, $F(1,54) = 4.70$, $p = .035$, $\eta p^2 = .08$. The four-way interaction reflects differential responding to fear-relevant CSs in the US-reactivation group, on block 1, $F(1,54) = 10.90$, $p = .002$, $\eta p^2 = .17$, but not on block 2, $F(1,54) = 2.01$, $p = .163$, $\eta p^2 = .04$. Differential responding was not evident to fear-irrelevant CSs or in the control group, all $F(1,54) \leq 1.18$, $p \geq .283$, $\eta p^2 \leq .02$. Closer inspection of the data indicated that enhanced responding to fear-relevant CSs+ in the US-reactivation group may reflect on enhanced responses to spider pictures. Examination of baseline CS valence ratings revealed that participants assigned to sequences starting with spider pictures used as CS+ rated spiders as less pleasant ($M = 2.63$, $SD = 1.19$) than snakes ($M = 4.38$, $SD = 1.92$), which may have enhanced orienting responses. The larger dislike of spiders may have resulted in a significant increase in electrodermal responding on the first block of habituation. However, this difference across CS conditions and groups was absent on the last block of habituation.

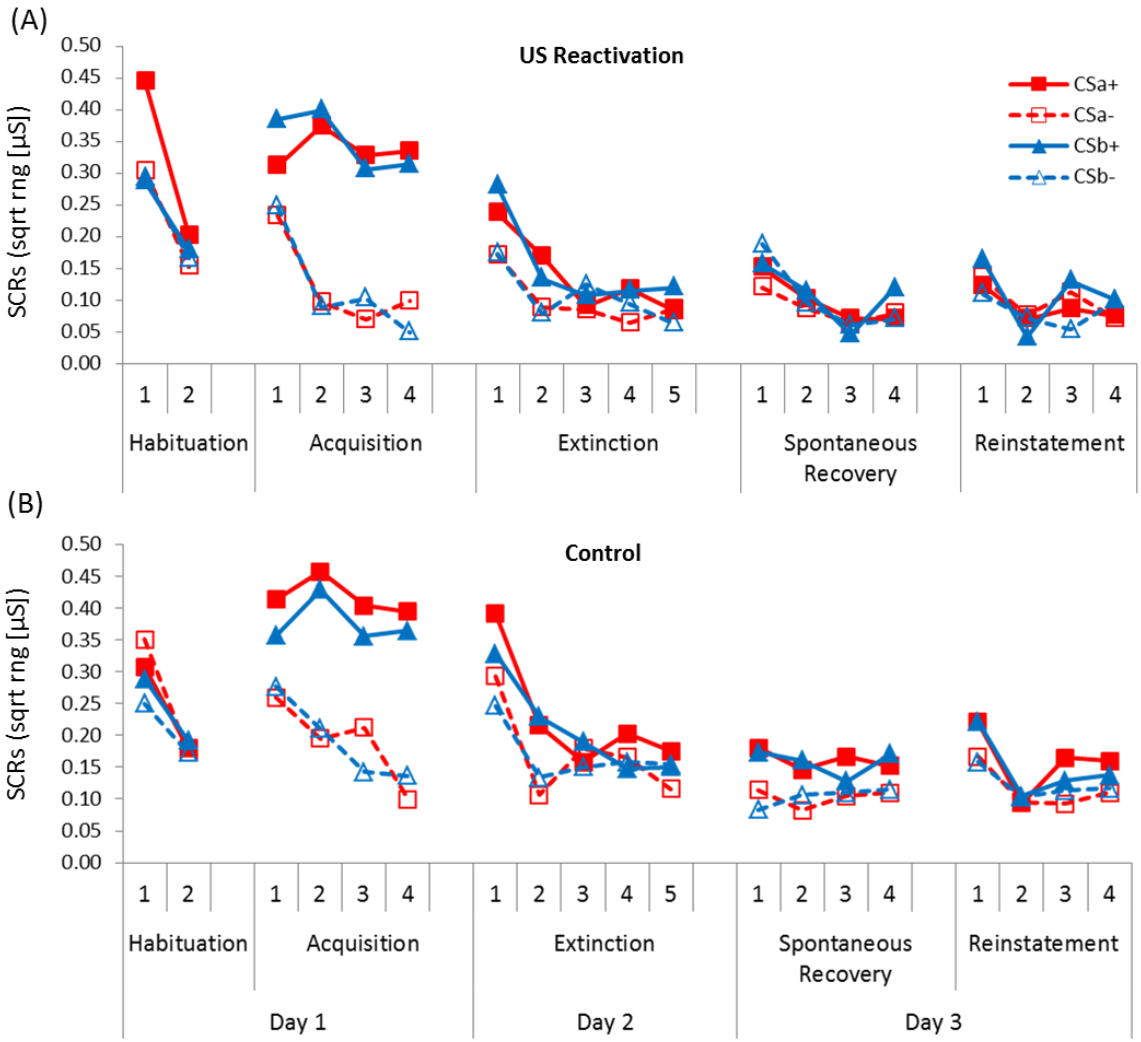


Figure 1. Mean skin conductance responses (SCRs) to fear-relevant (CSa+/-) and fear-irrelevant (CSb+/-) conditioned stimuli in the US-reactivation **(A)** and control **(B)** group. SCRs are presented in blocks of two consecutive trials.

In line with Figure 1, statistical analyses revealed that conditioned responding was acquired to fear-relevant and fear-irrelevant CSs on Day 1, conditioning, $F(1,54) = 56.18$, $p < .001$, $\eta p^2 = .51$, block, $F(3,52) = 8.69$, $p < .001$, $\eta p^2 = .33$, conditioning x block, $F(3,52) = 7.52$, $p < .001$, $\eta p^2 = .30$. The interaction reflects an increase in differential responding to both types of CSs, across blocks of acquisition, all $F(1,54) \geq 11.60$, $p \leq .001$, $\eta p^2 \geq .18$. The main effect of fear-relevance and remaining interactions did not attain significance, largest effect (fear-relevance x conditioning x group), $F(1,54) = 2.90$, $p = .094$, $\eta p^2 = .05$.

On Day 2, differential responding was evident in both groups during the early phase of extinction training, but decreased thereafter, conditioning, $F(1,54) = 14.14$, $p < .001$, $\eta p^2 = .21$, block,

$F(4,51) = 11.81$, $p < .001$, $\eta p^2 = .48$, conditioning x block, $F(4,51) = 4.56$, $p = .003$, $\eta p^2 = .26$. The interaction reflects differential responding on block 1 and 2, both $F(1,54) \geq 16.73$, $p < .001$, $\eta p^2 = .24$, but not on blocks 3 to 5, $F(1,54) \leq 1.55$, $p \geq .219$, $\eta p^2 \leq .03$. The main effect of fear-relevance and remaining interactions did not attain significance, largest effect (fear-relevance x block x group), $F(4,51) = 1.72$, $p = .160$, $\eta p^2 = .12$. Please note that although Figure 1 seems to indicate that there were group differences in differential responding during early extinction (block 1-2), this was not supported by the analysis, all interactions involving the factor group, $F(1,54) \leq 0.44$, $p \geq .508$, $\eta p^2 \leq .01$.

Tests of fear recovery, conducted on Day 3, revealed spontaneous recovery of previously extinguished differential responding in the control group, but not in the US-reactivation group. Analyses yielded a main effect of conditioning, $F(1,54) = 4.74$, $p = .034$, $\eta p^2 = .08$, as well as interactions of time x group, $F(1,54) = 4.39$, $p = .041$, $\eta p^2 = .08$, and fear-relevance x conditioning x time x group, $F(1,54) = 5.06$, $p = .029$, $\eta p^2 = .09$. The remaining main effects and interactions did not attain significance, largest effect (main effect of time), $F(1,54) = 2.19$, $p = .145$, $\eta p^2 = .04$. The significant four-way interaction reflects group differences in electrodermal responding to the non-reinforced fear-irrelevant CSb-, on the last block of extinction training and first block of spontaneous recovery. Differential responding to fear-relevant or fear-irrelevant CSs was not evident on the last block of extinction training in either group, all $F(1,54) \leq 2.58$, $p \geq .114$, $\eta p^2 \leq .05$. In contrast, spontaneous recovery of differential responding to both types of CSs was observed in the control group, both $F(1,54) \geq 4.26$, $p \leq .044$, $\eta p^2 \geq .07$, but not in the US-reactivation group, both $F(1,54) \leq 0.95$, $p \geq .334$, $\eta p^2 \leq .02$.

Analysis of reinstatement data yielded main effects of conditioning, $F(1,54) = 5.69$, $p = .021$, $\eta p^2 = .10$, and time, $F(1,54) = 7.50$, $p = .008$, $\eta p^2 = .12$, reflecting overall larger SCRs to reinforced than to non-reinforced stimuli and overall larger SCRs on the first block of reinstatement testing than on the last block of spontaneous recovery in both groups. The main effect of fear-relevance and remaining interactions did not attain significance, largest effect (fear-relevance x conditioning), $F(1,54) = 1.50$, $p = .226$, $\eta p^2 = .03$. The main effect of conditioning suggests that differential responding was still present during the last block of spontaneous recovery testing. This may have

masked a between group difference in differential responding on the first block of reinstatement. To test this proposition, we conducted follow-up comparisons to test whether differential responding was observed in either group on the first block of reinstatement. Differential responding recovered on the first block of reinstatement testing in the control group, $F(1,54) = 5.06$, $p = .029$, $\eta p^2 = .09$, but not in the US-reactivation group, $F(1,54) = 0.62$, $p = .434$, $\eta p^2 = .01$.

4.4.3. Conditioned Stimulus Valence Ratings

Analysis of CS valence ratings was based on data from 28 participants in the US-reactivation group and 27 participants in the control group, as ratings from one participant were lost due to a recording error. Participants in both groups rated fear-irrelevant CSs as more pleasant than fear-relevant CSs and reinforced CSs as less pleasant than non-reinforced CSs (Figure 2). Statistical analyses revealed main effects of fear-relevance, $F(1,53) = 132.31$, $p < .001$, $\eta_p^2 = .71$, conditioning, $F(1,53) = 34.57$, $p < .001$, $\eta_p^2 = .40$, and time, $F(3,51) = 11.10$, $p < .001$, $\eta_p^2 = .40$. These main effects were qualified by interactions of fear-relevance x time, $F(3,51) = 4.87$, $p = .005$, $\eta_p^2 = .22$, conditioning x time, $F(3,51) = 16.70$, $p < .001$, $\eta_p^2 = .50$, and fear-relevance x conditioning x time, $F(3,51) = 6.38$, $p = .001$, $\eta_p^2 = .27$. Follow-up analyses assessing whether the three-way interaction reflects differences in conditioning as a function of fear-relevance and time failed to find significant results. While differential evaluations for both types of CSs were smaller on Day 3 than after acquisition on Day 1, they were nevertheless significant across all conditions, except at baseline, all $F(1,53) \geq 8.21$, $p \leq .006$, $\eta_p^2 \geq .13$. The three-way interaction reflects differences in the time course of evaluation patterns across the four stimuli (fear-relevant and fear-irrelevant CS+/-). Whereas evaluations of non-reinforced fear-relevant and fear-irrelevant CSs became more positive across measurement points, both $F(3,51) \geq 3.70$, $p \leq .018$, $\eta_p^2 \geq .18$, evaluations of fear-relevant CSa+ became more negative after acquisition, but recovered thereafter and exceeded baseline ratings, $F(3,51) = 10.25$, $p < .001$, $\eta_p^2 = .38$. Evaluations of the fear-irrelevant CSb+ became more negative after acquisition and increased thereafter, but did not recover to baseline levels, $F(3,51) = 17.25$, $p < .001$, $\eta_p^2 = .50$.

Statistical analyses also yielded a significant fear-relevance x group interaction, $F(1,53) = 5.04$, $p = .029$, $\eta_p^2 = .09$, however, follow-up comparisons did not attain significance, both $F(1,53) \leq$

3.48, $p \geq .068$, $\eta_p^2 \leq .06$. None of the remaining interactions were significant, largest effect (fear-relevance x conditioning), $F(1,53) = 2.62$, $p = .112$, $\eta_p^2 = .05$.

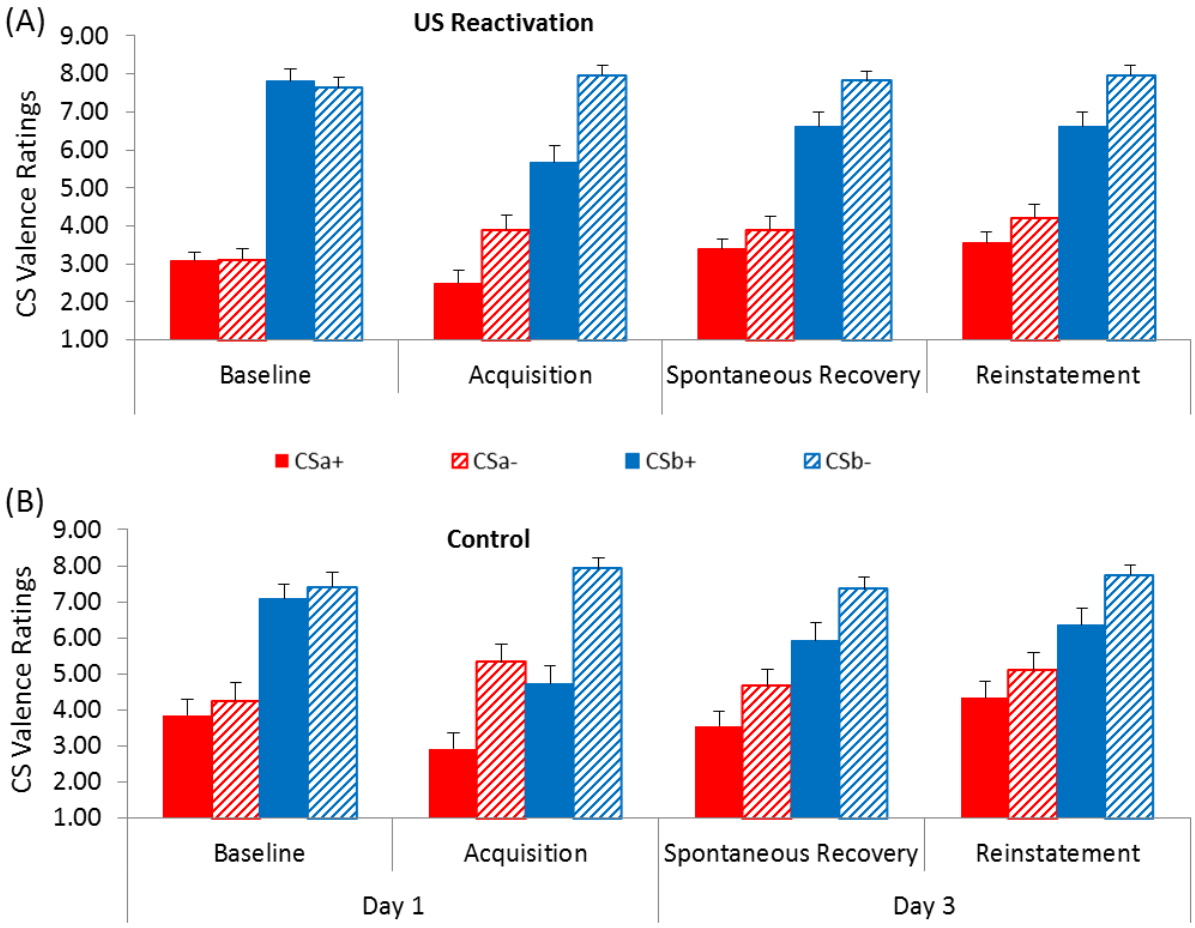


Figure 2. Mean stimulus valence ratings for fear-relevant (CSa+/-) and fear-irrelevant (CSb+/-) conditioned stimuli at baseline and after acquisition, spontaneous recovery, and reinstatement testing, in the US-reactivation **(A)** and control **(B)** group. Error bars represent standard errors.

4.4.4. Summary of Results

Analysis of electrodermal data revealed that differential responding to both types of CSs was acquired on Day 1 and extinguished on Day 2 in the US-reactivation and control group. Group differences emerged on Day 3, showing spontaneous recovery and reinstatement of previously extinguished differential responding in the control group, but not in the US-reactivation group. In contrast to the electrodermal responses, subjective evaluations of fear-relevant and fear-irrelevant CSs did not differ across groups. Following acquisition, reinforced stimuli were rated as less pleasant than non-reinforced stimuli. While these differential evaluations were also evident during tests of fear recovery, they were significantly smaller than ratings obtained after acquisition.

It should be also noted that we did not observe differences in fear acquisition or extinction between fear-relevant and fear-irrelevant CSs. However, this may reflect on the use of a 100% reinforcement schedule in a differential fear conditioning paradigm, which typically results in rapid acquisition of differential responding and may, therefore, mask differences in fear learning between stimuli (Ho & Lipp, 2014; Lissek, Pine, & Grillon, 2006). As the present investigation examined post-reconsolidation recovery of fear, we selected a strong conditioning protocol, to facilitate acquisition of differential responding, which is a prerequisite for subsequent reactivation and destabilization of conditioned fear memories.

4.5. Discussion

The aim of this investigation was to apply the US-reactivation procedure (Liu et al., 2014) to two distinct types of conditioned fears and to examine whether post-reconsolidation recovery of fear differs as a function of CS fear-relevance. Contrary to our predictions, we did not find significant differences between fear-relevant and fear-irrelevant CSs, as differential electrodermal responding to both CS pairs was absent during spontaneous recovery and reinstatement tests in the US-reactivation group. In contrast, fear recovery was observed in the control group, which received traditional, non-reminded extinction training. Thus, our findings indicate that extinction training, delivered 10 minutes after administration of a brief reminder trial involving the US, at half the physical intensity used during acquisition, can eliminate spontaneous recovery and reinstatement of differential responding.

These results are consistent with previous observations (Dębiec, Díaz-Mataix, Bush, Doyère, & LeDoux, 2010; Liu et al., 2014; Luo et al., 2015) that memory reactivation procedures involving the US are capable of destabilizing cue-dependent associations and, thereby, facilitate disruption of the reconsolidation process and reduce recovery of conditioned responding. The present results provide further support for the utility of the US-reactivation procedure, suggesting it may facilitate the simultaneous destabilization of multiple, distinct fear memories, conditioned to fear-irrelevant and fear-relevant CSs, thereby allowing subsequent modification of both fear memories through extinction training, delivered during a period of memory reconsolidation.

4.5.1. Persistent Reduction of Differential Electrodermal Responding

The current results stand in stark contrast to previous research (Fricchione et al., 2016; Golkar et al., 2012; Kindt & Soeter, 2013; Meir Drexler et al., 2014; Soeter & Kindt, 2011), which reported a return of conditioned electrodermal responding to fear-relevant CSs, subsequent to post-reactivation extinction training. Similar to our study, Kindt and Soeter (2013) observed no group differences in differential electrodermal responding and fear potentiated startle during acquisition and extinction of fear, conditioned to pictures of spiders. However, Kindt and Soeter failed to find group differences during spontaneous recovery, reinstatement and reacquisition, reporting that previously extinguished fear had recovered in participants who received a reminder trial prior to extinction training and in those who received traditional, non-reminded extinction training. These results contrast our findings which showed spontaneous recovery and reinstatement of differential electrodermal responding in the control group, but not in the US-reactivation group.

It should be noted that the current findings do not reflect on differences in data analysis, as we followed Kindt and Soeter's (2013) recommendations to avoid potential confounds from non-associative processes on conditioned responding that may be misinterpreted as effects of disrupted reconsolidation. Rather than analyzing difference scores (subtracting responses to the CS- from responses to the CS+) or omitting the first CS+ and CS- trial during tests of fear recovery, we report responses to CS+ and CS- and retained all data. Thus, our data analysis resembled the approaches employed in previous studies which did not find evidence that behavioral interventions can disrupt reconsolidation of fear, conditioned to fear-relevant CSs (Fricchione et al., 2016; Golkar et al., 2012; Kindt & Soeter, 2013). Hence, it appears that previous failed attempts to replicate the seminal work of Schiller et al. (2010) with fear-relevant stimuli may not reflect on differences in approaches to data analysis, but on the method used to reactivate fear memories.

The key difference between the current and past research was the use of the US-reactivation procedure, in lieu of the more commonly employed CS-reactivation (e.g. Fricchione et al., 2016; Kindt & Soeter, 2013). Memory reactivation procedures involving the US have received little attention, relative to CS-reactivation procedures. While the majority of investigations employing US-reactivation were conducted on animals (Alfei, Ferrer Monti, Molina, Bueno, & Urcelay, 2015;

Dębiec et al., 2010; Díaz-Mataix et al., 2013; Luo et al., 2015), the outcomes of the present study and previous findings reported by Liu et al. (2014) indicate that US-reactivation also facilitates disruption of reconsolidation in humans and may persistently block recovery of fear.

However, the question remains as to how exactly a US-only reactivation trial generates prediction errors, as traditional models of associative learning (e.g. Pearce & Hall, 1980; Rescorla & Wagner, 1972) do not readily account for prediction errors generated by presentations of the US, in the absence of the CS. Indeed, advances in reconsolidation research have sparked renewed interest into the role of prediction errors in associative learning, suggesting that prediction errors are not only implicated in fear acquisition (Holland & Schiffino, 2016; W. Schultz & Dickinson, 2000), but also in the modification of previously consolidated fear memories during the reconsolidation period (Exton-McGuinness et al., 2015; Fernández et al., 2016; Sevenster et al., 2012; Sevenster, Beckers, & Kindt, 2013, 2014). A large body of evidence points to the role of the amygdala in the processing of CS and US properties (Bentz & Schiller, 2015; LeDoux, 2000), including prediction errors related to the unexpected presentation or omission of the CS/US, as well as prediction errors pertaining to changes in US value (Belova, Paton, Morrison, & Salzman, 2007; Bentz & Schiller, 2015; Dębiec et al., 2010; Díaz-Mataix, Tallot, & Doyère, 2014; McNally, Johansen, & Blair, 2011).

Consistent with this argument, Díaz-Mataix et al. (2013) reported that the amygdala encodes the timing of US onset and detects deviations from the CS-US interval used during acquisition. In an auditory fear conditioning experiment, rats were presented with a foot shock US 30 seconds after CS onset. During memory reactivation, the US was either presented at the same time or 20 seconds earlier. Subsequent administration of the protein synthesis inhibitor anisomycin into the lateral amygdala was found to impair reconsolidation of the conditioned fear memory only when the CS-US interval had been altered during reactivation. These results indicate that prediction errors required for destabilization of fear memories are not necessarily the consequence of a mere absence or presence of the US, but may reflect on violations of other aspects of the learning history. This proposition is further supported by recent reports that memory reactivation can be achieved through presentation of cues which had not been paired with the US, but which are categorically related to the CSs (Soeter & Kindt, 2015b).

In addition to encoding timing of US onset (Díaz-Mataix et al., 2013; Díaz-Mataix et al., 2014; Harnett et al., 2016), the amygdala also appears to process specific sensory features of an aversive US and, thereby, may be involved in the reactivation of fear memories which are associated with a discrete US. Consistent with the results of the present study, Dębiec et al. (2010) reported that a memory reactivation procedure involving an unsignalled presentation of the US destabilized multiple fear memories that were associated with the reactivating US. Furthermore, Dębiec and colleagues found that reconsolidation was selective to the reactivated US, whereby conditioned responding to CSs which had not been paired with the reactivating US was not diminished through pharmacological manipulations of reconsolidation. These findings were replicated by Liu et al. (2014) in a series of fear conditioning experiments conducted with animals and humans. In contrast to Dębiec et al., Liu and colleagues halved the physical intensity of the US during reactivation and employed extinction training to disrupt reconsolidation. Liu et al.'s results were consistent with our findings, showing that US-reactivation destabilized multiple fear memories, which were associated with the reactivating US. Extending Liu et al.'s findings, the present results further suggest that US-reactivation is capable of destabilizing multiple, distinct fear associations, conditioned to fear-irrelevant and fear-relevant CSs.

It remains to be investigated whether a decrease in US intensity is necessary for memory reactivation. Past research indicates that a discrepancy between actual and expected US intensity during reactivation is required to facilitate behavioral manipulations of reconsolidation (Liu et al., 2014). A reduction in US intensity during reactivation may create a stronger prediction error, which appears to be necessary for behavioral interventions, as indicated by a comparison of pharmacological and behavioral manipulations of reconsolidation, following CS-reactivation (Soeter & Kindt, 2011).

Of interest for future investigations is also the role of verbal instructions in memory reactivation. In the present study, participants were instructed to remember what they had learned during acquisition before receiving the reminder trial (adapted from Kindt & Soeter, 2013; Sevenster et al., 2012; Soeter & Kindt, 2010). It could be argued that these instructions created a prediction error; however, this appears unlikely, as the control group received identical instructions prior to extinction training, but displayed spontaneous recovery and reinstatement of conditioned responding on Day 3. Previous research further indicates that instructions prior to memory reactivation trials are

not sufficient to create the prediction error necessary for reactivation of fear memories, conditioned to fear-relevant CSs (Kindt & Soeter, 2013). Given the limited scope of literature pertaining to US-reactivation procedures, more research is required to determine the necessary and sufficient conditions for US-mediated memory reactivation and destabilization. It also remains to be investigated whether interventions based on the present reactivation-extinction protocol could be employed in the treatment of anxiety and related disorders.

Reconsolidation-based interventions have been used successfully to reduce symptoms of post-traumatic stress disorder (Brunet et al., 2008; Brunet et al., 2011; Kindt & van Emmerik, 2016; Kredlow & Otto, 2015), but see Wood et al. (2015), phobias (Björkstrand et al., 2016; Soeter & Kindt, 2015a), drug cravings in substance-dependent individuals (Lonergan et al., 2016; Xue et al., 2012), and cocaine seeking in animals (Luo et al., 2015). Yet, an obvious challenge for clinical applications of the US-reactivation procedure is the identification of the US that contributed to the development of cue-dependent associations, as well as the adaptation of the US to resemble the reactivation procedure used in the present study. With regards to the modification of US intensity, it remains to be investigated whether this is a prerequisite for memory reactivation, as prediction errors can be generated through other avenues, such as modification of contingencies (US presented without CS) or by changing the timing of US onset (Dębiec et al., 2010; Díaz-Mataix et al., 2013).

Identification of the US, on the other hand, is a key component of and a prerequisite for exposure-based therapies for anxiety disorders (Craske, Treanor, Conway, Zbozinek, & Vervliet, 2014). It would be reasonable to expect that therapists and clients would encounter similar challenges pertaining to the identification of the US in the context of reconsolidation-based interventions, as they do in the design of exposure-based therapies. Yet, the advantage of reconsolidation-based interventions is the potential for long-lasting reduction of fear and prevention of fear relapse, as reconsolidation, in contrast to extinction learning, alters the original fear memory trace (Nader, 2015; Nader et al., 2000a). Another potential advantage is the reactivation and destabilization of multiple memories that are associated with the reactivating US. In contrast to CS-reactivation, which results in destabilization of a single CS-US association (e.g. Schiller et al., 2010), a single US-reactivation session may destabilize multiple CS-US associations. All reactivated associations could be

subsequently disrupted through extinction training or through pharmacological interventions, such as propranolol (for a detailed discussion of reconsolidation-based interventions, see Dunbar & Taylor, 2017). Overall, extant literature suggests that reconsolidation-based interventions, including US-reactivation procedures (e.g. Liu et al., 2014; Luo et al., 2015), may be beneficial in the treatment of anxiety, stress, and substance dependence disorders. Areas requiring further investigation are applications of US-reactivation in clinical samples and the optimization of methods that disrupt reconsolidation following US-reactivation. The latter should address comparisons of imaginal and in vivo reactivation-extinction (Agren, Björkstrand, & Fredrikson, 2017) and of extinction training and pharmacological interventions (e.g. propranolol; Soeter & Kindt, 2011).

4.5.2. Subjective Evaluations of CS Valence

In contrast to electrodermal responding, subjective evaluations of CS valence did not differ across groups. These results are in accordance with fear conditioning research which suggests that response systems which are governed largely by conscious, cognitive processes, such as verbal indices of US expectancy, and physiological indices of conditioned responding are differentially sensitive to extinction training (Gawronski, Gast, & De Houwer, 2015; Lipp & Edwards, 2002; Lipp, Oughton, & LeLievre, 2003) as well as manipulations of reconsolidation (Kindt & Soeter, 2013; Soeter & Kindt, 2011), but see Das, Lawn, and Kamboj (2015) and Pine, Mendelsohn, and Dudai (2014). On the other hand, our results showed that differential evaluations were significantly smaller on Day 3 than after acquisition on Day 1- a pattern that is characteristic of extinction learning. The absence of group differences could indicate that reminded as well as non-reminded extinction training facilitated reduction of conditioned negative valence. However, this proposition requires further investigation as we cannot rule out the influence of demand characteristics on post-test ratings.

The double dissociation between implicit and explicit response systems has been reported previously (e.g. Lipp et al., 2003; D. H. Schultz et al., 2013), yet does not necessarily indicate that acquired negative valence is not sensitive to manipulations of reconsolidation. Das et al. (2015) investigated counterconditioning during the reconsolidation period as a means of decreasing the liking of alcohol-related cues. Hazardous drinkers who received a reactivation trial triggering a prediction error prior to counterconditioning showed decreased liking of the CSs as well as a generalization of

the conditioned valence to novel alcohol cues. These findings indicate that subjective ratings of CS valence are sensitive to behavioral manipulations of reconsolidation, but may require administration of post-reactivation training protocols which specifically target CS valence, such as counterconditioning, in lieu of non-reinforced CS presentations. Altering conditioned valence has potential clinical applications for the treatment of alcohol and drug addictions as well as anxiety disorders, as residual negative valence has been associated with reinstatement of fear (Zbozinek, Hermans, Prenoveau, Liao, & Craske, 2015). Further research is necessary to determine which manipulations of reconsolidation are most effective in the persistent reduction of conditioned negative valence.

In conclusion, the results of this study demonstrate that behavioral manipulations of reconsolidation are sufficient for the persistent elimination of fear conditioned to fear-irrelevant and fear-relevant CSs. Our findings also indicate that the memory reactivation procedure employed in this study is capable of destabilizing multiple, distinct fear memories, associated with the reactivating US. It is conceivable that a modified version of the US reactivation-extinction procedure could be employed in clinical settings, as fears and phobias are typically associated with fear-relevant stimuli (Mineka & Öhman, 2002). However, more research is necessary to determine how the US-reactivation extinction procedure employed here could be adapted to the treatment of anxiety and related disorders.

Chapter 4: Study 2 – Addendum

To examine if the reduction of spontaneous recovery and reinstatement of fear observed 24 hours after the delivery of the reactivation-extinction procedure was long-lasting, follow-up tests of fear recovery were conducted for the US-reactivation group 8 to 12 months after initial testing.

4.6. Method

4.6.1. Participants

Participants who received the US reactivation-extinction procedure and completed all three days of testing were invited to the laboratory for follow-up tests. Out of 28 participants, 12 were able to return for further testing (4 male, 8 female; M = 23.08 years, SD = 5.78) and received a modest financial compensation of 15 AUD. Participants who were unable to return to the laboratory (11 male, 5 female; M = 23.88 years, SD = 8.04) either did not respond to the email invitation or stated they were unable to attend due to time constraints or because they had relocated and no longer lived in close proximity to the university. Ethical approval for this study was obtained from the Curtin University Human Research Ethics Committee.

4.6.2. Procedure

Follow-up tests involved the same apparatus, materials, and trial sequences as those used on Day 3 of initial testing**.** After providing written consent, participants were asked to wash their hands and were then fitted with the skin conductance electrodes, respiration belt, and shock electrode. At the start of spontaneous recovery tests, participants were asked to pay attention to the pictures on the computer screen; no further instructions were provided. Spontaneous recovery was assessed with eight non-reinforced presentations of all conditioned stimuli (CSs). Subsequently, participants provided CS valence ratings and calibrated the intensity of the unconditioned stimulus (US) by selecting a shock intensity that was perceived as “unpleasant, but not painful” in a shock work-up procedure. Of note, US calibration was conducted at the conclusion of the first test phase, as the presentation of electrotactile stimuli during the shock work-up procedure may have induced reinstatement-like effects during tests of spontaneous recovery (Haaker, Golkar, Hermans, & Lonsdorf, 2014; Lonsdorf et al., 2017). Reinstatement testing commenced with three unsignaled presentations of the US, which was followed by a 10-second break, and eight non-reinforced

presentations of all CSs. Thereafter, participants were asked to rate CS valence and indicate the CS-US relationship learned during acquisition training on Day 1 of initial testing.

Scoring and response definition of skin conductance responses (SCRs), as well as data transformation methods, were identical to the approaches detailed in the published manuscript. The range correction (Lykken, 1972) in the present sample was conducted by dividing each SCR by the largest SCR displayed by the participant during follow-up tests.

4.6.3. Statistical Analyses

We conducted preliminary analyses to examine if participants who took part in follow-up tests (n = 12) and those who did not (n = 16) differed on variables that may affect conditioned responding (see Table A4.1). Group differences were assessed through independent samples t tests or, in case of violations of normality, through the non-parametric Mann-Whitney U test. Normality was assessed through the Shapiro-Wilk test and a visual inspection of histograms. In accordance with the analytical approach adopted for the analysis of fear recovery on Day 3 of initial training, spontaneous recovery and reinstatement of differential SCRs were assessed through separate 2 (fear-relevance: fear-relevant vs. fear-irrelevant CSs) x 2 (conditioning: CS+ vs. CS-) x 2 (time; spontaneous recovery of fear: block 4 of reinstatement [Day3] vs. block 1 of spontaneous recovery [Day 4]; reinstatement of fear: block 4 of spontaneous recovery [Day 4] vs. block 1 of reinstatement [Day 4]) repeated measures analyses of variance (ANOVAs). CS valence ratings were analysed through a 2 (fear-relevance) x 2 (conditioning) x 2 (time: spontaneous recovery, reinstatement) repeated measures ANOVA. Multivariate F values (Pillai's Trace) and partial eta squared values were reported for all main effects and interactions. Statistical significance was assessed at α = .05; Bonferroni corrections were used for follow-up analyses to guard against the accumulation of a Type 1 error.

4.7. Results

4.7.1. Preliminary Analyses

There were no significant differences in age, selected US intensity, baseline CS and US valence ratings, level of fear acquisition, as indexed by mean differential SCRs to fear-relevant and fear-irrelevant CSs during late acquisition (trials 5-8), or level of differential SCRs at the end of testing on Day 3 (i.e., reinstatement tests, block 4) between participants who returned to the laboratory

for follow-up tests and those who were unable to do so (Table A4.1). US intensity selected on Day 4 (i.e., 8-12 month follow-up) ranged from 35 to 80 Volts (M = 52.50, SD = 14.85) and did not significantly differ from the US intensity participants selected on the first day of the experiment (range: 28-70 Volts, M = 50.38, SD = 14.44), as reflected in the non-significant results of a paired samples t test: $t(12) = 0.79$, $p = .448$, $d = 0.14$. Finally, inspection of CS-US contingency questionnaires indicated that 11 participants recalled at least one of the CS-US relationships learned 8-12 months prior, while eight participants recalled both.

Table A4.1

Comparison of Means (M) and Standard Deviations (SD) for Age, Baseline Valence Ratings, US Intensity, and Differential Skin Conductance Responses (SCRs) During Late Acquisition and Reinstatement for Sub-groups of the US-reactivation Group

	Follow-up (n = 12)		No follow-up (n = 16)		
	M	*SD*	*M*	*SD*	Test
Age	23.08	5.78	23.88	8.04	$U = 87.50$, exact $p = .698$
CS valence	5.12	1.13	5.63	0.65	$U = 75.50$, exact $p = .347$
US valence	3.67	1.61	3.38	1.89	$U = 85.00$, exact $p = .631$
US intensity	50.38	14.44	45.84	14.55	$t(26) = 0.82$, $p = .421$
SCRs					
Acquisition (Day 1)					
CSa	0.17	0.20	0.31	0.27	$t(26) = 1.53$, $p = .139$
CSb	0.20	0.22	0.26	0.27	$t(26) = 0.70$, $p = .491$
Reinstatement (Day 3)					
CSa	0.04	0.22	-0.02	0.18	$U = 94.50$, exact $p = .945$
CSb	<0.01	0.10	<0.01	0.33	$U = 86.00$, exact $p = .664$

Note. CS = conditioned stimulus, US = unconditioned stimulus. The data presented here were collected between Day 1 and Day 3 of testing. Differential SCRs were computed by subtracting mean SCRs to the non-reinforced CSs- from SCRs to the reinforced CSs+ during late acquisition (trials 5-8) and on block 4 of reinstatement (i.e., the final block of the experiment on Day 3); separate calculations were performed for fear-relevant (CSa) and fear-irrelevant CSs (CSb). Square root transformed and range corrected SCRs are reported in micro Siemens. US intensity is reported in Volts.

4.7.2. Electrodermal Responding

4.7.2.1. Spontaneous Recovery

SCRs and CS valence ratings during follow-up tests are displayed in Figure A4.1. While it appears that differential SCRs to fear-irrelevant CSs may have recovered after the passage of time, the small increase in differential SCRs to fear-irrelevant CSs observed in Figure A4.1 was not statistically

significant. The results of the 2 (fear-relevance) x 2 (conditioning) x 2 (time: Day 3 reinstatement, block 4 vs. Day 4 spontaneous recovery, block 1) repeated measures ANOVA yielded only a main effect of time, $F(1, 11) = 10.70$, $p = .007$, $\eta p^2 = .49$, but no other significant main effects or interactions that would indicate recovery of extinguished differential SCRs, largest effect (main effect of conditioning), $F(1, 11) = 0.57$, $p = .468$, $\eta p^2 = .05$. The significant main effect of time reflects overall larger SCRs during follow-up tests, on block 1 of spontaneous recovery ($M = 0.18$, $SE = 0.04$), than on the last block of reinstatement tests conducted on Day 3 ($M = 0.06$, $SE = 0.04$). Hence, the present results reflect an overall increase in electrodermal responding between Day 3 and follow-up tests, but no recovery of differential SCRs. Furthermore, a comparison of the present effect sizes of the non-significant main effects and interactions (all $\eta p^2 \leq .05$) with the magnitude of fear recovery observed in the control group on Day 3 (fear-relevance x conditioning x time x group interaction: $\eta p^2 = .09$; follow-up comparisons for CSa/b: $\eta p^2 \geq .07$) and in the control group in the occasionally reinforced extinction study (Chapter 3; conditioning x group: $\eta p^2 = .09$; follow-up comparison: $\eta p^2 = .16$), suggests that even with a larger sample, meaningful levels of fear recovery are unlikely to be found. Nevertheless, due to the small size of the follow-up group, this proposition requires further examination in future research.

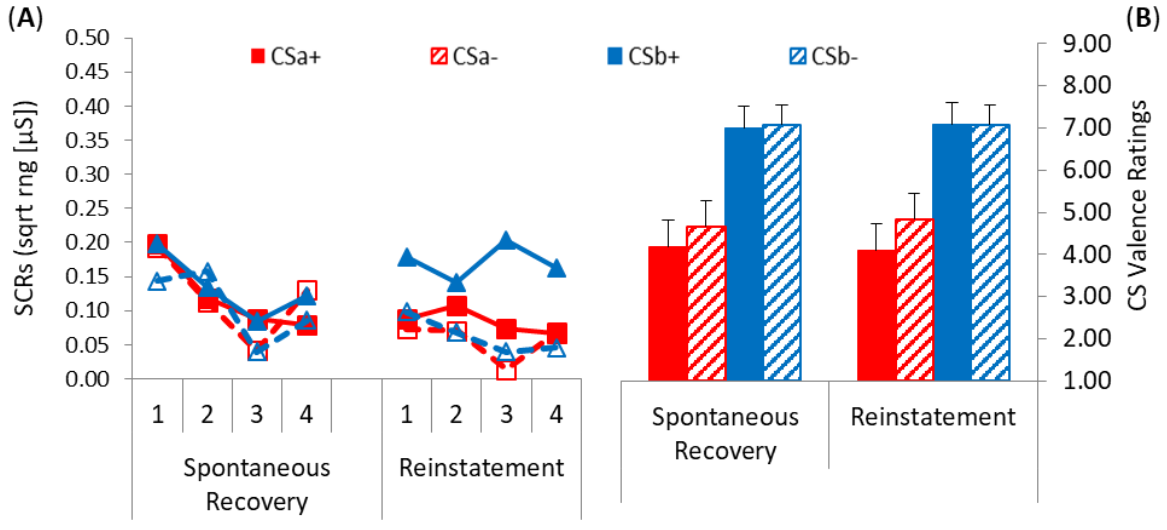


Figure A4.1. Results of follow-up tests in the US-reactivation group. Left panel (**A**): Mean skin conductance responses (SCRs) to fear-relevant (CSa+/-) and fear-irrelevant (CSb+/-) conditioned stimuli (CSs). SCRs are presented in blocks of two consecutive trials. Right panel (**B**): Mean rated valence of fear-relevant (CSa+/-) and fear-irrelevant (CSb+/-) CSs. Valence was rated on a scale ranging from 1 (unpleasant) to 9 (pleasant). Error bars represent standard errors.

4.7.2.2. Reinstatement

A visual inspection of differential SCRs on block 1 of reinstatement tests (Figure A4.1) suggests possible reinstatement of fear to fear-irrelevant, but not to fear-relevant, CSs after the presentation of three unsignaled USs. The results of a 2 (fear-relevance) x 2 (conditioning) x 2 (time [Day 4 only]: spontaneous recovery block 4 vs. reinstatement, block 1) repeated measures ANOVA, however, did not confirm the initial observations, yielding no significant main effects or interactions, largest effect (fear-relevance x time interaction), $F(1, 11) = 2.68$, $p = .130$, $\eta p^2 = .20$. Moreover, the non-significant fear-relevance x conditioning x time interaction, $F(1, 11) = 0.02$, $p = .882$, and its small effect size of $\eta p^2 < .01$ further support the absence of statistically significant reinstatement of fear to fear-relevant or fear-irrelevant CSs. This being said, whilst the combined results of follow-up tests indicate absence of statistically significant differential SCRs, these results need to be interpreted with caution due to the small sample size of the follow-up group.

4.7.3. CS Valence Ratings

In accordance with the pattern of data displayed in Figure A4.1, fear-irrelevant CSs were rated as more pleasant than fear-relevant CSs. Statistical analyses yielded a main effect of fear-relevance, $F(1, 11) = 16.56$, $p = .002$, $\eta p^2 = .60$, reflecting more positive evaluations of the fear-irrelevant CSs ($M = 7.06$, $SE = 0.37$) than the fear-relevant CSs ($M = 4.44$, $SE = 0.57$). The remaining main effects and interactions did not attain significance, largest effect (fear-relevance x conditioning x time), $F(1, 11) = 1.16$, $p = .305$, $\eta p^2 = .10$, indicating that conditioned negative evaluations of the CS+, relative to the CS-, did not recover during follow-up tests.

4.8. Discussion

The key aim of the reconsolidation study was the examination of the effects of extinction training that was delivered 10 minutes after an unsignaled presentation of the US, at half the intensity as that employed during acquisition, on the recovery of extinguished responding. Results of the 3-day study showed reduced spontaneous recovery and reinstatement of differential SCRs 24 hours after the delivery of the reactivation-extinction procedure, but not subsequent to extinction training that was delivered without prior exposure to the US-reactivation trial. Results of follow-up tests further

indicated that the reduction of fear was long-lasting, as reflected in the absence of spontaneous recovery and reinstatement of differential SCRs and CS valence ratings 8 to 12 months after initial testing. Manipulation tests further indicated that this absence of fear recovery did not reflect on participants' inability to recall the trained CS-US associations, as inspection of CS-US contingency questionnaires, completed during follow-up tests, showed that the vast majority of participants were able to recall which CSs had been paired with the US during initial training.

The broad findings of the present study are consistent with previous research, which showed that fears can be eliminated through extinction training that is delivered during the memory reconsolidation period – a time-dependent process that is engaged through the reactivation and destabilisation of previously consolidated memories (Liu et al., 2014; Schiller et al., 2010). However, in contrast to past research that employed extinction training to disrupt the reconsolidation process of fears conditioned to fear-relevant CSs (e.g., Golkar, Bellander, Olsson, & Öhman, 2012; Kindt & Soeter, 2013), there was no post-reconsolidation recovery of fear to fear-relevant CSs in the present study.

The present study thus replicated and extended previous findings (Liu et al., 2014; Schiller et al., 2010) to fears conditioned to fear-relevant CSs and provided the first application of the US reactivation-extinction procedure to the concurrent manipulation of the reconsolidation process of two distinct fear memories, conditioned to different classes of CS fear-relevance (cf. Liu et al., 2014). Furthermore, the present findings indicate that the effects of the US reactivation-extinction procedure may be long-lasting, as reflected in the absence of fear recovery during the 8 to 12-month follow-up. Of note, while the findings of the follow-up tests are in line with those reported in past research, which showed that behavioural manipulations of the reconsolidation process can yield persistent (6-18 months) reduction of fear (Björkstrand et al., 2015; Liu et al., 2014; Schiller et al., 2010), they must be nevertheless interpreted with caution due to the small sample size of the follow-up group. This being said, recent reports corroborate the present findings, showing that behavioural manipulations of the reconsolidation process can yield superior reduction of experimentally induced (Agren, Björkstrand, & Fredrikson, 2017; Fernandez-Rey, Gonzalez-Gonzalez, & Redondo, 2018; Grégoire &

Greening, 2019) and naturally occurring fears (Björkstrand et al., 2017; Telch, York, Lancaster, & Monfils, 2017), relative to extinction training that is delivered outside the reconsolidation window.

4.8.1. Limitations

In addition to the small sample size of the follow-up group, a key limitation of the present follow-up study included the lack of return of fear assessments in the control group. In line with previous research (Schiller et al., 2010), the aim was to conduct tests of fear recovery in both groups 12 months after initial training. However, this was not possible due to many participants no longer being available for testing. As such, the present follow-up results must be interpreted with caution. Due to the small sample size, there is also the possibility of selection bias. Selection bias may be present if there are significant differences on variables that may affect conditioned responding between participants who took part in follow-up tests and those who did not. To address this possible confound, extensive preliminary tests were conducted. The respective results (Table A4.1) did not yield evidence of a selection bias, showing no differences in differential responding or selected US intensities between participants who took part in follow-up tests and those who did not.

Finally, it could be argued that the recalibration of US intensity during follow-up tests represents a limitation, as the presentation of unsignaled USs during the shock work-up procedure may induce reinstatement-like effects in conditioned responding (Haaker et al., 2014). To minimise such effects, the shock work-up procedure was carried out at the conclusion of tests of spontaneous recovery. Strictly speaking, this still leaves the possibility of the shock work-up procedure to influence conditioned responding during tests of reinstatement, for instance by creating a more threatening context (Haaker et al., 2014). However, this supposition is not in line with the present results, which showed no reinstatement of fear.

4.8.2. Conclusion

Despite limitations, the present follow-up study made an important contribution to human reconsolidation research by providing preliminary evidence for the effectiveness of the US reactivation-extinction procedure in the long-lasting reduction of fear to fear-irrelevant and fear-relevant CSs. The present findings further showed that a single memory reactivation trial is capable of destabilising multiple fear memories, thereby allowing for the concurrent disruption of the

reconsolidation process of multiple fear memories. More research is needed, however, to investigate how the present US-reactivation procedure could be adapted for the use in the treatment of naturally occurring fears and whether a reduction of US intensity is required to generate the prediction error required for the reactivation and destabilisation of the fear memory trace. The implications of the present findings for the basic and applied setting are further discussed in the general discussion of findings (Chapter 6).

Chapter 5: Study 3 – US Devaluation

Effects of Unconditioned Stimulus Devaluation on Immediate and Delayed Conditioned Responding to Fear-Relevant and Fear-Irrelevant Conditioned Stimuli

Unpublished Manuscript

5.1. Abstract

Background: Building on the reconsolidation study conducted as part of this thesis (Chapter 4) and on past *US devaluation* research, the present study investigated whether persistent reduction of fear can be achieved through a procedure that resembles US reactivation-extinction, but is not delivered during the memory reconsolidation period. In accordance with reconsolidation research, it was also examined whether prediction errors during the post-acquisition presentation of the unsignaled USs are required for subsequent reduction of fear. **Method:** On Day 1, 96 participants (68 women, 28 men; *M* age = 24.33 years, *SD* = 7.12) underwent differential fear conditioning to fear-relevant and fear-irrelevant conditioned stimuli (CSs), before taking part in *instructed* or *uninstructed* US devaluation (or no US devaluation), followed by extinction training. Verbal instructions were employed to either enhance or reduce the prediction error generated by the presentation of three USs, at reduced physical intensity, during the US devaluation phase. Spontaneous recovery and reinstatement of extinguished fear were assessed 24 hours later. **Results:** Assessment of differential SCRs and conditioned negative CS valence ratings, conducted 10 minutes (during early extinction training) and 24 hours after the US devaluation phase, indicated absence of conditioned responding in both US devaluation groups, but also in the control group. Further complicating the interpretation of findings was the lack of equal acquisition of fear across groups. **Conclusion:** Due to differences in fear acquisition across groups and absence of fear recovery in the control group, no definitive conclusions can be drawn about the role of US devaluation or prediction errors in the persistent reduction of fear.

5.2. 1Introduction

Fears are learned through association of neutral cues with naturally aversive outcomes (unconditioned stimuli [USs]; Davey, 1992). Following such pairings, the previously neutral cue becomes a predictor (conditioned stimulus [CS]) of the US and comes to elicit the conditioned fear response (Davey, 1992). While associative learning is the key mechanism underlying the development of fears, phobias, and anxiety disorders (Craske, Treanor, Conway, Zbozinek, & Vervliet, 2014; Pittig, Treanor, LeBeau, & Craske, 2018), it has been suggested that it does not readily account for fears and phobias in individuals who are unable to recall events that precipitated the onset of their fears (Davey, De Jong, & Tallis, 1993). At the same time, many individuals who have experienced anxiety-arousing or traumatic events do not develop clinically significant fears (Davey, 1989, 1992), which suggests that factors beyond CS-US pairings contribute to the development of fears or influence the strength of the fear response. One such factor, and the focus of the present investigation, is *US revaluation* (for comprehensive reviews of additional factors involved in the development of fears, phobias, and anxiety disorders, see Craske & Stein, 2016; Mineka & Zinbarg, 2006; Pittig et al., 2018).

US revaluation refers to the post-acquisition modification of the motivational value of the US (Davey, 1989; Storsve, McNally, & Richardson, 2012). US revaluation is carried out after the successful acquisition of the CS-US association, by modifying the subjective evaluation of the US that had been associated with the CS (Davey, 1989), for instance by reducing the physical intensity of an electrotactile US (Schultz, Balderston, Geiger, & Helmstetter, 2013). In contrast to the initial acquisition of the CS-US association, US revaluation is conducted in the absence of the CS and its effects on subsequent conditioned responding are mediated by non-associative processes, such as habituation (Davey, 1989; Haesen & Vervliet, 2015; Leer & Engelhard, 2015; but see Storsve et al., 2012 for a discussion of a potential dual role of associative and non-associative processes). In fear conditioning research, the US can be revalued by either increasing (*US inflation*; Rescorla, 1974) or decreasing (*US devaluation*; Rescorla, 1973) its aversiveness. Several methods have been employed for this purpose, which can be broadly summarised into those involving (a) direct and (b) indirect experience with the US.

Methods involving direct experience with the US include repeated administrations of the US, either at the same intensity as that employed during acquisition (i.e., *US habituation*; Haesen & Vervliet, 2015; Laborda & Miller, 2011; Rescorla, 1973; Siddle, Power, Bond, & Lovibond, 1988; Storsve, McNally, & Richardson, 2010; Storsve et al., 2012), or at an increased/decreased intensity, relative to acquisition (Davey & McKenna, 1983; Hosoba, Iwanaga, & Seiwa, 2001; Leer & Engelhard, 2015; Schultz et al., 2013; White & Davey, 1989).[6] Research conducted with animals showed that US devaluation consisting of repeated, unsignaled presentations of an aversive US reduced the strength of the conditioned fear response, as reflected in decreased conditioned suppression (Rescorla, 1973) and decreased freezing (Storsve et al., 2010, 2012). However, extant literature also indicates that this reduction of conditioned responding is not necessarily long-lasting, whereby findings from research conducted with animals showed that US devaluation does not guard against spontaneous recovery (Laborda & Miller, 2011), renewal, or reinstatement of fear (Storsve et al., 2010, 2012; but see Costanzi et al., 2014 for a different pattern of results).

US devaluation has also been employed in research conducted with humans: Poulos, Furedy, and Heslegrave (1979) reported reduction of differential SCRs subsequent to 70 presentations of an aversive auditory US, delivered at the same intensity as that employed during acquisition; yet, a later replication attempt involving aversive electrotactile USs (Siddle et al., 1988) failed to corroborate these findings. On the other hand, recent reports indicate that three (Schultz et al., 2013) or five (Hosoba et al., 2001) US presentations at decreased intensity, relative to the US intensity employed during acquisition, are sufficient to decrease subsequent differential SCRs. Examination of US expectancy ratings in past research (Haesen & Vervliet, 2015; Leer & Engelhard, 2015; Schultz et al., 2013) further indicates that US devaluation does not decrease US expectancy ratings, meaning that participants still expect the CS to be followed by the US after the US devaluation procedure. These findings suggest that the reduction of conditioned responding subsequent to US devaluation is not

[6] The terms *US habituation* and *US devaluation* have been used interchangeably in some reports (Rescorla, 1973; Storsve et al., 2010), but not in others (Hosoba et al., 2001). Whenever the term *US habituation* is used in the present chapter, it is to highlight the use of a specific type of US devaluation procedure, involving presentations of the US at the same intensity as that employed during acquisition.

mediated by a decrease in the strength of the CS-US association (Davey, 1989; Haesen & Vervliet, 2015; Leer & Engelhard, 2015), but by the revaluation of the motivational value of the US.

Interestingly, in contrast to findings reported in research conducted with animals (e.g., Storsve et al., 2010), repeated post-acquisition presentations of the US in experiments conducted with humans appear to prevent fear renewal (Haesen & Vervliet, 2015; Leer & Engelhard, 2015). It remains to be investigated whether these divergent results reflect cross-species differences in the devaluation of the US or, alternatively, the nature of single- versus multi-day experiments. In multi-day studies, which are more commonly conducted in animal than human research, fear acquisition may be separated in time from the US devaluation/habituation phase (e.g., Storsve et al., 2012), thereby allowing sufficient time for the consolidation of fear learning, yielding a more robust fear memory that may be more difficult to modify (Auber, Tedesco, Jones, Monfils, & Chiamulera, 2013; Dudai, Karni, & Born, 2015). At the same time, as the majority of human US devaluation studies have been conducted in a single test session (e.g., Haesen & Vervliet, 2015; Schultz et al., 2013), it is presently not known whether the post-devaluation reduction of fear observed in single-day studies is indeed long-lasting. This question has been addressed in the present 2-day US devaluation study.

As an alternative to US revaluation methods involving direct experience with the US, US revaluation in experiments conducted with humans may be carried out through methods that capitalise on higher order cognitive functions, such as our ability to process verbal instructions or our use of mental imagery (e.g., Dibbets, Lemmens, & Voncken, 2018). As such, US inflation and US devaluation in humans may not require direct exposure to the US. Examples of such indirect methods include: (a) verbal instructions aimed at inflating the aversiveness of the US (de Jong, Merckelbach, Koertshuis, & Muris, 1994); (b) revaluation of the model's fear response (US) in vicarious fear learning (Reynolds, Field, & Askew, 2015); (c) imagery rescripting (Dibbets et al., 2018; Dibbets, Poort, & Arntz, 2012); (d) or eye movement desensitisation and reprocessing (EMDR; Dibbets et al., 2018; Leer, Engelhard, Dibbets, & van den Hout, 2013). However, at present, there is limited support for the effectiveness of indirect US devaluation methods.

For instance, laboratory-based studies have shown that imagery rescripting and EMDR reduced subjective ratings of US aversiveness (Dibbets et al., 2018), reflecting effects of US

devaluation (Baeyens, Eelen, Van den Bergh, & Crombez, 1992; Davey & McKenna, 1983), yet did not reduce conditioned physiological responding, as indexed by SCRs and fear potentiated startle (Dibbets et al., 2018; Dibbets et al., 2012; Leer et al., 2013). In an observational learning study, a reduction in fear beliefs and avoidance preferences has been observed in children who underwent vicarious US devaluation (Reynolds et al., 2015), whilst hybrid methods combining verbal instructions, aimed at devaluing the US, with the delivery of the US either decreased (Davey & McKenna, 1983) or increased (Pile, Barnhofer, & Wild, 2015) SCRs to the CS+. It should be noted, however, that the interpretation of these findings is complicated by cross-study methodological variations, such as the use of trauma film (Dibbets et al., 2018; Pile et al., 2015) or verbal instructions used for the imaginal devaluation of the US (Dibbets et al., 2012).

To date, the phenomenon of US devaluation remains poorly understood and it is not known if the mixed results reported in past research reflect methodological limitations or true boundary conditions of US devaluation. It appears that past research raised more questions than it answered. For instance, it remains to be investigated whether post-acquisition conditioned responding is differentially sensitive to US devaluation procedures based on direct (e.g., Schultz et al., 2013) or indirect (e.g., Dibbets et al., 2018) experience with the US and whether the number of US presentations and the intensity of the US (i.e., reduced vs. not reduced, relative to acquisition) are important determinants of subsequent conditioned responding. Assuming that US intensity is reduced to decrease the perceived aversiveness of the US, questions arise about adequate control procedures to rule out alternative explanations, such as the effects of prediction errors generated by the reduction of US intensity and/or by the unsignaled presentation of the US (Fernández, Boccia, & Pedreira, 2016; Liu et al., 2014). Further, due to the lack of delayed assessments of post-devaluation conditioned responding (e.g., after 24 hours), it is presently not known if the reduction of conditioned responding is long-lasting and, therefore, if US devaluation could indeed explain why some individuals who have experienced traumatic events do not develop fears or anxiety disorders, as suggested by Davey (1992). There are, of course, many additional questions arising from past research, for example pertaining to the role of verbal instructions (Davey & McKenna, 1983; Dibbets et al., 2018; Mertens, Boddez, Sevenster, Engelhard, & De Houwer, 2018) or the exact mechanisms mediating fear

reduction subsequent to US devaluation (Laborda & Miller, 2011; Storsve et al., 2012). The present investigation was designed to address two of these questions: (a) whether the effects of US devaluation on conditioned responding are long-lasting and (b) whether they are mediated by prediction errors.

The prediction error hypothesis is based on the premise that acquisition and reduction of fear are facilitated by a mismatch between expected and actual events (Fernández et al., 2016; Holland & Schiffino, 2016). Prediction errors pertaining to the US have been shown to enhance extinction learning (e.g., Brown, LeBeau, Chat, & Craske, 2017; Thompson, McEvoy, & Lipp, 2018) and to facilitate the disruption of the memory reconsolidation process by reactivating and destabilising previously consolidated fear memories (Lee, Nader, & Schiller, 2017; Liu et al., 2014; Thompson & Lipp, 2017). Past reconsolidation research that utilised a memory reactivation approach that was procedurally identical to US devaluation (Liu et al., 2014; Thompson & Lipp, 2017) demonstrated that an unsignaled presentation of the US, delivered at half the physical intensity used during acquisition (i.e., *US-reactivation*), 10 minutes prior to extinction training, facilitated the disruption of the reconsolidation processes and, thereby, allowed for elimination of the conditioned fear response. Given the similarity of US-reactivation and US devaluation procedures, and previous findings indicating that prediction errors pertaining to the US facilitate fear reduction (e.g., Fernández et al., 2016), it is conceivable that prediction errors mediate the effects of US devaluation on conditioned responding.

The present study utilised an adapted version of the multi-day differential fear conditioning paradigm used in the reconsolidation study conducted as part of this thesis (Chapter 4), to examine effects of US devaluation on conditioned responding, assessed after a delay of 10 minutes (at the start of extinction training) and after 24 hours (during tests of fear recovery). SCRs and CS valence ratings were recorded as primary and secondary dependent measures of conditioned responding, respectively. In contrast to the reconsolidation study (Chapter 4), acquisition, US devaluation, and extinction training in the present study were conducted during the same test session, to prevent accidental reactivation of fear memories. The aim of this approach was to test whether persistent reduction of fear can be achieved through a procedure that resembles US reactivation-extinction, but is not

delivered during the memory reconsolidation period. To clarify, in reconsolidation research, a delay of at least 24 hours is inserted between acquisition training and memory reactivation (e.g., Schiller et al., 2010). This break allows the new and vulnerable fear memory to consolidate into a stable form, a long-term memory, before it is reactivated and returned to a labile state (Nader et al., 2000b). This process of memory consolidation starts immediately after the acquisition of new learning, but the formation of a stable long-term memory is not completed until several hours after acquisition (Visser, Lau-Zhu, Henson, & Holmes, 2018), whereby some learning may require 24 hours to consolidate (Bekinschtein et al., 2008; for a detailed description of memory consolidation, see Chapter 1). As US devaluation in the present study was delivered immediately after acquisition training, the procedure is not expected to target the reconsolidation process, because the memory is yet to consolidate.

Following differential Pavlovian fear conditioning (e.g., Lipp, 2006a) to fear-relevant and fear-irrelevant CSs, participants in the US devaluation groups received three presentations of the electrotactile US, at half the physical intensity employed during acquisition (Schultz et al., 2013), followed by extinction training; participants in the control group received extinction training without prior US devaluation. To examine if prediction errors mediate the effects of US devaluation on conditioned responding, participants in the US devaluation groups received verbal instructions pertaining to the upcoming US devaluation procedure: Participants in the *instructed* and *uninstructed* US devaluation groups were informed that several stimuli would be presented, but only participants in the instructed group were informed of the nature, frequency, and intensity of the stimuli. The aim of these instructions was to maximise prediction errors generated during the US devaluation phase in the uninstructed group, but decrease prediction errors in the instructed group. Specifically, the uninstructed US devaluation procedure would generate prediction errors through the mismatch between expectations based on prior learning (i.e., CS is followed by the US) and actual events (i.e., the US is presented by itself, at decreased intensity).

The hypotheses in the present investigation were as follows: Based on past reports of reduced post-devaluation differential SCRs at the start of extinction training (Schultz et al., 2013) and during tests of renewal (Haesen & Vervliet, 2015), it was predicted that differential SCRs would be lower in the US devaluation groups than in the control group, both 10 minutes after the US devaluation

procedure as well as during tests of fear recovery, conducted after a delay of 24 hours. With regards to prediction errors, if the effects of US devaluation are mediated by prediction errors, post-devaluation differential SCRs would be lower in the uninstructed than in the instructed US devaluation group. Conversely, if prediction errors do not mediate US devaluation effects, there should be no differences in differential SCRs between the instructed and uninstructed US devaluation group. No directional predictions were made about the effects of US devaluation on conditioned CS valence ratings, due to the mixed findings reported in past research (Hosoba et al., 2001; Jensen-Fielding, Luck, & Lipp, 2017).

5.3. Materials and Methods

5.3.1. Participants

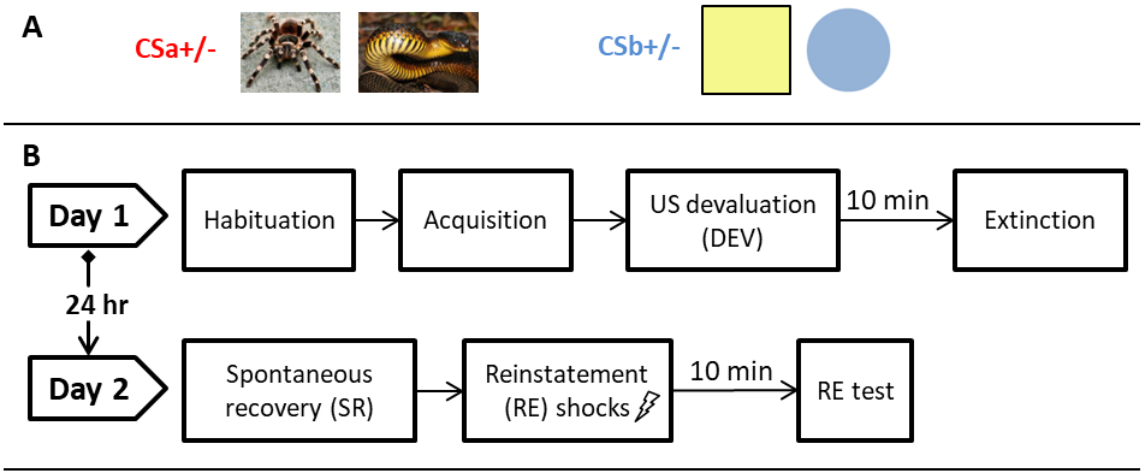
Healthy volunteers who met inclusion criteria (i.e., no cardiovascular disease, seizure disorder, or pregnancy) were recruited from a sample of community members and university students. Participants were compensated through partial course credit or a modest financial compensation of 30 AUD. After exclusion of eight participants who failed to verbalise the CS-US relationship (participants excluded per group: control: $n = 2$; instructed: $n = 2$; uninstructed: $n = 4$) and four participants who did not present for testing on day 2, data from 96 participants were included in the analyses (68 women, 28 men; female:male ratio per group: 23:9 [control and uninstructed group], 22:10 [instructed group]). The age range of participants was 18 to 55 years (*M* age = 24.33 years, *SD* = 7. 12). Ethical approval for this study was obtained from the Curtin University Human Research Ethics Committee.

5.3.2. Apparatus and Materials

5.3.2.1. Stimuli

Conditioned stimuli were adapted from previous work conducted in our laboratory (Chapter 4). Fear-relevant conditioned stimuli (CSa+/-) consisted of a spider and a snake picture, while a yellow square and blue circle served as fear-irrelevant CSs (CSb+/-; Figure 5.1). The pictures were presented for 6 seconds (s), in the centre of a 17-inch colour LCD screen, over a black background, with an inter-trial interval of 10 to 14 s. To control for order effects, the assignment of snake and spider pictures as CSa+ or CSa-, the assignment of yellow and blue shapes as CSb+ or CSb-, and

whether the first trial of each phase was a CSa+/- or CSb+/- were counterbalanced across participants. Stimuli were presented in a pseudo-randomised order, whereby each CS was presented twice within blocks of eight trials. The US consisted of a mild electric shock, which was generated with a Grass SD9 stimulator (Grass Technologies, Middleton, WI) and delivered to the wrist of the dominant hand via a concentric electrode. The shock was presented for 200 milliseconds (ms), was pulsed at 50 Hertz (Hz), and coincided with the CSs+ offset; the CSs- were never paired with the US. The delivery of the US and CSs was controlled with DMDX 5.0.5 software (Forster & Forster, 2003).



C

Habituation	Acquisition		DEV	Extinction	SR	RE	RE Test
4 CSa+/-	6 CSa+/-	6 US	3 US*	10 CSa+/-	8 CSa+/-	3 US	8 CSa+/-
4 CSb+/-	6 CSb+/-	6 US		10 CSb+/-	8 CSb+/-		8 CSb+/-

Figure 5.1. Schematic representation of the experimental paradigm of the US devaluation study. Fear-relevant (CSa+/-) and fear-irrelevant (CSb+/-) conditioned stimuli (CSs), which were either reinforced (+) or non-reinforced (-) during acquisition, are presented in panel **A,** followed by the sequential order of experimental conditions (**B**), and number of CS and unconditioned stimulus (US) presentations per experimental phase (**C**).
*****The physical intensity of the US was halved for the purpose of US devaluation, relative to US intensity employed during acquisition.

5.3.2.2. Electrodermal Activity (SCRs)

Electrodermal activity was recorded through two self-adhesive, pre-gelled electrodes (Biopac Systems EL507), attached to the thenar and hypothenar eminences of the non-dominant hand. Electrodermal activity was DC amplified at a gain of 5 micro Siemens (µS) per volt and recorded with a Biopac MP150 system at a sampling frequency of 1000 Hz, using AcqKnowledge 4 (Biopac

Systems, Goleta, CA). A Biopac respiration belt was fitted around each participant's waist to control for respiration-induced artefacts in SCRs.

5.3.2.3. Subjective Evaluation of Stimulus Valence

Participants provided post-test CS and US valence ratings on a 9-point scale (from 1 [unpleasant] to 9 [pleasant]) at baseline, after acquisition, US devaluation (CS ratings only), extinction training, spontaneous recovery (CS ratings only), and test of reinstatement. Valence ratings were obtained electronically through a custom-made Microsoft Access application (Thompson et al., 2018), whereby the stimuli were presented on the computer screen in randomised order and participants were instructed to rate stimulus valence on the scale located below the picture.

5.3.2.4. Self-report Questionnaires

To assess the degree of spider and snake fear across groups, participants completed the spider (SPQ) and snake (SNAQ) questionnaire (Klorman, Weerts, Hastings, Melamed, & Lang, 1974). Both measures demonstrated excellent internal consistency in the current sample ($\alpha \geq .88$). Participants were also asked to complete the short form of the Intolerance of Uncertainty Scale (IUS-12; Carleton, Norton, & Asmundson, 2007). The 12-item IUS measures beliefs about and reactions to uncertainty, ambiguous situations, and the future and comprises two subscales: prospective IU (measures cognitive appraisals of future uncertainty) and inhibitory IU (indicative of behavioural inhibition and avoidance). Participants also completed the short form of the Depression, Anxiety, and Stress Scales (DASS-21; Henry & Crawford, 2005; Lovibond & Lovibond, 1995). All questionnaires were completed electronically at the start of the experiment.

5.3.2.5. Manipulation Checks

Following acquisition, participants were presented with a CS-US contingency questionnaire, containing pictures of the CSs and two control stimuli, and were asked to indicate which stimuli had been paired with the US. As inability to verbalise the correct contingency may reflect a genuine failure to learn the CS-US relationship (Lipp, 2006a), data from participants who failed this test (n = 8) were excluded from statistical analyses. Participants were also asked to indicate if they believed the instructions presented during the US devaluation stage.

5.3.3. Procedure

A schematic representation of the experimental paradigm is presented in Figure 5.1. Testing was conducted across two consecutive days. Participants completed each stage of the experiment individually, while seated in front of a 17-inch colour LCD screen. At the start of the first test phase on Day 1 and Day 2, participants were asked to wash their hands and were then fitted with the skin conductance electrodes, respiration belt, and the shock electrode.

Upon arriving in the laboratory on Day 1, participants were informed about the experimental procedures and had the opportunity to ask questions, before providing information about current medication use and medical history. Individuals who met inclusion criteria provided written consent and completed the self-report measures. Thereafter, participants were asked to relax and look at the computer screen while a 2-minute baseline of their electrodermal activity was recorded. Subsequently, participants provided baseline CS valence ratings, set the US intensity to a level that was perceived as "unpleasant, but not painful," and rated US valence.

5.3.3.1. Day 1: Acquisition

Participants were asked to pay attention to the computer screen and to learn which CSs were followed by the US. Conditioning commenced with a habituation phase, consisting of four non-reinforced presentations of each CS, and was immediately followed by acquisition, which involved six presentations of CSa+/- and CSb+/-. The US was presented on all CS+ trials. Thereafter, participants completed the CS-US contingency questionnaire, rated CS and US valence, and were assigned to groups in the order they presented for testing, with the restriction that an approximately equal number of women and men were assigned to each group.

5.3.3.2. Day 1: US Devaluation

At the start of the US devaluation phase, participants received the following instructions about the upcoming experimental phase:

- Instructed group: "You will receive three presentations of the electrical stimulus, at half the intensity used in the previous part of this experiment. Please pay attention to the computer screen at all times."

- Uninstructed group: “In this task you will be presented with several stimuli. Please pay attention to the computer screen at all times.”
- Control group: “In this task, you are required to look at the computer screen while your breathing is recorded. No stimuli will be presented. Please pay attention to the computer screen at all times.” (These instructions were similar to those presented to participants prior to the 2-minute recording of baseline electrodermal activity.)

Subsequently, participants in the instructed and uninstructed group received three presentations of the US, at half the physical intensity that was employed during acquisition (Schultz et al., 2013), while participants in the control group were asked to look at the computer screen for an equal amount of time. The computer screen remained switched on and displayed a black background. At the conclusion of the US devaluation phase, the shock electrode was removed and participants were offered magazines to read during a 10-minute break. The break was included to allow electrodermal activity to return to a stable baseline before the start of extinction training (Schultz et al., 2013). The choice of the US devaluation procedure was guided by two primary concerns, being (a) demonstrated effectiveness in the reduction of conditioned responding and (b) demands on participants. Hence, the procedure used by Schultz and colleagues was adapted for the purpose of the present study, as it was shown that three presentations of the US, at half the intensity used during acquisition, were sufficient to reduce subsequent conditioned responding, and because a relatively brief US devaluation procedure (cf. Leer & Engelhard, 2015) reduces demands on participants.

5.3.3.3. Day 1: Extinction Training

After the break, the shock electrode was reattached and participants were asked to rate CS valence and were then instructed to pay attention to the pictures on the computer screen; no further instructions were provided. Extinction training consisted of 10 non-reinforced presentations of the CSa+/- and the CSb+/-. Thereafter, participants rated CS and US valence and were asked to indicate whether they believed the instructions received during the US devaluation stage. While the post-devaluation test phase (i.e., CS-only presentations) typically consists of a relatively small number of CS+/- trials (e.g., 4; Hosoba et al., 2001), in the present investigation each CS was presented 10 times, to allow for extinction of differential responding to fear-relevant and fear-irrelevant CSs in all groups

(i.e., in line with previous research conducted in our laboratory; see Chapter 4). The recovery of extinguished responding was then assessed after a delay of 24 hours.

5.3.3.4. Day 2: Tests of Fear Recovery

Participants were asked to pay attention to pictures presented on the computer screen; no further instructions were provided. Assessment of spontaneous recovery involved eight non-reinforced presentations of the CSa+/- and the CSb+/- and was followed by CS valence ratings. Participants then received three unsignaled presentations of the US, with a duration of 200 ms and an ITI of 6 s. The US intensity was identical to that used during acquisition. The computer screen remained switched on and displayed a black background (in line with previous training). After a 10-minute break (identical to Day 1), participants underwent reinstatement testing, consisting of eight non-reinforced presentations of CSa+/- and CSb+/-, followed by CS and US valence ratings.

5.3.4. Scoring and Response Definition

Skin conductance responses were scored offline in AcqKnowledge 4. Participants' baseline electrodermal activity was determined by counting all spontaneous responses that occurred during a 2-minute rest period (Dawson, Schell, & Filion, 2007). A visual inspection of electrodermal data was conducted to check if any SCRs were influenced by movement- or respiration-induced artefacts: Nineteen SCRs (across groups) were discarded due to the presence of artefacts. In accordance with past research (e.g., Kindt & Soeter, 2013; Pineles, Orr, & Orr, 2009), SCRs elicited by the CSs were calculated by subtracting the mean skin conductance level during the 2 s baseline preceding CS onset from the largest skin conductance level occurring 1 to 6 s after CS onset. SCRs were range corrected to control for individual differences in electrodermal activity (Lykken, 1972) and then square root transformed to reduce the skew of the distribution (Dawson et al., 2007). The range correction was obtained by dividing each response by the largest response displayed by the participant. Electrodermal responses were averaged into blocks of two consecutive trials to reduce the influence of trial by trial variability.

5.3.5. Statistical Analyses

5.3.5.1. SCRs

Electrodermal data were analysed through mixed analyses of variance (ANOVAs) for repeated measures, with group (instructed, uninstructed, control) as between-groups factor and fear-relevance (fear-relevant vs. fear-irrelevant), conditioning (CS+ vs. CS-) and block/time as within-groups factor. The following factors of block/time were included in the analyses: habituation: block 1-2; acquisition: block 1-3; post-devaluation reduction of differential responding: acquisition block 3 vs. extinction block 1; extinction of differential SCRs: extinction block 1-5; spontaneous recovery: extinction block 5 vs. spontaneous recovery block 1; reinstatement: spontaneous recovery block 4 vs. reinstatement block 1.

5.3.5.2. Valence Ratings

Conditioning effects in evaluative conditioning are reflected in changes in liking of the CSs+ that were paired with a pleasant or unpleasant US (De Houwer, 2007). In the present fear conditioning paradigm, fear acquisition would be reflected in increased negative evaluations of the CSs+, relative to the CSs-. For the purpose of data analysis, differential negative evaluation scores were computed for each CS type by subtracting valence ratings of the CS+ from ratings of the CS-. Larger values indicate more negative differential evaluations of the CS+ (a similar approach has been employed in past research; Gawronski, Gast, & De Houwer, 2015). The differential negative evaluation scores were subjected to a 3 (group) x 2 (fear-relevance) x 6 (time: baseline, acquisition, devaluation, extinction, spontaneous recovery, and reinstatement) mixed ANOVA for repeated measures. US valence ratings were analysed through a 3 (group) x 4 (time: baseline, acquisition, extinction, and reinstatement) mixed ANOVA for repeated measures.

Multivariate *F* values (Pillai's Trace) and partial eta squared values were reported for all main effects and interactions. Statistical significance was assessed at $\alpha = .05$; Bonferroni corrections were used for follow-up analyses to guard against the accumulation of Type 1 errors.

5.4. Results

5.4.1. Preliminary Analyses

The groups did not differ in age, selected US intensity, baseline CS and US valence ratings, self-reported snake or spider fear, IUS-12 scores, or baseline electrodermal activity (Table 5.1). However, a one-way between-groups ANOVA showed that groups differed on the scores of the DASS-21 anxiety sub-scale. Follow-up *t* tests revealed significantly higher levels of self-reported anxiety in the uninstructed (M = 9.37, SD = 8.85) than in the instructed group (M = 4.56, SD = 4.07), $t(62) = 2.80$, $p = .007$, $d = 0.74$. There were no significant differences in levels of anxiety between the control (M = 6.63, SD = 4.72) and instructed group, $t(62) = 1.55$, $p = .126$, $d = 0.41$, or between the control and uninstructed group, $t(62) = 1.87$, $p = .066$, $d = 0.47$. Considering that random assignment was used in the present study and no attempts were made to recruit clinical populations, it can be concluded that this group difference arose by chance. Furthermore, logistic regression analyses indicated that anxiety was not a significant predictor of fear acquisition in the present sample (fear-relevant CSs: $p = .291$, Nagelkerke $R^2 = .02$; fear-irrelevant CSs: $p = .163$, Nagelkerke $R^2 = .03$), whereby fear acquisition was operationalised as differential SCRs ≥ 0.01 μS during the second half of acquisition. As such, no attempts were made to control for the group difference in anxiety (for a discussion of the use of statistical methods as a means of controlling for group differences in psychological research, see Miller & Chapman, 2001).

Selected US intensities ranged from 26 to 80 Volts, with a mean of 58.47 Volts (SD = 13.17). Inspection of responses from the manipulation check conducted at the end of testing on Day 1 confirmed that the vast majority of participants believed the instructions received prior to the US devaluation or control phase, with the exception of 2 participants in the control group and one each in the instructed and uninstructed group. Removal of data from these participants did not change the pattern of results, hence, data from all participants have been retained in the analyses.

Table 5.1

Means (M) and Standard Deviations (SD) for Age, US Intensity, Baseline Valence Ratings (VR), Baseline Electrodermal Activity (EDA), and Self-Report Questionnaires

	Control		Instructed		Uninstructed		
	M	*SD*	*M*	*SD*	*M*	*SD*	Test
Age	23.72	7.38	24.84	7.18	24.44	6.99	$F(2,93)=0.20$, $p=.818$
US intensity	57.50	14.75	60.63	11.45	57.28	13.25	$F(2,93)=0.64$, $p=.529$
Baseline VR							
CSa	4.23	1.67	4.03	1.73	4.70	2.00	$F(2,93)=1.16$, $p=.317$
CSb	7.20	1.67	7.72	1.37	7.69	1.39	$F(2,93)=1.21$, $p=.302$
US	3.31	1.38	2.97	1.09	3.09	1.47	$F(2,93)=0.55$, $p=.576$
Baseline EDA	8.72	6.65	10.44	9.81	12.00	8.66	$F(2,93)=1.20$, $p=.305$
SPQ	8.16	4.95	8.94	5.22	9.25	7.11	$F(2,93)=0.30$, $p=.743$
SNAQ	10.25	6.50	9.94	7.06	10.50	8.08	$F(2,93)=0.05$, $p=.953$
IUS-12							
Prospective	19.28	5.04	20.16	4.95	20.38	5.45	$F(2,93)=0.40$, $p=.669$
Inhibitory	9.88	3.41	10.22	4.05	11.22	5.10	$F(2,93)=0.87$, $p=.424$
DASS-21							
Depression	6.50	6.51	6.31	6.24	7.25	6.89	$F(2,93)=0.18$, $p=.833$
Anxiety	6.63	4.72	4.56	4.07	9.37	8.85	**$F(2,93)=4.78$, $p=.011$**
Stress	9.75	6.09	9.25	6.44	11.44	8.49	$F(2,93)=0.84$, $p=.436$

Note. CSa = fear-relevant conditioned stimulus; CSb = fear-irrelevant conditioned stimulus; US = unconditioned stimulus; SPQ = Spider Phobia Questionnaire; SNAQ = Snake Phobia Questionnaire; IUS-12 = Intolerance of Uncertainty Scale (short version); DASS-21 = Depression, Anxiety, and Stress Scales (short version). Baseline EDA refers to the number of spontaneous responses that occurred during a 2-minute rest period. US intensity is reported in Volts.

5.4.2. Electrodermal Responding

5.4.2.1. Habituation

Electrodermal responding across groups and experimental stages is presented in Figure 5.2. Analysis of electrodermal data during habituation yielded main effects of fear-relevance, $F(1, 93) = 35.83$, $p < .001$, $\eta p^2 = .28$, and block, $F(1, 93) = 122.54$, $p < .001$, $\eta p^2 = .57$, as well as interactions of fear-relevance x block, $F(1, 93) = 8.89$, $p = .004$, $\eta p^2 = .09$, and fear-relevance x conditioning x group, $F(2, 93) = 3.61$, $p = .031$, $\eta p^2 = .07$. The main effect of conditioning and remaining interactions did not attain significance, largest effect (conditioning x group interaction), $F(2, 93) = 2.03$, $p = .137$, $\eta p^2 = .04$. In line with the representation of results in Figure 5.2, the significant fear-relevance x block interaction reflects overall larger SCRs to fear-relevant than to fear-irrelevant CSs during habituation, although this difference was larger on the first block ($M = 0.11$, $SD = 0.19$) than on the last block ($M = 0.04$, $SD = 0.14$) of habituation, $t(95) = 2.99$, $p = .004$, $d = 0.42$. The significant fear-relevance x

conditioning x group interaction does not reflect group differences in differential SCRs, but reflects larger SCRs to the fear-irrelevant CSb- than to the CSb+, in the instructed group only, $F(1, 93) = 8.80$, $p = .004$, $\eta p^2 = .09$. Nevertheless, as group differences in electrodermal responding may affect fear acquisition, follow-up analyses were conducted to assess electrodermal responding on the first trial of acquisition. The results of a 2 (fear-relevance) x 2 (conditioning) x 3 (group) ANOVA yielded no significant main effects or interactions, largest effect (main effect of fear-relevance), $F(1, 93) = 3.44$, $p = .067$, $\eta p^2 = .04$, showing that electrodermal responding did not differ across groups at the start of acquisition.

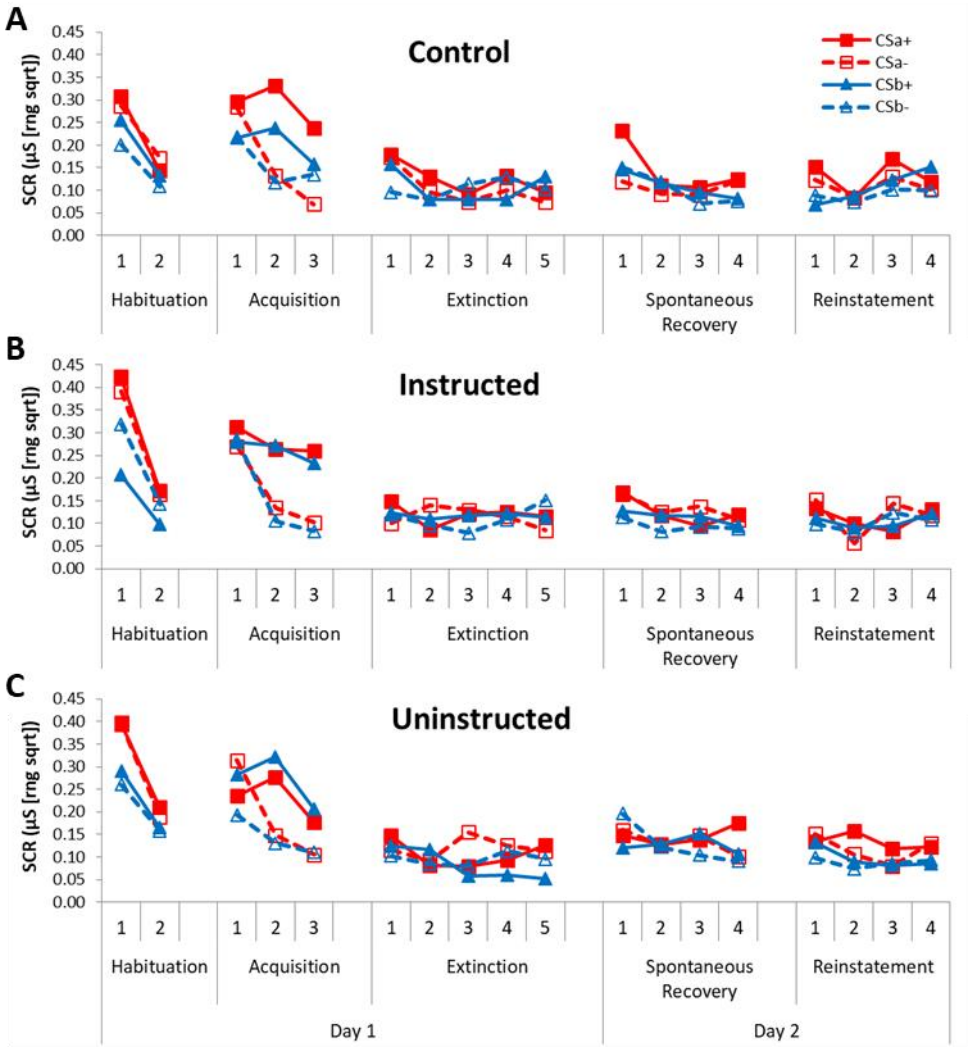


Figure 5.2. Mean skin conductance responses (SCRs) to fear-relevant (CSa+/-) and fear-irrelevant (CSb+/-) conditioned stimuli (CSs) in the control (**A**), instructed (**B**), and uninstructed (**C**) group. SCRs are presented in blocks of two consecutive trials.

5.4.2.2. Acquisition

Analysis of acquisition data revealed main effects of conditioning, $F(1, 93) = 45.16$, $p < .001$, $\eta p^2 = .33$, block, $F(2, 92) = 25.92$, $p < .001$, $\eta p^2 = .36$, and fear-relevance, $F(1, 93) = 4.55$, $p = .036$, $\eta p^2 = .05$, which were qualified by interactions of conditioning x block, $F(2, 92) = 15.75$, $p < .001$, $\eta p^2 = .26$, as well as fear-relevance x conditioning x group, $F(2, 93) = 5.71$, $p = .005$, $\eta p^2 = .11$. The remaining interactions did not attain significance, largest effect (fear-relevance x conditioning x block), $F(2, 92) = 1.81$, $p = .169$, $\eta p^2 = .04$. The significant conditioning x block interaction reflects acquisition of differential SCRs across blocks of training, showing differential SCRs on block 2 and 3, both $F(1, 93) \geq 36.36$, $p < .001$, $\eta p^2 \geq .28$, but not at the start of acquisition, on block 1, $F(1, 93) = 0.33$, $p = .570$, $\eta p^2 = .01$. However, an examination of the significant fear-relevance x conditioning x group interaction indicated that acquisition of differential SCRs differed across groups: There were no significant differential SCRs to fear-relevant CSs in the uninstructed group ($M = 0.04$, $SE = 0.03$) or to fear-irrelevant CSs in the control group ($M = 0.05$, $SE = 0.03$), both $F(1, 93) \leq 2.64$, $p \geq .108$, $\eta p^2 \leq .03$. As the divergent pattern of results reflected in the two-way and three-way interaction point to potential group differences in the acquisition of differential SCRs, follow-up comparisons were conducted with data from block 3 only, to test if the prerequisites for further testing had been met, being the acquisition of differential SCRs to both CS types at the end of acquisition training.

The results of a 3 (group) x 2 (fear-relevance) x 2 (conditioning) ANOVA revealed a main effect of conditioning, $F(1, 93) = 36.36$, $p < .001$, $\eta p^2 = .28$, which was qualified by a fear-relevance x conditioning x group interaction, $F(2, 93) = 3.35$, $p = .039$, $\eta p^2 = .07$. The interaction reflects differential SCRs to both CS types in the instructed (CSa: $M = 0.16$, $SE = 0.04$; CSb: $M = 0.15$, $SE = 0.05$) and uninstructed group (CSa: $M = 0.07$, $SE = 0.04$; CSb: $M = 0.10$, $SE = 0.05$), all $F(1, 93) \geq 4.24$, $p \leq .042$, $\eta p^2 \geq .04$. In the control group, differential SCRs were acquired to fear-relevant CSs ($M = 0.17$, $SE = 0.04$), $F(1, 93) = 22.99$, $p < .001$, $\eta p^2 = .20$, but not to fear-irrelevant CSs ($M = 0.02$, $SE = 0.05$), $F(1, 93) = 0.27$, $p = .606$, $\eta p^2 = .01$. While the absence of significant differential SCRs at the end of acquisition training does not necessarily reflect lack of fear acquisition (e.g., Holland & Rescorla, 1975; Lonsdorf et al., 2017), in particular when considering that participants who were included in the present analyses were able to verbalise the CS-US contingency, the lack of equal

acquisition of differential SCRs across groups poses a problem for the assessment of changes in differential SCRs as a function of US devaluation, extinction, spontaneous recovery, and reinstatement.

Despite the lack of equal acquisition of differential SCRs to fear-irrelevant CSs, all subsequent analyses have been conducted with the complete dataset and no performance-based acquisition criteria have been employed in the present paper. While performance-based acquisition criteria have been used in past research (e.g., Kindt & Soeter, 2013) to ensure that only data from participants who demonstrated successful acquisition of fear were included in the analyses, such selection criteria have the potential to introduce selection bias (Lonsdorf et al., 2017). However, as US devaluation is contingent on prior fear acquisition, one could argue that the use of performance-based selection criteria is warranted. As such, the interested reader is referred to the Supplementary Materials for supplementary analyses, conducted with data from only those participants who met acquisition criteria (for full details of selection criteria, see Supplementary Materials). In brief, despite including only those participants who exhibited differential SCRs to both CS types in the second half of acquisition training, conditioned responding did not differ as a function of US devaluation. Hence, the results of the supplementary analyses were in line with the pattern of results reported here.

5.4.2.3. Post-devaluation Reduction of Differential SCRs

To assess the effects of the US devaluation procedures on differential electrodermal responding, a 3 (group) x 2 (fear-relevance: fear-relevant vs. fear-irrelevant) x 2 (conditioning: CS+ vs. CS-) x 2 (time: acquisition block 3 vs. extinction block 1) ANOVA was conducted. Results revealed significant main effects of conditioning, $F(1, 93) = 31.89$, $p < .001$, $\eta p^2 = .26$ and time, $F(1, 93) = 4.92$, $p = .029$, $\eta p^2 = .05$. These main effects were qualified by significant interactions of conditioning x time, $F(1, 93) = 17.68$, $p < .001$, $\eta p^2 = .16$, and fear-relevance x conditioning x time x group, $F(2, 93) = 4.49$, $p = .014$, $\eta p^2 = .09$. The main effect of fear-relevance and remaining interactions did not attain significance, largest effect (main effect of fear-relevance), $F(1, 93) = 2.34$, $p = .129$, $\eta p^2 = .03$.

The significant four-way interaction reflects group differences in differential SCRs to fear-irrelevant CSs, but not to fear-relevant CSs: Differential SCRs to fear-relevant CSs were present on

block 3 of acquisition, all $F(1, 93) \geq 4.24$, $p \leq .042$, $\eta p^2 \geq .04$, but not on block 1 of extinction training, all $F(1, 93) \leq 1.99$, $p \geq .161$, $\eta p^2 \leq .02$, reflecting a decrease in differential SCRs to fear-relevant CSs between block 3 of acquisition and block 1 of extinction training across groups. Conversely, differential SCRs to fear-irrelevant CSs decreased in the instructed and uninstructed US devaluation groups, but increased in the control group, as reflected in significant differential SCRs to fear-irrelevant CSs on block 3 of acquisition in the US devaluation groups, both $F(1, 93) \geq 4.64$, $p \leq .034$, $\eta p^2 \geq .05$, but not in the control group, $F(1, 93) = 0.27$, $p = .606$, $\eta p^2 = .01$, as well as in the absence of significant differential SCRs to fear-irrelevant CSs on block 1 of extinction training in the US devaluation groups, both $F(1, 93) \leq 0.50$, $p \geq .479$, $\eta p^2 \leq .01$, but not in the control group, which exhibited an increase in differential SCRs, $F(1, 93) = 4.31$, $p = .041$, $\eta p^2 = .04$. However, due to the lack of fear acquisition to fear-irrelevant CSs in the control group, these results cannot be interpreted as reflecting effects of US devaluation.

5.4.2.4. Extinction of Differential SCRs

To assess extinction of differential SCRs across blocks of extinction training, a 3 (group) x 2 (fear-relevance) x 2 (CS type) x 5 (block: extinction block 1-5) ANOVA was conducted. Results yielded a significant main effect of block, $F(4, 90) = 2.60$, $p = .041$, $\eta p^2 = .10$, which was qualified by a significant fear-relevance x block x group interaction, $F(8, 182) = 2.22$, $p = .028$, $\eta p^2 = .09$. The remaining main effects and interactions did not attain significance, largest effect (main effect of fear-relevance), $F(1, 93) = 2.79$, $p = .098$, $\eta p^2 = .03$. The significant fear-relevance x block x group interaction reflects larger SCRs to fear-relevant than fear-irrelevant CSs+/- on block 1 of extinction training in the control group (CSa: $M = 0.18$, $SE = 0.03$; CSb: $M = 0.13$, $SE = 0.02$), $F(1, 93) = 4.64$, $p = .034$, $\eta p^2 = .05$. In the uninstructed group, the same pattern of results was evident on block 3 of extinction training (CSa: $M = 0.12$, $SE = 0.03$; CSb: $M = 0.07$, $SE = 0.02$), $F(1, 93) = 7.22$, $p = .009$, $\eta p^2 = .07$; there were no other significant differences between SCRs to fear-relevant and fear-irrelevant CSs+/-, all $F(1, 93) \leq 3.63$, $p \geq .060$, $\eta p^2 \leq .04$. Finally, the absence of a significant main effect of conditioning, $F(1, 93) = 0.04$, $p = .849$, $\eta p^2 < .01$, or significant interactions that may reflect differential SCRs across blocks of extinction training, such as the conditioning x block interaction,

$F(4, 90) = 1.81$, $p = .134$, $\eta p^2 = .07$, reflects an absence of differential SCRs during extinction training.

5.4.2.5. Spontaneous Recovery

Recovery of differential SCRs was assessed after a delay of 24 hours. A visual inspection of electrodermal data on block 1 of spontaneous recovery (Figure 5.2) suggests that differential SCRs to fear-relevant CSs recovered in the control group, but not in the instructed or uninstructed group. This observation was, however, not supported by the results of a 3 (group) x 2 (fear-relevance) x 2 (conditioning) x 2 (time: extinction block 5 vs. spontaneous recovery block 1) ANOVA. The results yielded a main effect of time, $F(1, 93) = 6.14$, $p = .015$, $\eta p^2 = .06$, as well as significant interactions of conditioning x group, $F(2, 93) = 4.33$, $p = .016$, $\eta p^2 = .09$, fear-relevance x conditioning, $F(1, 93) = 5.14$, $p = .026$, $\eta p2 = .05$, and fear-relevance x time x group, $F(2, 93) = 4.56$, $p = .013$, $\eta p2 = .09$. The remaining main effects and interactions did not attain significance, largest effect (fear-relevance x conditioning x time x group interaction), $F(2, 93) = 2.29$, $p = .107$, $\eta p^2 = .05$.

The significant conditioning x group interaction reflects overall larger SCRs to the CSs+ ($M = 0.15$, $SE = 0.02$) than to the CSs- ($M = 0.11$, $SE = 0.02$) in the control group, $F(1, 93) = 5.69$, $p = .019$, $\eta p^2 = .06$, but not in the uninstructed, $F(1, 93) = 3.14$, $p = .080$, $\eta p^2 = .03$, or instructed group, $F(1, 93) = 0.01$, $p = .911$, $\eta p^2 < .01$. Follow-up comparisons conducted for the significant fear-relevance x conditioning interaction failed to yield significant results, both $F(1, 93) \leq 3.37$, $p \geq .069$, $\eta p^2 \leq .04$. Finally, follow-up comparisons conducted for the significant fear-relevance x time x group interaction showed larger SCRs to fear-relevant CSs on the first block of spontaneous recovery ($M = 0.18$, $SE = 0.04$) than on the last block of extinction training ($M = 0.09$, $SE = 0.02$) in the control group, $F(1, 93) = 5.40$, $p = .022$, $\eta p^2 = .06$; in the uninstructed group, SCRs to fear-irrelevant CSs were larger on the first block of spontaneous recovery ($M = 0.16$, $SE = 0.03$) than on the last block of extinction training ($M = 0.07$, $SE = 0.02$), $F(1, 93) = 5.25$, $p = .024$, $\eta p^2 = .05$. In summary, these results indicate that conditioned responding differed across groups, but there were no significant interactions to indicate that differential SCRs to fear-relevant or fear-irrelevant CSs increased between the last block of extinction training and the first block of spontaneous recovery: conditioning x time x group, $F(2, 93) = 0.92$, $p = .404$, $\eta p^2 = .02$, fear-relevance x conditioning x time, $F(1, 93) = 0.20$, $p =$

.657, ηp^2 = .01; fear-relevance x conditioning x time x group, $F(2, 93) = 2.29$, $p = .107$, $\eta p^2 = .05$. Hence, the results indicate that differential SCRs to fear-relevant or fear-irrelevant CSs did not recover 24 hours after extinction training.

5.4.2.6. Reinstatement

In line with the results observed during tests of spontaneous recovery, statistical analyses of electrodermal data during tests of reinstatement showed no differences in SCRs as a function of conditioning or group: The results of a 3 (group) x 2 (fear-relevance) x 2 (conditioning) x 2 (time: spontaneous recovery block 4 vs. reinstatement block 1) ANOVA revealed only a main effect of fear-relevance, $F(1, 93) = 15.01$, $p < .001$, $\eta p^2 = .14$, which reflected larger SCRs to fear-relevant (M = 0.13, SE = 0.02) than to fear-irrelevant CSs (M = 0.10, SE = 0.01). The remaining main effects and interactions did not attain significance, largest effect (fear-relevance x conditioning x block x group interaction), $F(2, 93) = 1.46$, $p = .238$, $\eta p^2 = .03$. The present results indicate that differential SCRs were not reinstated in any of the groups subsequent to the presentation of three unsignaled USs, at the intensity employed during acquisition.

5.4.2.7. Post-hoc Comparisons: Unconditioned Response (UR)

Given the unexpected absence of group differences in differential SCRs subsequent to US devaluation-extinction or stand-alone extinction, further comparisons were conducted to test whether URs were reduced subsequent to US devaluation and whether these effects were evident during reinstatement, conducted on Day 2. Two repeated measures ANOVAs were conducted to test changes in unconditioned electrodermal responding between the last trial of acquisition training, the US devaluation phase, and the reinstatement trial. The respective URs were scored as the largest response starting in the 1 to 3 s window following US offset (Prokasy & Raskin, 1967) and were subsequently range corrected (Lykken, 1972) and square root transformed (Dawson et al., 2007). The largest UR displayed by the participant was used for the purpose of range correction.

A 2 (group: instructed vs. uninstructed US devaluation) x 4 (time: acquisition trial 6, US devaluation trial 1-3) repeated measures ANOVA was conducted to test changes in unconditioned electrodermal responding between the last trials of acquisition training and the US devaluation phase. The results yielded a main effect of time, $F(3, 60) = 11.48$, $p < .001$, $\eta p^2 = .37$, reflecting a decrease in

unconditioned electrodermal responding across trials (for means and standard deviations, see Table 5.2), but no significant group differences, as reflected in the non-significant time x group interaction, $F(3, 60) = 0.95$, $p = .422$, $\eta p^2 = .05$. These results, therefore, indicate that the US devaluation procedures reduced the perceived aversiveness of the US in both groups, as reflected in reduced electrodermal responding to the USs presented during the US devaluation phase.

Table 5.2

Means (M) and Standard Deviations (SD) for Unconditioned Skin Conductance Responses during Acquisition, US Devaluation, and Reinstatement

	Control		Instructed		Uninstructed	
	M	*SD*	*M*	*SD*	*M*	*SD*
Acquisition						
Trial 3	0.61	0.30	0.65	0.31	0.52	0.28
US devaluation						
Trial 1	-	-	0.52	0.35	0.49	0.32
Trial 2	-	-	0.39	0.30	0.39	0.32
Trial 3	-	-	0.34	0.26	0.35	0.28
Reinstatement						
Trial 1	0.90	0.15	0.71	0.28	0.80	0.24
Trial 2	0.77	0.24	0.72	0.25	0.76	0.24
Trial 3	0.80	0.16	0.66	0.22	0.72	0.24

Note. Unconditioned responses are presented in micro Siemens (square root transformed and range corrected).

Next, it was examined whether the reduction in unconditioned electrodermal responding was long-lasting. For this purpose, a 3 (group: instructed, uninstructed, control) x 4 (time: acquisition trial 6, reinstatement trial 1-3) repeated measures ANOVA was conducted. US devaluation effects would be reflected in a greater reduction of unconditioned responding in the US devaluation groups than in the control group. Furthermore, if prediction errors mediated effects of US devaluation, this would be reflected in greater reduction of the UR in the uninstructed than in the instructed US devaluation group. The results yielded a significant main effect of time, $F(3, 91) = 9.25$, $p < .001$, $\eta p^2 = .23$, which reflected larger unconditioned electrodermal responding at the end of acquisition training than during tests of reinstatement (for means and standard deviations, see Table 5.2). The time x group interaction, however, did not attain significance, $F(6, 184) = 1.93$, $p = .079$, $\eta p^2 = .06$, indicating absence of group differences in URs during reinstatement. These results further suggest that the

attenuation of unconditioned electrodermal responding that was observed during the US devaluation phase was not sustained during the delivery of reinstatement shocks (cf. Storsve et al., 2012).

5.4.3. CS Valence Ratings

A visual inspection of mean CS valence ratings (Figure 5.3) and differential negative evaluations (Figure 5.4) suggests that differential negative evaluations of the CSs+, relative to the CSs-, did not differ as a function of US devaluation. Statistical analyses confirmed this observation, showing that differential negative evaluations differed across experimental stages, but did not differ as a function of fear-relevance or group. Results of statistical analyses yielded a main effect of time, $F(5, 89) = 7.38$, $p < .001$, $\eta p^2 = .29$, but the main effect of fear-relevance and interactions did not attain significance, largest effect (fear-relevance x time interaction), $F(5, 89) = 2.21$, $p = .060$, $\eta p^2 = .11$. The significant main effect of time reflects larger differential negative evaluations after acquisition ($M = 0.93$, $SE = 0.22$) and after administration of the US devaluation (or control) procedures ($M = 0.64$, $SE = 0.16$) than at baseline ($M = -0.11$, $SE = 0.14$), after extinction ($M = 0.23$, $SE = 0.14$), after spontaneous recovery ($M = 0.22$, $SE = 0.13$), or after tests of reinstatement ($M = 0.23$, $SE = 0.12$). While differential negative evaluations differed across experimental stages, the absence of significant group interactions suggests that changes in valence ratings were not a function of US devaluation, largest group interaction (time x group), $F(10, 180) = 1.09$, $p = .373$, $\eta p2 = .06$.

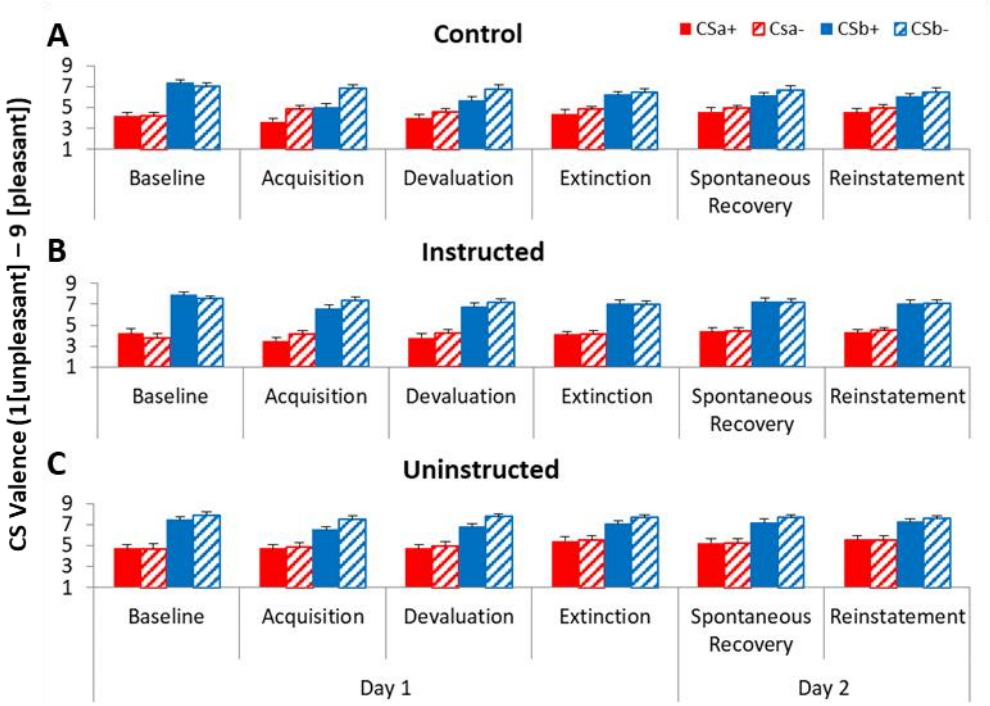


Figure 5.3. Mean conditioned stimulus (CS) valence ratings of fear-relevant (CSa+/-) and fear-irrelevant (CSb+/-) CSs in the control (**A**), instructed (**B**), and uninstructed (**C**) group. CS valence was rated on a scale ranging from 1 (unpleasant) to 9 (pleasant). Error bars represent standard errors.

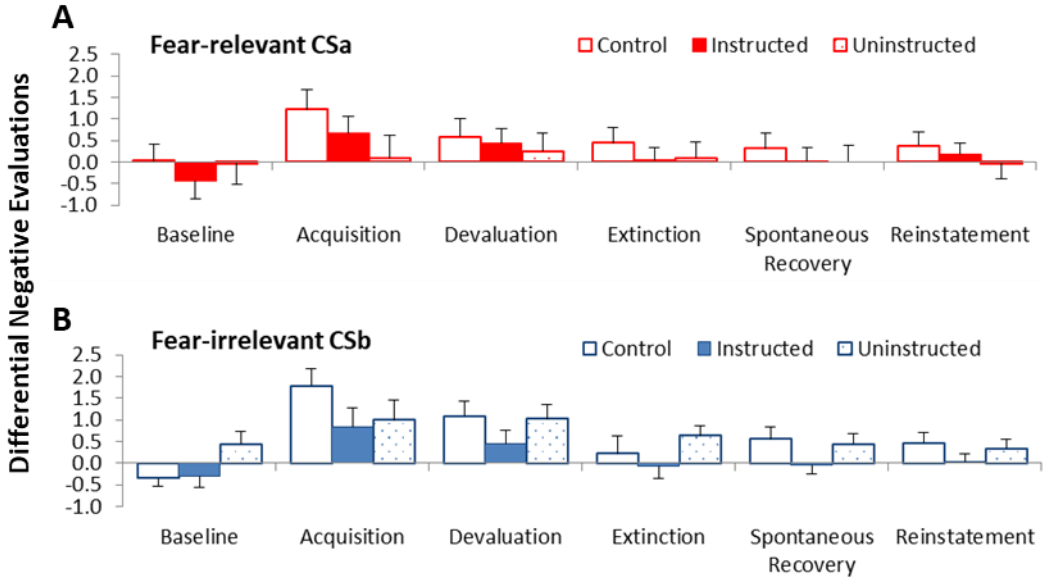


Figure 5.4. Mean differential negative evaluations of the CS+, relative to the CS-, for fear-relevant (**A**) and fear-irrelevant (**B**) conditioned stimuli (CSs). CS valence was rated on a scale ranging from 1 (unpleasant) to 9 (pleasant). Differential negative evaluations were calculated as the difference between CS- and CS+ valence ratings; larger values indicate more negative evaluations of the CS+, relative to the CS-. Error bars represent standard errors.

5.4.4. US Valence Ratings

Ratings of US valence (Figure 5.5) were obtained after the shock work-up procedure (i.e., baseline), after acquisition, after extinction, and after tests of reinstatement. In line with the pattern of US valence ratings presented in Figure 5.5, results of a 3 (group) x 4 (time) ANOVA yielded a main effect of time, $F(3, 91) = 37.79$, $p < .001$, $\eta p2 = .56$, reflecting more negative evaluations of US valence at baseline ($M = 3.13$, $SE = 0.14$) and after acquisition ($M = 3.41$, $SE = 0.17$) than after extinction training ($M = 5.74$, $SE = 0.25$) and after tests of reinstatement ($M = 5.41$, $SE = 0.24$). There were no group differences in evaluations of US valence, as reflected in the non-significant time x group interaction, $F(6, 184) = 1.00$, $p = .428$, $\eta p2 = .03$.

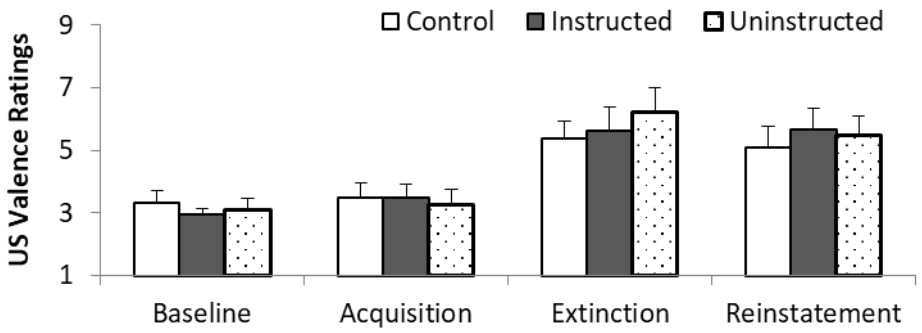


Figure 5.5. Mean unconditioned stimulus (US) valence ratings in the control, instructed and uninstructed group. US valence was rated on a scale ranging from 1 (unpleasant) to 9 (pleasant), at baseline, as well as after acquisition, extinction training, and tests of reinstatement. Error bars represent standard errors.

5.5. Discussion

The key aim of the present study was to examine whether long-lasting reduction of conditioned fear can be achieved through US devaluation and if these effects are mediated by prediction errors. The findings were as follows: Analysis of acquisition data revealed acquisition of differential SCRs to fear-relevant CSs across groups, but there was no acquisition of differential SCRs to fear-irrelevant CSs in the control group. Assessment of differential SCRs, conducted 10 minutes after the US devaluation phase, showed that differential SCRs to fear-irrelevant and fear-relevant CSs were eliminated in the instructed and uninstructed US devaluation groups. Differential SCRs were

also eliminated in the control group, but only to fear-relevant CSs, while differential SCRs to fear-irrelevant CSs increased between the last block of acquisition and the first block of extinction training. However, due to the absence of differential SCRs to both CS types at the end of acquisition training in the control group, these group differences cannot be interpreted as reflecting US devaluation effects.

Spontaneous recovery and reinstatement of differential SCRs were not observed in any of the groups. Furthermore, differential SCRs across experimental test phases did not differ between the instructed and uninstructed US devaluation groups, indicating that manipulating prediction errors during the US devaluation phase through the use of verbal instructions did not affect subsequent conditioned responding. With regards to the analysis of post-test CS valence ratings, whilst ratings differed across experimental stages, they did not differ across groups, indicating that CS valence ratings were not sensitive to US devaluation. As there were no differences in conditioned responding between the US devaluation groups and the control group, the present results indicate that US devaluation, followed by extinction training, did not result in enhanced reduction of fear, relative to extinction training conducted without prior US devaluation. As such, the hypotheses were not supported by the results of this study.

5.5.1. Skin Conductance Responses

The electrodermal results contrast those reported in past US devaluation research (Hosoba et al., 2001; Schultz et al., 2013; White & Davey, 1989). For instance, Schultz et al. (2013) reported that administration of three USs at reduced intensity subsequent to acquisition decreased SCRs to fear-irrelevant CSs, relative to a control group that received the same number of US presentations at the intensity used during acquisition. However, Schultz and colleagues reported using a shock intensity that was perceived as “painful, but tolerable.” For ethical reasons, and to minimise discomfort to participants, the shock intensity in the present study was set to a level that was perceived as “uncomfortable, but not painful.” It is conceivable that a higher shock intensity allowed for group differences to emerge, as the US devaluation procedure would have created a larger discrepancy between the US intensity used during acquisition and the US intensity employed during the US devaluation phase. Furthermore, a painful US intensity would also elicit larger SCRs to the CS+ than

a US intensity that was merely unpleasant (Rescorla & Wagner, 1972), thereby maximising differences in conditioned SCRs between the group that was expecting to receive further presentations of the painful US and the US devaluation group that would expect to receive the US at a reduced and, presumably, no longer painful intensity.

It should be also noted that Schultz et al. (2013) observed decreased US intensity ratings subsequent to US devaluation. While intensity ratings were not recorded in the present study, examination of US valence ratings showed more negative evaluations of US valence after the shock work-up procedure and after acquisition than after extinction training and reinstatement, in all groups. These results indicate that the mere absence of the US during extinction training may have reduced the perceived unpleasantness of the US (for similar findings, see Leer & Engelhard, 2015). While it is possible that the reduction in perceived US aversiveness, indexed by US valence ratings, accounted for the reduced recovery of differential SCRs across groups in the present study, past research indicates that ratings of US aversiveness do not necessarily mediate conditioned physiological responding (Leer, Haesen, & Vervliet, 2018): Leer and colleagues reported less renewal of fear potentiated startle, but not differential SCRs, after US habituation than after conventional extinction training; however, ratings of US aversiveness did not differ across groups. Similarly, research conducted in our laboratory showed that ratings of US valence obtained after extinction training, conducted with or without occasional presentations of the US, did not predict subsequent recovery of differential SCRs (see Chapter 3, results section).

These findings are of interest to the examination of mechanisms underlying fear reduction subsequent to US devaluation, as Davey and McKenna (1983) proposed that reduction of conditioned responding is contingent upon a more positive revaluation of the aversive US. Indeed, Davey and McKenna found reduced SCRs only in participants who showed a substantial increase in rated US valence; similar findings were reported by Hosoba et al. (2001), although the reduction of US aversiveness and SCRs was larger after US devaluation that involved US presentations at decreasing intensity than after US habituation. In saying that, the literature also indicates that US devaluation may reduce US aversiveness ratings, without the corresponding reduction in conditioned SCRs (Dibbets et al., 2018; Dibbets et al., 2012).

Due to these mixed findings, it is difficult to draw conclusions about the role of the subjective devaluation of US aversiveness in the reduction of conditioned physiological responding. There is also the possibility that the mixed findings reflect methodological limitations or boundary conditions of US devaluation. Aspects that should be examined in future research to gain a better understanding of factors underlying fear reduction through US devaluation include: (a) the optimal number of US presentations required to reduce subsequent conditioned responding (e.g., 70 US trials used by Poulos et al., 1979; vs. 3 trials used by Schultz et al., 2013); (b) whether a reduction of US intensity is required and if so, whether it should be reduced gradually (Hosoba et al., 2001) or instantaneously (Schultz et al., 2013); (c) which instruments or approaches are best suited to capture post-devaluation changes in perceived US aversiveness (cf. Leer & Engelhard, 2015; Leer et al., 2018); (d) comparison of the effectiveness of different US devaluation methods in the long-lasting reduction of conditioned fear (e.g., methods involving direct vs. indirect experience with the US), even though such comparisons may pose challenges to the selection of adequate control procedures; and (e) examination of fear recovery after US devaluation conducted with (Dibbets et al., 2012; Leer & Engelhard, 2015; Leer et al., 2013) and without (Schultz et al., 2013) extinction training.

Further examination of US devaluation that is conducted in conjunction with extinction training would be of particular importance for the treatment of fears, as exposure-based therapies for anxiety disorders involve real or imaginal exposure to the CS *and* the US (e.g., Craske et al., 2014; Scheveneels, Boddez, Vervliet, & Hermans, 2016; Vervliet, Craske, & Hermans, 2013) and recent human fear conditioning research suggests that extinction training which involves CS and US presentations leads to superior reduction of fear recovery, relative to conventional extinction training (Culver, Stevens, Fanselow, & Craske, 2018; Leer et al., 2018; Thompson et al., 2018; Vervliet, Vansteenwegen, & Hermans, 2010). At the same time, the findings of the present study showed no differences in fear recovery between conventional extinction training and extinction training that was preceded by US devaluation.

The absence of group differences during tests of fear recovery was unexpected, given the well-documented recovery from extinction phenomena that occur subsequent to CS-only extinction (Bouton, 2000, 2002). Nonetheless, it has been proposed that CS-only extinction contains aspects of

US devaluation, whereby the presentation of the CS is proposed to activate the mental representation of the US (Rescorla & Heth, 1975). The repeated mental activation of the US memory is akin to imaginal US devaluation (e.g., Dibbets et al., 2018) and, as such, could result in the devaluation of the US. Based on this proposition, all participants in the present study would have undergone US devaluation, which might have abolished the recovery of extinguished responding. Although lack of group differences in conditioned SCRs has also occurred in previous US devaluation research (Dibbets et al., 2012; Leer et al., 2018), it would be difficult to argue that these effects were caused by an extinction-driven devaluation of the mental representation of the US.

First, if conditioned responding in the extinction only group decreased as a result of US devaluation, reinstatement of differential responding should have occurred on Day 2. This is because the post-devaluation presentation of three unsignaled USs at the intensity used during acquisition should result in US inflation and increase differential SCRs (Rescorla & Heth, 1975). Differential responding was, however, not reinstated in any of the groups – an outcome that requires further examination. Second, past research showed that return of fear was larger after extinction training than after US devaluation (Haesen & Vervliet, 2015; Leer et al., 2018 [fear potentiated startle]). These differences in levels of fear recovery subsequent to US devaluation procedures and CS-only extinction training do not offer support for the proposition that conventional CS-only extinction training devalues the aversiveness of the US. Finally, recovery of extinguished responding is a well-documented phenomenon (Bouton, 2002) and has been observed in previous research conducted in our laboratory, utilising a similar experimental paradigm (Chapter 4). Hence, it appears unlikely that the absence of fear recovery in the control group was mediated by extinction-driven US devaluation.

Alternatively, the absence of fear recovery may indicate that the administration of extinction training shortly after acquisition interfered with the consolidation (Dudai et al., 2015) of fear learning. Extant literature indicates that extinction training can reduce recovery of extinguished responding when it is delivered 10 minutes after acquisition, but not when delivered after a delay of 72 hours (Myers, Ressler, & Davis, 2006; Norrholm et al., 2008), suggesting that extinction training delivered in close temporal proximity to acquisition may interfere with the consolidation of fear learning. Conversely, other investigations reported the opposite pattern of results, namely recovery of

extinguished responding after immediate, but not after delayed, extinction training (Huff, Hernandez, Blanding, & LaBar, 2009; Merz, Hamacher-Dang, & Wolf, 2016; Schiller et al., 2008). Taken together, it appears that immediate extinction does not necessarily interfere with the consolidation of fear learning (for a review, see Maren, 2014), yet the absence of spontaneous recovery and reinstatement of differential SCRs in the present study is consistent with previous research showing deficits in fear memory consolidation subsequent to immediate extinction training (Myers et al., 2006; Norrholm et al., 2008). Whether consolidation was impaired through extinction training *per se* is debatable, as differential SCRs did not generalise from the acquisition to the extinction context in any of the groups.

Whilst lack of fear generalisation from the acquisition to the extinction context has been observed in previous US devaluation research (Haesen & Vervliet, 2015; Leer et al., 2018), it did not affect renewal of conditioned responding. In the present study, no explicit change in context was introduced after acquisition, however, it could be argued that the break between acquisition and extinction training was perceived as a change in context (Lonsdorf et al., 2017) or, more precisely, a change in temporal context (Bouton, 2002). However, fear acquisition, as opposed to extinction learning, is relatively context-independent (Dunsmoor & Paz, 2015), as such, it is not clear why a change in context should abolish differential SCRs. Further, past research employed breaks between experimental phases (Thompson et al., 2018) and used filler tasks (Schultz et al., 2013) without subsequent loss of differential SCRs. Therefore, it is unlikely that the 10-minute break between US devaluation and extinction training eliminated differential SCRs. Taken together, the present electrodermal results are indeed difficult to reconcile with published literature and will require further examination in future work.

5.5.2. CS Valence Ratings

Results of CS valence ratings are in line with those reported by Hosoba et al. (2001), who found no effects of US devaluation on post-test ratings of CS valence. In contrast, findings from an evaluative conditioning study conducted in our laboratory (Jensen-Fielding et al., 2017) suggest otherwise: Evaluations of geometrical shapes (CSs) that had been paired with happy or angry faces (USs) were found to be sensitive to US revaluation conducted through the use of verbal instructions.

Similar findings were reported in previous evaluative conditioning research (Walther, Gawronski, Blank, & Langer, 2009), indicating that CS valence may be modified through US revaluation. However, the methodological differences between fear conditioning paradigms involving pairings of CSs with aversive USs, such as electric shocks, and evaluative conditioning protocols involving pairings of neutral and positively/negatively valanced pictures do not readily permit for a comparison of findings. To get a better understanding of US devaluation effects on CS valence ratings in human fear conditioning, future research specifically designed to examine changes in CS valence as a result of US devaluation could examine whether online valence ratings, in lieu of the presently employed post-test ratings, might be more sensitive to US devaluation effects (for a comparison of post-test and online valence ratings, see Lipp, Oughton, & LeLievre, 2003).

5.5.3. Limitations

One of the main limitations of this study was the lack of equal acquisition of differential SCRs to both CS types across groups. As such, it was not possible to examine whether US devaluation differentially affects fear conditioned to fear-relevant and fear-irrelevant CSs. This lack of fear acquisition was unexpected, given the use a strong conditioning protocol, whereby each CS+ presentation was followed by the electrotactile US. Furthermore, fear acquisition to both CS types was observed in a previous study conducted in our laboratory, utilising a comparable acquisition protocol (Chapter 4). It is important to note, however, that the lack of equal fear acquisition across participants is not uncommon (e.g., Kindt & Soeter, 2013; Schiller et al., 2010), although presently it is not known which exact factors contribute to inter-individual differences in fear acquisition (for a discussion of individual difference factors, see Lonsdorf et al., 2017; Lonsdorf & Merz, 2017).

An additional limitation pertains to the use of verbal instructions to manipulate the degree of prediction error generated by the US devaluation procedures. The instructions participants received during the US devaluation phase may have failed to generate the desired level of prediction error. Specifically, the aim was to minimise prediction errors in the instructed US devaluation and in the control group by informing participants of the nature, type, and frequency of stimuli to be presented (or the absence of stimuli [control group]). Participants in the uninstructed group, on the other hand, were merely informed that some stimuli would be presented, but were not given any further details.

Manipulation checks indicated that the vast majority of participants believed these instructions. Based on prior learning (i.e., CS-US pairings during acquisition), participants in the uninstructed group should have experienced the greatest discrepancy between actual (i.e., presentation of an unsignaled US, at reduced intensity) and expected events (i.e., further CS-US pairings) and, consequently, the greatest degree of prediction error. At the same time, it is possible that participants in the instructed group experienced some degree of mismatch between expected and actual US aversiveness during the US devaluation phase, due to lack of prior experience with the reduced US intensity. Whether such a prediction error would be sufficient to enhance learning during the US devaluation phase could be examined in future research.

This being said, examination of URs during the US devaluation phase confirmed that both US devaluation procedures resulted in the devaluation of US aversiveness, as reflected in decreased unconditioned electrodermal responding between the last trial of acquisition training and the subsequent US devaluation phase. These results suggest that US devaluation was effective in the reduction of electrodermal responding. However, because electrodermal responding in the US devaluation groups did not significantly differ from that observed in the control group, this decrease in electrodermal responding cannot be readily attributed to US devaluation effects and requires further examination in future research.

Finally, in contrast to past US devaluation research (e.g., Schultz et al., 2013), US expectancy ratings were not recorded in the present study. This was done due to (a) the majority of past research indicating that US expectancy ratings are not sensitive to US devaluation, as reflected in the double dissociation between US expectancy ratings and physiological indices of conditioned responding (e.g., Leer et al., 2018; Schultz et al., 2013), and (b) due to the results of pilot testing conducted prior to this study, which showed that the operation of a physical US expectancy scale (Variable Assessment Transducer; Biopac Systems), interfered with participants' attention to the computer screen. Perhaps the use of a digital US expectancy scale, which is presented on the computer screen (pending availability), would reduce some confounds that might be introduced by other types of instruments.

5.5.4. Conclusion

In line with previous US devaluation research, the present study has raised more questions than it has answered. A pertinent one is that of boundary conditions of US devaluation, as the results indicate that conditioned responding was not a function of US devaluation. These findings contrast those observed in previous research that used a similar US devaluation procedure (Schultz et al., 2013). Furthermore, because post-devaluation conditioned responding in the present US devaluation groups did not differ from conditioned responding in the control group, it is difficult to draw conclusions about the effects of US devaluation on fear recovery, or to determine whether the effects of US devaluation are mediated by prediction errors. Another question arising from this research is that of determinants of human fear learning. The lack of fear acquisition to fear-irrelevant CSs in the control group indicates that there are factors beyond US devaluation that can explain why some individuals do not develop fears or anxiety disorders subsequent to CS-US pairings (see also Lonsdorf & Merz, 2017). As the present results indicate, pairings of CSs with unpleasant electric shocks are not sufficient to establish a robust fear response. Hence, the lack of fear acquisition to fear-irrelevant CSs and the absence of group differences during extinction training, spontaneous recovery, and reinstatement tests mean that no definitive conclusions can be made about the role of US devaluation or prediction errors in the persistent reduction of fear to fear-irrelevant or fear-relevant CSs.

Chapter 5: Study 3 – Supplementary Materials

5.6. Materials and Methods

5.6.1. Fear Acquisition Criteria

Data from only those participants who exhibited successful acquisition of differential skin conductance responses (SCRs) to fear-relevant *and* fear-irrelevant conditioned stimuli (CSs) have been included in the present statistical analyses. Acquisition of differential SCRs was determined based on differential electrodermal responding during the second half of acquisition (trials 4 to 6). Successful acquisition of conditioned responding was defined as a differential SCR ≥ 0.01 micro Siemens (µS; for response thresholds and acquisition criteria employed in other laboratories, see Kredlow, Orr, & Otto, 2018b; Lonsdorf et al., 2017; Schiller et al., 2018). Differential SCRs were calculated for each CS type (fear-relevant and fear-irrelevant) by subtracting the mean SCR to the CS- from the mean SCR to the CS+; calculations were performed on raw, non-transformed data. It should be noted that no agreed-upon conventions exist for the definition of successful acquisition of differential responding and that the use of performance-based inclusion and exclusion criteria differs across laboratories (for a review, see Lonsdorf et al., 2017).

The present acquisition criteria are based on theoretical and methodological considerations, being that (a) acquisition of conditioned responding is the result of pairings of the CS with the unconditioned stimulus (US) and (b) that acquisition of fear in a differential fear conditioning paradigm is reflected in larger responding to the reinforced CS+ than to the non-reinforced CS- (e.g., Lipp, 2006a). Given the 100 % reinforcement schedule employed during acquisition and the results from previous work conducted in our laboratory (e.g., Thompson & Lipp, 2017), acquisition of differential SCRs has been assessed on trials during which robust conditioning effects are expected to emerge, being trials during the second half of acquisition.

5.6.2. Statistical Analyses and Interpretation of Findings

The statistical analyses employed here are identical to those used in the primary analysis of data. It should be noted that results from the present supplementary analyses must be interpreted with caution, as the small sample size may increase the influence of individual differences and of unusually

large values on conditioned responding. Another aspect that should be acknowledged is that performance-based inclusion criteria, such as the one employed here, may result in selection bias, as participants who were excluded may differ from those who met inclusion criteria (Lonsdorf et al., 2017; Lonsdorf & Merz, 2017). Preliminary analyses indicated that this proposition is supported by the present data. Examination of individual difference factors in the present sample showed that participants who met inclusion criteria exhibited larger baseline electrodermal responding and more negative evaluations of the US than participants who did not meet inclusion criteria (Table S5.1). At present, the influence of individual differences on indices of conditioned responding is still poorly understood (Lonsdorf & Merz, 2017), hence, the present results should be interpreted with caution.

Table S5.1

Included vs. Excluded Participants: Means (M) and Standard Deviations (SD) for Age, US Intensity, Baseline Valence Ratings (VR), Baseline Electrodermal Activity (EDA), and Questionnaires

	Included (n = 42)		Excluded (n = 54)		
	M	*SD*	*M*	*SD*	Test
Age	23.76	7.93	24.78	6.47	$t(94) = -0.69$, $p = .491$
US intensity	57.90	13.26	58.91	13.20	$t(94) = -0.37$, $p = .713$
Baseline VR					
CSa	4.37	1.78	4.29	1.85	$t(94) = 0.22$, $p = .827$
CSb	7.35	1.78	7.69	1.22	$t(94) = -1.11$, $p = .269$
US	2.79	1.26	3.39	1.31	**$t(94) = -2.28$, $p = .025$**
Baseline EDA	12.86	9.12	8.31	7.58	**$t(94) = 2.66$, $p = .009$**
SPQ	8.12	5.54	9.30	6.00	$t(94) = -0.99$, $p = .326$
SNAQ	9.45	6.95	10.83	7.35	$t(94) = -0.94$, $p = .352$
IUS-12					
Prospective	20.00	5.49	19.89	4.87	$t(94) = 0.11$, $p = .917$
Inhibitory	10.55	4.72	10.35	3.86	$t(94) = 0.22$, $p = .824$
DASS-21					
Depression	7.05	7.77	6.41	5.36	$t(94) = 0.48$, $p = .634$
Anxiety	7.00	8.09	6.74	5.00	$t(94) = 0.19$, $p = .847$
Stress	10.90	8.10	9.56	6.18	$t(94) = 0.93$, $p = .357$

Note. CSa = fear-relevant conditioned stimulus; CSb = fear-irrelevant conditioned stimulus; US = unconditioned stimulus; SPQ = Spider Phobia Questionnaire; SNAQ = Snake Phobia Questionnaire; IUS-12 = Intolerance of Uncertainty Scale (short version); DASS-21 = Depression, Anxiety, and Stress Scales (short version). Baseline EDA refers to the number of spontaneous responses that occurred during a 2-minute rest period. US intensity is reported in Volts.

5.7. Results

5.7.1. Preliminary Analyses

The groups did not differ in age, selected US intensity, baseline CS and US valence ratings, self-reported snake or spider fear, IUS-12 scores, or baseline electrodermal activity (Table S5.2). The female-to-male ratio did not differ across groups (control: 9:5; instructed: 8:5; uninstructed: 10:5), χ^2 (2, N = 42) = 0.08, p = .961. Selected US intensities ranged from 28 to 80 Volts, with a mean of 57.90 Volts (SD = 13.26).

Table S5.2

Included Participants: Means (M) and Standard Deviations (SD) for Age, US Intensity, Baseline Valence Ratings (VR), Baseline Electrodermal Activity (EDA), and Questionnaires

	Control (n = 14)		Instructed (n = 15)		Uninstructed (n = 13)		
	M	*SD*	*M*	*SD*	*M*	*SD*	Test
Age	24.93	10.12	21.60	4.84	25.00	8.21	$F(2,39)=0.86$, $p=.430$
US intensity	54.71	16.53	59.07	11.07	60.00	11.99	$F(2,39)=0.61$, $p=.547$
Baseline VR							
CSa	4.46	1.66	4.13	1.90	4.54	1.86	$F(2,39)=0.20$, $p=.817$
CSb	6.86	2.16	7.57	1.71	7.62	1.37	$F(2,39)=0.79$, $p=.462$
US	3.14	1.56	2.40	0.74	2.85	1.34	$F(2,39)=1.30$, $p=.284$
Baseline EDA	10.14	8.03	13.07	9.63	15.54	9.48	$F(2,39)=1.20$, $p=.313$
SPQ	6.50	3.44	8.80	4.89	9.08	7.71	$F(2,39)=0.90$, $p=.414$
SNAQ	9.50	6.30	8.87	6.21	10.08	8.72	$F(2,39)=0.10$, $p=.904$
IUS-12							
Prospective	20.21	5.38	19.53	5.13	20.31	6.36	$F(2,39)=0.08$, $p=.922$
Inhibitory	10.93	3.58	9.93	4.74	10.85	5.94	$F(2,39)=0.19$, $p=.827$
DASS-21							
Depression	5.57	7.73	7.60	8.32	8.00	7.53	$F(2,39)=0.38$, $p=.689$
Anxiety	6.71	6.01	4.27	3.92	10.46	12.00	$F(2,39)=2.17$, $p=.127$
Stress	10.71	6.35	10.13	7.50	12.00	10.61	$F(2,39)=0.18$, $p=.833$

Note. CSa = fear-relevant conditioned stimulus; CSb = fear-irrelevant conditioned stimulus; US = unconditioned stimulus; SPQ = Spider Phobia Questionnaire; SNAQ = Snake Phobia Questionnaire; IUS-12 = Intolerance of Uncertainty Scale (short version); DASS-21 = Depression, Anxiety, and Stress Scales (short version). Baseline EDA refers to the number of spontaneous responses that occurred during a 2-minute rest period. US intensity is reported in Volts.

5.7.2. Electrodermal Responding

5.7.2.1. Habituation

Electrodermal responding across groups is presented in Figure S5.1. Analysis of electrodermal data during habituation yielded main effects of fear-relevance, $F(1, 39) = 20.01$, $p <$

.001, ηp^2 = .34, and block, $F(1, 39) = 45.69$, $p < .001$, ηp^2 = .54, which were qualified by a fear-relevance x block interaction, $F(1, 39) = 8.20$, $p = .007$, ηp^2 = .17. The main effect of conditioning and remaining interactions did not attain significance, largest effect (fear-relevance x block x group interaction), $F(2, 39) = 2.42$, $p = .102$, ηp^2 = .11. In line with the pattern of results presented in Figure S5.1, the significant interaction reflects larger SCRs to fear-relevant CSs than to fear-irrelevant CSs on block 1, $F(1, 39) = 24.20$, $p < .001$, ηp^2 = .38, but not on block 2 of habituation, $F(1, 39) = 3.01$, $p = .091$, ηp^2 = .07, showing that electrodermal responding did not differ as a function of fear-relevance at the end of habituation.

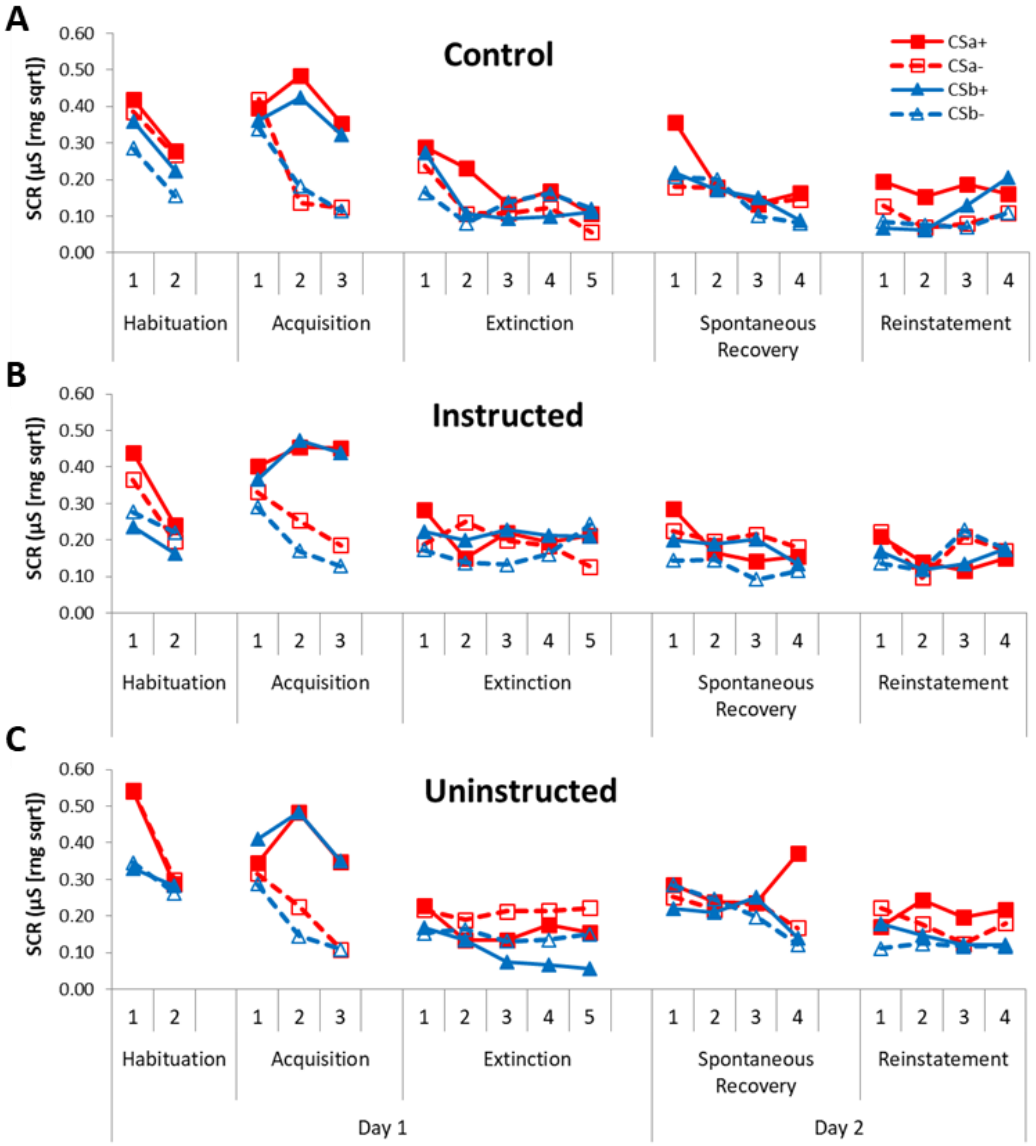


Figure S5.1. Mean skin conductance responses (SCRs) to fear-relevant (CSa+/-) and fear-irrelevant (CSb+/-) conditioned stimuli (CSs) in the control (**A**), instructed (**B**), and uninstructed (**C**) group. SCRs are presented in blocks of two consecutive trials.

5.7.2.2. Acquisition

Analysis of acquisition data revealed main effects of conditioning, $F(1, 39) = 117.44$, $p < .001$, $\eta p^2 = .75$, and block, $F(2, 38) = 11.21$, $p < .001$, $\eta p^2 = .37$, which were qualified by a conditioning x block interaction, $F(2, 38) = 28.95$, $p < .001$, $\eta p^2 = .60$. The main effect of fear-relevance and remaining interactions did not attain significance, largest effect (main effect of fear-relevance), $F(1, 39) = 2.20$, $p = .146$, $\eta p^2 = .05$. The significant two-way interaction reflects a different pattern of responding to the reinforced and non-reinforced CSs, more specifically larger responding to the reinforced CSa/b+ on block 2 than on block 1 and 3, $F(2, 38) = 6.44$, $p = .004$, $\eta p^2 = .25$, but a decrease in responding to the non-reinforced CSa/b- across blocks of acquisition, $F(2, 38) = 25.91$, $p < .001$, $\eta p^2 = .58$. Significant differential responding was evident on all blocks of acquisition, all $F(1, 39) \geq 5.91$, $p \leq .020$, $\eta p^2 \geq .13$, providing evidence of fear acquisition to fear-relevant and fear-irrelevant CSs. The absence of a significant group interaction further indicates that acquisition of differential SCRs did not significantly differ across groups, largest group interaction (fear-relevance x conditioning x group), $F(2, 39) = 0.81$, $p = .454$, $\eta p^2 = .04$.

5.7.2.3. Post-devaluation Reduction of Differential SCRs

To assess the effects of the US devaluation procedures on differential electrodermal responding, a 3 (group) x 2 (fear-relevance: fear-relevant vs. fear-irrelevant) x 2 (conditioning: CS+ vs. CS-) x 2 (time: acquisition block 3 vs. extinction block 1) ANOVA was conducted. Results revealed a significant main effect of conditioning, $F(1, 39) = 65.09$, $p < .001$, $\eta p^2 = .63$, which was qualified by a conditioning x time interaction, $F(1, 39) = 40.57$, $p < .001$, $\eta p^2 = .51$. The interaction reflects a decrease of differential SCRs between block 3 of acquisition ($M = 0.25$, $SE = 0.02$) and block 1 of extinction training ($M = 0.06$, $SE = 0.02$), $t(41) = 6.40$, $p < .001$, $d = 1.24$. However, the absence of a significant group interaction indicates that this decrease in conditioned responding was not a function of the US devaluation procedures; largest group interaction (fear-relevance x conditioning x time x group), $F(2, 39) = 1.18$, $p = .319$, $\eta p^2 = .06$. Finally, the results also yielded a main effect of fear-relevance, $F(1, 39) = 4.45$, $p = .041$, $\eta p^2 = .10$, reflecting larger SCRs to fear-relevant ($M = 0.25$, $SE = 0.03$) than to fear-irrelevant CSs ($M = 0.22$, $SE = 0.02$). The main effect of

time and remaining interactions did not attain significance, largest effect (main effect of time), $F(1, 39) = 3.85$, $p = .057$, $\eta p^2 = .09$.

5.7.2.4. Extinction of Differential Responding

To assess extinction of differential SCRs across blocks of extinction training, a 3 (group) x 2 (fear-relevance) x 2 (conditioning) x 5 (block: extinction block 1-5) ANOVA was conducted. Results yielded a main effect of fear-relevance, $F(1, 39) = 4.66$, $p = .037$, $\eta p^2 = .11$, reflecting overall larger SCRs to fear-relevant ($M = 0.18$, $SE = 0.03$) than to fear-irrelevant CSs ($M = 0.15$, $SE = 0.02$). The results also yielded a significant conditioning x group interaction, $F(2, 39) = 5.57$, $p = .007$, $\eta p^2 = .22$. The remaining main effects and interactions did not attain significance, largest effect (main effect of block), $F(4, 36) = 2.37$, $p = .071$, $\eta p^2 = .21$. Follow-up comparisons conducted for the significant conditioning x group interaction showed that participants in the uninstructed group exhibited larger SCRs to the non-reinforced CSs- ($M = 0.18$, $SE = 0.04$) than to the previously reinforced CSs+ ($M = 0.13$, $SE = 0.04$), $F(1, 39) = 5.57$, $p = .023$, $\eta p^2 = .13$. There were no significant differences between SCRs to the CSs- and CSs+ in the control or instructed groups, both $F(1, 39) \leq 3.40$, $p \geq .073$, $\eta p^2 \leq .08$. Finally, the absence of a significant main effect of conditioning or conditioning x block (x group) interaction, all Fs ≤ 1.15, $p \geq .348$, $\eta p^2 \leq .11$, indicates that there were no significant differences between SCRs to the CSs+ and the CSs-, meaning there was no evidence of differential responding during extinction training.

5.7.2.5. Spontaneous Recovery

A visual inspection of Figure S5.1, spontaneous recovery (block 1), suggests that differential SCRs to fear-relevant CSs recovered in the control group, but not in the instructed or uninstructed group. This observation was, however, not supported by the results of statistical analyses. The results yielded a significant main effect of time, $F(1, 39) = 5.89$, $p = .020$, $\eta p^2 = .13$, which was qualified by a fear-relevance x time x group interaction, $F(2, 39) = 4.08$, $p = .025$, $\eta p^2 = .17$. The interaction reflects an increase in SCRs to fear-relevant CSs from the last block of extinction training ($M = 0.08$, $SE = 0.05$) to the first block of spontaneous recovery ($M = 0.27$, $SE = 0.06$), $F(1, 39) = 7.11$, $p = .011$, $\eta p^2 = .15$, in the control group. In the uninstructed group, SCRs to fear-irrelevant CSs increased from the last block of extinction training ($M = 0.10$, $SE = 0.04$) to the first block of spontaneous recovery ($M =$

0.25, *SE* = 0.06), $F(1, 39) = 4.29$, $p = .045$, $\eta p^2 = .10$. There was no change in responding to fear-relevant or fear-irrelevant CSs in the instructed group, both $F(1, 39) \leq 1.54$, $p \geq .222$, $\eta p^2 \leq .04$.

The results further yielded significant interactions of conditioning x group, $F(2, 39) = 3.93$, $p = .028$, $\eta p^2 = .17$, and fear-relevance x conditioning, $F(1, 39) = 4.85$, $p = .034$, $\eta p^2 = .11$, as well as a trend towards significance in the conditioning x time interaction, $F(1, 39) = 3.94$, $p = .054$, $\eta p^2 = .09$. The remaining main effects and interactions did not attain significance, largest effect (fear-relevance x time interaction), $F(1, 39) = 3.04$, $p = .089$, $\eta p^2 = .07$. While the significant interactions reflect group differences in electrodermal responding, they do not provide evidence of group differences in the recovery of differential responding. Group differences in fear recovery would be reflected in interactions of conditioning, time, group, and, potentially, fear-relevance. As the respective interactions were not significant, it can be concluded that spontaneous recovery of extinguished differential responding did not differ across groups: conditioning x time x group, $F(2, 39) = 0.19$, $p = .829$, $\eta p^2 = .01$; fear-relevance x conditioning x time x group, $F(2, 39) = 1.09$, $p = .348$, $\eta p^2 = .05$. The small effect sizes ($\eta p^2 \leq .05$) indicate that it is unlikely that failure to detect group differences is the result of low statistical power.[7]

5.7.2.6. Reinstatement

Analysis of reinstatement data yielded a main effect of fear-relevance, $F(1, 39) = 18.51$, $p < .001$, $\eta p^2 = .32$ and a fear-relevance x conditioning x time x group interaction, $F(2, 39) = 3.53$, $p = .039$, $\eta p^2 = .15$. The remaining main effects and interactions did not attain significance, largest effect (main effect of conditioning), $F(1, 39) = 1.78$, $p = .190$, $\eta p^2 = .04$. The significant interaction reflects a decrease in differential SCRs to fear-relevant CSs from the last block of spontaneous recovery to the first block of tests of reinstatement in the uninstructed group, $F(1, 39) = 7.89$, $p = .008$, $\eta p^2 = .17$. There were no other significant changes in differential responding, all $F(1, 39) \leq 1.52$, $p \geq .225$, $\eta p^2 \leq .04$. As the differential responding on the last block of tests of spontaneous recovery may have masked group differences in differential responding on the first block of reinstatement, follow-up

[7] A 3 (group) x 2 (fear-relevance) x 2 (conditioning) ANOVA conducted with data from the first block of spontaneous recovery yielded a similar pattern of results, showing a main effect of fear-relevance, $F(1, 39) = 4.57$, $p = .039$, $\eta p^2 = .11$, but no other significant main effects or interactions.

comparisons were conducted to assess differential responding on the first block of reinstatement. The results of the 3 (group) x 2 (fear-relevance) x 2 (conditioning) ANOVA yielded a main effect of fear-relevance, $F(1, 39) = 16.22$, $p < .001$, $\eta p^2 = .29$, reflecting larger SCRs to fear-relevant ($M = 0.19$, $SE = 0.03$) than to fear-irrelevant CSs ($M = 0.13$, $SE = 0.02$). The remaining main effects and interactions did not attain significance, largest effect (fear-relevance x conditioning x group interaction), $F(2, 39) = 1.60$, $p = .216$, $\eta p^2 = .08$. In line with the pattern of results obtained from the analysis of spontaneous recovery data, results of reinstatement testing showed no significant reinstatement of differential SCRs in any of the groups.

5.7.3. CS Valence Ratings

In line with a visual inspection of mean CS valence ratings in Figure S5.2 and the differential negative evaluations of the CS+, relative to the CS-, presented in Figure S5.3, the ANOVA results showed overall larger differential negative evaluations of fear-irrelevant CSs ($M = 0.54$, $SE = 0.17$) than fear-relevant CSs ($M = -0.16$, $SE = 0.27$), main effect of fear-relevance, $F(1, 39) = 6.69$, $p = .014$, $\eta p^2 = .15$, but no significant group differences in differential negative evaluations: fear-relevance x group, $F(2, 39) = 0.49$, $p = .615$, $\eta p^2 = .03$; time x group, $F(10, 72) = 0.72$, $p = .707$, $\eta p^2 = .09$, fear-relevance x time x group, $F(10, 72) = 0.26$, $p = .988$, $\eta p^2 = .04$. The fear-relevance x time interaction was also non-significant, $F(5, 35) = 0.73$, $p = .607$, $\eta p^2 = .09$, although there was a significant main effect of time, $F(5, 35) = 3.40$, $p = .013$, $\eta p^2 = .33$. This main effect reflects overall larger differential negative evaluations after acquisition ($M = 0.77$, $SE = 0.33$) than at baseline ($M = -0.25$, $SE = 0.18$), $t(41) = 3.58$, $p = .001$, $d = 1.61$. Therefore, analysis of CS valence ratings showed that differential negative evaluations did not differ across groups.

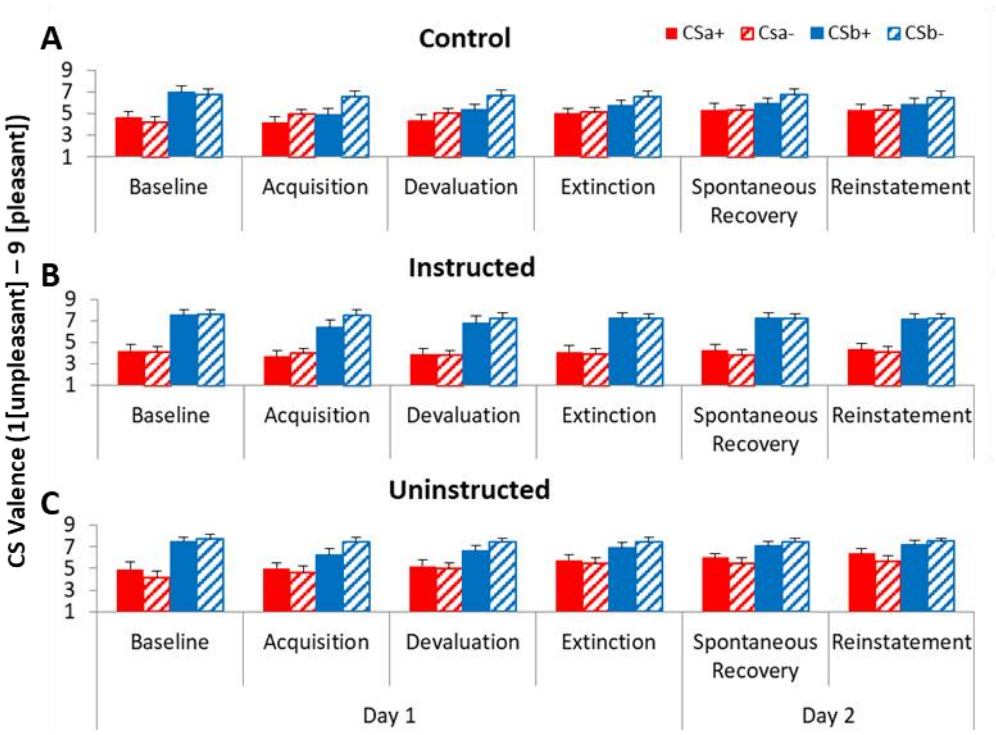


Figure S5.2. Mean conditioned stimulus (CS) valence ratings of fear-relevant (CSa+/-) and fear-irrelevant (CSb+/-) CSs in the control (**A**), instructed (**B**), and uninstructed (**C**) group. CS valence was rated on a scale ranging from 1 (unpleasant) to 9 (pleasant). Error bars represent standard errors.

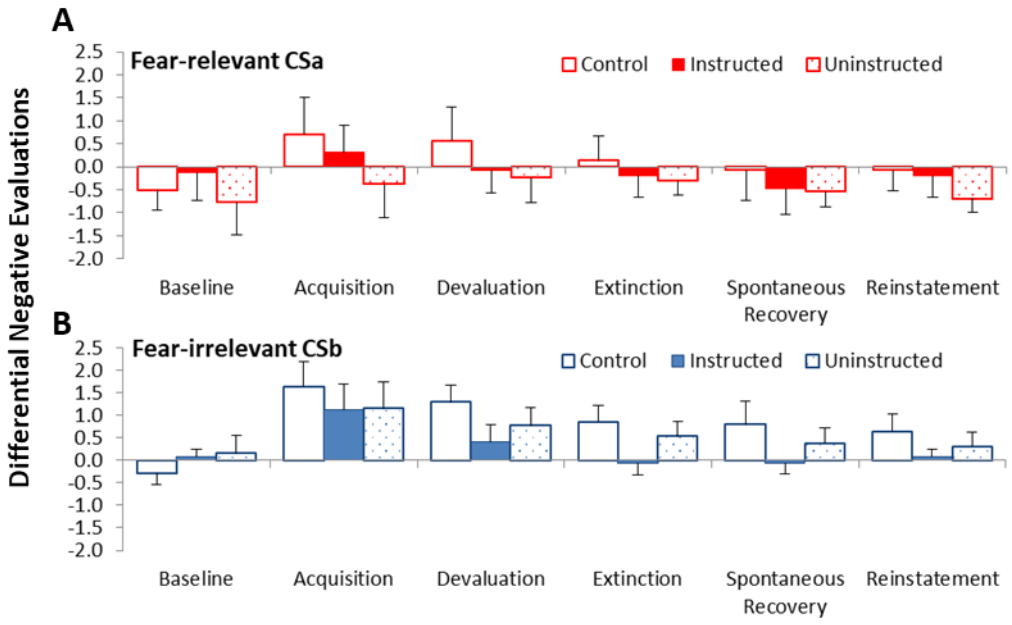


Figure S5.3. Mean differential negative evaluations of the CS+, relative to the CS-, for fear-relevant (**A**) and fear-irrelevant (**B**) conditioned stimuli (CSs). CS valence was rated on a scale ranging from 1 (unpleasant) to 9 (pleasant). Differential negative evaluations were calculated as the difference between CS- and CS+ valence ratings; larger values indicate more negative evaluations of the CS+, relative to the CS-. Error bars represent standard errors.

5.7.4. US Valence Ratings

Ratings of US valence (Figure S5.4) were obtained after the shock work-up procedure (baseline), after acquisition, after extinction, and reinstatement. In line with the pattern of data displayed in Figure S5.4, results of a 3 (group) x 4 (time) ANOVA yielded a main effect of time, $F(3, 37) = 18.88$, $p < .001$, $\eta p^2 = .61$, reflecting more negative evaluations of US valence at baseline ($M = 2.80$, $SE = 0.19$) and after acquisition ($M = 3.20$, $SE = 0.28$), than after extinction training ($M = 5.61$, $SE = 0.41$) and after tests of reinstatement ($M = 5.80$, $SE = 0.38$). There were no group differences in evaluations of US valence, as reflected in the non-significant time x group interaction, $F(6, 76) = 0.21$, $p = .974$, $\eta p^2 = .02$.

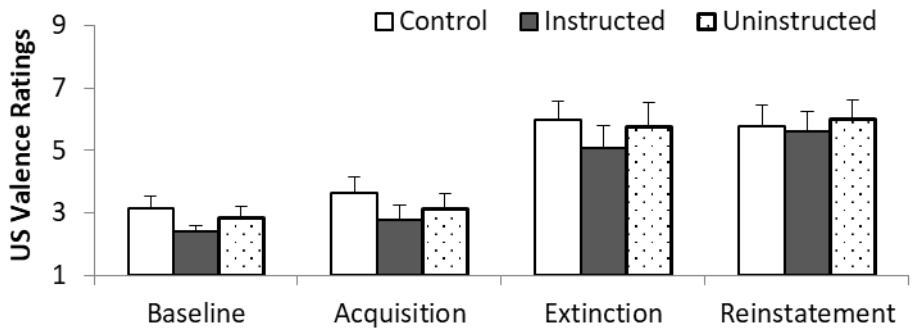


Figure S5.4. Mean unconditioned stimulus (US) valence ratings in the control, instructed, and uninstructed group. US valence was rated on a scale ranging from 1 (unpleasant) to 9 (pleasant), at baseline, as well as after acquisition, extinction training, and tests of reinstatement. Error bars represent standard errors.

5.7.5. Summary of Results

Analysis of electrodermal data provided evidence of fear acquisition to fear-relevant and fear-irrelevant CSs in all groups. However, differential SCRs did not differ across groups during extinction training, spontaneous recovery, or tests of reinstatement. Examination of CS valence ratings yielded similar results, showing no group differences in differential evaluations of CS valence during any of the test phases. Taken together, the analysis of data obtained from a sample of participants who exhibited successful acquisition of differential SCRs to both CS types showed that US devaluation did not affect immediate (extinction training) or delayed conditioned responding (tests of fear recovery [Day 2]), as reflected in differential SCRs and differential negative evaluations of CS+ valence.

Chapter 6: General Discussion

The central aim of the present thesis was to investigate whether approaches to fear reduction that involve presentations of the CS and the US could yield superior reduction of fear recovery, relative to conventional CS-only extinction training. Whilst conventional extinction training, which involves repeated presentations of the CS in the absence of the US, is effective in the reduction of fear, a large body of evidence suggests that it does not prevent fear recovery (e.g., Bouton, 2002; Craske, Hermans, & Vervliet, 2018; Craske, Treanor, Conway, Zbozinek, & Vervliet, 2014). The recovery of extinguished fear is problematic from a clinical perspective, as many individuals relapse after the successful conclusion of treatments for anxiety disorders (Craske & Mystkowski, 2006). A possible reason for this recovery of extinguished fear is the nature of extinction training, which involves the acquisition of a novel, inhibitory CS-no US association, leaving the original fear association intact and allowing for the return of fear during future CS encounters (Bouton, 2002). In order to reduce the recovery of extinguished fear, past research has proposed a range of methods that may be suited to the long-lasting reduction of fear, ranging from methods aimed at enhancing extinction learning to those focusing on the elimination of the original fear memory trace (Agren et al., 2012; Bouton, Woods, & Pineño, 2004; Craske et al., 2014).

In the studies conducted as part of this thesis, three approaches to fear reduction have been examined and contrasted with conventional extinction training, being (a) occasionally reinforced extinction training with paired and explicitly unpaired US presentations (Chapter 3; Bouton et al., 2004); (b) disruption of the memory reconsolidation process through extinction training that was delivered after US-induced memory reactivation (Chapter 4; Liu et al., 2014); and (c) extinction training administered after US devaluation (Chapter 5; Schultz, Balderston, Geiger, & Helmstetter, 2013). Electrodermal responding and CS valence ratings were recorded as primary and secondary dependent measures of conditioned fear, respectively. Based on the reviewed literature (e.g., Liu et al., 2014), it was hypothesised that these methods would yield superior reduction of fear recovery, relative to conventional CS-only extinction training.

Study 1 (Chapter 3) examined the effects of extinction training that was conducted with occasional presentations of the US, either paired with the CS (partially reinforced extinction training)

or presented in the inter-trial interval (unpaired extinction training), on the recovery of fear to fear-irrelevant CSs. Results showed that partially reinforced and unpaired extinction training provided enhanced protection from fear recovery, as indexed by differential SCRs, relative to conventional, CS-only extinction. Both types of reinforced extinction training reduced spontaneous recovery of fear, but only unpaired extinction training prevented the reacquisition of fear. The rate of reacquisition did not differ between partially reinforced and conventional extinction training. Unpaired extinction training, on the other hand, prevented the recovery of differential SCRs during all stages of testing. It should be noted, however, that there was no statistically significant reinstatement of differential SCRs in any of the groups. Finally, there was no benefit of occasionally reinforced extinction training on the reduction of conditioned negative evaluations of CS valence. These results demonstrate that extinction training that is conducted with occasional presentations of the US, either paired with the CS or presented in the inter-trial interval, can yield superior reduction of fear recovery, as indexed by SCRs, relative to conventional CS-only extinction training.

Study 2 (Chapter 4) examined the recovery of fear to fear-irrelevant and fear-relevant CSs subsequent to extinction training that was conducted with or without prior memory reactivation. Memory reactivation consisted of a single presentation of the US, at half the physical intensity as that employed during acquisition training (US-reactivation). The results showed that the delivery of extinction training 10 minutes after US-reactivation yielded superior reduction of fear to both classes of CSs, as indexed by differential SCRs, relative to extinction training that was delivered without prior memory reactivation. Follow-up tests, conducted in the US-reactivation group 8 to 12 months after initial testing, indicated that this reduction of fear was long-lasting, whereby participants were able to verbalise the trained CS-US contingencies, but did not exhibit spontaneous recovery or reinstatement of fear. Contrasting previous reports (e.g., Kindt & Soeter, 2013), conditioned responding in the present study was not a function of fear-relevance, indicating that even strong fears, conditioned to fear-relevant CSs, can be reduced through safe and non-invasive behavioural manipulations of the memory reconsolidation process. The present results further indicate that, in contrast to research employing a memory reactivation procedure involving a brief presentation of the CS (e.g., Schiller et al., 2010), a single US-reactivation trial is capable of reactivating and

destabilising multiple fear memories, conditioned to fear-irrelevant and fear-relevant CSs. This study thus replicated and extended previous findings (Liu et al., 2014), showing that multiple, distinct fear memories can be modified through a single US reactivation-extinction procedure. No group differences were observed in the evaluation of CS valence.

Study 3 (Chapter 5) examined the effects of US devaluation on the spontaneous recovery and reinstatement of fear to fear-irrelevant and fear-relevant CSs. In line with extant reconsolidation literature, it was also examined whether prediction errors generated by the unsignaled presentation of the USs during the US devaluation phase mediate the post-devaluation reduction of fear. While US devaluation reduced conditioned responding, assessed after a delay of 10 minutes and 24 hours, results showed that there were no statistically significant differences in differential SCRs or conditioned negative evaluations of CS valence between the US devaluation groups and the control group. The overall interpretation of findings was further complicated by the absence of equal levels of fear acquisition across groups. As such, no definitive conclusions can be drawn about the role of US devaluation in the persistent reduction of fear.

In summary, the overall pattern of electrodermal results demonstrates that recovery of extinguished fear can be reduced, and even prevented, through methods that involve exposure to the CS and the US. In this regard, occasionally reinforced extinction training and the US reactivation-extinction procedure were shown to yield superior reduction of fear recovery, relative to conventional extinction training, conducted in the absence of the US. Areas requiring further investigation are (a) the effects of US devaluation on the recovery of fear and (b) the double dissociation between SCRs and CS valence ratings. In contrast to SCRs, CS valence ratings, employed as a secondary measure of conditioned responding, were not sensitive to the present experimental manipulations. However, this double dissociation between SCRs and CS valence ratings is not uncommon in fear conditioning research (e.g., Lipp & Edwards, 2002) and will be discussed at a later stage in this chapter.

6.1. Implications for Theory and Research

The role of the US in fear acquisition has been well established (e.g., Rescorla & Wagner, 1972), but its role in the reduction of fear remains poorly understood. Already in 1920, Watson and Rayner observed that fear of an initially neutral stimulus could be conditioned by pairing the stimulus

with an aversive sound. Extensive research into the acquisition of associations between a CS and a US has culminated in the proposition of various models of associative learning (e.g., Pearce & Hall, 1980; Rescorla & Wagner, 1972). The Rescorla-Wagner model (Rescorla & Wagner, 1972), for instance, focuses on the role of the US in the acquisition and extinction of conditioned responding, including the role of prediction errors pertaining to the presence, absence, or the value of the US (for a detailed discussion, see Chapter 1). While acquisition of conditioned responding involves the repeated pairing of the CS with the US in order to establish a CS-US association (Rescorla & Wagner, 1972), extinction of conditioned responding in appetitive and aversive preparations typically involves the repeated presentation of the CS in the absence of the US (e.g., Bouton, 2002). This type of extinction training is effective in the within-session reduction of conditioned responding, but does not prevent the recovery of extinguished responding, as demonstrated in past research (e.g., Bouton, 2002; Bouton et al., 2004) and in the studies conducted as part of this thesis (Chapters 3-4).

Despite methods that are based on CS-only extinction training being the most commonly employed approaches to fear reduction, alternatives do exist and have been suggested to yield better long-term reduction of fear (Craske et al., 2014). In 1977, Frey and Butler suggested that extinction training and exposure therapy that involve exposure to the CS and the US may yield superior reduction of fear compared to CS-only extinction. Similarly, a review of exposure-based practices for the treatment of anxiety disorders proposed that occasional CS-US pairings during extinction training could be employed to enhance extinction learning and reduce subsequent recovery of fear (Craske et al., 2014). Propositions such as these appear counterintuitive, in particular since it is known that fears can be acquired even on relatively low reinforcement schedules (e.g., 38%; Oyarzún et al., 2012; Schiller et al., 2010) or after a single CS-US pairing (Izquierdo, Barros, Medina, & Izquierdo, 2000; Öhman, Eriksson, & Olofsson, 1975). It may, therefore, appear questionable why and how additional post-acquisition CS-US pairings, such as the ones employed in the partially reinforced extinction procedure in the present research (Chapter 3), should facilitate long-lasting reduction of fear.

Extant literature, albeit limited, does support the use of the US for the purpose of fear reduction on empirical and theoretical grounds: The methods examined as part of this thesis are based on several lines of research, specifically early reinforced extinction research (Frey & Butler, 1977)

and subsequent studies of occasionally reinforced extinction (Bouton et al., 2004; Culver, Stevens, Fanselow, & Craske, 2018); reconsolidation research (Liu et al., 2014); and US devaluation research (Schultz et al., 2013). Past research indicates that these methods reduce the recovery of extinguished fear by either eliminating the fear memory trace in the human amygdala (Agren et al., 2012), weakening the CS-US association (Frey & Butler, 1977; Vervliet, Vansteenwegen, & Hermans, 2010), or by decreasing the subjective aversiveness of the US (Schultz et al., 2013). Additional mechanisms have been proposed in the literature, such as 'sequential learning' (Bouton et al., 2004), and will be discussed in the following paragraphs. To preface the general discussion of findings, it should be noted that a comprehensive discussion of the empirical findings and potential underlying mechanisms has been provided in the respective empirical chapters (Chapters 3-5). The present general discussion focuses on the key contributions of the present findings to human extinction and reconsolidation research, as well as providing a discussion of potential mechanisms that may mediate fear reduction in approaches utilising CS and US exposure.

6.1.1. The Role of the US in Fear Reduction

The recovery of fear in the studies conducted as part of this thesis was reduced through the use of occasionally reinforced extinction training (Chapter 3) and behavioural manipulations of the memory reconsolidation process (Chapter 4). Occasionally reinforced extinction training involved the presentation of five USs amongst 24 CS+ and 24 CS- trials (for a schematic representation of the experimental paradigm, see Chapter 3, Figure 1), whereby the US was either paired with a CS+ (partially reinforced extinction) or presented in the middle of the ITI, either preceding or following a CS+ (unpaired extinction). The only difference between the experimental groups and the control group was the administration of the USs during extinction training. The experimental groups received an identical number of US presentations, which did not differ in their intensity or rated unpleasantness. In contrast to past research (Culver et al., 2018), only five, instead of six, USs were presented during extinction training, to facilitate the extinction of conditioned responding. While Culver et al.'s findings indicated that extinction of fear was not necessary to reduce the rate of reacquisition subsequent to partially reinforced extinction training, lack of fear extinction does

complicate the assessment of fear recovery from a conceptual point of view, as fear cannot recover, if it is not extinguished.

Analysis of electrodermal data in the present study showed that all extinction procedures resulted in the extinction of fear, as reflected in the absence of differential SCRs at the end of extinction training. The reduction of the reinforcement schedule has, therefore, allowed for the extinction of fear and subsequent assessment of fear recovery. Results showed absence of spontaneous recovery in the partially reinforced and unpaired extinction groups, but not in the control group. Reacquisition of extinguished fear, on the other hand, was only prevented through unpaired, but not through partially reinforced or conventional extinction training. The superior reduction of fear reacquisition subsequent to unpaired extinction training is in accordance with previous animal research conducted in the appetitive setting (Bouton et al., 2004), showing that an extinction procedure involving occasional presentations of unpaired USs can be successfully applied to the extinction of fear in humans. Contrasting Culver et al.'s (2018) findings, however, partially reinforced extinction did not interfere with the reacquisition of fear in the present study, although it prevented spontaneous recovery of fear.

The results of the occasionally reinforced extinction study, therefore, demonstrate that fear recovery can be reduced, or eliminated, if extinction training is conducted with occasional presentations of the US, whereby unpaired US presentations were more effective in the reduction of fear recovery than occasional CS-US pairings. Conventional CS-only extinction training did not prevent fear recovery, corroborating past research (e.g., Culver et al., 2018). Considering that both experimental groups received an identical number of CS and US presentations and no differences were evident in the levels of differential SCRs at the conclusion of extinction training, the question arises as to which factors mediated the reduction of fear recovery and accounted for the differences in electrodermal responding during reacquisition.

Broadly speaking, fear recovery could have been reduced through an enhancement of extinction learning (Craske et al., 2014) or through the weakening of the CS-US association (Frey & Butler, 1977; Rescorla, 1967; Rescorla & Wagner, 1972), although alternative explanations might apply (i.e., US habituation; Rescorla, 1973). For instance, the repeated presentation of the US during

unpaired extinction training led to the proposition that fear reduction may be mediated through US habituation (Rauhut, Thomas, & Ayres, 2001). While there is evidence to suggest that US habituation in human fear conditioning may occur through a relatively low number of US presentations (8 US trials; Haesen & Vervliet, 2015; but see Siddle, Power, Bond, & Lovibond, 1988 for failure to induce US habituation through 30 US trials), US habituation would be unlikely to occur through the five US presentations employed during extinction training in the present research. Indeed, examination of URs and ratings of US unpleasantness showed that these did not differ between the experimental groups, neither were there differences in unconditioned electrodermal responding between acquisition and extinction training. As such, the present results do not support the US habituation explanation, despite previous suggestions that US habituation reduces the rate of reacquisition of extinguished fear (Rauhut et al., 2001; but see Thomas, Longo, & Ayres, 2005). The present results do, however, provide support for the enhancement of extinction learning and/or the weakening of the CS-US association through occasional presentations of the US during extinction training.

6.1.1.1. Enhanced Extinction Learning

A large body of evidence supports the importance of prediction errors in the acquisition and extinction of conditioned responding (Holland & Schiffino, 2016; Pearce & Hall, 1980; Rescorla & Wagner, 1972; Vurbic & Bouton, 2014), as well as in the updating of consolidated memories (Exton-McGuinness, Lee, & Reichelt, 2015; Fernández, Boccia, & Pedreira, 2016; Sevenster, Beckers, & Kindt, 2012, 2013, 2014). Extant literature further indicates that associative learning stops when there is nothing new to be learned; this proposition is supported by learning models (Pearce & Hall, 1980; Rescorla & Wagner, 1972) as well as by neuroscientific evidence showing that prediction errors modulate neural and molecular activity underlying associative learning (for reviews, see Fernández et al., 2016; Holland & Schiffino, 2016). During extinction training, prediction errors are generated by the unexpected absence of the US (Rescorla & Wagner, 1972), because the absence of reinforcement creates a mismatch between expected (CS-US) and actual (CS-no US) events.

To enhance extinction learning, the partially reinforced and unpaired extinction procedures in the present research were designed in a manner that would maximise prediction errors. Specifically, the first US presentation during extinction training occurred on/after the fourth CS+ trial, as opposed

to the second (Culver et al., 2018) or first trial (Gershman, Jones, Norman, Monfils, & Niv, 2013). Consequently, the unexpected omission of the US, relative to acquisition training, would have sustained learning during early extinction training, while the unexpected presentations of paired or unpaired USs would have sustained learning on subsequent trials. In this regard, the unpaired extinction procedure would have generated a stronger prediction error than the partially reinforced procedure, due to the presentation of the US in the ITI, as opposed to the US coinciding with CS-offset. Accordingly, if extinction learning was enhanced through the presence of prediction errors, there should be less fear recovery after unpaired than after partially reinforced or conventional extinction training.

Based on the present results, it indeed appears that extinction learning was enhanced through increased prediction errors, as unpaired extinction training not only prevented spontaneous recovery but also reacquisition of extinguished fear, while partially reinforced extinction only prevented spontaneous recovery and conventional extinction did not interfere with spontaneous recovery or rapid reacquisition of fear. Still, while the prediction error hypothesis supports the role of occasional US presentations during extinction in the enhancement of extinction learning, it does not explain what exactly is learned during partially reinforced or unpaired extinction training or how this learning reduces fear recovery.

6.1.1.2. Generalisation of Extinction Learning Across Contexts

Conventional CS-only extinction training results in the acquisition of an inhibitory CS-no US association, but does not eliminate the original CS-US association (e.g., Bouton, 2002). Consequently, the meaning of the CS becomes ambiguous, as the CS can signal the arrival or the absence of the US. Bouton (2004) proposed that the fate of the CS after extinction is determined by contextual information: If the CS is presented in the acquisition context, the memory of acquisition training is retrieved and conditioned responding returns. If, however, the CS is presented in the extinction context, the memory of the CS-no US learning can be activated, thereby reducing the return of conditioned responding. As extinction is context-dependent (Bouton, 2004), fear recovery should be reduced through a procedure that allows for easier retrieval of extinction learning in the test context.

It should be noted, however, that the definition of context is broad and may encompass an actual physical context, such as a laboratory, but also a temporal context (e.g., the mere passage of time), a context of reinforcement or non-reinforcement (e.g., acquisition or extinction), or interoceptive context (e.g., drug-induced changes, such as increased heart rate; for a review, see Bouton, 2004). The studies conducted as part of this thesis did not entail an explicit change in context. Bouton et al.'s (2004) use of context is based on the context-retrieval view of extinction (Bouton, 1993) and Capaldi's sequential theory (1994), and refers to a trial sequence that retrieves the memory of the learning that occurred during acquisition or extinction training.

In this regard, Bouton et al. (2004) proposed that extinction training that is conducted with occasional presentations of the US may break the US's exclusive association with the acquisition context. What is essentially learned through the presentation of USs during extinction training is that the US does not only occur in the presence of other CS-US trials (acquisition context), but also in the presence of CS-no US trials (extinction context). When applied to partially reinforced extinction training, participants would learn that a CS-US trial is followed by several CS-no US trials (Bouton et al., 2004; Capaldi, 1994). Following such learning, post-extinction CS-US presentations should slow the rate of reacquisition, as a CS-US trial would signal the arrival of CS-no US trials. This explanation is readily applicable to Bouton et al.'s findings, as the rate of reacquisition was slowed through partially reinforced extinction training. In the present research, however, partially reinforced extinction did not interfere with the reacquisition of fear, although it prevented spontaneous recovery of fear.

Hence, the present findings could be viewed as yielding partial support for Bouton et al.'s (2004) proposition that US presentations during extinction training facilitate retrieval of extinction learning in the test context, as partially reinforced extinction training prevented spontaneous recovery of fear. It is possible that the present extinction session was too short to create a robust extinction memory that would facilitate the reduction of reacquisition. More research is required to determine whether extended extinction training, or repeated extinction sessions, in line with Bouton et al.'s study, would reduce reacquisition of fear subsequent to partially reinforced extinction.

It also remains to be investigated which mechanisms mediate the effects of unpaired extinction training on conditioned responding. In accordance with partially reinforced extinction training, it could be argued that the presentation of unpaired USs has broken the US's exclusive association with the acquisition context and facilitated the retrieval of extinction learning in the test context (Bouton et al., 2004). However, this proposition does not account for the differences in fear reacquisition between unpaired and partially reinforced extinction training. Similarly, if occasionally reinforced extinction training involves the learning of a trial sequence (e.g., CS-US trial is followed by several CS-no US trials; Bouton et al., 2004), it is not clear how unpaired extinction training could prevent reacquisition, as unpaired extinction training did not involve presentations of CS-US pairings. As such, the first reacquisition trial should have activated the memory of the acquisition context and resulted in rapid reacquisition of fear. The present findings, therefore, only yield partial support for the proposition that occasionally reinforced extinction training is mediated by sequential learning, which is proposed to facilitate the generalisation of extinction learning from the extinction to the test context (Bouton et al., 2004). In contrast, a mechanism that may underlie unpaired extinction, is a *weakening* of the CS-US association (Frey & Butler, 1977; Rescorla, 1967; Rescorla & Wagner, 1972; Vervliet et al., 2010).

6.1.1.3. Weakening of the CS-US Association

The present argument must be prefaced by acknowledging that it is generally recognised that extinction is a form of new learning, not weakening of the CS-US association, which would be synonymous with the "unlearning" of the acquisition memory (Bouton, 2002, 2004). A large body of evidence shows that CS-only extinction training does not prevent the return of conditioned responding, thereby supporting the premise that extinction training does not eliminate the original CS-US association (Bouton, 2002, 2004). This view is also supported by the results of the CS-only extinction groups in the present occasionally reinforced extinction and reconsolidation studies. At the same time, there is evidence to suggest that unpaired extinction training (e.g., Frey & Butler, 1977; Vervliet et al., 2010), as well as manipulations of the memory reconsolidation process (e.g., Lee, Nader, & Schiller, 2017), reduce fear recovery by modifying the original fear learning.

Simply put, weakening of the acquired CS-US association through unpaired extinction training may be achieved by learning that the US no longer follows the CS (in line with CS-only extinction), but also that US presentations are no longer contingent on CS presentations (i.e., the US occurs in an unsignaled manner), thereby reducing the likelihood of post-extinction CS presentations eliciting the fear response (Frey & Butler, 1977; Vervliet et al., 2010). Hence, the explicit unpairing of the CS and the US may provide a stronger violation of the previously learned CS-US contingency than that achieved through exposure to the CS only (Frey & Butler, 1977; Vervliet et al., 2010). Furthermore, Rescorla (1967) proposed that the removal of the US during CS-only extinction eliminates any non-associative effects the US may exert on responding. An extinction procedure that involves US presentations that are not reliably predicted by the CS, controls for the effects of non-associative processes, while allowing for "examination of the loss of contingency-dependent learning" (Rescorla, 1967, p. 75).

The unpaired extinction procedure employed in the present research is closely aligned with Rescorla's (1967) notion of a "truly random" procedure, to the extent that the presence or absence of the US would not be reliably predicted by the CS. This is because the US was presented only five times among 24 CS+ and 24 CS- trials; was presented in the middle of the ITI, whereby the length of the ITI varied between 13 and 17 seconds; and either followed or preceded a CS+ trial, thereby reducing the risk of participants acquiring a new temporal relationship between the CS+ and the US, namely that the US occurs within a specific time after a CS+ presentation. Arguably, the present unpaired procedure was not truly random, as no random CS-US pairings were allowed to occur and because the US was presented in the middle of the ITI. Nevertheless, a similar unpaired extinction procedure employed by Vervliet and colleagues (2010), albeit one that entailed a higher US:CS ratio than that used here, induced uncertainty about US presentations, as reflected in online US expectancy ratings. This uncertainty has been proposed to indicate that participants perceived the unpaired US presentations as truly random, which in turn would reflect the absence of a CS-US contingency (Vervliet et al., 2010).

Similarly, the present unpaired extinction procedure would allow participants to learn that US presentations are no longer contingent on the CS, thereby potentially weakening or "breaking" the

learned CS-US contingency and eliminating conditioned responding to the CS+. Examination of electrodermal responding during spontaneous recovery, reinstatement, and reacquisition indeed confirms that conditioned responding did not recover. The lack of reacquisition of differential SCRs, in particular, may further indicate that participants learned that the "CS is irrelevant to the US" (Rescorla, 1967, p. 76). Taken together, the present findings are in accordance with previous observations (Frey & Butler, 1977; Vervliet et al., 2010), although more research is required to identify the exact mechanisms underlying unpaired extinction.

In this regard, it should be noted that the proposition of unpaired extinction training weakening the CS-US association is compatible with the Rescorla-Wagner model of associative learning (Rescorla & Wagner, 1972) and with the previously discussed prediction error hypothesis, to the extent that US-induced prediction errors facilitate extinction learning and reduce the associative strength of the CS. It is also possible, albeit unlikely, that the presentation of unpaired USs during extinction training increased the associative strength of the context, which would subsequently increase the loss of the associative strength of the CS+, when the CS+ is presented in compound with the context (Bouton, 2004; Rescorla & Wagner, 1972; see also Bouton et al., 2004). Due to this enhanced loss of associative strength, subsequent responding to the CS+ would be reduced; some scholars would even propose that the CS+ was transformed into a conditioned inhibitor through the present unpaired extinction procedure (personal communication, F. Westbrook, July 12, 2018).

Briefly, a conditioned inhibitor has a negative associative value (as opposed to the positive associate value that develops through CS-US pairings during excitatory conditioning); it is a predictor for the absence of the US, and decreases the likelihood of a CS presentation eliciting a conditioned response (Rescorla & Wagner, 1972). It appears though that the 'conditioned inhibitor hypothesis' is not readily applicable to the present findings. First, it is unlikely that the five US presentations, which were presented in a pseudo-random order across 48 CS trials, would allow for the development of an excitatory context-US association, as any gains in associative strength would be extinguished when the context (i.e., the black computer background between CS presentations) was presented without the US. Second, a negative associative value would not predict absence of responding during reacquisition, merely a slower rate of reacquisition, as the CS+ would gain excitatory properties while

being paired with the US. Finally, tests for conditioned inhibition conducted in animal research (Thomas et al., 2005) showed that the CS+ had retained some excitation subsequent to unpaired extinction training that was conducted across multiple days with a US:CS ratio of 1:2 (see also Rauhut et al., 2001). Hence, it appears unlikely that the reduction of fear recovery and reacquisition subsequent to unpaired extinction training is the result of conditioned inhibition.

To summarise the discussion of mechanisms that may mediate fear reduction through occasionally reinforced extinction training, the results of the present research showed that the reduction of fear recovery in the partially reinforced and unpaired extinction groups was not the result of US habituation. The results are, however, consistent with previous reports that indicate prediction errors are important for the reduction of fear (Craske et al., 2014; Exton-McGuinness et al., 2015; Fernández et al., 2016; Rescorla & Wagner, 1972; Sevenster et al., 2012, 2013, 2014). In this regard, the present findings indicate that increasing prediction errors through the occasional presentation of the US during extinction training may enhance extinction learning and be beneficial for the long-lasting reduction of fear.

The results of the unpaired extinction procedure corroborated previous findings from animal (Frey & Butler, 1977) and human (Vervliet et al., 2010) fear conditioning research, showing that unpaired extinction training provides superior protection from fear recovery, compared to CS-only extinction training. The present findings also appear to support previous suggestions that unpaired US presentations during extinction training may weaken, or "break," the CS-US association (Frey & Butler, 1977; Rescorla, 1967; Vervliet et al., 2010). Although this proposition contradicts the dominant view of extinction learning as a type of new learning, not unlearning (Bouton, 2002), the absence of spontaneous recovery, reinstatement, and reacquisition of fear subsequent to unpaired extinction training, as well as the differential pattern of fear recovery across the different types of extinction training employed in the present research indicate that the effects of each extinction procedure may be mediated by distinct mechanisms. The present results yield stronger support for the proposition that unpaired extinction breaks the CS-US association than for the alternative explanations discussed in the present thesis, and warrants further examination in future research.

Broadly speaking, unpaired US presentations during extinction training not only maximise prediction errors, but also allow participants to learn that the US no longer follows the CS *and* that the US occurs in the absence of the CS (Vervliet et al., 2010). Such learning may result in greater reduction of fear recovery than learning that US presentations are context-dependent (i.e., CS-only extinction; Bouton, 2002). The results of the present occasionally reinforced extinction research, therefore, contributed to the human fear conditioning literature by presenting two alternative methods that may yield better long-term reduction of fear than conventional CS-only extinction training.

6.1.1.4. Devaluation of Subjective US Aversiveness

In contrast to the results of the occasionally reinforced extinction and reconsolidation studies, the results of the US devaluation study (Chapter 5) did not provide any insights into the role of US devaluation in the persistent reduction of fear. While there was no spontaneous recovery or reinstatement of differential SCRs subsequent to the US devaluation-extinction procedure, these results did not significantly differ from those of the control group, which received extinction training without prior US devaluation. An aspect that complicated the interpretation of findings in this study was the lack of equal levels of fear acquisition across groups, whereby differential SCRs to fear-irrelevant CSs (geometric shapes) were not evident in the control group on the last block of acquisition training. Although there is some evidence to suggest that a reduction of perceived US aversiveness can reduce the renewal of fear (Haesen & Vervliet, 2015; Leer & Engelhard, 2015), the overall field of US devaluation research provides only weak support for the utility of US devaluation in the long-lasting reduction of fear (for a comprehensive discussion, see Chapter 5). Whether a procedure that combines US devaluation with subsequent delivery of extinction training could yield persistent reduction of fear, for instance by reducing conditioned responding through a combination of associative and non-associative processes (Frey & Butler, 1977; see also Haesen & Vervliet, 2015), requires further examination.

Perhaps the key contribution of the US devaluation study to extant human fear conditioning research is the observation that the acquisition of differential SCRs in a differential fear conditioning paradigm may be influenced by factors other than the use of a strong conditioning protocol, involving CS-US pairings on a 100% reinforcement schedule. It is interesting to note that the use of an almost

identical conditioning protocol resulted in the acquisition of differential SCRs to fear-relevant and fear-irrelevant CSs in both groups of the reconsolidation study (Chapter 4). Nevertheless, the lack of fear acquisition across all participants is not limited to the present study, but has been reported in past research as well (e.g., Kindt & Soeter, 2013; Schiller et al., 2018), and represents a common challenge for human fear conditioning research that relies on the acquisition of differential responding as a prerequisite for further testing. More research is required to gain a better understanding of the influence of non-associative factors on the acquisition of differential electrodermal responding in humans (e.g., see Lonsdorf & Merz, 2017 for a review of individual differences in fear conditioning).

6.1.1.5. Effects of US Presentations on the Extinction of Conditioned Valence

Similar to the results of the US devaluation study, the pattern of results of conditioned negative evaluations of the CS+, relative to the CS-, was difficult to interpret, predominantly because the pattern of results diverged from that obtained in the analysis of electrodermal data. Of note, the present discussion focuses on the key findings; a detailed discussion of the effects of experimental manipulations on conditioned negative valence is presented in Chapters 3 to 5. With the exception of the US devaluation study, the pairing of fear-irrelevant or fear-relevant CSs with an aversive electrotactile US resulted in the acquisition of negative valence in all groups, meaning the self-reported pleasantness of the CSs+ that had been paired with the US decreased, relative to the rated pleasantness of the CSs-. Contrasting past research (Luck & Lipp, 2015), there was evidence of extinction of conditioned valence in the reconsolidation and occasionally reinforced extinction studies (for a review of relevant evaluative conditioning literature, see De Houwer, Thomas, & Baeyens, 2001).

The results of the reconsolidation study were consistent with previous suggestions that evaluative conditioning is more pronounced during post-acquisition than post-extinction ratings (Hofmann, De Houwer, Perugini, Baeyens, & Crombez, 2010). In the present research, evaluations of the CS+, relative to the CS-, were more negative after acquisition training than after the subsequent exposure to CS-only trials (i.e., extinction training and tests of spontaneous recovery). While this reduction of conditioned negative valence is consistent with an extinction explanation (Hofmann et al., 2010), CS valence was not measured immediately after extinction training, but at the conclusion

of spontaneous recovery tests, 24 hours after the administration of extinction training with or without prior memory reactivation. As such, it is possible that the actual conditioned negative valence after extinction training differed from that reported after tests of spontaneous recovery.

The results of the occasionally reinforced extinction study also provided evidence of extinction of conditioned negative valence in the conventional and unpaired extinction groups, but not in the partially reinforced extinction group. These results suggest that it is not the presence of an aversive US per se that may maintain significant levels of conditioned negative valence between post-acquisition and post-extinction ratings, but that CS-US pairings play a role in evaluative conditioning (for a review of theoretical accounts of evaluative conditioning, see Hofmann et al., 2010). This proposition is further supported by the reacquisition of conditioned negative valence in all groups after administration of additional CS-US pairings. Notably, the reacquisition of conditioned negative valence in the unpaired extinction group has important implications for the understanding of mechanisms underlying evaluative conditioning.

Extant literature suggests that evaluative conditioning may differ from other forms of Pavlovian conditioning. Several theoretical accounts exist, however, the dominant view holds that evaluative conditioning is not simply a reflection of the statistical contingency between the CS and the US, but is influenced by higher order cognitive processes, such as conceptual categorisation and formation of propositions (for comprehensive reviews, see De Houwer, 2007; Hofmann et al., 2010). Although it is not possible to exclude the possibility that the evaluative conditioning effects in the present occasionally reinforced extinction study reflect participants' use of cognitive strategies (Gawronski, Gast, & De Houwer, 2015), the overall pattern of results indicates that CS-US pairings, rather than the mere co-occurrence of the US close in time to the CS (i.e., unpaired extinction) may mediate evaluative conditioning (cf. Baeyens, Eelen, Crombez, & van den Bergh, 1992).

Examination of CS valence ratings in human fear conditioning is of importance to our understanding of pathways to fear recovery. It has been proposed that residual negative valence is associated with the reinstatement of fear and, therefore, may interfere with persistent fear reduction (Hermans et al., 2005; Zbozinek, Hermans, Prenoveau, Liao, & Craske, 2015). However, these findings are contrasted by the results of the present research, which did not find a clear link between

residual negative valence and reinstatement of fear. For instance, reinstatement of fear, as indexed by differential SCRs, was observed in the control group of the reconsolidation study, but not in the US-reactivation group, despite both groups evidencing residual negative valence after tests of spontaneous recovery. Similarly, partially reinforced extinction training interfered with the extinction of conditioned negative valence, but prevented spontaneous recovery of differential SCRs. As a limitation, however, it should be noted that reinstatement of fear was not a robust phenomenon in the present studies (see Limitations and Future Directions for further discussion). Therefore, more research is required to examine the correlation between residual negative valence and the reinstatement of fear.

Finally, the divergent effects of experimental manipulations on physiological and verbal indices of conditioned responding observed in the present research may represent a challenge for the overall interpretation of findings. However, this double dissociation is in accordance with previous fear conditioning research, suggesting that response systems which are governed largely by conscious, cognitive processes, such as ratings of CS valence or US expectancy, and physiological indices of conditioned responding are differentially sensitive to extinction training (e.g., Culver et al., 2018; Lipp & Edwards, 2002) and manipulations of reconsolidation (e.g., Kindt & Soeter, 2013). However, a limitation of the present research was the recording of offline valence ratings, which do not permit assessment of trial-by-trial changes in conditioned valence, as well as being potentially susceptible to the influence of demand characteristics (Lipp, 2006a). Online ratings were not recorded, as these could have resulted in movement-induced artefacts in SCRs due to the handling of a ratings scale, and thereby interfered with the recording of the primary dependent measure of conditioned responding. Future research specifically designed to examine changes in CS valence as a result of occasionally reinforced extinction or reactivation-extinction may provide further insight into the exact underlying mechanisms.

6.1.1.6. US-induced Memory Destabilisation

The final point of discussion pertains to the role of the US in the reactivation and destabilisation of fear memories prior to behavioural manipulations of the memory reconsolidation process (Liu et al., 2014). A key difference between the present and past reconsolidation research,

which did not find that extinction training was able to disrupt the reconsolidation of fears conditioned to fear-relevant CSs (e.g., Golkar, Bellander, Olsson, & Öhman, 2012; Kindt & Soeter, 2013), was the use of the US, instead of the CS, for the reactivation and destabilisation of fear memories. The reactivation procedure used to destabilise fear memories is a key determinant of successful manipulation of reconsolidation (for reviews, see Rodriguez-Ortiz & Bermúdez-Rattoni, 2017; Wideman, Jardine, & Winters, 2018), as the reconsolidation process is not initiated through the mere retrieval of a memory, but through the presence of novel information that creates a mismatch between expected and actual events and warrants memory updating (i.e., prediction errors; Agustina López et al., 2016; Faliagkas, Rao-Ruiz, & Kindt, 2018; Fernández et al., 2016; Monti et al., 2017; Sevenster et al., 2012, 2013, 2014). Thus, the purpose of memory reactivation is to return the consolidated memory trace to an active and malleable state (De Oliveira Alvares et al., 2013; Monti et al., 2017; Pineyro, Monti, Alfei, Bueno, & Urcelay, 2014), thereby allowing for the modification of the destabilised fear memory through the subsequent administration of extinction training (e.g., Liu et al., 2014; Schiller et al., 2010) or pharmacological agents (e.g., Kindt, Soeter, & Vervliet, 2009; Nader, Schafe, & LeDoux, 2000a; for a comprehensive overview of determinants of memory destabilisation and reconsolidation interference, see Chapter 1).

Based on the results of the present reconsolidation study and past research (Liu et al., 2014), a key advantage of US-reactivation appears to be the concurrent destabilisation of multiple fear memories that are associated with the reactivated US. Findings from Liu et al.'s study indicate that an unsignaled presentation of the US, delivered at half the intensity as that employed during acquisition, destabilised two fear memories, conditioned to fear-irrelevant CSs. In the present research, the same US-reactivation procedure was employed to destabilise two fear memories that were conditioned to different classes of CS fear-relevance, being fear-irrelevant (geometric shapes) and fear-relevant CSs (pictures of snakes and spiders). In both studies, extinction training that was delivered 10 minutes after delivery of the US-reactivation trial prevented spontaneous recovery and reinstatement of fear to both CSs, as reflected in the absence of differential SCRs. The present findings further indicate that the reduction of fear was long-lasting, as reflected in the absence of fear recovery 8 to 12 months after initial testing (see also Liu et al., 2014 for a 6-month follow-up).

The long-lasting reduction of fear to fear-relevant CSs contrasts past research, which suggested that strong fear memories, conditioned to fear-relevant CSs, are not sensitive to behavioural manipulations of the memory reconsolidation process (Fricchione et al., 2016; Golkar et al., 2012; Kindt & Soeter, 2013). The present findings indicate that the strength of the fear memory is not necessarily a boundary condition of reconsolidation, but that reactivation and destabilisation of fears conditioned to fear-relevant CSs may require the use of a potent reactivation protocol that generates the prediction error required for the activation of neural and molecular processes underlying memory reconsolidation (for relevant reviews, see Fernández et al., 2016; Huang, Zhu, Zhou, Liu, & Ma, 2017; Wideman et al., 2018; Zhang, Haubrich, Bernabo, Finnie, & Nader, 2018).

Accordingly, US-reactivation may create a stronger prediction error than that generated through the unexpected absence of the US during CS-reactivation, due to the unsignaled presentation of the US and the reduction of the physical US intensity, both of which would violate participants' expectations pertaining to the CS-US contingency and US intensity employed during fear conditioning. Although a brief presentation of the previously conditioned CS has been successfully employed in the destabilisation of fear memories (Agren et al., 2012; Oyarzún et al., 2012; Schiller et al., 2010), a non-reinforced CS-only trial resembles the first trial of extinction training and, depending on the learning history and experimental parameters, may lead to extinction learning rather than the destabilisation of the fear memory trace (for reviews, see Besnard, Caboche, & Laroche, 2012; Exton-McGuinness et al., 2015). Nevertheless, at present, US-reactivation remains underutilised and poorly understood, compared to reactivation procedures involving the CS. Consequently, more research is required to gain a better understanding of the exact mechanisms mediating US-induced memory reactivation and destabilisation in humans.

Currently, there is evidence from an auditory fear conditioning study conducted with animals (Huang et al., 2017) to suggest that CS- and US-induced memory reactivation generate a different pattern of activity in brain areas underlying fear learning. Huang and colleagues conditioned two distinct fear memories by pairing auditory CSs (CSa, CSb) with a foot shock. Memory reactivation either involved an unsignaled presentation of the foot shock, at its original intensity, or a non-reinforced presentation of the previously conditioned CSa/CSb. Subsequent administration of

propranolol interfered with the reconsolidation of the reactivated CS-US association(s), which was reflected in reduced freezing to both CSs in the US-reactivation group, while the rate of freezing in the CS-reactivation group was only reduced to the reactivated CSa/CSb.

In line with these behavioural observations, Huang et al. (2017) reported that US-induced memory reactivation, relative to CS-induced reactivation, resulted in greater activation of transcription factors that drive protein synthesis required for memory reconsolidation (cAMP responsive element binding protein [CREB]) in the hippocampus and lateral amygdala (see also reviews by Finnie & Nader, 2012; Kida, 2018). These results indicate that both CS- and US-reactivation activate brain areas implicated in auditory fear conditioning, although US-reactivation does so to a greater extent, which may reflect the destabilisation of multiple CS-US associations (see Zhu et al., 2018 for similar findings in the destabilisation of reward memories). Subsequent administration of propranolol suppressed CREB activation and interfered with memory reconsolidation.

Taken together, Huang et al.'s (2017) findings are in line with the results of the present reconsolidation study and those reported by Liu et al. (2014), showing that manipulation of the reconsolidation process subsequent to a US-reactivation trial prevents recovery of fear to multiple CSs. In contrast, CS-reactivation can be employed to target a single CS-US association and eliminate recovery of fear to the reactivated CS (Liu et al., 2014; Schiller et al., 2010; see also Chapter 1, Figure 1.2). Irrespective of which memory reactivation procedure is employed, fear reduction is contingent on the destabilisation of the fear memory trace (Wideman et al., 2018) and on the successful interference with the restabilisation of the active memory trace through pharmacological (e.g., Kindt et al., 2009) or behavioural means (e.g., Liu et al., 2014; see also Chapter 1, Figure 1.1).

In this regard, Huang et al.'s (2017) findings point to potential mechanisms that may mediate US-induced memory destabilisation in humans. Although findings from animal research may be difficult to translate to humans, insights gained from animal research can inform future human reconsolidation research, in particular when no safe and non-invasive methods exist to study the molecular processes underlying memory destabilisation in humans (for reviews of processes underlying memory destabilisation, see Vigil & Giese, 2018; Wideman et al., 2018). Many of the

methods employed in animal reconsolidation research, such as the infusion of the protein synthesis inhibitor anisomycin into the amygdala (Nader et al., 2000a), cannot be used in human research due to their invasive and toxic nature. Despite extensive research into the phenomenon of memory reconsolidation, presently no memory reactivation procedure has been identified that always destabilises fear memories (see also Kindt, 2018). As memory destabilisation is necessary for subsequent interference with memory reconsolidation, identification of effective memory reactivation and destabilisation procedures would considerably advance the development of reconsolidation-based interventions for the treatment of anxiety and stress-related disorders.

The findings from the reconsolidation study conducted as part of this thesis, therefore, contribute to a growing body of literature showing that a memory reactivation trial consisting of the US, either presented at its original (Dębiec, Díaz-Mataix, Bush, Doyère, & LeDoux, 2010; Huang et al., 2017) or reduced intensity (Liu et al., 2014), destabilises fear memories and, thereby, allows for the subsequent interference with the restabilisation of all reactivated memories. Recent advances in animal research further indicate that US-reactivation is not only suitable for the destabilisation of fear memories, but can also be applied to the destabilisation of reward memories (Luo et al., 2015) and in this regard, was shown to be more effective than CS-reactivation (Zhu et al., 2018).

Finally, the present findings also demonstrated that fear recovery subsequent to behavioural manipulations of reconsolidation is not a function of CS fear-relevance, thereby indicating that previously proposed boundary conditions of reconsolidation (e.g., Auber, Tedesco, Jones, Monfils, & Chiamulera, 2013) may actually reflect deficits in memory destabilisation (see also Kindt, 2018). At present, more research is required to enhance our understanding of mechanisms mediating US-induced memory destabilisation, and to examine how the present US-reactivation procedure could be adapted to make it suitable for the reactivation of naturally occurring fears in the clinical setting.

6.1.1.7. The Future of the US in Fear Reduction Research

To summarise the discussion of implications for theory and research, advances in reconsolidation research (e.g., Lee et al., 2017), including the findings from the present research, showed that fear recovery can be eliminated through manipulations of the memory reconsolidation process. This discovery has offered exciting avenues for the development of new methods of long-

lasting fear reduction. The present findings further suggest that persistent reduction of fear may not require the manipulation of the reconsolidation process, but could also be achieved through extinction training that is conducted with occasional presentations of unpaired USs. Admittedly, due to the limited amount of research available in the area of occasionally reinforced extinction, this proposition requires further examination in pre-clinical and clinical studies. Animal research would be valuable in this regard, as it allows for the study of neural and molecular processes underlying fear reduction.

Of the fear reduction methods tested in the present studies, those combining exposure to unpaired US(s) with CS-extinction training reduced fear recovery to a greater extent than partially reinforced or conventional extinction training, the US devaluation-extinction procedure being the exception. The results of the unpaired extinction procedure are consistent with previous research that proposed unpaired US presentations during extinction training break the CS-US association (Frey & Butler, 1977; Rescorla, 1967; Vervliet et al., 2010). Similarly, medical imaging studies demonstrated that reactivation-extinction procedures, but not conventional extinction training, eliminate the reactivated fear memory trace in the human amygdala (Agren et al., 2012; Björkstrand et al., 2016; Björkstrand et al., 2015; but see Klucken et al., 2016). Both procedures have, therefore, the potential to persistently reduce conditioned fear by targeting the original CS-US association. Beyond this similarity, however, the mechanisms underlying unpaired extinction training and US reactivation-extinction are likely to differ, most notably because occasionally reinforced extinction training, as conducted in the present research, was not delivered during the memory reconsolidation period (it was delivered in close temporal proximity to acquisition training).

To enhance our understanding of mechanisms underlying long-lasting fear reduction, more research is required to examine which exact mechanisms mediate the effects of occasionally reinforced extinction training and reactivation-extinction procedures on conditioned responding. Reactivation-extinction, for instance, has been proposed to enhance extinction learning, rather than interfere with the reconsolidation process (Carpenter, Pinaire, & Hofmann, 2019; Kindt, 2018; but see Monfils, Cowansage, Klann, & LeDoux, 2009). This proposition is based on the presently limited understanding of mechanisms mediating fear reduction through manipulations of the memory reconsolidation process (for a discussion, see Beckers & Kindt, 2017), as well as on failed replications

of landmark studies (e.g., Luyten & Beckers, 2017). However, two lines of evidence oppose this proposition, being medical imaging studies showing that reactivation-extinction procedures eliminate the fear memory trace in the human amygdala (e.g., Agren et al., 2012), and the observation that fear recovery to two distinct CSs is prevented through a US reactivation-extinction procedure that involves extinction of one CS only (Liu et al., 2014).

Findings reported by Liu et al. (2014) indicate that fear reduction subsequent to CS reactivation-extinction is contingent on the extinction of both reactivated CSs. In contrast, fear reduction subsequent to US reactivation-extinction does not appear to require extinction of all CSs that are associated with the reactivated US. Specifically, Liu and colleagues observed that extinction training that was conducted with only one of the previously conditioned CSs, resulted in spontaneous recovery and reinstatement of fear to the non-extinguished CS after CS but not after US reactivation-extinction, despite CS-reactivation involving presentations of both CSs. Similar findings were reported in the extinction of reward memories in rats subsequent to CS- and US-reactivation (Luo et al., 2015).

As extinction training in Liu et al.'s (2014) study was conducted with one CS only, but fear recovery was reduced to both CSs that were associated with the reactivated US, it would be difficult to argue that the effects of the reactivation-extinction procedure are mediated by enhanced extinction learning. At the same time, further research is required to determine if fear reduction subsequent to reactivation-extinction that is preceded by a brief presentation of the CS or the US is mediated by distinct processes, as discussed previously. Notwithstanding the need for further research into the exact underlying mechanisms, the findings from the present and past reconsolidation research (e.g., Liu et al., 2014; Schiller et al., 2010) indicate that superior reduction of fear can be achieved through extinction training that is preceded by a memory reactivation trial and delivered during the reconsolidation period, relative to conventional extinction training. Yet, the advantage of the US-reactivation procedure, relative to CS-reactivation, lies in its ability to concurrently destabilise multiple CS-US associations (e.g., Huang et al., 2017; Liu et al., 2014).

While these findings are promising, it should be noted that outcomes from reconsolidation research remain mixed, both from studies utilising pharmacological (e.g., Chalkia, Weermeijer, Van

Oudenhove, & Beckers, 2019; Prado-Alcalá, Medina, Bello-Medina, & Quirarte, 2017; Schroyens, Beckers, & Kindt, 2017; Thome et al., 2016; Wood et al., 2015) and those employing behavioural manipulations of the reconsolidation process (e.g., Fricchione et al., 2016; Goode, Holloway-Erickson, & Maren, 2017; Ishii et al., 2015; Klucken et al., 2016; Luyten & Beckers, 2017). More research is required to gain a better understanding of determinants of memory destabilisation and reconsolidation. In this regard, the present general discussion of findings highlighted the need for the development of reliable memory reactivation procedures, as manipulations of reconsolidation are contingent on prior memory reactivation and destabilisation (e.g., Wideman et al., 2018).

With regards to the role of occasionally reinforced extinction training in the long-lasting reduction of fear, the present findings await replication in future human fear conditioning research. It is interesting to note that already in the 1960s and 70s animal research indicated that persistent reduction of fear is best achieved through approaches that involve exposure to the CS and the US, such as unpaired extinction training (Frey & Butler, 1977; Rescorla, 1967). Similar to the fate of early reconsolidation research (Misanin, Miller, & Lewis, 1968), however, the study of unpaired extinction training has been largely abandoned in favour of CS-only extinction research. Nevertheless, recent investigations of unpaired (Vervliet et al., 2010) and partially reinforced extinction (Culver et al., 2018), present study included, are in line with early animal research, supporting the utility of this procedure in the reduction of fear recovery in humans.

More research is required to determine if the reduction of fear observed in the present study could be sustained over long periods of time. While both occasionally reinforced extinction procedures evidenced superior reduction of fear recovery, compared to conventional extinction training, it is presently not known if these effects would persist during delayed tests of fear recovery, such as the ones conducted in the present reconsolidation study (but see Culver et al., 2018 for tests conducted a week after delivery of partially reinforced extinction). It also remains to be investigated how the present occasionally reinforced extinction procedures could be adapted for the use in clinical research. A key challenge pertains to the use of in vivo exposure to the US, at its original "intensity." It is conceivable that imaginal US exposure could be utilised in lieu of in vivo exposure (Agren, Björkstrand, & Fredrikson, 2017; Grégoire & Greening, 2019) and that reduction of fear recovery

could be achieved even when the US is not presented at its original intensity (Burhans & Schreurs, 2019; but seeFrey & Butler, 1977).

6.1.1.7.1. Examination of Shared Mechanisms

Due to the procedural similarities across studies conducted as part of this thesis, most notably the exposure to the CS and the US for the purpose of fear reduction, it might appear feasible to assume that the reduction of fear recovery in the reconsolidation and occasionally reinforced extinction study was mediated by similar mechanisms. Such a supposition, however, would not be in accordance with past research or the results of the present studies.

Based on the current discussion of findings, and past research (e.g., Craske et al., 2014; Sevenster et al., 2012), prediction errors would have contributed to the long-lasting reduction of fear in the reconsolidation and occasionally reinforced extinction study. Prediction errors play an important role in the acquisition and reduction of fear (e.g., Rescorla & Wagner, 1972; see Chapter 1 for a detailed discussion), but they neither fully account for the present results, nor is the presence of prediction errors sufficient for the long-lasting reduction of fear. For instance, prediction errors are required for the reactivation of previously consolidated fear memories (e.g., Sevenster et al., 2012; Chapter 4), but fear reduction requires the administration of an experimental manipulation that is capable of interfering with the restabilisation of the reactivated memory (e.g., Liu et al., 2014). In the present reconsolidation study (Chapter 4), the prediction error was generated through the US-reactivation procedure. Extinction training was then administered to interfere with the reconsolidation of the reactivated memory. The absence of fear recovery 24 hours after administration of US reactivation-extinction, is consistent with previous reconsolidation research showing that extinction training that is delivered during the memory reconsolidation period results in superior reduction of fear recovery, relative to extinction training that was delivered without prior memory reactivation (e.g., Liu et al., 2014; Schiller et al., 2010).

In contrast to the reconsolidation study, extinction training in the occasionally reinforced extinction study (Chapter 3) was delivered in close temporal proximity to acquisition training, leaving insufficient time for the fear memory to fully consolidate (e.g., Visser, Lau-Zhu, Henson, & Holmes, 2018). As memory consolidation is a prerequisite for memory reconsolidation (e.g., Visser et al.,

2018; see Chapter 1 for a detailed discussion), the role of *reconsolidation update mechanisms* (see Chapter 1, Figure 1.1) in the reduction of fear recovery in the present occasionally reinforced extinction study can be ruled out.

The prediction error hypothesis, on the other hand, can be readily applied to the occasionally reinforced extinction study. Specifically, prediction errors would have been generated during extinction training through the unexpected absence of the US on CS-only trials (Rescorla & Wagner, 1972), occasional presentation of the US amongst many CS-only trials, and/or through the explicit unpairing of the CS/US. Hence, prediction errors would have been enhanced in the paired and unpaired extinction groups, relative to the conventional extinction group, leading to enhanced extinction learning and reduced fear recovery (see also Craske et al., 2014). Whilst prediction errors would drive learning, the prediction error hypothesis does not explain what exactly is learned during occasionally reinforced extinction training or how this learning prevents fear recovery.

In this regard, the general discussion of findings indicates that different theoretical frameworks may account for the differential pattern of results observed between the paired and unpaired extinction procedure. Briefly, the paired and unpaired extinction procedures yielded different patterns of fear recovery, despite extinction training involving the same number of US presentations, and despite there being no group differences in US intensity or perceived US unpleasantness. The only difference between these groups was the paired or unpaired nature of US presentations during extinction training. Bouton et al.'s (2004) sequential learning hypothesis was found to represent a poor fit to the present results, as it cannot account for the differential pattern of results between the paired and unpaired extinction groups. The results of the unpaired extinction group appeared to be consistent with previous suggestions that unpaired extinction training weakens the CS-US association (Frey & Butler, 1977; Vervliet et al., 2010), thereby indicating that unpaired extinction training, similar to the behavioural manipulation of the memory reconsolidation process, may prevent fear recovery by altering the original fear association.

Notwithstanding this similarity, behavioural manipulations of the memory reconsolidation process, in contrast to occasionally reinforced extinction training, would not be expected to reduce fear recovery by enhancing extinction learning, but by altering or eliminating the original fear

memory trace (e.g., Agren et al., 2012; Monfils et al., 2009; see previous paragraphs for the argument against the 'enhanced extinction learning' hypothesis). Similar research in the area of occasionally reinforced extinction training is currently lacking; hence, it is not known if delivery of occasionally paired or unpaired extinction training 24 hours after acquisition training could yield long-lasting reduction of fear, and if so, whether a potential reduction of fear recovery would be mediated by changes to the original fear memory trace. Animal research would be particularly useful in this regard, as it allows for examination of neural and molecular mechanisms between reconsolidation- and extinction-based experimental manipulations (e.g., Besnard et al., 2012).

Another potential mechanism that could have accounted for fear reduction in all studies conducted as part of this thesis is US devaluation (Davey, 1989). Although the US devaluation study itself did not yield conclusive results and it is presently not known if persistent reduction of fear can be achieved through US devaluation (see Chapter 5 for a comprehensive review of literature), the post-acquisition devaluation of US aversiveness has reduced differential SCRs to the CS+ in past research (e.g., Schultz et al., 2013). US devaluation can occur through the repeated presentation of the US at its original (Haesen & Vervliet, 2015) or reduced intensity (e.g., Schultz et al., 2013). Consequently, it could be argued that the effects of the US-reactivation procedure in the reconsolidation study (Chapter 4) and those of the paired and unpaired extinction procedures (Chapter 3) were mediated by US devaluation. Such an explanation has been ruled out in the occasional reinforced extinction study, as the examination of URs and US valence ratings did not yield evidence of decreased US aversiveness as a result of repeated US presentations.

In the reconsolidation study (Chapter 4), the memory reactivation procedure, consisting of the unsignaled presentation of the US at half the physical intensity as that employed during acquisition training, resembled a US devaluation trial (e.g., Schultz et al., 2013). Therefore, one could argue that US devaluation could account for the observed reduction of fear. However, examination of SCRs during extinction training showed no statistically significant differences between the US-reactivation group, which received the memory reactivation trial, and the control group, which received extinction training without prior memory reactivation. Had US devaluation occurred, this would be reflected in group differences during extinction training, in line with the effects observed in past US devaluation

research (Schultz et al., 2013). Consequently, US devaluation can be ruled out as a potential alternative explanation for the fear reduction in the reconsolidation study. These findings are in line with past US reactivation-extinction research, which also ruled out US devaluation as an alternative explanation (Liu et al., 2014).

Although additional mechanisms could be discussed, such as Bouton et al.'s (2004) adaptation of sequential theory (Capaldi, 1994), there is no evidence originating from the research conducted as part of this thesis, or from the reviewed literature, to suggest that these frameworks would be readily applicable to all of the phenomena examined in the present studies. An aspect that may warrant further examination, however, is the role of cognitive factors in the reduction of fear recovery (Lovibond, 2004). Future research could examine to what extent the unpaired nature of CS and US presentations in the occasionally reinforced extinction study (Chapter 3) shaped participants' beliefs and expectancies about the CS-US relationship, or to what extent cognitive factors may have interacted with associative learning in the reduction of fear recovery (see Lovibond, 2004 for a detailed discussion). In this regard, it could be also investigated if fear reacquisition in the unpaired extinction group was reduced because the three unsignaled reinstatement shocks, followed by reinstatement tests, served as a confirmation of prior extinction learning, namely that the CS and US were not related. Such a proposition does, however, not explain why the first CS-US pairing during reacquisition did not serve as a reminder of acquisition training and, consequently, increased differential SCRs.

Taken together, there is little support for the supposition that the same mechanism mediated fear reduction across the distinct phenomena tested as part of this thesis, prediction errors being the exception. What is known from this and past research, however, is that approaches to fear reduction involving exposure to the CS and the US yield superior reduction of fear recovery, relative to conventional CS-only extinction training. Potential clinical applications, ethical considerations, and directions for future clinical research are discussed in the following paragraphs.

6.2. Implications for the Clinical Setting

The discussion of clinical applications will focus on the examination of the translational utility of occasionally reinforced extinction and US reactivation-extinction, meaning on the

approaches that yielded superior reduction of fear recovery, relative to CS-only extinction training. Specific clinical examples will be presented; however, it is important to note that fear memories underlie many psychopathologies, in particular anxiety and stress-related disorders (Foa & McLean, 2016; Mineka & Zinbarg, 2006). As such, research of the translational utility of the present methods would benefit from an approach that transcends the boundaries of diagnostic labels, although exceptions may apply (for a critical commentary, see Zoellner & Foa, 2016).

6.2.1. Challenges for Translational Research

Despite the promising findings from the occasionally reinforced extinction and reconsolidation studies conducted as part of the present thesis, the adaptation of the present methods for the use in the clinical setting faces several challenges. Most notably, there are ethical considerations which may prohibit the use of in vivo exposure to the US. Additional factors that require further consideration include: (a) the lack of a reactivation procedure that always destabilises fear memories (see also Kindt, 2018); (b) unpaired US presentations, whether during memory reactivation or unpaired extinction training, may pose a challenge to the treatment of some psychopathologies; (c) clinicians may be reluctant to deliberately expose clients to the feared US, and clients themselves may eschew exposure to feared cues and aversive consequences, preferring to engage in avoidance behaviours instead; and (d) exposure to the original US, at its original "intensity," may not be feasible (in addition to not being ethically permissible). Nevertheless, incorporation of the methods investigated in the present research into treatments of naturally occurring fears may require only minor modification of current therapeutic techniques (e.g., Maples-Keller et al., 2017; Telch, York, Lancaster, & Monfils, 2017), and a number of avenues exist to overcome the challenges listed here, as will be discussed in the following paragraphs.

6.2.2. Clinical Applications of Reactivation-extinction Procedures

The findings of the present reconsolidation study showed that fears that were thought to resist modification through reactivation-extinction procedures, such as those conditioned to fear-relevant CSs (e.g., Golkar et al., 2012; Kindt & Soeter, 2013), can be persistently reduced through safe and non-invasive behavioural interventions. Furthermore, the ability to disrupt the reconsolidation process of multiple fear memories in a single US reactivation-extinction session would make this procedure

particularly suited for the treatment of naturally occurring fears, which are often associated with multiple triggers (Carpenter et al., 2019; Schiller, 2014) and generalise from the fear predictive CS+ to stimuli that were not paired with the aversive US (e.g., Dunsmoor & Paz, 2015; Dymond, Dunsmoor, Vervliet, Roche, & Hermans, 2015; Lissek, 2012). Interventions based on the present US reactivation-extinction procedure may, therefore, reduce the duration of treatment. The translational utility of reactivation-extinction procedures is further supported by recent reports, published after the conceptualisation and/or implementation of the present reconsolidation study, which showed that behavioural manipulations of the reconsolidation process, relative to extinction-based methods that are delivered outside the reconsolidation window, can yield superior reduction of experimentally induced (Agren et al., 2017; Björkstrand et al., 2015; Fernandez-Rey, Gonzalez-Gonzalez, & Redondo, 2018; Grégoire & Greening, 2019) and naturally occurring fears (Björkstrand et al., 2016, 2017).

Recent clinical research indicates that only minor adaptations to current exposure-based practices may be required to achieve long-lasting reduction of fear. Telch et al. (2017) demonstrated that exposure therapy that is delivered during a period of memory reconsolidation provides greater reduction of self-reported fear of spiders and snakes than exposure therapy conducted without prior memory reactivation (the "reactivation trial" in the control group was delivered after, not before, exposure). During reactivation of the fear memory, participants were exposed to a live snake or spider for 10 seconds, while mentally rehearsing an actual or imagined encounter with the feared animal. Hence, the reactivation procedure contained elements of the CS (snake/spider) and, presumably, the US (mental reliving of feared animal encounter). Thirty minutes later, participants received six in vivo exposure trials, which required close proximity (within 30.5 cm) to the feared animal for a duration of three minutes per trial. Fear ratings obtained during a behavioural approach test conducted one month later, reflected lower self-rated animal fear in participants who received exposure therapy subsequent to memory reactivation, relative to participants who underwent exposure treatment without prior memory reactivation. These findings provide preliminary evidence for the translational utility of reactivation-extinction procedures, indicating that behavioural manipulations of the memory reconsolidation process can be used in the treatment of naturally occurring fears (see also Björkstrand

et al., 2017). The question remains though whether such an approach might be applicable to more complex fear memories, other than those underlying specific phobias.

There is indeed evidence to suggest that an intervention that is based on the principles of US reactivation-extinction may reduce intrusive memories and nightmares in male veterans with a diagnosis of post-traumatic stress disorder (PTSD; Gray, Budden-Potts, & Bourke, 2019). The *Reconsolidation of Traumatic Memories* intervention employed by Gray and colleagues has two core components, one being the memory reactivation phase focusing on distressing aspects of the trauma, and the other being the delivery of corrective information intended to update the traumatic memory with non-threatening information, akin to that provided by CS-only extinction training (for full details of the protocol, see Gray et al., 2019; Gray & Liotta, 2012).

Memory reactivation was based on the client's trauma narrative, consisting of a brief retelling of the targeted traumatic event until the client exhibited signs of sympathetic arousal, such as changes in breathing or tone of voice. At this stage, the reactivation trial was terminated and corrective information was delivered to update the reactivated fear memory. Gray et al. (2019) reported that the reactivation trial has been derived from Liu et al.'s (2014) work, and therefore deliberately focuses on the US, rather than the CS. However, it appears that the reactivation procedure may contain aspects of CS- and US-reactivation, given that these stimuli might be difficult to separate when the client is verbalising details of the trauma (see also Kindt & van Emmerik, 2016 for a similar reactivation procedure). Treatment consisted of three 2-hour sessions, delivered over a period of two weeks. Gray and colleagues reported that 71% of participants who completed treatment no longer met diagnostic criteria of PTSD, with 65% being in complete remission, as assessed two and four weeks after the conclusion of treatment.

These findings are promising and indicate that a brief reconsolidation-interfering intervention may be applicable to the treatment of symptoms underlying PTSD. However, there are a number of limitations that call for further research to replicate Gray et al.'s (2019) findings. While similar findings were reported in a previous study (Tylee, Gray, Glatt, & Bourke, 2017), a key limitation of both studies is the use of a wait-list controlled design, which does not permit for comparison of Gray et al.'s protocol with established treatments for PTSD, such as trauma-focused cognitive behavioural

therapies (National Institute for Health and Care Excellence, 2018), or with a group that received the same intervention without prior memory reactivation. Furthermore, the use of multiple, unprotected *t* tests means that the results must be interpreted with caution. Nevertheless, Gray et al.'s present and past research (Tylee et al., 2017) provides preliminary support for the effectiveness of the intervention in the treatment of PTSD symptoms and may encourage future research into clinical applications of behavioural manipulations of the memory reconsolidation process.

6.2.2.1. Advancing Clinical Applications of US Reactivation-extinction

The studies conducted by Telch et al. (2017) and Gray et al. (2019) provide preliminary evidence for the translational utility of reactivation-extinction procedures to the treatment of naturally occurring fears. In contrast to the present reconsolidation study, the memory reactivation procedures in both studies involved exposure to the CS and the US, although Gray et al.'s reactivation procedure was designed to focus on the US during reactivation, while Telch et al.'s procedure is more closely aligned with CS-reactivation. Although both procedures appeared to destabilise the target fear memory, as reflected in the reduced recovery of fear or loss of PTSD diagnosis, the development of a memory reactivation procedure that always destabilises the target memory will remain a challenge for future research.

Kindt (2018), a key contributor to the field of human reconsolidation research, aptly describes the challenges involved in the pilot testing of an effective memory reactivation procedure for the treatment of subclinical spider fear through the pharmacological disruption of reconsolidation (Soeter & Kindt, 2015a). In line with the literature reviewed in the present thesis, the main challenges identified were the need for the reactivation procedure to generate a prediction error that leads to the destabilisation of the target memory, which subsequently allows for interference with the restabilisation of the active and malleable memory (see also Chapter 1, Figure 1.1). Further complicating the development of reconsolidation-interfering interventions is the limited knowledge of the participant's conditioning history, which means that it is difficult to determine how the fear was acquired and how a memory reactivation procedure should be tailored to the needs of the participant (or client), in order to successfully destabilise the fear memory (Kindt, 2018).

In this regard, it is conceivable that memory reactivation procedures focusing on the feared outcome (US), rather than the feared cue (CS), may be better suited to the destabilisation of naturally occurring fears, as US-reactivation by design does not require the identification of the CSs, and has been shown to destabilise multiple CS-US associations (Liu et al., 2014; see also Chapter 4). Past research indicates that US-reactivation in the clinical setting may be achieved through the reactivation of hotspots, meaning the most distressing aspects of a traumatic event (Kindt & van Emmerik, 2016), or through brief retelling of traumatic memories (Gray et al., 2019). Memory reactivation in these examples is conducted in an imaginal manner (see also Grégoire & Greening, 2019), and in the case of PTSD treatment (e.g., Gray et al., 2019), in vivo exposure to the US would be neither feasible nor desirable or ethically permissible. While systematic investigations into the role of the US in the reactivation of naturally occurring fears are lacking, it is conceivable that the US could be isolated during the memory reactivation trial, for instance by instructing clients to focus exclusively on the feared outcome, rather than on the entire traumatic event. In this instance, future research could compare the effectiveness of US-reactivation in the destabilisation of fear memories with reactivation procedures that are likely to involve aspects of the CS and the US, such as the reactivation procedures involving script-driven imagery (Brunet et al., 2018).

As an alternative to memory reactivation induced by mental imagery, in vivo US-reactivation may be employed in the destabilisation of some naturally occurring fears, such as those underlying social anxiety disorder (SAD; see Chapter 3 for detailed example of potential CSs and USs in SAD). Briefly, US-reactivation in SAD could consist of brief exposure to a feared outcome, such as negative feedback (US), which would then be followed by exposure therapy, meaning exposure to the feared CSs, in the absence of the US. Such an approach may only require minor modification of techniques that are already employed in the treatment of SAD, such as shame attacks (i.e., clients intentionally elicit or imagine disapproval from others to assess the true cost and capacity to cope; Craske et al., 2014; McEvoy, Erceg-Hurn, Saulsman, & Thibodeau, 2015).

Of importance for the success of reconsolidation-interfering interventions is the presence of a prediction error during memory reactivation, in order to destabilise the target memory (Lee et al., 2017). This step may appear counterintuitive to clinicians, as prediction errors (violations of

expectancies) are integral to the actual exposure treatment, which involves the disconfirmation of clients' beliefs about the occurrence, intensity, and consequences of feared outcomes (Craske et al., 2018). The prediction error required for the destabilisation of fear memories, on the other hand, is not aimed at changing clients' beliefs about future threat encounters, but serves to initiate the neural and molecular processes that return the consolidated fear memory trace to an active and labile state (i.e., *destabilisation*), which allows for subsequent manipulation of the reconsolidation (i.e., *restabilisation*) process (e.g., Lee, 2009; Lee et al., 2017; Wideman et al., 2018).

To date, no systematic investigations have been conducted into how exactly different types of memory reactivation procedures generate the prediction error required for memory destabilisation (see also Kindt, 2018). What is known, however, is that prediction errors are generated by presenting information that is of relevance to the learning history (e.g., exposure to the CS or US), but creates a mismatch between expected and actual events (Lee, 2009; Lee et al., 2017). In contrast to CS-reactivation, which may require identification of all CSs that are associated with the feared outcome, US-reactivation could be employed even if the client cannot recall which specific CSs had been originally paired with the feared US.

The prediction error in US-induced memory reactivation could be generated through a number of pathways: (a) through the unsignaled presentation of the US, as opposed to the US being predicted by encounters of the CS; (b) by terminating exposure to the US after a brief period of time, which may violate the client's expectation that exposure to trauma-related stimuli is dangerous and will lead to a loss of control or that "anxiety will last forever when thinking about the trauma" (i.e., as applicable to PTSD; Foa & McLean, 2016, p. 7); or (c) by asking clients to mentally reduce (i.e., devalue) the perceived aversiveness of the US (Dibbets, Lemmens, & Voncken, 2018; Dibbets, Poort, & Arntz, 2012; see also Chapter 5), akin to the reduction of US intensity in the present US-reactivation procedure (Chapter 4). Whether the perceived "intensity," or aversiveness of the US, needs to be altered for the purpose of memory reactivation remains to be investigated.

Liu et al.'s (2014) findings indicate that a reduction of US intensity was required for memory destabilisation in humans. However, animal research suggests that the mere unsignaled nature of a US-reactivation trial may be sufficient to destabilise fear memories (Dębiec et al., 2010; Huang et al.,

2017). Hence, more work is required to determine which types of US-reactivation procedures yield optimal destabilisation of naturally occurring fears. In saying this, as reconsolidation is not a technique used in the treatment of fears, but a process that maintains memory relevance through the updating of existing memories (e.g., Lee, 2009), it is unlikely that the client's current mental representation of the US perfectly matches that of the original fear acquisition episode (see also Davey, 1989 for the role of US revaluation in the inflation and devaluation of perceived US aversiveness; and Fernández, Pedreira, & Boccia, 2017 for the role of reconsolidation in the maintenance of anxiety disorders). Consequently, brief exposure to the feared US may be sufficient to generate the prediction error required for memory destabilisation, pending further investigation in clinical research.

Of further interest to future research are two open questions, being whether the duration of the US-reactivation trial should be relatively short, in line with CS-induced reactivation procedures (e.g., 15 s; Maples-Keller et al., 2017), and whether extinction of all CSs is required. In general, it is recognised that relatively brief (e.g., 1-4 s), rather than longer (e.g., 30 s; 3 min), reactivation trials facilitate memory destabilisation (Hu et al., 2018; but see Agren et al., 2012), whereas prolonged exposure to the CS, or repeated presentations of the CS, may induce extinction learning rather than memory destabilisation (Merlo, Milton, Goozée, Theobald, & Everitt, 2014). Whether similar boundary conditions would apply to US-reactivation requires further examination. However, it may be in the client's best interest to restrict the duration of the US-reactivation procedure, to minimise discomfort and prevent premature cessation of treatment.

With regards to the second question, an aspect awaiting further examination is whether the treatment of naturally occurring fears requires extinction of all CSs that are associated with the reactivated US, or whether fear reduction to multiple CSs could be achieved through extinction of one CS only, as demonstrated in the experimental setting (Liu et al., 2014). This point of interest relates to a key determinant of successful fear reduction through the manipulation of the reconsolidation process. It is not only the destabilisation of the fear memory that is required for subsequent fear reduction, but also the delivery of an intervention, whether behavioural (e.g., Telch et al., 2017) or pharmacological (e.g., Soeter & Kindt, 2015a), that is capable of disrupting the reconsolidation

process. The delivery of a suitable intervention is important, as past research demonstrated that manipulations of the reconsolidation process can result in the reduction (e.g., Schiller et al., 2010) or enhancement (Chan, Leung, Westbrook, & McNally, 2010; Lee, 2008; Tay, Flavell, Cassini, Wimber, & Lee, 2019) of conditioned fear, whereby the latter may contribute to the maintenance or exacerbation of fears. Therefore, destabilisation of the fear memory trace by itself is not sufficient for the long-lasting reduction of fear – fear reduction also requires the use of a protocol that is capable of interfering with memory reconsolidation (see also Chapter 1, Figure 1.1).

It should be noted, however, that at present no indices of successful memory destabilisation in humans exist. In line with other phenomena in human fear conditioning research, successful memory destabilisation (and manipulation of reconsolidation) is inferred from the behavioural expression of fear, typically tested after a delay of 24 hours (e.g., Liu et al., 2014). While such an approach allows for comparison of fear recovery between the control and experimental groups, failure to observe fear reduction subsequent to the manipulation of reconsolidation cannot be unequivocally linked to deficits in memory destabilisation, but may also reflect the limited effectiveness of the method used to disrupt reconsolidation, or indeed boundary conditions of reconsolidation (e.g., Zuccolo & Hunziker, 2019). Hence, the development of clinical markers of memory destabilisation would substantially advance the development of reconsolidation-interfering interventions for the treatment of naturally occurring fears. This might indeed be achievable in the near future, with the respective research being conducted in the Kindt laboratory (Kindt, 2018).

6.2.2.1.1. Addressing Misconceptions about Reconsolidation

Since the conception and implementation of the reconsolidation study conducted as part of this thesis, many advances in pre-clinical (e.g., Björkstrand et al., 2016, 2017) and clinical reconsolidation research (e.g., Gray et al., 2019; Maples-Keller et al., 2017; Telch et al., 2017) have been made, indicating that reconsolidation-interfering interventions may be successfully applied in the treatment of psychopathologies. In this regard, extant literature suggests that interventions involving pharmacological (e.g., Brunet et al., 2018; Kindt & van Emmerik, 2016) or behavioural (e.g., Telch et al., 2017) manipulations of the reconsolidation process can be employed in the treatment of anxiety and stress-related disorders (Beckers & Kindt, 2017; Gray et al., 2019; Kida, 2018; Kindt, 2018;

Visser et al., 2018; Walsh, Das, Saladin, & Kamboj, 2018; Weisman & Rodebaugh, 2018), specific phobias (Björkstrand et al., 2016, 2017; Maples-Keller et al., 2017; Soeter & Kindt, 2015a; Telch et al., 2017), depression (Högberg & Hällström, 2018; Post & Kegan, 2017), and substance use disorders (Das, Gale, Hennessy, & Kamboj, 2018; Exton-McGuinness & Milton, 2018; Germeroth et al., 2017; Hon, Das, & Kamboj, 2016; Lonergan et al., 2016; Treanor, Brown, Rissman, & Craske, 2017; Walsh et al., 2018).

These reports are indeed encouraging and a healthy degree of optimism is beneficial for future research. At the same time, it has to be stressed that despite all advances made in human reconsolidation research, our understanding of memory reconsolidation processes is still advancing and interventions based on the disruption of the reconsolidation process, or treatments that are inspired by reconsolidation research (Iyadurai et al., 2018), should not be viewed as a panacea for the treatment of psychological disorders or as a "cognitive therapeutic vaccine" (Iyadurai et al., 2018, p. 7) for the prevention of clinically significant anxiety or stress-related disorders (for a detailed critique of such claims, see Cristea, Naudet, Shanks, & Hardwicke, 2018). To date, outcomes of reconsolidation studies remain mixed (as discussed in the Implications for Theory and Research section of the general discussion) and further pre-clinical and clinical research is required to develop reconsolidation-interfering interventions that can be successfully employed in the treatment of naturally occurring fears.

A key misconception that may pose a challenge to the translation of experimental findings to the clinical setting pertain to the role of prediction errors in the initiation of the reconsolidation process. As discussed previously, the reactivation procedure needs to generate a mismatch between expected and actual events to destabilise the fear memory (e.g., Lee, 2009); mere retrieval is not sufficient for memory destabilisation (e.g., Sevenster et al., 2012). The type of prediction error required to destabilise fear memories differs from the prediction errors used in exposure therapies, which are aimed at enhancing extinction learning by violating clients' threat expectancies (e.g., Craske et al., 2014). The goal of reconsolidation interference, on the other hand, is to alter the original fear memory (e.g., Agren et al., 2012), rather than creating a novel, inhibitory association, which would be the result of extinction learning (e.g., Bouton, 2002; Craske et al., 2014). In contrast to the

violations of expectancies utilised in exposure therapies, memory reactivation procedures in pre-clinical research do not require participants to verbalise their threat expectancies, although these can be measured throughout the experiment (Sevenster et al., 2013), nor are verbal instructions required to generate prediction errors (e.g., the US-reactivation procedure is not based on verbal instructions; Liu et al., 2014).

A typical reactivation procedure in pre-clinical research involves a brief presentation of the previously conditioned CS+, in the absence of the US, whereas the less commonly employed US-reactivation entails a brief presentation of the US, in the absence of the CS (see Chapter 1, Figure 1.1 for further information). Both procedures can generate the prediction error required for memory destabilisation, for instance by violating the expectation that the US follows a CS (US-reactivation) or that the CS signals the imminent arrival of the US (CS-reactivation; for a comprehensive discussion of prediction errors, see Chapter 1); both reactivation procedures violate the CS-US contingency.

However, the prediction error generated by the reactivation procedure is not intended to "replace the original memory" (Manfield, Lovett, Engel, & Manfield, 2017, p. 197). While our understanding of mechanisms underlying fear reduction through manipulations of reconsolidation is still advancing (e.g., Beckers & Kindt, 2017), extant literature demonstrates that manipulation of the reconsolidation process in humans can be achieved through the oral administration of propranolol (e.g., Kindt et al., 2009), thereby demonstrating that *reconsolidation interference* (unlike memory *destabilisation*) is not contingent on the delivery of an intervention that generates prediction errors (for relevant reviews of pre-clinical and clinical research, see Paulus, Kamboj, Das, & Saladin, 2019; Walsh et al., 2018).

To conclude the discussion of the translational utility of US reactivation-extinction, the information presented in the preceding paragraphs indicates that only minor modifications of existing techniques may be required to destabilise naturally occurring fears and interfere with their reconsolidation. Key determinants of successful reconsolidation interference include (a) a reactivation procedure that generates the prediction error required to destabilise the fear memory and (b) the delivery of an intervention, such as exposure therapy, that can interfere with reconsolidation of the active fear memory trace. Importantly, the continuing development of reconsolidation-interfering

interventions for the treatment of anxiety and stress-related disorders will require close collaboration between basic and applied researchers, which not only serves to overcome perceived challenges in the translation of basic research findings to the clinical setting (as discussed in this chapter), but also allows clinical researchers (e.g., Gray et al., 2019; Manfield et al., 2017; Telch et al., 2017) to inform future experimental research.[8]

6.2.3. Clinical Applications of Occasionally Reinforced Extinction Training

In contrast to the preceding discussion of reconsolidation-interfering interventions, at present no clinical research exists which may inform the discussion of clinical applications of occasionally reinforced extinction training. Nevertheless, the present findings corroborate previous suggestions (Craske et al., 2014) that occasional presentations of the US during extinction training, either paired with the CS or presented in the middle of the ITI, may yield superior reduction of fear recovery compared to conventional CS-only extinction training. The key advantage of occasionally reinforced extinction is the low number of US presentations, which may be better tolerated by clients than the relatively high US:CS ratio employed in past unpaired extinction research (Rauhut et al., 2001; Thomas et al., 2005; Vervliet et al., 2010). Thus, it is not the occasional exposure to the US that may pose a challenge for translational research, but the adaptation of the unpaired extinction procedure to the treatment of naturally occurring fears.

In contrast to the partially reinforced extinction procedure, which can be readily adapted to the use in the clinical setting in the treatment of some anxiety disorders (see Chapter 3 for clinical example), the unsignaled presentation of the US poses obvious challenges, as it may be difficult to separate CS and US exposure. However, as was illustrated in the discussion of reconsolidation-interfering interventions, it may be possible to conduct imaginal exposure to the US (e.g., Dibbets et al., 2018; Dibbets et al., 2012), which will be illustrated in the following hypothetical scenario. It is important to mention, however, that this example serves to illustrate the potential use of the present partially reinforced and unpaired extinction procedure in the clinical setting, not to suggest that these

[8] I would like to thank the clinicians and clinical researchers who have provided me with a deep appreciation of the importance of and difficulties in translating experimental research findings to the clinical setting. I am particularly grateful to Prof. Peter McEvoy, A/Prof. Melissa Norberg, and Ms Andrea Pauquet for their generous contribution of their time, expertise, and ideas for future research.

procedures are readily applicable to the treatment of naturally occurring fears, without further pre-clinical and clinical research. Please also note that this is one of many possible scenarios. In the clinical setting, treatment would commence with the identification of feared cues and feared outcomes (for an examination of common feared outcomes, see Lovibond & Rapee, 1993). As such, the present example has been annotated, to highlight the CS and US in this particular example, and to explain how paired and unpaired US presentations could be employed in the reduction of fear in the present scenario.

Scenario: Susan is seeking treatment due to recurrent panic attacks (US) she is experiencing while driving (CS) to work, which made her afraid of losing control of her car and causing an accident. As a result, Susan developed a fear of driving (conditioned response). During the intake interview, Susan disclosed that the panic attacks originate from ongoing workplace bullying, whereby the first panic attack occurred when she was preparing for a meeting (CS) during which the bullying typically occurs. At the beginning of treatment, Susan and her therapist define treatment goals and Susan receives psychoeducation pertaining to the development, maintenance, and treatment of her debilitating anxiety. Psychoeducation may be of particular benefit to reduce the risk of drop out, for instance by explaining to Susan that experiencing occasional panic attacks outside of therapy, for instance while preparing for a meeting (unpaired US) or during a meeting at work (paired US), does not indicate that the treatment is not working, but may be actually beneficial for the long-term reduction of fear.

Application of the occasionally reinforced extinction procedure to Susan's example may be conducted through imaginal exposure to the feared CSs (driving her car; attending a meeting) while occasionally asking her to recall a time when the panic attack (US) occurred. Such an approach is similar in its application to the previously discussed US-driven memory reactivation through hotspots (Kindt & van Emmerik, 2016) or through the retelling of traumatic memories (Gray et al., 2019). The challenge here lies in the therapist's and client's willingness or ability to engage in intentional exposure to the US, as such exposure may cause temporary distress to the client. At the same time, this distress would also be experienced during traditional exposure therapy, during which clients

mentally relive feared situations, in order to disconfirm unhelpful beliefs about the intensity or frequency of the expected aversive outcome (Craske et al., 2014; Foa & McLean, 2016).

In the present example, the occasional, unexpected presentation of the US may help to disconfirm the belief (i.e., create a violation of expectancies/prediction error) that panic attacks are dangerous and will lead to a loss of control and injury. In this sense, the intentional imaginal exposure to the US may aid the client in developing greater resilience and allow her to better cope with real-life panic attacks (see also Culver et al., 2018 for the "physiological toughness" hypothesis). Furthermore, if an unpaired extinction procedure is employed, for instance through imaginal exposure to the US between brief CS-exposure trials (e.g., as an adaptation of Telch et al.'s, 2017 protocol), a greater violation of expectancies may occur (e.g., that driving triggers a panic attack) than that achieved through CS-only exposure or through a partially reinforced extinction-based procedure, which would facilitate the reduction of her fear of driving.

In order to attenuate fear of all cues that are associated with the panic attack, exposure to all CSs is required, in line with extinction training employed in the present research and in exposure therapy in clinical practice (Craske et al., 2014). However, based on the results of the present occasionally reinforced extinction study and past research (Culver et al., 2018), the key determinant of enduring fear reduction is the occasional exposure to the US, rather than repeated exposure to the feared cues in the absence of the US. Taken together, it appears that such a procedure could be implemented into existing exposure-based therapies, whereby the key modification would involve a shift in focus from rapid distress reduction through repeated CS exposure, to the enhancement of extinction learning through maximisation of prediction errors, generated by occasional US presentations (see also Craske et al., 2014). Notwithstanding the potential benefits of occasionally reinforced extinction training in the treatment of naturally occurring fears, further research is required to determine if such a procedure is suitable for the use with clinical populations.

An aspect that will require further examination is whether US intensity, or the perceived aversiveness of the US, modulates treatment outcomes. Findings from early animal research suggest that the US should be presented at the same intensity as that employed during acquisition training, in order to maximise the reduction of the CS-US association through unpaired extinction training; the

presentation of the US at reduced intensity also weakened the CS-US association, but to a lesser extent (Frey & Butler, 1977). While a recent animal study has shown that presenting the US at reduced intensity reduced conditioned fear (Burhans & Schreurs, 2019), it is presently not known whether such an approach would yield the same reduction of fear recovery as that observed in the present unpaired extinction procedure. This aspect requires further examination, as treatment of naturally occurring fears may require the use of a "weaker" US to minimise distress to clients.

6.2.4. Ethical Considerations

The key ethical concerns that pose a challenge to the translational utility of the present findings pertain to the distress US exposure may evoke in clients, as well as to the consequences of memory updating. The discussion of possible ramifications of memory updating is warranted, as extant literature shows that reconsolidation interference alters the original fear memory trace (e.g., Björkstrand et al., 2017). Concerns have been expressed about potential implications for the person's identity and personality through the updating of memories during the memory reconsolidation period (Lavazza, 2019). While such concerns are understandable from an ethical point of view, the suggestion that the modification of fear memories during the reconsolidation period may change a person's attachment style and consequently their identity and interactions with the world (Lavazza, 2019) is not supported by empirical evidence. While reconsolidation interference can eliminate the fear memory trace in the human amygdala (Agren et al., 2012), it is not the case that the entire memory trace is erased, as the declarative component of the fear memory remains intact (Kindt et al., 2009; see Chapter 1 for further details).

What is actually "erased" is the emotional expression of fear (Kindt et al., 2009), which means that participants can be exposed to a CS without exhibiting a physiological fear response, despite remembering that the CS used to signal the arrival of an aversive US (for further discussion of ethical concerns, see Beckers & Kindt, 2017). Such treatments may, therefore, serve an important adaptive function, allowing clients to reduce distress and anxiety while still remembering which cues may be potentially threatening (e.g., it would be of advantage to our survival to remember that dangerous animals in the wild are best avoided).

The other concern pertains to the distress US exposure may evoke in clients. It should be noted that the exposure to the US is not conducted to cause distress, but to reduce debilitating symptoms of anxiety and stress-related disorders. As discussed in the present thesis and in past research (e.g., Craske et al., 2014), the occasional presentations of the US during extinction training may improve long-term treatment outcomes and, thereby, reduce the distress associated with symptoms of anxiety and stress related disorders, such as that caused by intrusions and nightmares in PTSD patients (Foa & McLean, 2016). The gold-standard treatments for anxiety and stress-related disorders already entail exposure to the feared CSs (Craske et al., 2018) and at times to the US, for instance during *shame attacks* employed in the treatment of SAD (e.g., Craske et al., 2014; McEvoy et al., 2015). Although exposure-based practices may cause temporary discomfort, the exposure to the CS and the US serves to disconfirm clients' beliefs about threat expectancies, thereby reducing debilitating fear and anxiety when the fear-predictive CS is encountered outside of therapy.

Clients who are unable or unwilling to tolerate occasional in vivo or imaginal exposure to the US are able to draw on alternative, evidence-based psychological (e.g., for PTSD treatments, see National Institute for Health and Care Excellence, 2018) or pharmacological treatments (for a brief overview, see Craske & Stein, 2016). Irrespective of the treatment employed, potential risks (e.g., temporary discomfort and distress) and benefits of exposure-based therapies (e.g., reduction of anxiety and increase in quality of life) should be explained to the clients and informed consent should be obtained before treatment commences, in accordance with established practice.

In summary of the discussion of implications for the clinical setting, the key findings of the research conducted as part of this thesis show that superior reduction of fear recovery can be achieved through fear reduction methods that involve exposure to the CS and the US, compared to conventional CS-only extinction training. Translation of these methods to the treatment of anxiety and stress-related disorders requires further research, although behavioural manipulations of the memory reconsolidation process have been already successfully employed in the treatment of naturally occurring fears (Telch et al., 2017). The continuing development of reconsolidation-interfering interventions could be greatly enhanced through the identification of a reliable memory destabilisation procedure and/or through identification of clinical markers of memory destabilisation, which would

allow clinicians to devise memory reactivation procedures that are applicable to each individual client (see also Kindt, 2018). In this regard, the benefits of the US-reactivation procedure employed in the present and past (Liu et al., 2014) research have been discussed in the present chapter, including its ability to concurrently destabilise multiple fear memories, including those which were thought to resist modification through behavioural manipulations of reconsolidation (e.g., Kindt & Soeter, 2013).

6.3. Limitations and Future Directions

In addition to the limitations already addressed in this and the preceding chapters (3-5), a general limitation of the present research is the generalisability of experimental research findings to clinical populations, as only healthy volunteers, consisting largely of university students, were recruited for the studies conducted as part of this thesis. To examine the translational utility of the present occasionally reinforced extinction and reactivation-extinction procedures, more research is required to determine if these procedures can yield long-lasting reduction of fear in individuals with clinically significant anxiety or in those who exhibit heightened risk factors for the development of anxiety. In this regard, two variables that have received some research attention to date and warrant further investigation are trait anxiety and intolerance of uncertainty (Lonsdorf & Merz, 2017).

In a recent review of individual difference literature, Lonsdorf and Merz (2017) reported that trait anxiety affects several domains of human fear learning, including fear acquisition and fear generalisation, although the findings remain mixed and differ across indices of conditioned responding (e.g., SCRs vs. US expectancy ratings) and conditioning protocols (e.g., strong vs. weak protocols; Lissek, Pine, & Grillon, 2006). Nevertheless, conditioned responding during extinction training is somewhat larger in clinically anxious than non-anxious individuals, indicating that clinically anxious individuals exhibit greater levels of fear when exposed to CSs that used to signal the arrival of aversive events (Lissek et al., 2005). At present, more research is required to examine which factors mediate this increased responding in clinically anxious individuals, for instance whether it is the result of intolerance of uncertainty (Morriss, Christakou, & van Reekum, 2016).

Intolerance of uncertainty (IU), or the fear of the unknown (Carleton, 2012), is a dispositional risk factor for the development and maintenance of clinically significant anxiety and depression (Carleton, 2012, 2016; McEvoy & Mahoney, 2012). IU is also a transdiagnostic process that is

associated with a broad range of clinical presentations, including symptoms of obsessive-compulsive disorder, social anxiety, panic disorder, health anxiety, and post-traumatic stress disorder (for a review, see Shihata, McEvoy, Mullan, & Carleton, 2016). Compared to clinical research (e.g. McEvoy & Erceg-Hurn, 2016), the role of IU in the development, maintenance, and reduction of fears has received relatively little attention in the experimental setting.

Findings from experimental research indicate that individuals higher in IU, relative to those lower in IU, are more likely to view ambiguous stimuli as a source of threat (Oglesby, Raines, Short, Capron, & Schmidt, 2016), show an impaired ability to discriminate between conditioned threat and safety cues (Morriss, Macdonald, & van Reekum, 2016), engage in increased avoidance behaviours (Flores, Lopez, Vervliet, & Cobos, 2018), are slower to extinguish conditioned avoidance (Flores et al., 2018) and conditioned SCRs (Morriss, Christakou, et al., 2016), show greater spontaneous recovery of extinguished SCRs (Dunsmoor, Campese, Ceceli, LeDoux, & Phelps, 2015), exhibit increased physiological arousal (Chin, Nelson, Jackson, & Hajcak, 2016; Greco & Roger, 2003; Tanovic, Gee, & Joormann, 2018), and are more anxious in anticipation of potential threat (Oglesby & Schmidt, 2017). These findings, therefore, indicate that individuals who are high in IU, and hence at risk for developing mental health problems, may exhibit a different pattern of conditioned responding, relative to individuals low in IU. Nevertheless, the findings remain mixed and the role of IU in human fear conditioning and extinction remains poorly understood, and requires further investigation (Lonsdorf & Merz, 2017).

In addition to the need for further research with clinical populations, another aspect limiting the overall interpretation of findings and requiring further examination is the absence of group differences in differential SCRs during tests of reinstatement. Reinstatement in all studies involved the unsignaled presentation of three USs, delivered at the same intensity as that employed during acquisition training, and was followed by six or eight non-reinforced presentations of each CS type; each reinstatement phase was preceded by tests of spontaneous recovery. Reinstatement of differential SCRs, however, was only observed in the control group of the reconsolidation study, but not in any of the other groups in the studies conducted.

Reinstatement of fear is proposed to occur after re-exposure to the aversive US, in the absence of the CS (Bouton, 2004), and is the experimental analogue of clients unexpectedly encountering a feared outcome, such as a panic attack, which increases subsequent fear of cues that were predictive of aversive events (for this and other applied examples of phenomena investigated in the basic research setting, see Vervliet, Craske, & Hermans, 2013). In contrast to animal research, reinstatement of fear in humans has received little research attention and remains poorly understood (Haaker, Golkar, Hermans, & Lonsdorf, 2014). In this regard, the present findings offer insight into boundary conditions and methodological variations that may affect the reinstatement of fear.

Haaker et al. (2014) proposed that assessment of true reinstatement effects is contingent on sufficiently controlling extraneous factors that may affect conditioned responding, such as the orienting response or spontaneous recovery effects. As each reinstatement procedure in the present research was preceded by tests of spontaneous recovery and the use of a differential fear conditioning paradigm controls for the effects of the orienting response (e.g., Lipp, 2006a), it appears that the present results reflect the true extent of fear reinstatement, or lack thereof.

Another important consideration addressed in Haaker et al.'s (2014) review paper, is the effect of time delays between the administration of reinstatement shocks and subsequent tests of conditioned responding, as well as time delays between experimental phases, such as those between acquisition, extinction, and tests of fear recovery. Time delays between the administration of reinstatement shocks and tests of conditioned responding have been proposed to create a more threatening context due to the uncertainty created by the experimental break, and consequently have been proposed to increase conditioned responding (Haaker et al., 2014). However, this hypothesis is not readily reconciled with the present findings, as all studies employed a brief break between the delivery of reinstatement shocks and subsequent tests ($\leq$ 10 minutes), but reinstatement of differential SCRs occurred in one study only.

Another possibility is that reinstatement of fear is more pronounced when extinction is administered 24 hours after fear acquisition (Golkar & Öhman, 2012; Haaker et al., 2014). There is some evidence in support of this hypothesis, as reinstatement occurred only in the control group of the reconsolidation study, which underwent extinction training 24 hours after acquisition. At the same

time, follow-up tests, conducted 8 to 12 months later, showed no clear evidence of fear reinstatement. It appears, therefore, that the reinstatement of fear in humans requires further examination in studies specifically designed to test reinstatement. An experimental parameter that may be useful in this regard is the inclusion of a control group that does not receive reinstatement shocks, to control for the influence of non-associative factors, such as the uncertainty induced by an experimental break (Haaker et al., 2014).

6.4. Strengths

The present research addressed previous suggestions for the optimisation of exposure-based therapies, which are based on the principles of extinction learning (Craske et al., 2014), and investigated three alternative methods of fear reduction. All methods tested in the present studies involved exposure to the CS and the US that had been used during fear acquisition. In contrast to conventional CS-only extinction training or counterconditioning, which involves presentations of positively valanced (Haubrich et al., 2015) or neutral (Dunsmoor et al., 2015) stimuli in place of the aversive US, the present approaches were not based on the omission of the US that contributed to fear acquisition, but involved the deliberate exposure to the aversive US.

There are several advantages to such an approach, one of them being that the occasional exposure to the US may allow individuals to gain information about the future likelihood of being exposed to feared outcomes in the presence of fear predictive cues. Based on the results from the occasionally reinforced extinction study (Chapter 3), it appears that such learning is more effective in the reduction of fear recovery than merely learning that the US occurs in some contexts (i.e., the acquisition context), but not in others (i.e., the extinction context; Bouton, 2004). Additionally, the results of the reconsolidation study (Chapter 4) showed that the US is an important reminder of the conditioning history, capable of reactivating multiple CS-US associations. The reconsolidation of the reactivated memories was subsequently disrupted through extinction training, which was reflected in the absence of fear recovery after a delay of 24 hours and 8-12 months (Chapter 4: Addendum). Hence, these results provided important empirical support for the role of the US in the long-lasting reduction of fear and proposed three alternative pathways to the reduction of fear recovery, namely US-reactivation-extinction, partially reinforced extinction training, and unpaired extinction training.

Whether US devaluation-extinction (Chapter 5) might represent a fourth alternative pathway, as an alternative to conventional extinction training, requires further investigation.

With regards to methodological aspects, key strengths of the present studies included (a) the use of a Pavlovian fear conditioning and extinction paradigm and (b) the approach adopted to the scoring of electrodermal data. An aspect supporting the robustness of the present findings is the scoring of responses elicited by the CSs as entire interval SCRs (Kindt & Soeter, 2013; Pineles, Orr, & Orr, 2009), meaning SCRs were scored in the entire response window, between 1 to 6 s after CS-onset. This approach contrasts the frequently employed scoring of first interval, and to a lesser extent, second interval SCRs, occurring 1 to 4 s and 4 to 7 s after CS-onset, respectively (Pineles et al., 2009; Prokasy & Kumpfer, 1973). The scoring of SCRs represents a key methodological variation across studies which involve the recording of electrodermal activity, as well as being a point of ongoing debate (e.g., Luck & Lipp, 2016; Pineles et al., 2009).

Luck and Lipp (2016) suggested that scoring of electrodermal data in multiple response windows can be of advantage, for instance when experimental manipulations reflect a dissociation between the orienting response (proposed to be reflected in first interval responses) and the anticipation of the US (proposed to be reflected in second interval responses), while others do not support the separation of SCRs into first and second interval responses, but instead advocate the scoring of entire interval SCRs (e.g., Kindt & Soeter, 2013; Pineles et al., 2009). A key advantage of using entire interval SCRs, in lieu of alternative methods, is their ability to capture the maximum increase in electrodermal activity, irrespective of where it occurs within the CS-US interval (Pineles et al., 2009). This method makes it less likely that the true extent of conditioned responding will be underestimated (Pineles et al., 2009). Given that the recovery of extinguished fear was the key outcome of interest in the present studies, the use of entire interval SCRs also reduces the risk of underestimating the true extent of fear recovery and contributes to the robustness of the present findings.

Another strength of the studies conducted as part of this thesis was the use of a Pavlovian fear conditioning and extinction paradigm, which are laboratory-based analogues for the study of the development and treatment of clinically significant fear and anxiety (Craske et al., 2018; Grillon,

2008; Vervliet et al., 2013). While experimental research is often criticised by clinical researchers for its selection of "arbitrary stimuli like shapes or inanimate objects" (Carpenter et al., 2019, p. 6) or for the use of paradigms that are "too simple to model the learning and unlearning of complex fear memories" (Kunze, Arntz, & Kindt, 2015, p. 42), it is precisely the use of validated and well-controlled methods that allows us to study the mechanisms underlying fear learning and fear reduction. Admittedly, few naturally occurring fears will be based on blue and yellow squares, the type of stimuli often employed in experimental research (e.g., Schiller et al., 2010), but it is this type of research that allows for the identification of mechanisms underlying fear reduction (for a review, see Craske et al., 2018), including mechanisms underlying memory reconsolidation (for reviews, see Beckers & Kindt, 2017; Fernández et al., 2016; Lee et al., 2017). At the same time, if the goal of experimental research is to inform the treatment of clinically significant fears and anxiety disorders, the concerns surrounding ecological validity are warranted.

To enhance ecological validity of the present research, biologically fear-relevant CSs have been employed in addition to fear-irrelevant CSs (with the exception of the occasionally reinforced extinction study). Fear-relevant CSs, such as snakes, are stimuli that posed a threat to our ancestors' survival (Öhman & Mineka, 2001) and are preferentially associated with aversive outcomes (Seligman, 1971). There is evidence to suggest that the use of fear-relevant CSs leads to the acquisition of strong fears that are slower to extinguish than fears conditioned to fear-irrelevant CSs (Öhman, 2009; Öhman & Mineka, 2001). Hence, the use of fear-relevant CSs, such as the pictures of snakes and spiders employed in the present or in past research (Lipp, Cronin, Alhadad, & Luck, 2015) satisfies the suggestions of applied researchers (e.g., Carpenter et al., 2019) and allows for the comparison of fear recovery to fear-irrelevant and fear-relevant CSs. In contrast to past research (Öhman & Mineka, 2001), however, conditioned responding, as indexed by SCRs, was not a function of CS fear-relevance in the present research (see also Åhs et al., 2018). It is possible, however, that the use of a 100% reinforcement schedule masked potential differences in the rate of fear acquisition, as the use of a 100% reinforcement schedule typically results in rapid fear acquisition (for the effects of CS fear-relevance on the acquisition of fears on a 50% reinforcement schedule, see Ho & Lipp, 2014).

To advance the development of more efficacious treatments of anxiety and stress-related disorders, meaning treatments which are likely to provide long-lasting reduction of fear, findings from the experimental setting need to be translated to the applied setting. To facilitate research into the translational utility of occasionally reinforced extinction training and US reactivation-extinction, examples of potential clinical applications have been provided in the present thesis. It should be noted, however, that it was not the author's intention to provide an exhaustive list of examples, but to provide a few specific examples that can be used to inform future research and clinical practice.

6.5. Conclusion

Past research has provided us with a good understanding of mechanisms underlying fear learning and fear reduction. Effective methods of fear reduction, such as extinction training (Bouton, 2002), are available for the use in the basic and applied setting. Fear reduction is indeed easily achieved; it is the recovery of fear that poses a challenge for the treatment of naturally occurring fears (see also Vervliet et al., 2013). In the studies conducted as part of this thesis, three alternative methods of fear reduction have been tested, all of which involved exposure to the CS and the US.

The key findings of the present research demonstrated that the well-documented recovery of fear subsequent to CS-only extinction training (e.g., Bouton, 2002) can be reduced, and even eliminated, through fear reduction methods that involve exposure to the CS and the US. Specifically, fear recovery was reduced, or prevented, through (a) extinction training that was conducted with occasional presentations of the US, either paired with the CS or presented in the ITI, and (b) through administration of extinction training subsequent to US-induced memory reactivation. The findings from the US devaluation study, however, did not provide further clarification of the role of US devaluation in the persistent reduction of fear. Conventional extinction training was successful in the elimination of within-session conditioned responding, but did not prevent spontaneous recovery, reinstatement, or reacquisition of fear, thereby corroborating previous suggestions that within-session extinction of conditioned responding is not sufficient for the reduction of fear recovery (Bouton et al., 2004; Culver et al., 2018).

The key contributions of the present research to extant knowledge are as follows: Unpaired extinction training and US reactivation-extinction were more effective in the reduction of fear

recovery than partially reinforced extinction, to the extent that unpaired extinction interfered with the spontaneous recovery and reacquisition of fear, while the administration of extinction training subsequent to US-induced memory reactivation reduced spontaneous recovery and reinstatement of fear 24 hours and 8 to 12 months after administration of the procedure. Partially reinforced extinction training, on the other hand, showed greater reduction of spontaneous recovery compared to conventional extinction training, but did not prevent reacquisition of fear. As such, the approaches that involved unpaired US presentations appeared to be better suited to the persistent reduction of fear. However, this does not mean that fear reduction was mediated by the same underlying mechanisms.

Manipulations of the memory reconsolidation process, for instance, are proposed to modify the original fear memory trace (for a review, see Lee et al., 2017). The effects of unpaired and partially reinforced extinction training, on the other hand, may be mediated by an enhancement of extinction learning, although the determination of the exact underlying mechanisms requires further research; US habituation, however, has been eliminated as a possible underlying mechanism in the present research. Given the differences between unpaired and partially reinforced extinction training, both in their application and effects on fear recovery, it is conceivable that these methods are mediated by different mechanisms, as discussed in this chapter. Thus, the results of the occasionally reinforced extinction and the reconsolidation studies have provided important empirical support for the effectiveness of three alternative approaches to fear reduction, each involving exposure to the CS and the US, and demonstrating superior reduction of fear recovery, compared to the frequently employed CS-only extinction training.

Appendix A: Publication 1

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Enhancing extinction learning: Occasional presentations of the unconditioned stimulus during extinction eliminate spontaneous recovery, but not necessarily reacquisition of fear

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ABSTRACT

Background: Fears underlying anxiety disorders are commonly treated with exposure-based therapies, which are based on the principles of extinction learning. While these treatments are efficacious, fears may return after successful treatment. Past research suggested that post-extinction recovery of fear could be reduced through extinction training that involves occasional presentations of the aversive unconditioned stimulus (US), paired with the conditioned stimulus (CS). Here, we examined whether extinction training with occasionally paired or unpaired US presentations is superior in the reduction of fear recovery to non-reinforced extinction.

Method: Following differential fear conditioning to neutral cues, participants ($N = 72$; M age = 21.61 years, $SD = 3.95$) underwent either non-reinforced, partially reinforced, or unpaired extinction training.

Results: Extinction involving paired or unpaired US presentations, but not non-reinforced extinction, eliminated spontaneous recovery of differential skin conductance responses (SCRs). Results further suggested that unpaired, but not paired, US presentations may guard against rapid reacquisition of differential SCRs. No benefits of US presentations during extinction were found on the reinstatement of SCRs or recovery of differential negative CS + valence.

Conclusion: Presenting USs during extinction training was more effective than non-reinforced extinction in the reduction of fear recovery, as indexed by SCRs, with unpaired extinction being more effective than partially reinforced extinction.

1. Introduction

Past research has provided us with a good understanding of mechanisms underlying the development and reduction of fears, phobias, and anxiety disorders. Fears are acquired through association of neutral cues (conditioned stimuli, CSs), such as animals, with aversive outcomes (unconditioned stimuli [USs]; Davey, 1992), such as an animal bite. Through CS-US pairings we learn to predict which cues signal the arrival of aversive and potentially threatening events. While learning to fear cues which may pose a threat to our survival is an important adaptive mechanism that can protect us from harm and facilitate survival (Öhman & Mineka, 2001), fears may also become maladaptive and contribute to the development of anxiety and stress disorders, which can interfere with daily functioning (Foa & McLean, 2016). The current global prevalence rate of anxiety disorders is estimated at 7.3%, with approximately 11.6% of the population experiencing an anxiety disorder in a given year (Baxter, Scott, Vos, & Whiteford, 2013; Craske & Stein, 2016). Anxiety disorders are commonly treated with exposure-based therapies, which are based on the principles of extinction learning (Craske, Treanor, Conway, Zbozinek, & Vervliet, 2014). In its basic form, extinction training involves the repeated presentation of the CS, in the absence of the US, until a reduction of fear is achieved (Bouton, 2000).

While the efficacy of exposure therapies is well established, not all individuals respond to these treatments, while others experience a return of fear after successful treatment (Craske & Mystkowski, 2006; Weisman & Rodebaugh, 2018). Research suggests that extinguished fear may return, because extinction training does not result in the unlearning or persistent elimination of the original fear learning (i.e. the CS-US association), but creates a new, inhibitory association (CS-no US) that co-exists with the fear association (Bouton, 1993). As such, future CS presentations may activate the CS-no US or CS-US association,

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whereby the latter would allow for a return of fear (Bouton, 1993). Recovery from extinction phenomena are well-documented in the conditioning literature and include recovery of extinguished responding in a new context (renewal), after the passage of time (spontaneous recovery), after the unsignaled presentation of the US (reinstatement), or after additional post-extinction CS-US pairings (reacquisition; Bouton, 2002). Findings from animal research indicate that reacquisition after extinction may occur at a faster rate than de novo conditioning (Napier, Macrae, & Kehoe, 1992; but see; Ricker & Bouton, 1996), suggesting that the original fear learning is preserved during extinction and, thereby, may be retrieved through future cue encounters. Taken together, research has identified several pathways that may result in the return of fear following successful extinction training or the successful completion of exposure therapy.

When applied to an example of relapse in the clinical setting, for instance the return of social anxiety, which is characterized by fear of social situations in which individuals may be exposed to rejection, embarrassment, or negative evaluations by others (American Psychiatric Association, 2013), fear may recover when individuals are re-exposed to previously feared social situations (CS) or through exposure to additional CS-US pairings (reacquisition), such as receiving negative feedback (US) during a meeting at work (CS). Given the frequency with which feared cues and outcomes may be encountered in daily life, whether in a paired (CS-US) or unpaired manner (CS or US), the likelihood of fear recovery appears high - this may seem discouraging from a clinical point of view. However, recent evidence suggests that exposure therapy may be optimized in a way that would minimize recovery of extinguished fear, even in light of occasional post-extinction CS-US pairings.

A method of exposure therapy for reducing the return of fear proposed by Craske et al. (2014) involves *occasionally reinforced extinction*, meaning intentionally exposing clients to occasional presentations of the feared event (US) during exposure therapy. In the case of social anxiety, this may involve the delivery of rejection or "shame attacks" during exposure to social situations (Craske et al., 2014). While this idea appears counterintuitive as CS-US pairings are implicated in fear acquisition (Davey, 1992), extant literature suggests that occasional presentations of the US during extinction training may be superior to conventional, non-reinforced extinction in preventing recovery of extinguished responding (e.g. Bouton, Woods, & Pineño, 2004; Culver, Stevens, Fanselow, & Craske, 2018).

Specifically, experiments conducted with animal subjects demonstrated that partially reinforced extinction training, involving occasional delivery of CS-US pairings, interfered with the reacquisition of extinguished responding in appetitive (Bouton et al., 2004) and operant conditioning preparations (Woods & Bouton, 2007). Of particular interest was the observation that partially reinforced extinction slowed the reduction of responding during extinction, as would be expected from reinforced training, but protected against rapid reacquisition, relative to non-reinforced extinction. Additionally, compared to partially reinforced training, an unpaired extinction procedure, whereby reinforcers were not paired with the CS, but instead delivered in the inter-trial interval, further reduced the rate of reacquisition (Bouton et al., 2004 [Experiment 2]). Replication attempts with humans, however, have yielded mixed results in an appetitive conditioning study (van den Akker, Havermans, & Jansen, 2015), showing reduced reacquisition of US expectancies, but not self-rated *conditioned desires* for chocolate mousse, subsequent to partially reinforced and unpaired extinction training. That being said, the authors also reported group differences at baseline and differential effects of acquisition training on verbal (e.g. US expectancy) and physiological indices of conditioned responding (i.e. participants' rate of salivation in anticipation of food), making the overall interpretation of findings difficult.

An extension of Bouton et al's. (2004) findings to human fear conditioning, on the other hand, has yielded more promising results, suggesting that partially reinforced extinction may successfully reduce the reacquisition of extinguished fear responses (Culver et al., 2018). Following differential fear conditioning to neutral cues, participants underwent either non-reinforced or partially reinforced extinction training. Similar to Bouton and colleagues' work, a 2:8 reinforcement schedule was used during extinction in the partially reinforced group, translating to six reinforced and 18 non-reinforced CS+ trials and 24 non-reinforced CS- trials. Tests of fear recovery showed that partially reinforced extinction training, relative to non-reinforced extinction, interfered with subsequent reacquisition of conditioned fear, as indexed by electrodermal responding. An aspect requiring further investigation, however, is the effect of partially reinforced extinction on the spontaneous recovery of extinguished fear. While Culver and colleagues observed reduced recovery of electrodermal responding to the CS+ after partially reinforced extinction, relative to non-reinforced extinction, these results must be interpreted with caution, as conditioned responding failed to extinguish during partially reinforced extinction training and, consequently, could not "recover." Nevertheless, the results of the reacquisition test provide evidence for cross-species applicability of partially reinforced extinction. The aim of the present study was to replicate and extend previous findings (Bouton et al., 2004; Culver et al., 2018) to the spontaneous recovery, reinstatement, and reacquisition of extinguished conditioned responding in human fear conditioning, employing partially reinforced, unpaired, and non-reinforced extinction training. Furthermore, a direct comparison of occasionally paired and unpaired US presentations during extinction would also allow for examination of underlying mechanisms, which may differ across different types of reinforced extinction training (e.g. Bouton et al., 2004; Rauhut, Thomas, & Ayres, 2001; Rescorla & Skucy, 1969).

Several mechanisms have been proposed to account for the superior protection from fear recovery effects subsequent to reinforced and unpaired extinction training, compared to non-reinforced extinction, including: Weakening of the CS-US contingency through unpaired US presentations (Frey & Butler, 1977; Rescorla & Skucy, 1969; Vervliet, Vansteenwegen, & Hermans, 2010); US habituation (Rauhut et al., 2001; but see; Thomas, Longo, & Ayres, 2005); sequential learning (Bouton et al., 2004; Capaldi, 1966, 1994); and enhanced extinction learning through violation of expectancies, also referred to as prediction errors (Craske et al., 2014; Culver et al., 2018; Rescorla & Wagner, 1972; Vurbic & Bouton, 2014).

Prediction errors are implicated in the acquisition and extinction of conditioned responding (e.g. Pearce & Hall, 1980; Rescorla & Wagner, 1972; Vurbic & Bouton, 2014), whereby learning is proposed to cease when the CS reliably predicts the delivery of the US (or its absence, in the case of extinction learning). Extinction learning may be enhanced through the occasional presentation of the US during extinction training, due to the violation of expectancies regarding the frequency of US presentations or changes to the CS-US relationship (e.g. Craske et al., 2014). For instance, the omission of the US at the onset of extinction provides an opportunity for new learning due to the mismatch between current information (CS-no US) and past learning (CS-US), while the presentation of occasionally paired and unpaired USs on later trials would sustain learning through the presentation of novel information that needs to be reconciled with prior learning. Hence, the occasional presentation of USs during extinction would allow participants to learn about the likelihood of future threat encounters, such as the frequency of US presentations, relative to CS-only trials, or the relationship between the CS and the US (i.e. occasionally paired or unpaired). Subsequent fear recovery could be reduced because participants learned that the CS predicts the absence of the US (unpaired extinction; Rescorla & Skucy, 1969; Vervliet et al., 2010) or that occasional CS-US trials occur in the presence of many CS-no US trials (partially reinforced extinction). This proposition is also supported by Bouton et al's. (2004) adaptation of sequential theory (Capaldi, 1966, 1994).

Bouton et al. (2004) proposed that the key aspect learned during

partially reinforced extinction training is that CS-US trials do not occur exclusively in the "context" of other CS-US trials (i.e. acquisition), but may also occur in the context of extinction trials (i.e. a CS-US trial is followed by several CS-no US trials). Due to the association of reinforced trials with non-reinforced trials, reacquisition of extinguished responding may occur at a slower rate, as participants may expect a CS-US trial to be followed by further CS-no US trials. Bouton et al. further proposed that similar learning would occur during unpaired extinction, whereby unpaired US presentations would weaken the US's exclusive association with the acquisition context. Conversely, reacquisition subsequent to non-reinforced extinction is proposed to occur at a faster rate, as the omission of the US during training maintains the US's exclusive association with the acquisition context (i.e. CS-US trials occurring in the context of other CS-US trials). Hence, post-extinction presentations of reinforced trials would signal delivery of further reinforced trials and lead to rapid reacquisition. It should be noted that Bouton et al.'s model would also predict reduced reinstatement of fear, particularly in participants who received unpaired USs during extinction, but it is not readily applicable to spontaneous recovery, unless additional assumptions are made. Spontaneous recovery may depend, in part, on how easily the extinction memory can be retrieved, meaning the memory that a CS-only trial is more likely to signal further non-reinforced than reinforced trials. To summarize, there are several mechanisms that may account for reduction of fear recovery following reinforced extinction training, although, at present, they are still poorly understood and require further examination.

In the present study, we investigated whether occasional presentations of paired and unpaired USs during extinction training would result in superior reduction of spontaneous recovery, reinstatement, and reacquisition of fear, compared to non-reinforced extinction training. The fear association was established through differential Pavlovian conditioning (Culver et al., 2018; Lipp, 2006a), whereby one neutral cue (CS+) was continuously paired with an aversive electrotactile stimulus (US), while another cue (CS-) was presented by itself. Conditioned fear in differential paradigms is reflected in larger responding to the CS+, relative to the CS- (Lipp, 2006a). In line with Culver et al. (2018), electrodermal responding and CS valence ratings were recorded as primary and secondary dependent measures of conditioned responding, respectively. Based on the reviewed literature (Bouton et al., 2004; Craske et al., 2014; Culver et al., 2018), we predicted that extinction learning would be enhanced through occasional presentations of the US during extinction training, which would be reflected in reduced fear recovery and reacquisition of fear, relative to non-reinforced extinction. In line with Bouton et al.'s findings, we also predicted that unpaired extinction would result in slower reacquisition of extinguished fear than partially reinforced extinction.

2. Materials and methods

2.1. Participants

University students who met inclusion criteria (i.e. no cardiovascular disease, seizure disorder, or pregnancy) participated in exchange for partial course credit or a financial compensation of 30 AUD. After exclusion of one participant who failed to verbalize the CS-US contingency, data from 72 participants (44 females, 28 males; female-male ratio per group: 15:9 [non-reinforced and partially reinforced extinction group], 14:10 [unpaired extinction group]) were included in the analyses. The age range of participants was 18–38 years (M = 21.61 years, SD = 3.95). Ethical approval for this study was obtained from the Curtin University Human Research Ethics Committee.

2.2. Apparatus and materials

Stimuli. In line with previous research (Culver et al., 2018), non-fear relevant stimuli have been employed in the present study. Conditioned stimuli (CS) included four color images of animals, two birds and two fish (sourced from the internet). Each participant was presented with a subset of two pictures, comprising one bird and one fish picture; stimulus sets were counterbalanced across participants. The pictures measured between 700 × 467 pixels and 700 × 541 pixels and were presented for 6 s, in the center of a 17-inch color LCD screen, over a black background, with an inter-trial interval of 13–17 s. To control for order effects, the assignment of bird and fish pictures as CS + or CS- and the presentation order (whether the first trial of each phase was a CS+ or CS-) were counterbalanced across participants. Stimuli were presented in a pseudo-randomized order, whereby each CS was presented four times within blocks of eight trials, with the restriction that no more than two consecutive CS+ or CS- trials were presented. The unconditioned stimulus (US) consisted of a mild electric shock, which was generated with a Grass SD9 stimulator (Grass Technologies, Middleton, WI) and was delivered to the wrist of the dominant hand via a concentric electrode. The shock was presented for 200 ms (pulsed at 50 Hz) and coincided with the CS+ offset (unless otherwise indicated); the CS- was never paired with the US. The delivery of the US and CSs was controlled with DMDX 5.0.5 software (Forster & Forster, 2003).

Electrodermal activity (skin conductance responses, SCRs). Electrodermal activity was recorded through two self-adhesive isotonic gel electrodes (Biopac Systems EL507), attached to the thenar and hypothenar eminences of the non-dominant hand. Electrodermal activity was DC amplified at a gain of 5 micro Siemens (μS) per volt and recorded with a Biopac MP150 system at a sampling frequency of 1000 Hz, using AcqKnowledge 4 (Biopac Systems, Goleta, CA). A Biopac respiration belt was fitted around each participant's waist to control for respiration-induced artefacts in SCRs.

Subjective evaluation of stimulus valence. Participants provided post-test CS and US valence ratings on a 9-point scale (from 1 [unpleasant] to 9 [pleasant]) at baseline, after acquisition, extinction, spontaneous recovery (CS ratings only), reinstatement test, and reacquisition. US valence ratings were not obtained after spontaneous recovery to prevent potential interference with subsequent reinstatement, as reinstatement requires the unexpected presentation of the US (Haaker, Golkar, Hermans, & Lonsdorf, 2014). Valence ratings were obtained electronically through a custom-made Microsoft Access application, whereby the stimuli were presented on the computer screen in randomized order and participants were instructed to rate stimulus valence on the scale located below the picture.

Self-report questionnaires. To ensure groups did not differ on variables known to affect conditioned responding (Dunsmoor, Campese, Ceceli, LeDoux, & Phelps, 2015; Lonsdorf & Merz, 2017), participants were asked to complete the short version of the Intolerance of Uncertainty Scale (IUS-12; Carleton, Norton, & Asmundson, 2007). The 12-item IUS measures beliefs about and reactions to uncertainty, ambiguous situations, and the future (e.g. "Unforeseen events upset me greatly.") and comprises two subscales: prospective IU (measures anxiety about future events) and inhibitory IU (indicative of behavioral inhibition or avoidance). The scale has good psychometric properties (Carleton et al., 2007) and demonstrated excellent internal consistency in the current sample (α = .90). For exploratory purposes, participants also completed the short version of the Depression, Anxiety, and Stress Scales (DASS-21; Henry & Crawford, 2005; Lovibond & Lovibond, 1995). The questionnaires were completed electronically at the start of the experiment.

Manipulation checks. Following acquisition, participants were presented with a CS-US contingency questionnaire, containing the four stimulus pictures used in this study, and were asked to indicate which pictures had been paired with the US. As inability to verbalize the correct contingency may reflect a genuine failure to learn the CS-US relationship (Lipp, 2006a), data from one participant who failed this test were excluded from statistical analyses.

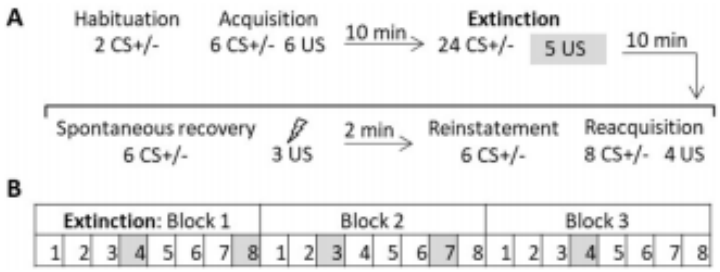

Fig. 1. Schematic representation of the experimental paradigm (A) and reinforcement schedule during extinction training (B). Panel A illustrates the sequential order of experimental stages with the respective number of conditioned (CS) and unconditioned stimulus (US) presentations. Boxes in panel B denote delivery of the previously conditioned CS + (CS- is not depicted here). The shaded boxes represent delivery of the US, which either coincided with the CS + offset (partially reinforced extinction group) or was presented in the middle of the inter-trial interval (unpaired extinction group), either after CS+ offset or before CS + onset.

2.3. Procedure

A schematic representation of the experimental paradigm and the reinforcement schedule employed during extinction training is presented in Fig. 1. Upon arrival in the laboratory, participants were informed about the experimental procedures and had the opportunity to ask questions, before providing information about current medication use and medical history. Participants were assigned to groups in the order they presented for testing, with the restriction that an approximately equal number of females and males were assigned to each group. Individuals who met inclusion criteria provided written consent, were seated in front of the computer screen, and were fitted with the skin conductance electrodes, respiration belt, and the shock electrode. After completing the self-report measures, participants were asked to relax and look at the blank computer screen while a 2-min baseline of their electrodermal activity was recorded. Subsequently, participants provided baseline CS valence ratings, set the US intensity to a level which was perceived as "unpleasant, but not painful," and rated US valence.

Acquisition. Participants were asked to pay attention to the computer screen and to learn which CSs were followed by the US. Conditioning commenced with a habituation phase, consisting of two non-reinforced presentations of each CS, and was immediately followed by acquisition, which involved six presentations of the CS+ and the CS-. The US was presented on all CS+ trials. Thereafter, participants completed the CS-US contingency questionnaire and rated CS and US valence. The shock electrode was removed and a 10-min rest period was inserted during which participants were offered magazines to read. The break was included to allow electrodermal activity to return to a stable baseline before the start of extinction training.

Extinction. The shock electrode was reattached and participants were informed that they would be presented with several stimuli and asked to pay attention to the computer screen at all times. No information was provided about the types of stimuli to be presented, their frequency, duration, or contingencies. Extinction training consisted of 24 CS +/- presentations; the CSs+ were non-reinforced in the non-reinforced extinction (EXT) and unpaired extinction (UNP) group and partially reinforced (5 CS-US pairings; see Fig. 1B) in the partially reinforced extinction (PRE) group. In the UNP group, five US presentations were delivered in the middle of the inter-trial interval (ITI). Within each block of eight trials, the US was delivered on trial 4 and 8 (block 1), 3 and 7 (block 2), and on trial 4 (block 3). The US coincided with the CS+ offset in the PRE group. In the UNP group, the US was presented in the middle of the ITI, either after CS+ offset or in the ITI before CS+ onset, with the restriction that the US was never presented between two CSs+. As previous reports indicated that a 2:8 reinforcement ratio maintained differential responding at the end of extinction training (Culver et al., 2018), we decreased the reinforcement ratio on the last block of training to a 1:8 ratio. This decrease was in line with past research (Bouton et al., 2004) and served to facilitate loss of conditioned responding. Following extinction training, participants were asked to rate CS and US valence. Subsequently, the shock electrode was removed and a 10-min break was inserted (identical to the post-acquisition break).

Test of fear recovery. The shock electrode was reattached and participants were informed that they would be presented with several stimuli and were asked to pay attention to the computer screen at all times (instructions were identical to those presented at the start of extinction training). Assessment of spontaneous recovery involved six non-reinforced presentations of the CS+/-, which were followed by post-test CS valence ratings. Subsequently, participants received three unsignaled presentations of the US, with a duration of 200 ms and an ITI of 6 s. The computer screen remained switched on and displayed a black background (in line with previous training). After a 2-min delay, reinstatement of extinguished responding was tested through six non-reinforced presentations of the CS+/-. After rating CS and US valence, participants underwent partially reinforced reacquisition, comprising eight presentation of the CS+/-. The US coincided with the CS+ offset on 50% of the trials (1st, 3rd, 5th and 6th CS); the remaining trials were not reinforced. The physical intensity of the US during reinstatement and reacquisition was identical to that employed during acquisition. We employed a partial reinforcement schedule (in line with Bouton et al., 2004) to permit emergence of group differences (Lissek, Pine, & Grillon, 2006). The partial reinforcement schedule further served to enhance the ecological validity of the reacquisition test, as individuals would be more likely to encounter occasional, than continuous, CS-US pairings in real life. At the conclusion of reacquisition, participants provided the final CS and US valence ratings.

2.4. Scoring and response definition

Electrodermal responses were scored offline in AcqKnowledge 4. Participants' baseline electrodermal activity was determined by counting all spontaneous responses that occurred during a 2-min rest period (Dawson, Schell, & Filion, 2007). A visual inspection of data was conducted to identify movement- or respiration-induced artefacts in SCRs. Eight SCRs (across groups) were discarded due to the presence of artefacts. In accordance with past research (Culver et al., 2018; Pineles, Orr, & Orr, 2009), SCRs elicited by the CSs were calculated by subtracting the mean skin conductance level during the 2 s baseline preceding CS onset from the largest skin conductance level occurring 1–6 s after CS onset. SCRs were range corrected to control for individual differences in electrodermal activity (Lykken, 1972) and then square root transformed to reduce the skew of the distribution (Dawson et al., 2007). The range correction was obtained by dividing each response by the largest response displayed by the participant. Electrodermal responses were averaged into blocks of two consecutive trials, to reduce the influence of trial by trial variability.

2.5. Statistical analyses

Electrodermal responding during habituation was analyzed through a repeated measures analysis of variance (ANOVA) with group (EXT, PRE, UNP) as a between-groups factor and CS type (CS+, CS-) as a

within-groups factor. Analysis of acquisition, extinction, and re-acquisition data was conducted through mixed ANOVAs for repeated measures, with group as a between-groups factor and CS type (CS+ vs. CS-) and block/time as within-groups factors (acquisition: block 1–3; extinction: early vs. late phase; reacquisition: block 1–3). Extinction of conditioned responding was assessed with data from the early (block 1–2) and late phase (block 11–12) of extinction training, during which no USs were presented in any of the groups (the US presented at the end of trial 4/block 2 in the PRE and UNP group would affect responding on the subsequent trials, but not on trial 4). In line with past research (Dunsmoor et al., 2015), recovery of extinguished responding was assessed during the early phase (block 1) of spontaneous recovery and reinstatement tests, through separate repeated measures ANOVAs, with CS type (CS+ vs. CS-) as a within-groups factor and group as a between-groups factor. CS valence ratings were analyzed through a series of mixed ANOVAs for repeated measures, with group as a between-groups factor and CS type (CS+ vs. CS-) and time as within-groups factors (acquisition: baseline vs. acquisition; extinction: acquisition vs. extinction; spontaneous recovery: extinction vs. spontaneous recovery; reinstatement: extinction vs. reinstatement). Reacquisition was assessed by means of a repeated measures ANOVA with group as a between-groups factor and CS type (CS+ vs. CS-) as a within-groups factor. Multivariate F values (Pillai's Trace) and partial eta squared values are reported for all main effects and interactions. Statistical significance was assessed at $\alpha = .05$; Bonferroni corrections were used for follow-up analyses to guard against the accumulation of a Type 1 error.

3. Results

3.1. Preliminary analyses

The groups did not differ in age, selected US intensity, baseline CS or US valence ratings, baseline electrodermal activity, IUS-12 scores, or DASS-21 scores (Table 1). Selected US intensities ranged from 34 to 80 V, with a mean of 66.19 V ($SD = 12.90$).

3.2. Electrodermal responding

Habituation. Electrodermal responding across groups is presented in Fig. 2. Analysis of SCRs during habituation indicated that SCRs to CS +/- did not differ across groups during habituation, as reflected in the non-significant main effect of CS type, F (1, 69) < 1, and the CS type × group interaction, F (2, 69) = 2.93, $p = .060$, $\eta p^2 = .08$. Follow-up comparisons conducted for the trend towards significance in the interaction revealed larger SCRs to the CS- ($M = 0.57$, $SD = 0.05$) than to the CS+ ($M = 0.47$, $SD = 0.05$) in the EXT group (Fig. 2A), F (1, 69) = 4.10, $p = .047$, $\eta p^2 = .06$, but not in the PRE or UNP group, both F (1, 69) ≤ 1.94, $p \geq .168$, $\eta p^2 \leq .03$. As the interaction indicates that groups may have differed on their level of electrodermal responding to CS ± at the start of acquisition, we conducted a separate 3 (group) x 2 (CS+, CS-) repeated measures ANOVA with data from the first trial of acquisition. The analysis revealed no significant group differences at the start of acquisition, as reflected in the non-significant main effect of CS type, F (1, 69) = 0.53, $p = .471$, $\eta p^2 = .01$ and the non-significant CS type × group interaction, F (2, 69) = 0.35, $p = .709$, $\eta p^2 = .01$.

Acquisition. Differential SCRs were evident across groups during acquisition, as reflected in main effects of CS type, F (1, 69) = 61.91, $p < .001$, $\eta p^2 = .47$; block, F (2, 68) = 12.18, $p < .001$, $\eta p^2 = .26$, and a CS type × block interaction, F (2, 68) = 6.85, $p = .002$, $\eta p^2 = .17$. The interaction reflects an increase in differential SCRs across blocks of acquisition, all F (1, 69) ≥ 13.16, $p \leq .001$, $\eta p^2 \geq .16$. The remaining interactions did not attain significance, with the largest of the non-significant effects suggesting that acquisition of differential SCRs did not significantly differ across groups, (CS type x block x group), F (4, 138) = 0.94, $p = .443$, $\eta p^2 = .03$.

Extinction. A visual inspection of Fig. 2 indicates that differential responding was larger during partially reinforced than during non-reinforced extinction. However, statistical analyses showed that differential responding during the reinforced stage of training did not interfere with the extinction of conditioned responding. Results of the ANOVA examining SCRs between the early (block 1–2) and late phase (block 11–12) of training revealed main effects of CS type, F (1, 69) = 18.84, $p < .001$, $\eta p^2 = .21$, and block, F (3, 67) = 7.96, $p < .001$, $\eta p^2 = .26$, which were qualified by a CS type × block interaction, F (3, 67) = 4.69, $p = .005$, $\eta p^2 = .17$. The interaction reflects differential electrodermal responding on block 1 and 2, both F (1, 69) ≥ 6.80, $p \leq .011$, $\eta p^2 \geq .09$, but not on block 11 or 12, both F (1, 69) ≤ 1.27, $p \geq .265$, $\eta p^2 \leq .02$, indicating that differential responding was extinguished in all groups. The remaining interactions did not attain significance, largest effect (CS type x block x group), F (6, 136) = 1.17, $p = .324$, $\eta p^2 = .05$. As Fig. 2 indicates that differential responding may have been present on the last block of training in the PRE group, we subjected the mean SCRs of the CS+ and the CS- to a t-test. In line with the between-groups comparisons, results confirmed that there were no significant differences between SCRs to the CS+ ($M = 0.20$, $SD = 0.22$) and the CS- ($M = 0.11$, $SD = 0.17$), t (23) = 1.68, $p = .106$, $d = 0.05$.

Spontaneous recovery. Our primary prediction was that

Table 1
Means (M) and Standard Deviations (SD) for Age, US Intensity, Baseline Valence Ratings (VR), Baseline Electrodermal Activity (EDA), and Self-Report Questionnaires.

	Non-reinforced extinction		Partially reinforced extinction		Unpaired extinction		Test
	M	SD	M	SD	M	SD	
Age	20.37	2.43	21.79	3.50	22.67	5.21	F (2,69) = 2.13, $p = .127$
US intensity	63.38	13.39	68.17	11.59	67.04	13.67	F (2,69) = 0.90, $p = .410$
Baseline VR							
CS+	7.25	1.51	6.71	2.05	7.38	1.35	F (2,69) = 1.09, $p = .342$
CS-	6.96	1.57	6.88	2.15	7.54	1.56	F (2,69) = 1.00, $p = .375$
US	3.88	1.92	3.83	1.88	3.42	1.74	F (2,69) = 0.45, $p = .639$
Baseline EDA	12.42	9.60	11.50	8.89	10.13	8.35	F (2,69) = 0.40, $p = .673$
IUS-12							
Prospective	18.46	5.12	19.62	5.45	18.96	5.49	F (2,69) = 0.29, $p = .752$
Inhibitory	9.54	3.56	11.38	4.75	10.92	4.69	F (2,69) = 1.14, $p = .324$
DASS-21							
Depression	8.67	10.05	7.92	7.99	9.00	7.55	F (2,69) = 0.10, $p = .905$
Anxiety	6.17	5.04	7.50	8.18	7.75	7.65	F (2,69) = 0.35, $p = .709$
Stress	10.75	7.73	13.75	9.57	14.25	9.35	F (2,69) = 1.08, $p = .345$

Note. CS = conditioned stimulus, US = unconditioned stimulus, IUS-12 = Intolerance of Uncertainty Scale (short version), DASS-21 = Depression, Anxiety, and Stress Scales (short version). Baseline EDA refers to the number of spontaneous responses that occurred during a 2-min rest period. US intensity is reported in Volt.

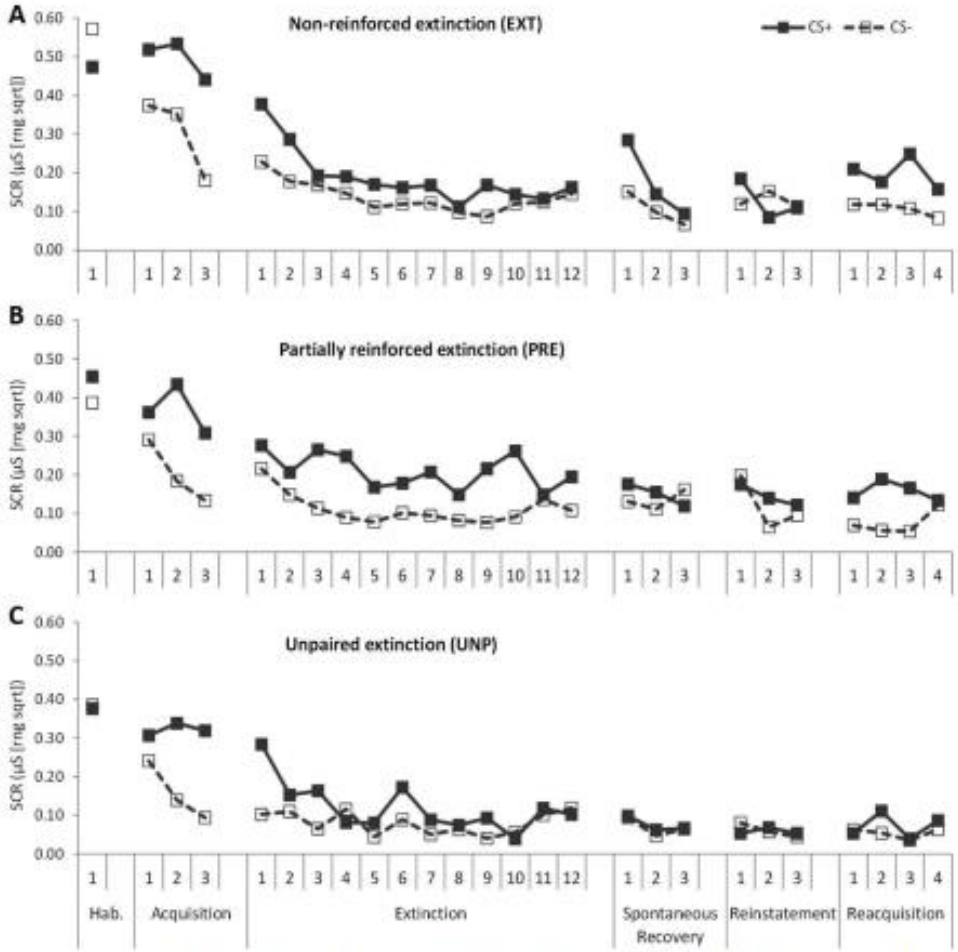

Fig. 2. Mean skin conductance responses (SCRs) to reinforced (CS+) and non-reinforced (CS-) conditioned stimuli in the EXT (A), PRE (B), and UNP (C) group. SCRs are presented in blocks of two consecutive trials.

occasional US presentations during extinction training would result in less recovery of fear than non-reinforced extinction training. Inspection of spontaneous recovery data in Fig. 2 suggests that differential responding recovered following non-reinforced extinction, but not after extinction conducted with occasionally paired or unpaired US presentations. This observation was confirmed by the results of statistical analyses, revealing a main effect of CS type, F (1, 69) = 8.22, p = .005, ηp^2 = .11, which was qualified by a CS type × group interaction, F (2, 69) = 3.25, p = .045, ηp^2 = .09. The interaction reflects differential electrodermal responding in the EXT group, F (1, 69) = 13.19, p = .001, ηp^2 = .16, but not in the PRE group, F (1, 69) = 1.51, p = .223, ηp^2 = .02, or UNP group, F (1, 69) = 0.01, p = .917, ηp^2 < .01.

Reinstatement. A visual inspection of differential responding on block 1 of reinstatement (Fig. 2) suggests that differential SCRs were reinstated in the EXT group, but not in the PRE or UNP group. However, these differences did not attain significance in the omnibus analysis. The results neither yielded a significant main effect of CS type, F (1, 69) = 0.04, p = .837, ηp^2 = .01, nor a CS type × group interaction, F (2, 69) = 1.48, p = .234, ηp^2 = .04, showing that differential SCRs did not differ across groups on the first block of reinstatement testing.

Reacquisition. Our first prediction was that occasional presentations of the US during extinction training would reduce reacquisition of extinguished responding, compared to non-reinforced extinction. To test this prediction, we conducted an ANOVA using data from block 1–3, which was the reinforced stage of reacquisition during which group differences would be expected to emerge (see also Fig. 2). The results revealed a main effect of CS type, F (1, 69) = 17.29, p < .001, ηp^2 = .20, as well as a trend towards significance in the CS type × group interaction, F (2, 69) = 2.57, p = .084, ηp^2 = .07. The interaction reflects increased differential responding to the CS+, relative to the CS-, in the EXT group, F (1, 69) = 10.18, p = .002, ηp^2 = .13, as well as in the PRE group, F (1, 69) = 11.94, p = .001, ηp^2 = .15, but not in the UNP group, F (1, 69) = 0.31, p = .580, ηp^2 = .01. The main effect of block and remaining interactions did not attain significance, largest effect (block × group interaction), F (4, 138) = 1.09, p = .362, ηp^2 = .03.

To follow up on Bouton et al's. (2004) findings, we also tested whether the rate of reacquisition would be slower after unpaired than partially reinforced extinction. The ANOVA conducted with data from the PRE and UNP group yielded a main effect of CS type, F (1, 46) = 9.87, p = .003, ηp^2 = .18, as well as a CS type × group

interaction, F (1, 46) = 5.15, p = .028, ηp^2 = .10, reflecting larger differential SCRs in the PRE group (M = 0.11, SD = 0.17) than in the UNP group (M = 0.02, SD = 0.08), t (46) = 2.23, p = .031, d = 0.68. The main effect of block and remaining interactions did not attain significance, largest effect (CS type × block interaction), F (2, 45) = 1.30, p = .282, ηp^2 = .06. These results show that the rate of reacquisition differed between the PRE and UNP group; although, contrasting Bouton et al.'s findings, we did not observe reduced reacquisition, but an absence of reacquisition in the UNP group.

Examination of underlying mechanisms: US habituation. The lack of reacquisition in the UNP group may indicate that the unpaired presentations of the US during extinction training resulted in US habituation. The reduced aversiveness of the US could have attenuated subsequent responding to the CS+ and slowed the rate of reacquisition (Rescorla, 1973). If US habituation occurred during extinction training in the UNP group, this would be reflected in smaller unconditioned responses (URs) in the UNP than in the PRE group.[1] The results of a 2 (group) x 5 (US presentations) ANOVA did not support the US habituation hypothesis. Results revealed a main effect of trial, F (4, 43) = 6.76, p < .001, ηp^2 = .39, but no significant trial × group interaction, F (4, 43) = 0.50, p = 736, ηp^2 = .04. The main effect reflects decreased URs across trials in both groups. However, the mean UR to the final US presentation in the PRE (M = 0.74, SD = 0.34) and UNP group (M = 0.67, SD = 0.29) resembled that on the last block of acquisition (PRE: M = 0.58, SD = 0.25; UNP: M = 0.65, SD = 0.30). Similarly, analysis of post-extinction US valence ratings showed that the US was rated as equally unpleasant in both groups (PRE: M = 3.38, SD = 1.66; UNP: M = 3.96, SD = 1.73), t (46) = 1.19, p = .240. The combined results, therefore, indicate that US habituation did not occur in either group.

3.3. CS valence ratings

Acquisition. Mean ratings of CS+ and CS- valence as well as negative evaluations of the CS+, relative to the CS-, are presented in Fig. 3. Assessment of acquisition (baseline vs. post-acquisition ratings) showed increased negative evaluations of the CS+, relative to the CS-, in all groups. The repeated measures ANOVA yielded main effects of CS type, F (1, 69) = 25.24, p < .001, ηp^2 = .27, and time, F (1, 69) = 46.98, p < .001, ηp^2 = .41, which were qualified by a CS type × time interaction, F (1, 69) = 53.67, p < .001, ηp^2 = .44. The interaction reflects a significant difference between evaluations of the CS+ and the CS- in all groups, after acquisition, F (1, 69) = 48.31, p < .001, ηp^2 = .41, but not at baseline, F (1, 69) = 0.01, p = .944, ηp^2 < .01.

Extinction. Assessment of extinction of differential evaluations revealed a main effect of CS type, F (1, 69) = 50.03, p < .001, ηp^2 = .42, as well as interactions of CS type x time, F (1, 69) = 9.26, p = .003, ηp^2 = .12, and time x group, F (2, 69) = 4.58, p = .014, ηp^2 = .12, which were qualified by a CS type x time × group interaction, F (2, 69) = 5.61, p = .006, ηp^2 = .14. The three-way interaction reflects a significant decrease in negative evaluations from post-acquisition to post-extinction as well as elimination of differential evaluations in the EXT and UNP group, but not in the PRE group. Differential negative evaluations in the PRE group remained significant after extinction training, F (1, 69) = 39.70, p < .001, ηp^2 = .37.

Tests of fear recovery. Assessment of spontaneous recovery and reinstatement data showed that this pattern was also reflected in ratings obtained after spontaneous recovery, CS type x group, F (2, 69) = 8.39, p = .001, ηp^2 = .20, and after reinstatement, CS type x group, F (2, 69) = 8.39, p = .001, ηp^2 = .20 (see also Fig. 3B). No group differences were found in valence ratings obtained after reacquisition; negative evaluations of the CS+, relative to the CS-, were observed in all groups, as reflected in the significant main effect of CS type, F (1, 69) = 62.76, p < .001, ηp^2 = .48, and non-significant CS type × group interaction, F (2, 69) = 1.68, p = .193, ηp^2 = .05. In summary, analysis of valence ratings showed that, compared to electrodermal responding, post-test ratings of CS valence exhibited a different pattern of conditioned responding, whereby occasional CS-US pairings, but not unpaired US presentations, maintained differential evaluations after extinction, spontaneous recovery and reinstatement. Additionally, all groups rated the CS+ as significantly more unpleasant than the CS- after reacquisition, indicating that differential evaluations were reacquired.

4. Discussion

Our primary aim was to investigate if occasional presentations of the US during extinction training would provide enhanced protection from fear recovery than non-reinforced extinction. The overall pattern of results supported this hypothesis, although there were important differences between the effects of partially reinforced and unpaired extinction training. The results showed no differences in the acquisition and extinction of differential SCRs across groups. Group differences emerged during tests of fear recovery, whereby spontaneous recovery of extinguished differential SCRs was observed after non-reinforced extinction, but not after extinction conducted with occasional presentations of paired or unpaired USs. Analysis of SCRs during reinstatement failed to show significant group differences, indicating that the presentation of three unsignaled shocks did not reinstate differential SCRs in any group. The reacquisition test yielded somewhat unexpected results, indicating reacquisition of differential SCRs subsequent to partially reinforced and non-reinforced extinction, but not after unpaired extinction training.

In contrast to electrodermal data, post-test ratings of CS+/- valence did not reflect any benefits of occasional presentations of the US during extinction over non-reinforced extinction. While acquisition of differential negative evaluations was in line with the electrodermal data, ratings obtained after the subsequent phases showed a different pattern of results. Negative evaluations of the CS+, relative to the CS-, were observed in the group that received partially reinforced extinction, but not in the groups that received non-reinforced or unpaired extinction, after extinction, spontaneous recovery, and reinstatement. Differential evaluations did not differ across groups after reacquisition, as the CS+ was rated as less pleasant than the CS- in all groups. The combined results demonstrate that partially reinforced and unpaired extinction training offer enhanced protection from fear recovery assessed by SCRs, relative to non-reinforced extinction. However, they also indicate that physiological and verbal indices of conditioned fear may be differentially sensitive to occasional presentations of the US during extinction, as reflected in the different pattern of results obtained from the analysis of electrodermal data and the analysis of valence ratings. Our results also indicate that occasional presentations of unpaired USs during extinction may be more effective in the long-lasting reduction of fear, as indexed by SCRs, than partially reinforced extinction, as unpaired extinction may prevent reacquisition of extinguished responding.

The overall pattern of results can be interpreted as being broadly consistent with past research to the extent that a) occasional presentations of the US during extinction resulted in superior attenuation of recovery from extinction effects than non-reinforced extinction (Bouton et al., 2004; Culver et al., 2018) and b) we observed a dissociation between physiological and verbal measures of fear (e.g. Culver et al., 2018; Luck & Lipp, 2015; Schultz, Balderston, Geiger, & Helmstetter, 2013; Thompson & Lipp, 2017). However, there were several important differences between our findings and those reported in past research. The results of the present study did not converge with previous reports (Bouton et al., 2004; Culver et al., 2018) that

[1] Respective unconditioned responses (URs) in the partially reinforced and unpaired extinction group were scored as the largest response starting in the 1–3 s window following US offset (Prokasy & Ebel, 1967). URs were range corrected and square root transformed prior to analysis.

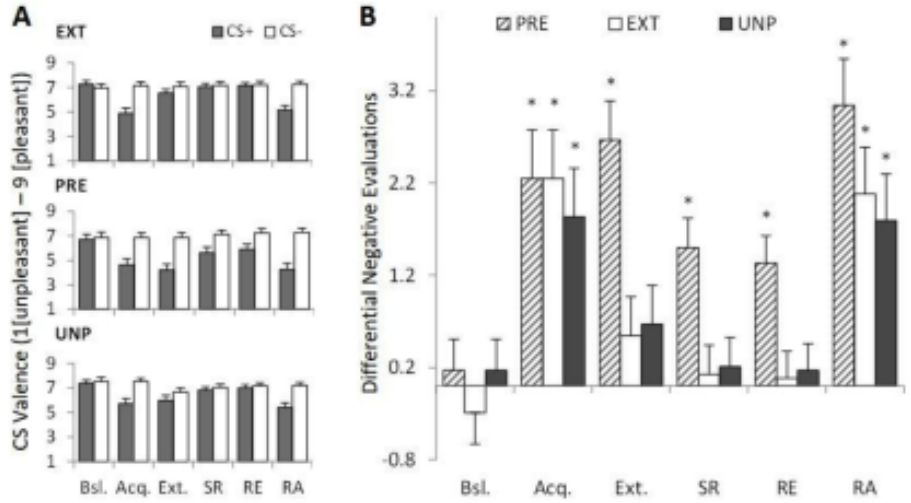

Fig. 3. Mean conditioned stimulus (CS) valence ratings (A) and differential negative evaluations of the CS+, relative to the CS- (B) at baseline (Bsl.), after acquisition (Acq.), extinction (Ext.), spontaneous recovery (SR), reinstatement (RE), and reacquisition (RA). CS valence was rated from 1 (unpleasant) to 9 (pleasant). Error bars represent standard errors. The asterisk in panel B indicates significant negative evaluation of the CS+, relative to the CS-, at $p \leq .001$.

interference with reacquisition was greater following partially reinforced than non-reinforced extinction – reacquisition in our study did not differ between the control and partially reinforced group. While this finding was unexpected, in particular since partially reinforced extinction prevented spontaneous recovery of extinguished responding, the divergent results between this and Bouton et al.'s study may reflect on the use of an aversive conditioning preparation, as opposed to appetitive conditioning, as well as on the larger number of extinction trials employed by Bouton et al.

Repeated extinction sessions, conducted across multiple days, may have strengthened the animal subjects' memory of occasional CS-US trials occurring in the presence of many CS-no US trials, which, according to Bouton et al. (2004) adaptation of sequential theory (Capaldi, 1966, 1994), would slow the rate of reacquisition. It is possible that the 24 extinction trials employed in our study were not sufficient to create a robust extinction memory. On the other hand, Culver et al. (2018) employed the same number of extinction trials, albeit with six, instead of five, paired US presentations, and reported decreased reacquisition after reinforced than non-reinforced extinction. However, a closer examination of Culver et al.'s data suggests that differential SCRs were initially larger in the partially reinforced extinction group than in the control group (after the 1st and 2nd CS-US trial) and only decreased during the second half of reacquisition. The authors suggested that physiological responding decreased because participants acquired "physiological toughness," meaning an improved ability to cope with repeated exposure to aversive stimuli, as a result of partially reinforced extinction training. There was no evidence of decreased SCRs during reacquisition in the partially reinforced extinction group in our data, although this may reflect on the use of a partial reinforcement schedule during reacquisition. In contrast to continuous reinforcement (Culver et al., 2018), alternating CS-US with CS-no US trials may create more uncertainty about future threat. From an evolutionary perspective, even occasional threats may pose a risk to our survival. Hence, it may be of advantage to adopt a "better safe than sorry" approach in response to occasional threat encounters. In this sense, partially reinforced extinction training may protect against spontaneous recovery of fear, as a CS-only trial does not signal imminent danger, but the fear response may return when the likelihood of future threat increases, as would be the case after exposure to a CS-US trial. This proposition could be explored in future research through a direct comparison of reacquisition on a partial and continuous reinforcement schedule.

Analysis of electrodermal data during reacquisition further indicated that the mere reduction of the reinforcement schedule during extinction training, relative to that used during acquisition, may not be sufficient to prevent reacquisition of differential SCRs. This proposition was supported by the differential pattern of reacquisition results observed between the partially reinforced and the unpaired extinction group. Our results indicated that, in contrast to partially reinforced extinction, an unpaired extinction procedure that involves the occasional presentations of the US in the inter-trial interval may not only reduce, but also prevent, reacquisition of differential SCRs. These results are in line with previous animal research conducted in the appetitive (Bouton et al., 2004) and aversive setting (Frey & Butler, 1977; Mickley et al., 2009; Rauhut et al., 2001; Thomas et al., 2005). Similarly, Vervliet et al. (2010) reported that extinction training consisting of eight non-reinforced CS trials and six unpaired electrotactile USs, presented during the inter-trial interval, reduced renewal of extinguished fear in humans. While the experimental methods differed across these studies, including the number of CS and US trials and the ratio of CS to US trials during extinction training, the outcomes of the present study and past research suggest that extinction training during which the US is retained, but no longer paired with the CS, is superior in the reduction of fear recovery to non-reinforced or partially reinforced extinction.

As a possible underlying mechanism, it has been proposed that unpaired presentations of the US weaken, or even eliminate, the CS-US association that was formed during acquisition (Frey & Butler, 1977; Rescorla & Skucy, 1969; Rescorla & Wagner, 1972; Vervliet et al., 2010). While the associative strength of the CS would also be reduced during non-reinforced extinction (Rescorla & Wagner, 1972), the presentation of unexpected unpaired USs would enhance extinction learning through increased prediction errors (e.g. Fernández, Boccia, & Pedreira, 2016; Todd, Vurbic, & Bouton, 2014). As the CS-US association is weakened, or eliminated, subsequent responding to the CS would be reduced.

A second mechanism proposed by Rauhut et al. (2001) involves US habituation. However, this proposition has not been supported through previous research (Thomas et al., 2005) or the results of the present study. Comparisons of unconditioned responses to the USs presented during extinction training showed no significant differences between

the partially reinforced and unpaired extinction groups. Similarly, analysis of US valence ratings showed that the US was rated as equally unpleasant in both groups, making a US habituation explanation unlikely.

Finally, Bouton et al. (2004) model would not readily account for the results observed in our unpaired extinction group. While the model proposes that unpaired extinction weakens the US's exclusive association with the acquisition context, it would make the same prediction about partially reinforced extinction. Therefore, it cannot account for the differences in responding during reacquisition between the paired and unpaired US group. Overall, the present results appear to be consistent with a weakened CS-US association explanation (Rescorla & Wagner, 1972; Vervliet et al., 2010). The reduced associative strength of the CS would explain the reduced recovery from extinction effects, but this hypothesis requires further examination, as extinction is generally recognized as a form of new learning, not unlearning (Bouton, 2002). Future fear conditioning research conducted with human participants may provide further insight into the mechanisms underlying extinction training with occasionally paired or unpaired USs through an examination of US expectancy ratings (Lovibond, 2006). Assessment of US expectancies on each trial of training may provide further information about what is learned during reinforced extinction training and how this learning may affect subsequent recovery of conditioned responding. It should be also noted that while the concurrent recording of multiple indices of conditioned responding may give us a better understanding of mechanisms underlying human fear learning (Lipp, 2006a), careful consideration must be given to the experimental parameters, to prevent response interference, such as movement-induced artefacts in SCRs due to the manual handling of a US expectancy scale.

As a limitation, we should note that the interpretation of reacquisition results must be treated with caution, as the respective group comparison only yielded a trend towards significance, indicating reacquisition of extinguished SCRs in the non-reinforced and partially reinforced extinction group, but not in the unpaired extinction group. Nevertheless, the results of spontaneous recovery showed that partially reinforced and unpaired extinction provided enhanced protection from fear recovery, as indexed by SCRs, compared to non-reinforced extinction training. Further, isolated comparisons of reacquisition data from the partially reinforced and unpaired extinction group supported previous observations (Bouton et al., 2004) that an unpaired extinction procedure results in stronger reduction of reacquisition of extinguished conditioned responding than partially reinforced extinction. Hence, the present extension of Bouton et al.'s findings provides further support for the utility of an extinction procedure involving occasional presentations of paired or unpaired USs in the reduction of fear recovery in humans and serves to inform future research.

It remains to be investigated why manipulations of extinction training showed no differential effects on the reinstatement of differential SCRs. It is possible that the large number of preceding CS-only trials, meaning CS trials during extinction training and those presented during tests of spontaneous recovery, reduced the effect of the reinstatement manipulation and, thus, prevented the observation of between group differences (for a review of reinstatement research, see Haaker et al., 2014). This proposition could be tested in future research, explicitly designed to assess the effect of occasional US presentations during extinction training on reinstatement. In this regard, tests of fear recovery, including reinstatement testing, could be conducted 24 h after the conclusion of extinction training (e.g. Schiller et al., 2010; Thompson & Lipp, 2017). Additional areas of examination involve the applicability of the present extinction procedures to fears conditioned to fear-relevant CSs, such as snakes (Lipp, 2006b; Öhman & Mineka, 2001), as well as to pre-existing fears and phobias in clinical populations. Recruitment of clinical populations will enable future research to test the translational utility of reinforced extinction, including the applicability of reinforced extinction procedures to well-established (i.e. consolidated) fear associations (Dudai, 2012; Dudai, Karni, & Born, 2015).

Another aspect requiring further examination pertains to the differential effects of extinction training with occasional US presentations on physiological and verbal indices of conditioned fear. This dissociation is in accordance with previous fear conditioning research, suggesting that response systems which are governed largely by conscious, cognitive processes, such as ratings of CS valence or US expectancy, and physiological indices of conditioned responding are differentially sensitive to (manipulations of) extinction (Culver et al., 2018; Lipp & Edwards, 2002; Lipp, Oughton, & LeLievre, 2003; Schultz et al., 2013; Thompson & Lipp, 2017). However, the differences between ratings in the partially reinforced and unpaired extinction group at the end of extinction training may also point to different underlying mechanisms, as discussed previously.

In conclusion, the counterintuitive suggestion that presentations of aversive events during extinction training may enhance extinction learning and thus reduce subsequent recovery of the extinguished fear response (Craske et al., 2014) has been supported through the results of the present study. In this regard, our findings further indicate that unpaired extinction training may be more effective in the reduction of recovery from extinction effects than partially reinforced extinction training, as unpaired extinction may guard against reacquisition of fear. When applied to the clinical setting, our results indicate that treatments focusing on the mere reduction of distress (in line with non-reinforced extinction) may be less effective in preventing fear recovery than treatments that provide clients with an opportunity to learn about the likelihood of future threat encounters (see also Craske et al., 2014; Weisman & Rodebaugh, 2018). Returning to our previous example of social anxiety disorder, during occasional exposure to a feared social situation and the feared outcome (CS-US pairing) clients may learn that the feared outcome occurs less often than expected. This learning may reduce the return of fear when the feared social situation (CS) is encountered between treatment sessions. For clients who are exposed to occasional CS-US pairings in daily life, such as negative feedback at work, it may be reassuring to know that such encounters may be beneficial for their overall treatment outcomes. In this sense, psychoeducation could be utilized to educate clients that occasional return of fear to cues in the environment is not an indicator of ineffective treatment, but an aspect that contributes to the reduction of maladaptive fears and, thereby, to the success of treatment.

We should add, however, that the translational utility of occasionally reinforced extinction requires further examination. It was not the authors' intention to suggest that a reinforced extinction procedure is readily applicable to the clinical setting, but to enhance our understanding of the mechanisms that affect extinction learning and to provide suggestions for future pre-clinical and clinical research (for a more detailed discussion of potential clinical applications, see: Craske, 2015; Craske et al., 2014; Krompinger, Van Kirk, Garner, Potluri, & Elias, 2018; Weisman & Rodebaugh, 2018). In this regard the procedure may lend itself more readily to the treatment of some, but not all, fears, phobias, or anxiety disorders. Conditions such as social anxiety disorder or specific phobias may benefit most from occasionally reinforced extinction, although the literature indicates the procedure may also be applicable to obsessive compulsive disorder (Krompinger et al., 2018). Clinical applications should also be guided by ethical considerations, in line with current exposure practices. As such, real life exposure to feared outcomes may be feasible from a pragmatic and ethical point of view in some, but not all, situations. Imaginal exposure may be considered in cases where real life exposure is not feasible; however, the application of occasionally reinforced extinction during imaginal exposure requires further investigation.

Translating the unpaired extinction procedure to the clinical setting may be slightly more challenging, but not impossible if we consider that unpaired extinction does not involve the elimination of the feared outcome, but the presentation of the feared outcome (US) at unexpected times, separate from the situation (CS) that typically predicts

the arrival of the US. It is conceivable that such training could be incorporated into imaginal exposure (e.g. Abramowitz & Arch, 2014), although more research is necessary to test this hypothesis.

Competing financial interests

The authors declare no competing financial interests.

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References

Abramowitz, J. S., & Arch, J. J. (2014). Strategies for improving long-term outcomes in cognitive behavioral therapy for obsessive-compulsive disorder: Insights from learning theory. *Cognitive and Behavioral Practice, 21*, 20–31. http://dx.doi.org/10.1016/j.cbpra.2013.06.004.

van den Akker, K., Havermans, R. C., & Jansen, A. (2015). Effects of occasional reinforced trials during extinction on the reacquisition of conditioned responses to food cues. *Journal of Behavior Therapy and Experimental Psychiatry, 48*, 50–58. http://dx.doi.org/10.1016/j.jbtep.2015.02.001.

American Psychiatric Association (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Washington, DC: Author.

Baxter, A. J., Scott, K. M., Vos, T., & Whiteford, H. A. (2013). Global prevalence of anxiety disorders: A systematic review and meta-regression. *Psychological Medicine, 43*, 897–910. http://dx.doi.org/10.1017/S003329171200147X.

Bouton, M. E. (1993). Context, time, and memory retrieval in the interference paradigms of Pavlovian learning. *Psychological Bulletin, 114*, 80–99. http://dx.doi.org/10.1037/0033-2909.114.1.80.

Bouton, M. E. (2000). A learning theory perspective on lapse, relapse, and the maintenance of behavior change. *Health Psychology, 19*(1S), 57–63. http://dx.doi.org/10.1037/0278-6133.19.Suppl1.57.

Bouton, M. E. (2002). Context, ambiguity, and unlearning: Sources of relapse after behavioral extinction. *Biological Psychiatry, 52*, 976–986. http://dx.doi.org/10.1016/S0006-3223(02)01546-9.

Bouton, M. E., Woods, A. M., & Pineño, O. (2004). Occasional reinforced trials during extinction can slow the rate of rapid reacquisition. *Learning and Motivation, 35*, 371–390. http://dx.doi.org/10.1016/j.lmot.2004.05.001.

Capaldi, E. J. (1966). Partial reinforcement: A hypothesis of sequential effects. *Psychological Review, 73*, 459–477. http://dx.doi.org/10.1037/h0023684.

Capaldi, E. J. (1994). The sequential view: From rapidly fading stimulus traces to the organization of memory and the abstract concept of number. *Psychonomic Bulletin & Review, 1*, 156–181. http://dx.doi.org/10.3758/BF03200771.

Carleton, R. N., Norton, M. A. P. J., & Asmundson, G. J. G. (2007). Fearing the unknown: A short version of the intolerance of uncertainty scale. *Journal of Anxiety Disorders, 21*, 105–117. http://dx.doi.org/10.1016/j.janxdis.2006.03.014.

Craske, M. G. (2015). Optimizing exposure therapy for anxiety disorders: An inhibitory learning and inhibitory regulation approach. *Verhaltenstherapie, 25*, 134–143. http://dx.doi.org/10.1159/000381574.

Craske, M. G., & Mystkowski, J. L. (2006). Exposure therapy and extinction: Clinical studies. In M. G. Craske, D. Hermans, & D. Vansteenwegen (Eds.). *Fear and learning: From basic processes to clinical implications* (pp. 37–52). Washington: American Psychological Association.

Craske, M. G., & Stein, M. B. (2016). Anxiety. *The Lancet, 388*, 3048–3059. http://dx.doi.org/10.1016/S0140-6736(16)30381-6.

Craske, M. G., Treanor, M., Conway, C. C., Zbozinek, T., & Vervliet, B. (2014). Maximizing exposure therapy: An inhibitory learning approach. *Behaviour Research and Therapy, 58*, 10–23. http://dx.doi.org/10.1016/j.brat.2014.04.006.

Culver, N. C., Stevens, S., Farsalinos, M. S., & Craske, M. G. (2018). Building physiological toughness: Some aversive events during extinction may attenuate return of fear. *Journal of Behavior Therapy and Experimental Psychiatry, 58*, 18–28. http://dx.doi.org/10.1016/j.jbtep.2017.07.003.

Davey, G. C. L. (1992). Classical conditioning and the acquisition of human fears and phobias: A review and synthesis of the literature. *Advances in Behaviour Research and Therapy, 14*, 29–66. http://dx.doi.org/10.1016/0146-6402(92)90010-I.

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

Dudai, Y. (2012). The restless engram: Consolidations never end. *Annual Review of Neuroscience, 35*, 227–247. http://dx.doi.org/10.1146/annurev-neuro-062111-150500.

Dudai, Y., Karni, A., & Born, J. (2015). The consolidation and transformation of memory. *Neuron, 88*, 20–32. http://dx.doi.org/10.1016/j.neuron.2015.09.004.

Dunsmoor, J. E., Campese, V. D., Ceceli, A. O., LeDoux, J. E., & Phelps, E. A. (2015). Novelty-facilitated extinction: Providing a novel outcome in place of an expected threat diminishes recovery of defensive responses. *Biological Psychiatry, 78*, 203–209. http://dx.doi.org/10.1016/j.biopsych.2014.12.008.

Fernández, R. S., Boccia, M. M., & Pedreira, M. E. (2016). The fate of memory: Reconsolidation and the case of prediction error. *Neuroscience & Biobehavioral Reviews, 68*, 423–441. http://dx.doi.org/10.1016/j.neubiorev.2016.06.004.

Foa, E. B., & McLean, C. P. (2016). The efficacy of exposure therapy for anxiety-related disorders and its underlying mechanisms: The case of OCD and PTSD. *Annual Review of Clinical Psychology, 12*, 1–28. http://dx.doi.org/10.1146/annurev-clinpsy-021815-093533.

Forster, K., & Forster, J. C. (2003). Dmdx: A windows display program with millisecond accuracy. *Behavior Research Methods, Instruments, & Computers, 35*, 116–124. http://dx.doi.org/10.3758/BF03195503.

Frey, P. W., & Butler, C. S. (1977). Extinction after aversive conditioning: An associative or nonassociative process? *Learning and Motivation, 8*, 1–17. http://dx.doi.org/10.1016/0023-9690(77)90063-7.

Haaker, J., Golkar, A., Hermans, D., & Lonsdorf, T. B. (2014). A review on human reinstatement studies: An overview and methodological challenges. *Learning & Memory, 21*, 424–440. http://dx.doi.org/10.1101/lm.036053.114.

Henry, J. D., & Crawford, J. R. (2005). The short-form version of the Depression Anxiety Stress Scales (DASS-21): Construct validity and normative data in a large non-clinical sample. *British Journal of Clinical Psychology, 44*, 227–239. http://dx.doi.org/10.1348/0144665053X29657.

Kroenpinger, J. W., Van Kirk, N. P., Garner, L. E., Potluri, S. I., & Elias, J. A. (2018). Hope for the worst: Occasional reinforced extinction and expectancy violation in the treatment of OCD. *Cognitive and Behavioral Practice*. http://dx.doi.org/10.1016/j.cbpra.2017.12.002.

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

Lipp, O. V. (2006b). Of snakes and flowers: Does preferential detection of pictures of fear-relevant animals in visual search reflect on fear-relevance? *Emotion, 6*, 296–308. http://dx.doi.org/10.1037/1528-3542.6.2.296.

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

Lipp, O. V., Oughton, N., & LeLievre, J. (2003). Evaluative learning in human Pavlovian conditioning: Extinct, but still there? *Learning and Motivation, 34*, 219–239. http://dx.doi.org/10.1016/S0023-9690(03)00011-0.

Lissek, S., Pine, D. S., & Grillon, C. (2006). The strong situation: A potential impediment to studying the psychobiology and pharmacology of anxiety disorders. *Biological Psychology, 72*, 265–270. http://dx.doi.org/10.1016/j.biopsycho.2005.11.004.

Lonsdorf, T. B., & Merz, C. J. (2017). More than just noise: Inter-individual differences in fear acquisition, extinction and return of fear in humans - biological, experiential, temperamental factors, and methodological pitfalls. *Neuroscience & Biobehavioral Reviews, 80*, 703–728. http://dx.doi.org/10.1016/j.neubiorev.2017.07.007.

Lovibond, P. F. (2006). Fear and avoidance: An integrated expectancy model. In M. G. Craske, D. Hermans, & D. Vansteenwegen (Eds.). *Fear and learning: From basic processes to clinical implications* (pp. 117–132). Washington: American Psychological Association.

Lovibond, P. F., & Lovibond, S. H. (1995). The structure of negative emotional states: Comparison of the depression anxiety stress scales (DASS) with the beck depression and anxiety inventories. *Behaviour Research and Therapy, 33*, 335–343. http://dx.doi.org/10.1016/0005-7967(94)00075-U.

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. http://dx.doi.org/10.1016/j.brat.2015.01.001.

Lykken, D. T. (1972). Range correction applied to heart rate and to GSR data. *Psychophysiology, 9*, 373–379. http://dx.doi.org/10.1111/j.1469-8986.1972.tb03222.x.

Mickley, G. A., DiSorbo, A., Wilson, G. N., Huffman, J., Bucik, S., Hosha, Z., ... Kim, Y.-H. (2009). Explicit disassociation of a conditioned stimulus and unconditioned stimulus during extinction training reduces both time to asymptotic extinction and spontaneous recovery of a conditioned taste aversion. *Learning and Motivation, 40*, 209–220. http://dx.doi.org/10.1016/j.lmot.2009.01.001.

Napier, R. M., Macrae, M., & Kehoe, E. J. (1992). Rapid reacquisition in conditioning of the rabbit's nictitating membrane response. *Journal of Experimental Psychology: Animal Behavior Processes, 18*, 182–192. http://dx.doi.org/10.1037/0097-7403.18.2.182.

Öhman, A., & Mineka, S. (2001). Fears, phobias, and preparedness: Toward an evolved module of fear and fear learning. *Psychological Review, 108*, 483–522. http://dx.doi.org/10.1037//0033-295x.108.3.483.

Pearce, J. M., & Hall, G. (1980). A model for Pavlovian learning: Variations in the effectiveness of conditioned but not of unconditioned stimuli. *Psychological Review, 87*, 532–552. http://dx.doi.org/10.1037/0033-295X.87.6.532.

Pineles, S. L., Orr, M. R., & Orr, S. P. (2009). An alternative scoring method for skin conductance responding in a differential fear conditioning paradigm with a long-duration conditioned stimulus. *Psychophysiology, 46*, 984–995. http://dx.doi.org/10.1111/j.1469-8986.2009.00852.x.

Prokasy, W. F., & Ebel, H. C. (1967). Three components of the classically conditioned GSR in human subjects. *Journal of Experimental Psychology, 73*, 247–256. http://dx.doi.org/10.1037/h0024108.

Rauhut, A. S., Thomas, B. L., & Ayres, J. J. B. (2001). Treatments that weaken Pavlovian conditioned fear and thwart its renewal in rats: Implications for treating human phobias. *Journal of Experimental Psychology: Animal Behavior Processes, 27*, 99–114.

http://dx.doi.org/10.1037/0097-7403.27.2.99.

Rescorla, R. A. (1973). Effects of US habituation following conditioning. *Journal of Comparative & Physiological Psychology, 82*, 137–143. http://dx.doi.org/10.1037/h0033815.

Rescorla, R. A., & Skucy, J. C. (1969). Effect of response-independent reinforcers during extinction. *Journal of Comparative & Physiological Psychology, 67*, 381–389. http://dx.doi.org/10.1037/h0026793.

Rescorla, R. A., & Wagner, A. W. (1972). A theory of Pavlovian conditioning: Variations in the effectiveness of reinforcement and nonreinforcement. In A. H. Black, & W. F. Prokasy (Eds.). *Classical conditioning II: Current research and theory* (pp. 64–99). New York: Appleton-Century-Crofts.

Ricker, S. T., & Bouton, M. E. (1996). Reacquisition following extinction in appetitive conditioning. *Animal Learning & Behavior, 24*, 423–436. http://dx.doi.org/10.3758/bf03199014.

Schiller, D., Monfils, M.-H., Raio, C. M., Johnson, D. C., LeDoux, J. E., & Phelps, E. A. (2010). Preventing the return of fear in humans using reconsolidation update mechanisms. *Nature, 463*, 49–53. http://dx.doi.org/10.1038/nature08637.

Schultz, D. H., Balderston, N. L., Geiger, J. A., & Helmstetter, F. J. (2013). Dissociation between implicit and explicit responses in postconditioning UCS revaluation after fear conditioning in humans. *Behavioral Neuroscience, 127*, 357–368. http://dx.doi.org/10.1037/a0032742.

Thomas, B. L., Longo, C. L., & Ayres, J. J. B. (2005). Thwarting the renewal (relapse) of conditioned fear with the explicitly unpaired procedure: Possible interpretations and implications for treating human fears and phobias. *Learning and Motivation, 36*, 374–407. http://dx.doi.org/10.1016/j.lmot.2004.11.005.

Thompson, A., & Lipp, O. V. (2017). Extinction during reconsolidation eliminates recovery of fear conditioned to fear-irrelevant and fear-relevant stimuli. *Behaviour Research and Therapy, 92*, 1–10. http://dx.doi.org/10.1016/j.brat.2017.01.017.

Todd, T. P., Vurbic, D., & Bouton, M. E. (2014). Behavioral and neurobiological mechanisms of extinction in Pavlovian and instrumental learning. *Neurobiology of Learning and Memory, 108*, 52–64. http://dx.doi.org/10.1016/j.nlm.2013.08.012.

Vervliet, B., Vansteenwegen, D., & Hermans, D. (2010). Unpaired shocks during extinction weaken the contextual renewal of a conditioned discrimination. *Learning and Motivation, 41*, 22–31. http://dx.doi.org/10.1016/j.lmot.2009.08.001.

Vurbic, D., & Bouton, M. E. (2014). A contemporary behavioral perspective on extinction. In F. K. McSweeney, & E. S. Murphy (Eds.). *The wiley blackwell handbook of operant and classical conditioning* (pp. 53–76). Oxford, UK: John Wiley & Sons, Ltd.

Weisman, J. S., & Rodebaugh, T. L. (2018). Exposure therapy augmentation: A review and extension of techniques informed by an inhibitory learning approach. *Clinical Psychology Review, 59*, 41–51. http://dx.doi.org/10.1016/j.cpr.2017.10.010.

Woods, A. M., & Bouton, M. E. (2007). Occasional reinforced responses during extinction can slow the rate of reacquisition of an operant response. *Learning and Motivation, 38*, 56–74. http://dx.doi.org/10.1016/j.lmot.2006.07.003.

Appendix B: Publication 2

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Extinction during reconsolidation eliminates recovery of fear conditioned to fear-irrelevant and fear-relevant stimuli

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ABSTRACT

Extant literature suggests that extinction training delivered during the memory reconsolidation period is superior to traditional extinction training in the reduction of fear recovery, as it targets the original fear memory trace. At present it is debated whether different types of fear memories are differentially sensitive to behavioral manipulations of reconsolidation. Here, we examined post-reconsolidation recovery of fear as a function of conditioned stimulus (CS) fear-relevance, using the unconditioned stimulus (US) to reactivate and destabilize conditioned fear memories. Participants ($N = 56$; 25 male; $M = 24.39$ years, $SD = 7.71$) in the US-reactivation and control group underwent differential fear conditioning to fear-relevant (spiders/snakes) and fear-irrelevant (geometric shapes) CSs on Day 1. On Day 2, participants received either reminded (US-reactivation) or non-reminded extinction training. Tests of fear recovery, conducted 24 h later, revealed recovery of differential electrodermal responding to both classes of CSs in the control group, but not in the US-reactivation group. These findings indicate that the US reactivation-extinction procedure eliminated recovery of extinguished responding not only to fear-irrelevant, but also to fear-relevant CSs. Contrasting previous reports, our findings show that post-reconsolidation recovery of conditioned responding is not a function of CS fear-relevance and that persistent reduction of fear, conditioned to fear-relevant CSs, can be achieved through behavioral manipulations of reconsolidation.

1. Introduction

The current focus of human memory reconsolidation research is on developing more efficient methods for long-lasting fear reduction. Research efforts in this area have increased since Schiller et al. (2010) demonstrated that fears, conditioned to fear-irrelevant stimuli (geometric shapes), can be permanently eliminated through safe and non-invasive behavioral interventions that target the memory reconsolidation process. These findings have since been replicated in other studies employing fear-irrelevant stimuli (Agren et al., 2012; Björkstrand et al., 2015; Johnson & Casey, 2015; Liu et al., 2014; Oyarzún et al., 2012; Schiller, Kanen, LeDoux, Monfils, & Phelps, 2013; Steinfurth et al., 2014), but see Golkar, Bellander, Olsson, and Öhman (2012 [experiment 2]) and Klucken et al. (2016). However, disruption of reconsolidation using behavioral interventions has not been demonstrated in studies employing fear-relevant stimuli (e.g. spiders; Fricchione et al., 2016; Golkar et al., 2012 [experiment 1]; Kindt & Soeter, 2013; Meir Drexler et al., 2014; Soeter & Kindt, 2011 [experiment 2]), leading to speculations that fear, conditioned to fear-relevant stimuli, may not be sensitive to behavioral manipulations of reconsolidation.

Reconsolidation is a time-dependent process that restabilizes reactivated memories (Nader, 2015). The purpose of reconsolidation is to update previously consolidated memories with novel information, in order to facilitate adaptation to the environment (Lee, 2009). Reconsolidation is initiated through reactivation and destabilization of the consolidated memory trace, by presenting cues associated with the original learning (Nader, 2013; Pineyro, Monti, Alfei, Bueno, & Urcelay, 2014). Once reactivated, memories become labile and are open to modification, before they reconsolidate and return to their inactive state (Nader, 2015; Nader, Schafe, & LeDoux, 2000). Although the exact time course of memory reconsolidation is not known, it is believed that reconsolidation is completed within six hours of memory reactivation (Agren et al., 2012; Alberini, 2011; Nader et al., 2000; Schiller et al., 2010).

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Interfering with reconsolidation during this period of lability through administration of pharmacological or behavioral interventions, such as extinction training, may modify the existing memory trace and persistently reduce the recovery of fear (Agren, 2014; Schiller et al., 2010). Conversely, when extinction training is administered without prior memory reactivation, fear may recover in a new context (renewal), after the passage of time (spontaneous recovery) or after re-exposure to the aversive event (reinstatement), as extinction learning involves the acquisition of a new, inhibitory association and not the unlearning of the original fear response (Bouton, 2002).

Reconsolidation studies are typically conducted over the course of three consecutive days and involve differential Pavlovian fear conditioning (e.g. Schiller et al., 2010), whereby a neutral conditioned stimulus (CS+) is paired with an intrinsically aversive stimulus (unconditioned stimulus, US), while another CS is presented by itself (CS-). Future presentations of the CS + result in anticipation of the US, which is reflected in increased differential responding to the CS+, relative to the CS-, on behavioral, verbal, and physiological indices of fear (Lipp, 2006a). During extinction (Day 2), typically delivered 10 min after administration of procedures which are thought to reactive and destabilize fear memories, the CSs are presented without the US until differential responding is extinguished. Successful disruption of reconsolidation is inferred from the absence of differential responding during tests of fear recovery (e.g. Liu et al., 2014; Schiller et al., 2010).

As fear is expressed on the verbal, behavioral, and physiological level (Lang, 1985), several methods exist to measure conditioned fear. The most commonly employed measure in humans is electrodermal activity (skin conductance responses, SCRs) which increases during conditioning as a result of increased sweat gland activity (Boucsein, 2012; Dawson, Schell, & Filion, 2007). Fear learning is also reflected on verbal indices of conditioned responding, such as increased negative valence of the reinforced CS+ (De Houwer, Thomas, & Baeyens, 2001). Physiological and verbal indices of fear learning are said to be governed by dissociable implicit (non-conscious) and explicit (conscious) processes respectively (LaBar & Cabeza, 2006; Schultz, Balderston, Geiger, & Helmstetter, 2013; but see; Sevenster, Beckers, & Kindt, 2012) and are differentially sensitive to manipulations of reconsolidation (e.g. Kindt & Soeter, 2013; Soeter & Kindt, 2010). By measuring multiple indices of conditioned responding, we can obtain a comprehensive understanding of processes underlying fear learning and fear reduction (Lipp, 2006a).

Extant literature indicates that post-reactivation extinction training is superior to extinction training alone in achieving lasting reduction of fear (Agren et al., 2012; Björkstrand et al., 2015; Liu et al., 2014; Schiller et al., 2010). However, at present, it is unknown whether all types of fear memories are sensitive to behavioral manipulations of reconsolidation. Relative to fear-irrelevant CSs, phylogenetically fear-relevant CSs, such as snakes, show superior conditioning which resists extinction (Mineka & Öhman, 2002). Accordingly, it is possible that fears conditioned to different classes of stimuli may be differentially sensitive to behavioral manipulations of reconsolidation. However, conclusive evidence is lacking, as past research has either employed only fear-relevant or fear-irrelevant CSs (e.g. Fricchione et al., 2016; Golkar et al., 2012; Kindt & Soeter, 2013). Cross-study comparisons are problematic, due to methodological variations, such as the reinforcement rate employed during fear conditioning, number of acquisition trials, type and duration of the US or the memory reactivation procedure (for reviews see Auber, Tedesco, Jones, Monfils, & Chiamulera, 2013; Finnie & Nader, 2012; Kredlow, Unger, & Otto, 2016).

Differences across memory reactivation procedures deserve further consideration, as successful reactivation and destabilization of memories is a prerequisite for memory reconsolidation (Pineyro et al., 2014). The vast majority of past fear conditioning research (e.g. Golkar et al., 2012; Kindt & Soeter, 2013; Schiller et al., 2010) has employed an unreinforced presentation of the previously conditioned CS + to reactivate fear memories ("CS-reactivation"). The success of this and other reactivation procedures is constrained by a number of boundary conditions. These include, but are not limited to, the age, strength and type of memory to be reactivated, type of reactivation procedure, and the 'prediction error' generated by the reactivating stimulus (for reviews of boundary conditions and prediction errors, please see Auber et al., 2013; Exton-McGuinness, Lee, & Reichelt, 2015; Fernández, Boccia, & Pedreira, 2016; Finnie & Nader, 2012; Lee, 2009).

The term 'prediction error' in reconsolidation research refers to a mismatch between past learning history, and actual events that are of relevance to prior learning and contain novel information that warrants updating or modification of memories (Exton-McGuinness et al., 2015; Fernández et al., 2016; Lee, 2009). An example of a manipulation that can generate a prediction error would be a change to the temporal CS-US relationship, by presenting the US 20 s earlier or later than expected, based on the trained CS-US interval (Díaz-Mataix, Ruiz Martinez, Schafe, LeDoux, & Doyère, 2013). An unreinforced presentation of the previously conditioned CS + may also generate a prediction error, for instance when the duration of the CS presentation is increased, relative to training conditions (Agren et al., 2012). It has also been observed that CS-reactivation may trigger memory reconsolidation when the consequences of the CS are not fully predictable, for instance following training on a partial reinforcement schedule (Oyarzún et al., 2012; Schiller et al., 2010), but see Golkar et al. (2012); Kindt and Soeter (2013). Overall, extinction training subsequent to CS-reactivation is more likely to disrupt the reconsolidation of fears conditioned to fear-irrelevant CSs (e.g. Schiller et al., 2010) than of those conditioned to fear-relevant CSs (Kindt & Soeter, 2013). There are several reasons as to why the CS-reactivation procedure may fail to destabilize these fear memories.

Briefly, it has been proposed that the strength of conditioned fears varies across training protocols. For instance, training with fear-relevant CSs is thought to result in strong fear associations which are resistant to extinction (Mineka & Öhman, 2002) and to behavioral manipulations of reconsolidation (Kindt & Soeter, 2013; Soeter & Kindt, 2011). It is also conceivable that the mere absence of the US during CS-reactivation does not create the prediction error necessary to facilitate behavioral manipulations of reconsolidation, even though the reactivation procedure is capable of supporting pharmacological manipulations (see Soeter & Kindt, 2011 for a comparison of these methods). It should be noted that there are a number of additional factors which may determine whether a manipulation results in memory destabilization. A comprehensive review of these factors is, however, beyond the scope of this paper. Readers interested in differences in prediction errors across memory types, training conditions, and computational models of associative learning may wish to consult Fernández et al. (2016); Holland and Schiffino (2016); or Schultz and Dickinson (2000).

Due to the mixed results from studies which used CS-reactivation, we employed a reactivation procedure that consisted of a single presentation of the US, at half the physical intensity used during acquisition ("US-reactivation"). This procedure has been successfully employed in past research (Liu et al., 2014), albeit only with fear-irrelevant CSs. Relative to CS-reactivation, the present reactivation procedure may be more likely to generate a prediction error, due to the mismatch between the actual and expected US intensity, as well as due to the unsignalled presentation of the US, in the absence of the CS. Based on previous reports, US-reactivation

appears to be capable of reactivating and destabilizing multiple fear memories that are associated with the reactivating US (Liu et al., 2014). This procedure may be of relevance to the treatment of real-life fears, as these involve multiple, often unknown, triggers (Schiller, 2014). It is conceivable that a single reactivation session could be sufficient to trigger the reconsolidation of multiple CS-US associations and facilitate the disruption of reconsolidation through extinction training, thereby preventing recovery of fear during future cue encounters (Dunbar & Taylor, 2016; Liu et al., 2014).

Here, we provided the first application of the US-reactivation procedure to fear, conditioned to fear-relevant stimuli, and investigated whether extinction training, delivered 10 min after reactivation, differentially affects the recovery of fear to fear-relevant and fear-irrelevant CSs. The study employed a mixed model design, whereby participants in the US-reactivation and control group were conditioned to fear-irrelevant and fear-relevant CSs (Olsson, Ebert, Banaji, & Phelps, 2005), but only participants in the US-reactivation group received a memory reactivation trial prior to extinction training. In line with previous research (Liu et al., 2014; Schiller et al., 2010), tests were conducted over the course of three consecutive days, involving differential Pavlovian conditioning (Day 1), reactivation-extinction/extinction-only (Day 2) and tests of fear recovery on Day 3. Electrodermal responding and CS valence ratings were recorded as primary and secondary dependent measures of conditioned fear, respectively. Based on the reviewed literature, which has not found that behavioral interventions affect reconsolidation of fear conditioned to fear-relevant stimuli, it was hypothesized that there would be a larger level of post-reconsolidation fear recovery for fear-relevant than for fear-irrelevant CSs during tests of spontaneous recovery and reinstatement.

2. Materials and methods

2.1. Participants

University students who met inclusion criteria (i.e. no cardiovascular disease, seizure disorder, or pregnancy) participated in exchange for partial course credit or a financial compensation of 45 AUD. After exclusion of three participants who failed to verbalize the CS-US relationship on Day 1 (US-reactivation group: $n = 1$; control group: $n = 2$) and three participants from the US-reactivation group, who did not present for testing on Day 3, 28 participants each remained in the US-reactivation (15 male; $M = 23.54$ years, $SD = 7.05$) and control group (10 male; $M = 25.25$ years, $SD = 8.36$). Ethical approval for this study was obtained from the Curtin University Human Research Ethics Committee.

2.2. Stimuli and measures

2.2.1. Stimuli

Fear-relevant CSs (CSa+/-) consisted of a spider (700 × 703 pixels) and snake (800 × 629 pixels) picture (Lipp, 2006b), while pictures of blue and yellow squares, measuring 700 × 700 pixels (Schiller et al., 2010), served as fear-irrelevant CSs (CSb+/-). The pictures were presented for 6 s, in the center of a 17-inch color LCD screen, over a black background, with an inter-trial interval of 10–14 s. To control for order effects, the assignment of snake and spider pictures as CSa + or CSa-, the assignment of yellow and blue squares as CSb + or CSb-, and whether the first trial of each phase was a CSa ± or CSb ± were counterbalanced across participants. Stimuli were presented in a pseudo-randomized order, whereby each CS was presented twice within blocks of eight trials. The US consisted of a mild electric shock, which was generated with a Grass SD9 stimulator (Grass Technologies, Middleton, WI) and delivered to the wrist of the dominant hand via a concentric electrode. The shock was presented for 200 ms (pulsed at 50 Hz) and coincided with the CSs + offset; the CSs-were never paired with the US. In line with Agren et al. (2012), we employed a 100% reinforcement schedule to facilitate acquisition of fear on Day 1 – a prerequisite for subsequent manipulations of reconsolidation. The delivery of the US and CSs was controlled with DMDX 5.0.5 software (Forster & Forster, 2003).

2.2.2. Electrodermal activity (skin conductance responses, SCRs)

Electrodermal activity was recorded through two self-adhesive isotonic gel electrodes (Biopac Systems EL507), attached to the thenar and hypothenar eminences of the non-dominant hand. Electrodermal activity was DC amplified at a gain of 5 micro Siemens (μS) per volt and recorded with a Biopac MP150 system at a sampling frequency of 1000 Hz, using AcqKnowledge 4 (Biopac Systems, Goleta, CA). A Biopac respiration belt was fitted around the participants' waist to control for respiration-induced artefacts in SCRs. Electrodermal responses were scored offline in AcqKnowledge 4. Following a visual inspection of data, 10 SCRs (across groups) were discarded, due to the presence of respiration-induced artefacts. In accordance with past research (Kindt & Soeter, 2013; Pineles, Orr, & Orr, 2009), SCRs elicited by the CSs were calculated by subtracting the mean skin conductance level during the 2 s baseline preceding CS onset from the largest skin conductance level occurring 1–6 s after CS onset. Responses below 0.02 μS were scored as zero and retained in the analysis (Kindt & Soeter, 2013). All SCRs were square root transformed and range corrected, to reduce the skew of the distribution as well as the influence of individual differences in electrodermal activity on conditioned responding (Lykken, 1972). The range correction was obtained by dividing each response by the largest response displayed by the participant. Electrodermal responses were averaged into blocks of two consecutive trials, to reduce the influence of trial by trial variability.

2.2.3. Valence ratings

Participants rated CS valence on a 9-point scale (from 1 [unpleasant] to 9 [pleasant]) at baseline, after acquisition, spontaneous recovery, and reinstatement testing. Online ratings were not obtained, as these may interfere with the measurement of SCRs (Kindt & Soeter, 2013; Oyarzún et al., 2012). US valence was measured in an identical manner, following acquisition (US-reactivation and control group) and post-reactivation extinction training (US-reactivation group). DMDX 5.0.5 software was used to control stimulus presentation and to record CS ratings.

2.2.4. Manipulation checks

Following acquisition, participants were presented with a CS-US contingency questionnaire, containing pictures of the CSs and two control stimuli, and were asked to indicate which stimuli had been paired with the US. As inability to verbalize the correct contingency may reflect a genuine failure to learn the CS-US relationship (Lipp, 2006a), data from participants who failed this test were excluded from statistical analyses. A second manipulation check was employed to assess whether participants in the US-reactivation group noticed the decrease in US intensity on Day 2: Participants were asked to rate US valence on a 9-point scale (from 1 [unpleasant] to 9 [pleasant]) and to indicate whether US intensity on Day 2 was lower, higher or the same as on Day 1.

2.3. Experimental procedure

Unless otherwise indicated, participants were fitted with the

skin conductance electrodes, respiration belt, and the shock electrode at the start of each session. Participants completed each stage of the experiment individually, while seated in front of a 17-inch color LCD screen. Please note that due to administrative limitations, data collection for the US-reactivation group was conducted six months before data collection in the control group. This separation in time has the potential to induce confounds reflecting non-associative factors that affect responding. However, the within subject nature of the design limits the extent to which these can affect the results. Moreover, extensive preliminary testing failed to reveal any between group differences in overall responsiveness, fear learning or other factors that could affect the results.

2.3.1. Day 1: acquisition

Participants were informed about the experimental procedures and had the opportunity to ask questions, before providing information about current medication use and medical history. Individuals who met the inclusion criteria provided written consent and set the US intensity to a level which was perceived as unpleasant, but not painful. After providing baseline CS valence ratings, participants were asked to pay attention to the computer screen and to learn which CSs were followed by the US. Conditioning commenced with a habituation phase, consisting of four presentations of each CS, and was immediately followed by acquisition, which involved eight presentations of CSa ± and CSb+/-. Thereafter, participants completed the CS-US contingency questionnaire and provided US and CS valence ratings.

2.3.2. Day 2: (post-reactivation) extinction training

Participants in the US-reactivation group were asked to remember what they had learned on Day 1, before receiving a memory reactivation trial, consisting of an unsignalled presentation of the US, at half the physical intensity employed on Day 1. The shock electrode was subsequently removed and participants were offered magazines to read during a 10-min break. Participants in the control group commenced the session by reading magazines for 10 min and were not fitted with the shock electrode, to prevent potential reactivation of fear memories. Prior to extinction training, all participants were fitted with the shock electrode and were instructed to pay attention to the computer screen and remember what they had learned during previous stages of the experiment. Extinction training consisted of 10 unreinforced presentations of all CSs. Subsequently, participants in the US-reactivation group were asked to rate the valence and intensity of the US.

2.3.3. Day 3: assessment of differential responding

Participants were asked to pay attention to the pictures on the computer screen; no further instructions were provided. Spontaneous recovery testing consisted of eight unreinforced presentations of all CSs. CS valence ratings were obtained thereafter and were followed by three unsignalled presentations of the US for 200 ms each, at the intensity used during acquisition, with an inter-trial interval of 6 s. The computer screen was switched on and displayed a black background. After a 10-min break (identical to Day 2), participants underwent reinstatement testing, consisting of eight unreinforced presentations of all CSs, followed by CS valence ratings.

2.4. Statistical analyses

Electrodermal data from the US-reactivation and control group were analyzed through mixed analyses of variance (ANOVAs) for repeated measures, with group (US-reactivation vs. control) as between-subjects factor and fear-relevance (fear-relevant vs. fear-irrelevant), conditioning (CS + vs. CS-) and block/time (habituation: block 1–2; acquisition: block 1–4; extinction: block 1–5; spontaneous recovery: block 5 extinction training vs. block 1 spontaneous recovery; reinstatement: block 4 spontaneous recovery vs. block 1 reinstatement) as within-subjects factors. CS valence ratings were analyzed in a similar manner, with group as between-subjects factor and fear-relevance (fear-relevant vs. fear-irrelevant), conditioning (CS + vs. CS-) and time (baseline, acquisition, spontaneous recovery, reinstatement) as within-subjects factors. Multivariate F values (Pillai's Trace) and partial eta squared values were reported for all main effects and interactions. Statistical significance was assessed at $\alpha = 0.05$; Bonferroni corrections were used for follow-up analyses to guard against the accumulation of a Type 1 error.

3. Results

3.1. Preliminary analyses

The groups did not differ in age, selected US intensity, baseline CS and US valence ratings or raw electrodermal responding during habituation (Table 1). A manipulation check confirmed that participants in the US-reactivation group registered the decrease in US intensity on Day 2, as the US was rated as less unpleasant on Day 2 ($M = 7.25$, $SD = 1.86$) than on Day 1 ($M = 3.50$, $SD = 1.75$), $t(27) = 7.50$, $p < 0.001$, $d = 2.08$, and was described as being of lower intensity than on Day 1.

3.2. Electrodermal responding

Electrodermal responding in the US-reactivation and control group is presented in Fig. 1. Analysis of electrodermal data during habituation yielded main effects of fear-relevance, $F(1,54) = 5.86$, $p = 0.019$, $\eta p^2 = 0.10$, and block, $F(1,54) = 55.12$, $p < 0.001$, $\eta p^2 = 0.51$, as well as fear-relevance x block, $F(1,54) = 8.87$, $p = 0.004$, $\eta p^2 = 0.14$, fear-relevance x conditioning x group, $F(1,54) = 6.82$, $p = 0.012$, $\eta p^2 = 0.11$, and fear-relevance x conditioning x block × group interactions, $F(1,54) = 4.70$, $p = 0.035$, $\eta p^2 = 0.08$. The four-way interaction reflects differential responding to fear-relevant CSs in the US-reactivation group, on block 1, $F(1,54) = 10.90$, $p = 0.002$, $\eta p^2 = 0.17$, but not on block 2, $F(1,54) = 2.01$, $p = 0.163$, $\eta p^2 = 0.04$. Differential responding was not evident to fear-irrelevant CSs or in the control group, all $F(1,54) \leq 1.18$, $p \geq 0.283$, $\eta p^2 \leq 0.02$. Closer inspection of the data indicated that enhanced responding to fear-relevant CSs+ in the US-reactivation group may reflect on enhanced responses to spider pictures. Examination of baseline CS valence ratings revealed that participants assigned to sequences starting with spider pictures used as CS + rated spiders as less pleasant ($M = 2.63$, $SD = 1.19$) than snakes ($M = 4.38$, $SD = 1.92$), which may have enhanced orienting responses. The larger dislike of spiders may have resulted in a significant increase in electrodermal responding on the first block of habituation. However, this difference across CS conditions and groups was absent on the last block of habituation.

In line with Fig. 1, statistical analyses revealed that conditioned responding was acquired to fear-relevant and fear-irrelevant CSs on Day 1, conditioning, $F(1,54) = 56.18$, $p < 0.001$, $\eta p^2 = 0.51$, block, $F(3,52) = 8.69$, $p < 0.001$, $\eta p^2 = 0.33$, conditioning x block, $F(3,52) = 7.52$, $p < 0.001$, $\eta p^2 = 0.30$. The interaction reflects an increase in differential responding to both types of CSs, across blocks of acquisition, all $F(1,54) \geq 11.60$, $p \leq 0.001$, $\eta p^2 \geq 0.18$. The main effect of fear-relevance and remaining interactions did not attain significance, largest effect (fear-relevance x conditioning x group), $F(1,54) = 2.90$, $p = 0.094$, $\eta p^2 = 0.05$.

On Day 2, differential responding was evident in both groups during the early phase of extinction training, but decreased

Table 1
Means (M) and standard deviations (SD) for age, baseline valence ratings, US intensity and electrodermal responding during habituation in the US-Reactivation and control group.

	US-reactivation		Control		t-Test
	M	*SD*	*M*	*SD*	
Age	23.54	7.05	25.25	8.36	t (54) = 0.83, p = 0.410
CS valence	5.41	0.90	5.60	1.39	t (54) = 0.60, p = 0.552
US valence	3.50	1.75	2.86	1.27	t (54) = 1.57, p = 0.122
US intensity	47.79	14.41	47.95	14.48	t (54) = 0.04, p = 0.967
Electrodermal responding	0.87	0.96	0.76	0.71	t (54) = 0.51, p = 0.616

Note. CS = conditioned stimulus, US = unconditioned stimulus. Electrodermal responding during habituation is reported in μSiemens; US intensity is reported in Volts.

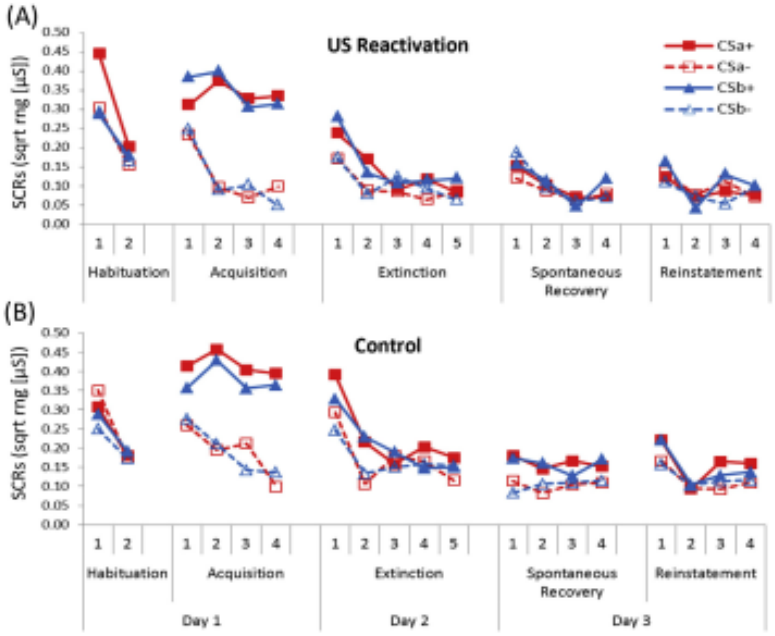

Fig. 1. Mean skin conductance responses (SCRs) to fear-relevant (CSa+/-) and fear-irrelevant (CSb+/-) conditioned stimuli in the US-reactivation (A) and control (B) group. SCRs are presented in blocks of two consecutive trials.

thereafter, conditioning, F (1,54) = 14.14, p < 0.001, ηp^2 = 0.21, block, F(4,51) = 11.81, p < 0.001, ηp^2 = 0.48, conditioning x block, F (4,51) = 4.56, p = 0.003, ηp^2 = 0.26. The interaction reflects differential responding on block 1 and 2, both F (1,54) ≥ 16.73, p < 0.001, ηp^2 = 0.24, but not on blocks 3 to 5, F (1,54) ≤ 1.55, p ≥ 0.219, ηp^2 ≤ 0.03. The main effect of fear-relevance and remaining interactions did not attain significance, largest effect (fear-relevance x block x group), F (4,51) = 1.72, p = 0.160, ηp^2 = 0.12. Please note that although Fig. 1 seems to indicate that there were group differences in differential responding during early extinction (block 1–2), this was not supported by the analysis, all interactions involving the factor group, F (1,54) ≤ 0.44, p ≥ 0.508, ηp^2 ≤ 0.01.

Tests of fear recovery, conducted on Day 3, revealed spontaneous recovery of previously extinguished differential responding in the control group, but not in the US-reactivation group. Analyses yielded a main effect of conditioning, F (1,54) = 4.74, p = 0.034, ηp^2 = 0.08, as well as interactions of time x group, F (1,54) = 4.39, p = 0.041, ηp^2 = 0.08, and fear-relevance x conditioning x time x group, F (1,54) = 5.06, p = 0.029, ηp^2 = 0.09. The remaining main effects and interactions did not attain significance, largest effect (main effect of time), F (1,54) = 2.19, p = 0.145, ηp^2 = 0.04. The significant four-way interaction reflects group differences in electrodermal responding to the non-reinforced fear-irrelevant CSb-, on the last block of extinction training and first block of spontaneous recovery. Differential responding to fear-relevant or fear-irrelevant CSs was not evident on the last block of extinction training in either group, all F (1,54) ≤ 2.58, p ≥ 0.114, ηp^2 ≤ 0.05. In contrast, spontaneous recovery of differential responding to both types of CSs was observed in the control group, both F (1,54) ≥ 4.26, p ≤ 0.044, ηp^2 ≥ 0.07, but not in the US-reactivation group, both F (1,54) ≤ 0.95, p ≥ 0.334, ηp^2 ≤ 0.02.

Analysis of reinstatement data yielded main effects of conditioning, F (1,54) = 5.69, p = 0.021, ηp^2 = 0.10, and time, F (1,54) = 7.50, p = 0.008, ηp^2 = 0.12, reflecting overall larger SCRs to reinforced than to non-reinforced stimuli and overall larger SCRs on the first block of reinstatement testing than on the last block of spontaneous recovery in both groups. The main effect of fear-relevance and remaining interactions did not attain significance, largest effect (fear-relevance x conditioning), F (1,54) = 1.50, p = 0.226, ηp^2 = 0.03. The main effect of conditioning suggests that differential responding was still present during the last block of

spontaneous recovery testing. This may have masked a between group difference in differential responding on the first block of reinstatement. To test this proposition, we conducted follow-up comparisons to test whether differential responding was observed in either group on the first block of reinstatement. Differential responding recovered on the first block of reinstatement testing in the control group, $F(1,54) = 5.06$, $p = 0.029$, $\eta p^2 = 0.09$, but not in the US-reactivation group, $F(1,54) = 0.62$, $p = 0.434$, $\eta p^2 = 0.01$.

3.3. Conditioned stimulus valence ratings

Analysis of CS valence ratings was based on data from 28 participants in the US-reactivation group and 27 participants in the control group, as ratings from one participant were lost due to a recording error. Participants in both groups rated fear-irrelevant CSs as more pleasant than fear-relevant CSs and reinforced CSs as less pleasant than non-reinforced CSs (Fig. 2). Statistical analyses revealed main effects of fear-relevance, $F(1,53) = 132.31$, $p < 0.001$, $\eta_p^2 = 0.71$, conditioning, $F(1,53) = 34.57$, $p < 0.001$, $\eta_p^2 = 0.40$, and time, $F(3,51) = 11.10$, $p < 0.001$, $\eta_p^2 = 0.40$. These main effects were qualified by interactions of fear-relevance x time, $F(3,51) = 4.87$, $p = 0.005$, $\eta_p^2 = 0.22$, conditioning x time, $F(3,51) = 16.70$, $p < 0.001$, $\eta_p^2 = 0.50$, and fear-relevance x conditioning x time, $F(3,51) = 6.38$, $p = 0.001$, $\eta_p^2 = 0.27$. Follow-up analyses assessing whether the three-way interaction reflects differences in conditioning as a function of fear-relevance and time failed to find significant results. While differential evaluations for both types of CSs were smaller on Day 3 than after acquisition on Day 1, they were nevertheless significant across all conditions, except at baseline, all $F(1,53) \geq 8.21$, $p \leq 0.006$, $\eta_p^2 \geq 0.13$. The three-way interaction reflects differences in the time course of evaluation patterns across the four stimuli (fear-relevant and fear-irrelevant CS+/-). Whereas evaluations of non-reinforced fear-relevant and fear-irrelevant CSs became more positive across measurement points, both $F(3,51) \geq 3.70$, $p \leq 0.018$, $\eta_p^2 \geq 0.18$, evaluations of fear-relevant CSa + became more negative after acquisition, but recovered thereafter and exceeded baseline ratings, $F(3,51) = 10.25$, $p < 0.001$, $\eta_p^2 = 0.38$. Evaluations of the fear-irrelevant CSb + became more negative after acquisition and increased thereafter, but did not recover to baseline levels, $F(3,51) = 17.25$, $p < 0.001$, $\eta_p^2 = 0.50$.

Statistical analyses also yielded a significant fear-relevance x group interaction, $F(1,53) = 5.04$, $p = 0.029$, $\eta_p^2 = 0.09$, however, follow-up comparisons did not attain significance, both $F(1,53) \leq 3.48$, $p \geq 0.068$, $\eta_p^2 \leq 0.06$. None of the remaining interactions were significant, largest effect (fear-relevance x conditioning), $F(1,53) = 2.62$, $p = 0.112$, $\eta_p^2 = 0.05$.

3.4. Summary of results

Analysis of electrodermal data revealed that differential responding to both types of CSs was acquired on Day 1 and extinguished on Day 2 in the US-reactivation and control group. Group differences emerged on Day 3, showing spontaneous recovery and reinstatement of previously extinguished differential responding in the control group, but not in the US-reactivation group. In contrast to the electrodermal responses, subjective evaluations of fear-relevant and fear-irrelevant CSs did not differ across groups. Following acquisition, reinforced stimuli were rated as less pleasant than non-reinforced stimuli. While these differential evaluations were also evident during tests of fear recovery, they were significantly smaller than ratings obtained after acquisition.

It should be also noted that we did not observe differences in fear acquisition or extinction between fear-relevant and fear-irrelevant CSs. However, this may reflect on the use of a 100% reinforcement schedule in a differential fear conditioning paradigm, which typically results in rapid acquisition of differential responding and may, therefore, mask differences in fear learning between stimuli (Ho & Lipp, 2014; Lissek, Pine, & Grillon, 2006). As the present investigation examined post-reconsolidation recovery of fear, we selected a strong conditioning protocol, to facilitate acquisition of differential responding, which is a prerequisite for

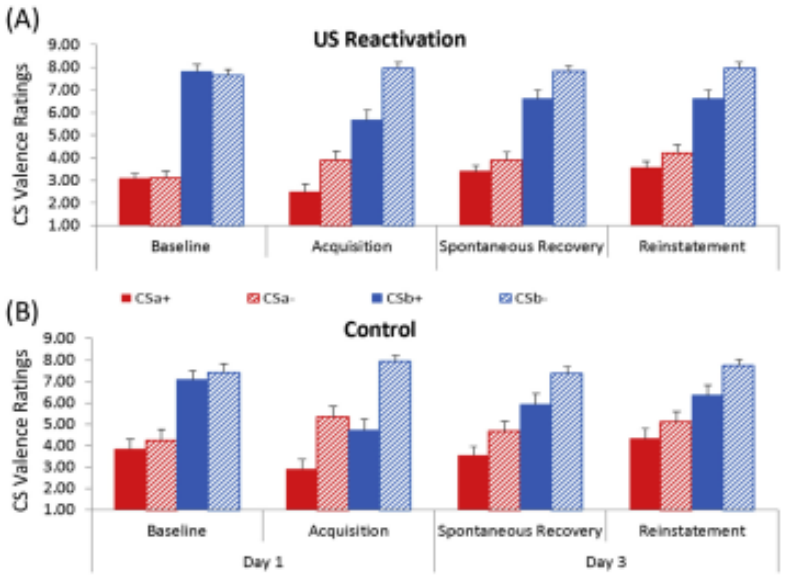

Fig. 2. Mean stimulus valence ratings for fear-relevant (CSa+/-) and fear-irrelevant (CSb+/-) conditioned stimuli at baseline and after acquisition, spontaneous recovery, and reinstatement testing, in the US-reactivation (A) and control (B) group. Error bars represent standard errors.

subsequent reactivation and destabilization of conditioned fear memories.

4. Discussion

The aim of this investigation was to apply the US-reactivation procedure (Liu et al., 2014) to two distinct types of conditioned fears and to examine whether post-reconsolidation recovery of fear differs as a function of CS fear-relevance. Contrary to our predictions, we did not find significant differences between fear-relevant and fear-irrelevant CSs, as differential electrodermal responding to both CS pairs was absent during spontaneous recovery and reinstatement tests in the US-reactivation group. In contrast, fear recovery was observed in the control group, which received traditional, non-reminded extinction training. Thus, our findings indicate that extinction training, delivered 10 min after administration of a brief reminder trial involving the US, at half the physical intensity used during acquisition, can eliminate spontaneous recovery and reinstatement of differential responding.

These results are consistent with previous observations (Dębiec, Díaz-Mataix, Bush, Doyère, & LeDoux, 2010; Liu et al., 2014; Luo et al., 2015) that memory reactivation procedures involving the US are capable of destabilizing cue-dependent associations and, thereby, facilitate disruption of the reconsolidation process and reduce recovery of conditioned responding. The present results provide further support for the utility of the US-reactivation procedure, suggesting it may facilitate the simultaneous destabilization of multiple, distinct fear memories, conditioned to fear-irrelevant and fear-relevant CSs, thereby allowing subsequent modification of both fear memories through extinction training, delivered during a period of memory reconsolidation.

4.1. Persistent reduction of differential electrodermal responding

The current results stand in stark contrast to previous research (Fricchione et al., 2016; Golkar et al., 2012; Kindt & Soeter, 2013; Meir Drexler et al., 2014; Soeter & Kindt, 2011), which reported a return of conditioned electrodermal responding to fear-relevant CSs, subsequent to post-reactivation extinction training. Similar to our study, Kindt and Soeter (2013) observed no group differences in differential electrodermal responding and fear potentiated startle during acquisition and extinction of fear, conditioned to pictures of spiders. However, Kindt and Soeter failed to find group differences during spontaneous recovery, reinstatement and reacquisition, reporting that previously extinguished fear had recovered in participants who received a reminder trial prior to extinction training and in those who received traditional, non-reminded extinction training. These results contrast our findings which showed spontaneous recovery and reinstatement of differential electrodermal responding in the control group, but not in the US-reactivation group.

It should be noted that the current findings do not reflect on differences in data analysis, as we followed Kindt and Soeter's (2013) recommendations to avoid potential confounds from non-associative processes on conditioned responding that may be misinterpreted as effects of disrupted reconsolidation. Rather than analyzing difference scores (subtracting responses to the CS- from responses to the CS+) or omitting the first CS+ and CS- trial during tests of fear recovery, we report responses to CS+ and CS- and retained all data. Thus, our data analysis resembled the approaches employed in previous studies which did not find evidence that behavioral interventions can disrupt reconsolidation of fear, conditioned to fear-relevant CSs (Fricchione et al., 2016; Golkar et al., 2012; Kindt & Soeter, 2013). Hence, it appears that previous failed attempts to replicate the seminal work of Schiller et al. (2010) with fear-relevant stimuli may not reflect on differences in approaches to data analysis, but on the method used to reactivate fear memories.

The key difference between the current and past research was the use of the US-reactivation procedure, in lieu of the more commonly employed CS-reactivation (e.g. Fricchione et al., 2016; Kindt & Soeter, 2013). Memory reactivation procedures involving the US have received little attention, relative to CS-reactivation procedures. While the majority of investigations employing US-reactivation were conducted on animals (Alfei, Ferrer Monti, Molina, Bueno, & Urcelay, 2015; Dębiec et al., 2010; Díaz-Mataix, Martinez, Schafe, LeDoux, & Doyère, 2013; Luo et al., 2015), the outcomes of the present study and previous findings reported by Liu et al. (2014) indicate that US-reactivation also facilitates disruption of reconsolidation in humans and may persistently block recovery of fear.

However, the question remains as to how exactly a US-only reactivation trial generates prediction errors, as traditional models of associative learning (e.g. Pearce & Hall, 1980; Rescorla & Wagner, 1972) do not readily account for prediction errors generated by presentations of the US, in the absence of the CS. Indeed, advances in reconsolidation research have sparked renewed interest into the role of prediction errors in associative learning, suggesting that prediction errors are not only implicated in fear acquisition (Holland & Schiffino, 2016; Schultz & Dickinson, 2000), but also in the modification of previously consolidated fear memories during the reconsolidation period (Exton-McGuinness et al., 2015; Fernández et al., 2016; Sevenster et al., 2012; Sevenster, Beckers, & Kindt, 2013, 2014). A large body of evidence points to the role of the amygdala in the processing of CS and US properties (Bentz & Schiller, 2015; LeDoux, 2000), including prediction errors related to the unexpected presentation or omission of the CS/US, as well as prediction errors pertaining to changes in US value (Belova, Paton, Morrison, & Salzman, 2007; Bentz & Schiller, 2015; Díaz-Mataix, Tallot, & Doyère, 2014; Dębiec et al., 2010; McNally, Johansen, & Blair, 2011).

Consistent with this argument, Díaz-Mataix et al. (2013) reported that the amygdala encodes the timing of US onset and detects deviations from the CS-US interval used during acquisition. In an auditory fear conditioning experiment, rats were presented with a foot shock US 30 s after CS onset. During memory reactivation, the US was either presented at the same time or 20 s earlier. Subsequent administration of the protein synthesis inhibitor anisomycin into the lateral amygdala was found to impair reconsolidation of the conditioned fear memory only when the CS-US interval had been altered during reactivation. These results indicate that prediction errors required for destabilization of fear memories are not necessarily the consequence of a mere absence or presence of the US, but may reflect on violations of other aspects of the learning history. This proposition is further supported by recent reports that memory reactivation can be achieved through presentation of cues which had not been paired with the US, but which are categorically related to the CSs (Soeter & Kindt, 2015b).

In addition to encoding timing of US onset (Díaz-Mataix et al., 2013, 2014; Harnett et al., 2016), the amygdala also appears to process specific sensory features of an aversive US and, thereby, may be involved in the reactivation of fear memories which are associated with a discrete US. Consistent with the results of the present study, Dębiec et al. (2010) reported that a memory reactivation procedure involving an unsignalled presentation of the US destabilized multiple fear memories that were associated with the reactivating US. Furthermore, Dębiec and colleagues found that reconsolidation was selective to the reactivated US, whereby conditioned responding to CSs which had not been paired with the reactivating US was not diminished through pharmacological

manipulations of reconsolidation. These findings were replicated by Liu et al. (2014) in a series of fear conditioning experiments conducted with animals and humans. In contrast to Dębiec et al., Liu and colleagues halved the physical intensity of the US during reactivation and employed extinction training to disrupt reconsolidation. Liu et al.'s results were consistent with our findings, showing that US-reactivation destabilized multiple fear memories, which were associated with the reactivating US. Extending Liu et al.'s findings, the present results further suggest that US-reactivation is capable of destabilizing multiple, distinct fear associations, conditioned to fear-irrelevant and fear-relevant CSs.

It remains to be investigated whether a decrease in US intensity is necessary for memory reactivation. Past research indicates that a discrepancy between actual and expected US intensity during reactivation is required to facilitate behavioral manipulations of reconsolidation (Liu et al., 2014). A reduction in US intensity during reactivation may create a stronger prediction error, which appears to be necessary for behavioral interventions, as indicated by a comparison of pharmacological and behavioral manipulations of reconsolidation, following CS-reactivation (Soeter & Kindt, 2011).

Of interest for future investigations is also the role of verbal instructions in memory reactivation. In the present study, participants were instructed to remember what they had learned during acquisition before receiving the reminder trial (adapted from Kindt & Soeter, 2013; Sevenster et al., 2012; Soeter & Kindt, 2010). It could be argued that these instructions created a prediction error; however, this appears unlikely, as the control group received identical instructions prior to extinction training, but displayed spontaneous recovery and reinstatement of conditioned responding on Day 3. Previous research further indicates that instructions prior to memory reactivation trials are not sufficient to create the prediction error necessary for reactivation of fear memories, conditioned to fear-relevant CSs (Kindt & Soeter, 2013). Given the limited scope of literature pertaining to US-reactivation procedures, more research is required to determine the necessary and sufficient conditions for US-mediated memory reactivation and destabilization. It also remains to be investigated whether interventions based on the present reactivation-extinction protocol could be employed in the treatment of anxiety and related disorders.

Reconsolidation-based interventions have been used successfully to reduce symptoms of post-traumatic stress disorder (Brunet et al., 2008, 2011; Kindt & van Emmerik, 2016; Kredlow & Otto, 2015), but see Wood et al. (2015), phobias (Björkstrand et al., 2016; Soeter & Kindt, 2015a), drug cravings in substance-dependent individuals (Lonergan et al., 2016; Xue et al., 2012), and cocaine seeking in animals (Luo et al., 2015). Yet, an obvious challenge for clinical applications of the US-reactivation procedure is the identification of the US that contributed to the development of cue-dependent associations, as well as the adaptation of the US to resemble the reactivation procedure used in the present study. With regards to the modification of US intensity, it remains to be investigated whether this is a prerequisite for memory reactivation, as prediction errors can be generated through other avenues, such as modification of contingencies (US presented without CS) or by changing the timing of US onset (Díaz-Mataix et al., 2013; Dębiec et al., 2010).

Identification of the US, on the other hand, is a key component of and a prerequisite for exposure-based therapies for anxiety disorders (Craske, Treanor, Conway, Zbozinek, & Vervliet, 2014). It would be reasonable to expect that therapists and clients would encounter similar challenges pertaining to the identification of the US in the context of reconsolidation-based interventions, as they do in the design of exposure-based therapies. Yet, the advantage of reconsolidation-based interventions is the potential for long-lasting reduction of fear and prevention of fear relapse, as reconsolidation, in contrast to extinction learning, alters the original fear memory trace (Nader, 2015; Nader et al., 2000). Another potential advantage is the reactivation and destabilization of multiple memories that are associated with the reactivating US. In contrast to CS-reactivation, which results in destabilization of a single CS-US association (e.g. Schiller et al., 2010), a single US-reactivation session may destabilize multiple CS-US associations. All reactivated associations could be subsequently disrupted through extinction training or through pharmacological interventions, such as propranolol (for a detailed discussion of reconsolidation-based interventions, see Dunbar & Taylor, 2016). Overall, extant literature suggests that reconsolidation-based interventions, including US-reactivation procedures (e.g. Liu et al., 2014; Luo et al., 2015), may be beneficial in the treatment of anxiety, stress, and substance dependence disorders. Areas requiring further investigation are applications of US-reactivation in clinical samples and the optimization of methods that disrupt reconsolidation following US-reactivation. The latter should address comparisons of imaginal and in vivo reactivation-extinction (Agren, Björkstrand, & Fredrikson, 2017) and of extinction training and pharmacological interventions (e.g. propranolol; Soeter & Kindt, 2011).

4.2. Subjective evaluations of CS valence

In contrast to electrodermal responding, subjective evaluations of CS valence did not differ across groups. These results are in accordance with fear conditioning research which suggests that response systems which are governed largely by conscious, cognitive processes, such as verbal indices of US expectancy, and physiological indices of conditioned responding are differentially sensitive to extinction training (Gawronski, Gast, & De Houwer, 2015; Lipp & Edwards, 2002; Lipp, Oughton, & LeLievre, 2003) as well as manipulations of reconsolidation (Kindt & Soeter, 2013; Soeter & Kindt, 2011), but see Das, Lawn, and Kamboj (2015) and Pine, Mendelsohn, and Dudai (2014). On the other hand, our results showed that differential evaluations were significantly smaller on Day 3 than after acquisition on Day 1 - a pattern that is characteristic of extinction learning. The absence of group differences could indicate that reminded as well as non-reminded extinction training facilitated reduction of conditioned negative valence. However, this proposition requires further investigation as we cannot rule out the influence of demand characteristics on post-test ratings.

The double dissociation between implicit and explicit response systems has been reported previously (e.g. Lipp et al., 2003; Schultz et al., 2013), yet does not necessarily indicate that acquired negative valence is not sensitive to manipulations of reconsolidation. Das et al. (2015) investigated counterconditioning during the reconsolidation period as a means of decreasing the liking of alcohol-related cues. Hazardous drinkers who received a reactivation trial triggering a prediction error prior to counterconditioning showed decreased liking of the CSs as well as a generalization of the conditioned valence to novel alcohol cues. These findings indicate that subjective ratings of CS valence are sensitive to behavioral manipulations of reconsolidation, but may require administration of post-reactivation training protocols which specifically target CS valence, such as counterconditioning, in lieu of non-reinforced CS presentations. Altering conditioned valence has potential clinical applications for the treatment of alcohol and drug addictions as well as anxiety disorders, as residual negative valence has been associated with reinstatement of fear (Zbozinek, Hermans, Prenoveau, Liao, & Craske, 2015). Further research is necessary to determine which manipulations of reconsolidation are most effective in the persistent reduction of conditioned negative valence.

In conclusion, the results of this study demonstrate that behavioral manipulations of reconsolidation are sufficient for the persistent elimination of fear conditioned to fear-irrelevant and fear-relevant CSs. Our findings also indicate that the memory reactivation procedure employed in this study is capable of destabilizing multiple, distinct fear memories, associated with the reactivating US. It is conceivable that a modified version of the US reactivation-extinction procedure could be employed in clinical settings, as fears and phobias are typically associated with fear-relevant stimuli (Mineka & Öhman, 2002). However, more research is necessary to determine how the US-reactivation extinction procedure employed here could be adapted to the treatment of anxiety and related disorders.

5. Competing financial interests

The authors declare no competing financial interests.

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References

Agren, T. (2014). Human reconsolidation: A reactivation and update. *Brain Research Bulletin, 105*, 70–82. http://dx.doi.org/10.1016/j.brainresbull.2013.12.010.

Agren, T., Björkstrand, J., & Fredrikson, M. (2017). Disruption of human fear reconsolidation using imaginal and in vivo extinction. *Behavioural Brain Research, 319*, 9–15. http://dx.doi.org/10.1016/j.bbr.2016.11.014.

Agren, T., Engman, J., Frick, A., Björkstrand, J., Larsson, E.-M., Furmark, T., et al. (2012). Disruption of reconsolidation erases a fear memory trace in the human amygdala. *Science, 337*, 1550–1552. http://dx.doi.org/10.1126/science.1223006.

Alberini, C. M. (2011). The role of reconsolidation and the dynamic process of long-term memory formation and storage. *Frontiers in Behavioral Neuroscience, 5*(10), 1–10. http://dx.doi.org/10.3389/fnbeh.2011.00012.

Alfei, J. M., Monti, F., R. I, Molina, V. A., Bueno, A. M., & Urcelay, G. P. (2015). Prediction error and trace dominance determine the fate of fear memories after post-training manipulations. *Learning & Memory, 22*, 385–400. http://dx.doi.org/10.1101/lm.038513.115.

Auber, A., Tedesco, V., Jones, C. E., Monfils, M.-H., & Chiamulera, C. (2013). Post-retrieval extinction as reconsolidation interference: Methodological issues or boundary conditions? *Psychopharmacology, 226*, 631–647. http://dx.doi.org/10.1007/s00213-013-3004-1.

Belova, M. A., Paton, J. J., Morrison, S. E., & Salzman, C. D. (2007). Expectation modulates neural responses to pleasant and aversive stimuli in primate amygdala. *Neuron, 55*, 970–984. http://dx.doi.org/10.1016/j.neuron.2007.08.004.

Bentz, D., & Schiller, D. (2015). Threat processing: Models and mechanisms. *Wiley Interdisciplinary Reviews-Cognitive Science, 6*, 427–439. http://dx.doi.org/10.1002/wcs.1353.

Björkstrand, J., Agren, T., Åhs, F., Frick, A., Larsson, E.-M., Hjorth, O., … Fredrikson, M. (2016). Disrupting reconsolidation attenuates long-term fear memory in the human amygdala and facilitates approach behavior. *Current Biology, 26*, 2690–2695. http://dx.doi.org/10.1016/j.cub.2016.08.022.

Björkstrand, J., Agren, T., Frick, A., Engman, J., Larsson, E.-M., Furmark, T., et al. (2015). Disruption of memory reconsolidation erases a fear memory trace in the human amygdala: An 18-month follow-up. *PLoS One, 10*, e0129393. http://dx.doi.org/10.1371/journal.pone.0129393.

Boucsein, W. (2012). *Electrodermal activity* (2nd ed.). New York: Springer.

Bouton, M. E. (2002). Context, ambiguity, and unlearning: Sources of relapse after behavioral extinction. *Biological Psychiatry, 52*, 976–986. http://dx.doi.org/10.1016/S0006-3223(02)01546-9.

Brunet, A., Orr, S. P., Tremblay, J., Robertson, K., Nader, K., & Pitman, R. K. (2008). Effect of post-retrieval propranolol on psychophysiologic responding during subsequent script-driven traumatic imagery in post-traumatic stress disorder. *Journal of Psychiatric Research, 42*, 503–506. http://dx.doi.org/10.1016/j.jpsychires.2007.05.006.

Brunet, A., Poundja, J., Tremblay, J., Bui, É., Thomas, É., Orr, S. P., … Pitman, R. K. (2011). Trauma reactivation under the influence of propranolol decreases posttraumatic stress symptoms and disorder: 3 open-label trials. *Journal of Clinical Psychopharmacology, 31*, 547–550. http://dx.doi.org/10.1097/JCP.0b013e318222f360.

Craske, M. G., Treanor, M., Conway, C. C., Zbozinek, T., & Vervliet, B. (2014). Maximizing exposure therapy: An inhibitory learning approach. *Behaviour Research and Therapy, 58*, 10–23. http://dx.doi.org/10.1016/j.brat.2014.04.006.

Das, R. K., Lawn, W., & Kamboj, S. K. (2015). Rewriting the valuation and salience of alcohol-related stimuli via memory reconsolidation. *Translational Psychiatry, 5*, e645. http://dx.doi.org/10.1038/tp.2015.132.

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

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

Dębiec, J., Díaz-Mataix, L., Bush, D. E. A., Doyère, V., & LeDoux, J. E. (2010). The amygdala encodes specific sensory features of an aversive reinforcer. *Nature Neuroscience, 13*, 536–537. http://dx.doi.org/10.1038/nn.2520.

Díaz-Mataix, L., Martinez, R., R. C, Schafe, G. E., LeDoux, J. E., & Doyère, V. (2013). Detection of a temporal error triggers reconsolidation of amygdala-dependent memories. *Current Biology, 23*, 467–472. http://dx.doi.org/10.1016/j.cub.2013.01.053.

Díaz-Mataix, L., Tallot, L., & Doyère, V. (2014). The amygdala: A potential player in timing CS–US intervals. *Behavioural Processes, 101*, 112–122. http://dx.doi.org/10.1016/j.beproc.2013.08.007.

Dunbar, A. B., & Taylor, J. R. (2016). Reconsolidation and psychopathology: Moving towards reconsolidation-based treatments. *Neurobiology of Learning and Memory*. http://dx.doi.org/10.1016/j.nlm.2016.11.005.

Exton-McGuinness, M. T. J., Lee, J. L. C., & Reichelt, A. C. (2015). Updating memories: The role of prediction errors in memory reconsolidation. *Behavioural Brain Research, 278*, 375–384. http://dx.doi.org/10.1016/j.bbr.2014.10.011.

Fernández, R. S., Boccia, M. M., & Pedreira, M. E. (2016). The fate of memory: Reconsolidation and the case of prediction error. *Neuroscience & Biobehavioral Reviews, 68*, 423–441. http://dx.doi.org/10.1016/j.neubiorev.2016.06.004.

Finnie, P. S. B., & Nader, K. (2012). The role of metaplasticity mechanisms in regulating memory destabilization and reconsolidation. *Neuroscience & Biobehavioral Reviews, 36*, 1667–1707. http://dx.doi.org/10.1016/j.neubiorev.2012.03.008.

Forster, K., & Forster, J. C. (2003). DMDX: A windows display program with millisecond accuracy. *Behavior Research Methods, Instruments, & Computers, 35*, 116–124. http://dx.doi.org/10.3758/BF03195503.

Fricchione, J., Greenberg, M. S., Spring, J., Wood, N., Mueller-Pfeiffer, C., Milad, M. R., … Orr, S. P. (2016). Delayed extinction fails to reduce skin conductance reactivity to fear-conditioned stimuli. *Psychophysiology, 53*, 1343–1351. http://dx.doi.org/10.1111/psyp.12687.

Gawronski, B., Gast, A., & De Houwer, J. (2015). Is evaluative conditioning really resistant to extinction? Evidence for changes in evaluative judgements without changes in evaluative representations. *Cognition & Emotion, 29*, 816–830. http://dx.doi.org/10.1080/02699931.2014.947919.

Golkar, A., Bellander, M., Olsson, A., & Öhman, A. (2012). Are fear memories erasable? Reconsolidation of learned fear with fear relevant and fear-irrelevant stimuli. *Frontiers in Behavioral Neuroscience, 6*, 80. http://dx.doi.org/10.3389/fnbeh.2012.00080.

Harnett, N. G., Shumen, J. R., Wagle, P. A., Wood, K. H., Wheelock, M. D., Baños, J. H., et al. (2016). Neural mechanisms of human temporal fear conditioning. *Neurobiology of Learning and Memory, 136*, 97–104. http://dx.doi.org/10.1016/j.nlm.2016.09.019.

Ho, Y., & Lipp, O. V. (2014). Faster acquisition of conditioned fear to fear-relevant than to nonfear-relevant conditional stimuli. *Psychophysiology, 51*, 810–813. http://dx.doi.org/10.1111/psyp.12223.

Holland, P. C., & Schiffino, F. L. (2016). Mini-review: Prediction errors, attention and associative learning. *Neurobiology of Learning and Memory, 131*, 207–215. http://dx.doi.org/10.1016/j.nlm.2016.02.014.

Johnson, D. C., & Casey, B. J. (2015). Extinction during memory reconsolidation blocks recovery of fear in adolescents. *Scientific Reports, 5*, 8863. http://dx.doi.org/10.1038/srep08863.

Kindt, M., & Soeter, M. (2013). Reconsolidation in a human fear conditioning study: A test of extinction as updating mechanism. *Biological Psychology, 92*, 43–50. http://dx.doi.org/10.1016/j.biopsycho.2011.09.016.

Kindt, M., & van Emmerik, A. (2016). New avenues for treating emotional memory disorders: Towards a reconsolidation intervention for posttraumatic stress disorder. *Therapeutic Advances in Psychopharmacology*, 1–13. http://dx.doi.org/10.1177/2045125316644541 (2045125316644541).

Klucken, T., Kruse, O., Schweckendiek, J., Kuepper, Y., Mueller, E. M., Hennig, J., et al. (2016). No evidence for blocking the return of fear by disrupting reconsolidation prior to extinction learning. *Cortex, 79*, 112–122. http://dx.doi.org/10.1016/j.cortex.2016.03.015.

Kredlow, M. A., & Otto, M. W. (2015). Interference with the reconsolidation of trauma-related memories in adults. *Depression and Anxiety, 32*, 32–37. http://dx.doi.org/10.1002/da.22343.

Kredlow, M. A., Unger, L. D., & Otto, M. W. (2016). Harnessing reconsolidation to weaken fear and appetitive memories: A meta-analysis of post-retrieval extinction effects. *Psychological Bulletin, 142*, 314–336. http://dx.doi.org/10.1037/bul0000034.

LaBar, K. S., & Cabeza, R. (2006). Cognitive neuroscience of emotional memory. *Nature Reviews Neuroscience, 7*, 54–64. http://dx.doi.org/10.1038/nrn1825.

Lang, P. J. (1985). The cognitive psychophysiology of emotion: Fear and anxiety. In A. H. Tuma, & J. D. Maser (Eds.), *Anxiety and the anxiety disorders*. Hillsdale, NJ: Erlbaum.

LeDoux, J. E. (2000). Emotion circuits in the brain. *Annual Review of Neuroscience, 23*, 155–184. http://dx.doi.org/10.1146/annurev.neuro.23.1.155.

Lee, J. L. C. (2009). Reconsolidation: Maintaining memory relevance. *Trends in*

Neurosciences, 32, 413–420. http://dx.doi.org/10.1016/j.tins.2009.05.002.
Lipp, O. V. (2006a). Human fear learning: Contemporary procedures and measurement. In M. G. Craske, D. Hermans, & D. Vansteenwegen (Eds.), *Fear and learning: From basic processes to clinical implications* (pp. 37–52). Washington: American Psychological Association.
Lipp, O. V. (2006b). Of snakes and flowers: Does preferential detection of pictures of fear-relevant animals in visual search reflect on fear-relevance? *Emotion, 6*, 296–308. http://dx.doi.org/10.1037/1528-3542.6.2.296.
Lipp, O. V., & Edwards, M. S. (2002). Effect of instructed extinction on verbal and autonomic indices of Pavlovian learning with fear-relevant and fear-irrelevant conditional stimuli. *Journal of Psychophysiology, 16*, 176–186. http://dx.doi.org/10.1027//0269-8803.16.3.176.
Lipp, O. V., Oughton, N., & LeLievre, J. (2003). Evaluative learning in human Pavlovian conditioning: Extinct, but still there? *Learning and Motivation, 34*, 219–239. http://dx.doi.org/10.1016/S0023-9690(03)00011-0.
Lissek, S., Pine, D. S., & Grillon, C. (2006). The strong situation: A potential impediment to studying the psychobiology and pharmacology of anxiety disorders. *Biological Psychology, 72*, 265–270. http://dx.doi.org/10.1016/j.biopsycho.2005.11.004.
Liu, J. F., Zhao, L. Y., Xue, Y. X., Shi, J., Suo, L., Luo, Y. X., … Lu, L. (2014). An unconditioned stimulus retrieval extinction procedure to prevent the return of fear memory. *Biological Psychiatry, 76*, 895–901. http://dx.doi.org/10.1016/j.biopsych.2014.03.027.
Lonergan, M., Saumier, D., Tremblay, J., Kieffer, B., Brown, T. G., & Brunet, A. (2016). Reactivating addiction-related memories under propranolol to reduce craving: A pilot randomized controlled trial. *Journal of Behavior Therapy and Experimental Psychiatry, 50*, 245–249. http://dx.doi.org/10.1016/j.jbtep.2015.09.012.
Luo, Y. X., Xue, Y. X., Liu, J. F., Shi, H. S., Jian, M., Han, Y., … Lu, L. (2015). A novel UCS memory retrieval-extinction procedure to inhibit relapse to drug seeking. *Nature Communications, 6*, 7675. http://dx.doi.org/10.1038/ncomms8675.
Lykken, D. T. (1972). Range correction applied to heart rate and to GSR data. *Psychophysiology, 9*, 373–379. http://dx.doi.org/10.1111/j.1469-8986.1972.tb03222.x.
McNally, G. P., Johansen, J. P., & Blair, H. T. (2011). Placing prediction into the fear circuit. *Trends in Neurosciences, 34*, 283–292. http://dx.doi.org/10.1016/j.tins.2011.03.005.
Meir Drexler, S., Merz, C. J., Hamacher-Dang, T. C., Marquardt, V., Fritsch, Otto, T., et al. (2014). Effects of postretrieval-extinction learning on return of contextually controlled cued fear. *Behavioral Neuroscience, 128*, 474–481. http://dx.doi.org/10.1037/a0036688.
Mineka, S., & Öhman, A. (2002). Phobias and preparedness: The selective, automatic, and encapsulated nature of fear. *Biological Psychiatry, 52*, 927–937. http://dx.doi.org/10.1016/S0006-3223(02)01669-4.
Nader, K. (2013). The discovery of memory reconsolidation. In C. M. Alberini (Ed.), *Memory reconsolidation* (pp. 1–13). San Diego: Academic Press.
Nader, K. (2015). Reconsolidation and the dynamic nature of memory. *Cold Spring Harbor Perspectives in Biology, 7*, a021782. http://dx.doi.org/10.1101/cshperspect.a021782.
Nader, K., Schafe, G. E., & LeDoux, J. E. (2000). Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature, 406*, 722–726. http://dx.doi.org/10.1038/35021052.
Olsson, A., Ebert, J. P., Banaji, M. R., & Phelps, E. A. (2005). The role of social groups in the persistence of learned fear. *Science, 309*, 785–787. http://dx.doi.org/10.1126/science.1113551.
Oyarzún, J. P., Lopez-Barroso, D., Fuentemilla, L., Cucurell, D., Pedraza, C., Rodriguez-Fornells, A., et al. (2012). Updating fearful memories with extinction training during reconsolidation: A human study using auditory aversive stimuli. *PLoS One, 7*, e38849. http://dx.doi.org/10.1371/journal.pone.0038849.
Pearce, J. M., & Hall, G. (1980). A model for Pavlovian learning: Variations in the effectiveness of conditioned but not of unconditioned stimuli. *Psychological Review, 87*, 532–552. http://dx.doi.org/10.1037/0033-295X.87.6.532.
Pineles, S. L., Orr, M. R., & Orr, S. P. (2009). An alternative scoring method for skin conductance responding in a differential fear conditioning paradigm with a long-duration conditioned stimulus. *Psychophysiology, 46*, 984–995. http://dx.doi.org/10.1111/j.1469-8986.2009.00852.x.
Pine, A., Mendelsohn, A., & Dudai, Y. (2014). Unconscious learning of likes and dislikes is persistent, resilient, and reconsolidates. *Frontiers in Psychology, 5*, 1051. http://dx.doi.org/10.3389/fpsyg.2014.01051.
Pineyro, M. E., Monti, R. I. F., Alfei, J. M., Bueno, A. M., & Urcelay, G. P. (2014). Memory destabilization is critical for the success of the reactivation-extinction procedure. *Learning & Memory, 21*, 46–54. http://dx.doi.org/10.1101/lm.032714.113.
Rescorla, R. A., & Wagner, A. W. (1972). A theory of Pavlovian conditioning: Variations in the effectiveness of reinforcement and nonreinforcement. In A. H. Black, & W. F. Prokasy (Eds.), *Classical conditioning II: Current research and theory* (pp. 64–99). New York: Appleton-Century-Crofts.
Schiller, D. (2014). A lighter shade of trauma. *Biological Psychiatry, 76*, 838–839. http://dx.doi.org/10.1016/j.biopsych.2014.09.016.
Schiller, D., Kanen, J. W., LeDoux, J. E., Monfils, M.-H., & Phelps, E. A. (2013). Extinction during reconsolidation of threat memory diminishes prefrontal cortex involvement. *Proceedings of the National Academy of Sciences, 110*, 20040–20045. http://dx.doi.org/10.1073/pnas.1320322110.
Schiller, D., Monfils, M.-H., Raio, C. M., Johnson, D. C., LeDoux, J. E., & Phelps, E. A. (2010). Preventing the return of fear in humans using reconsolidation update mechanisms. *Nature, 463*, 49–53. http://dx.doi.org/10.1038/nature08637.
Schultz, D. H., Balderston, N. L., Geiger, J. A., & Helmstetter, F. J. (2013). Dissociation between implicit and explicit responses in postconditioning UCS revaluation after fear conditioning in humans. *Behavioral Neuroscience, 127*, 357–368. http://dx.doi.org/10.1037/a0032742.
Schultz, W., & Dickinson, A. (2000). Neuronal coding of prediction errors. *Annual Review of Neuroscience, 23*, 473–500. http://dx.doi.org/10.1146/annurev.neuro.23.1.473.
Sevenster, D., Beckers, T., & Kindt, M. (2012). Retrieval per se is not sufficient to trigger reconsolidation of human fear memory. *Neurobiology of Learning and Memory, 97*, 338–345. http://dx.doi.org/10.1016/j.nlm.2012.01.009.
Sevenster, D., Beckers, T., & Kindt, M. (2013). Prediction error governs pharmacologically induced amnesia for learned fear. *Science, 339*, 830–833. http://dx.doi.org/10.1126/science.1231357.
Sevenster, D., Beckers, T., & Kindt, M. (2014). Prediction error demarcates the transition from retrieval, to reconsolidation, to new learning. *Learning & Memory, 21*, 580–584. http://dx.doi.org/10.1101/lm.035493.114.
Soeter, M., & Kindt, M. (2010). Dissociating response systems: Erasing fear from memory. *Neurobiology of Learning and Memory, 94*, 30–41. http://dx.doi.org/10.1016/j.nlm.2010.03.004.
Soeter, M., & Kindt, M. (2011). Disrupting reconsolidation: Pharmacological and behavioral manipulations. *Learning & Memory, 18*, 357–366. http://dx.doi.org/10.1101/lm.2148511.
Soeter, M., & Kindt, M. (2015a). An abrupt transformation of phobic behavior after a post-retrieval amnesic agent. *Biological Psychiatry, 78*, 880–886. http://dx.doi.org/10.1016/j.biopsych.2015.04.006.
Soeter, M., & Kindt, M. (2015b). Retrieval cues that trigger reconsolidation of associative fear memory are not necessarily an exact replica of the original learning experience. *Frontiers in Behavioral Neuroscience, 9*(122), 1–10. http://dx.doi.org/10.3389/fnbeh.2015.00122.
Steinfurth, E. C. K., Kanen, J. W., Raio, C. M., Clem, R. L., Huganir, R. L., & Phelps, E. A. (2014). Young and old Pavlovian fear memories can be modified with extinction training during reconsolidation in humans. *Learning & Memory, 21*, 338–341. http://dx.doi.org/10.1101/lm.033589.113.
Wood, N. E., Rosasco, M. L., Suris, A. M., Spring, J. D., Marin, M.-F., Lasko, N. B., … Pitman, R. K. (2015). Pharmacological blockade of memory reconsolidation in posttraumatic stress disorder: Three negative psychophysiological studies. *Psychiatry Research, 225*, 31–39. http://dx.doi.org/10.1016/j.psychres.2014.09.005.
Xue, Y.-X., Luo, Y.-X., Wu, P., Shi, H.-S., Xue, L.-F., Chen, C., … Lu, L. (2012). A memory retrieval-extinction procedure to prevent drug craving and relapse. *Science, 336*, 241. http://dx.doi.org/10.1126/science.1215070.
Zbozinek, T. D., Hermans, D., Prenoveau, J. M., Liao, B., & Craske, M. G. (2015). Post-extinction conditional stimulus valence predicts reinstatement fear: Relevance for long-term outcomes of exposure therapy. *Cognition & Emotion, 29*, 654–667. http://dx.doi.org/10.1080/02699931.2014.930421.

Appendix C: Confirmation of Author Contribution

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To whom it may concern,

I, Alina Thompson, was the major contributor to the conceptualisation and implementation of the following papers:

Thompson, A., & Lipp, O. V. (2017). Extinction during reconsolidation eliminates recovery of fear conditioned to fear-irrelevant and fear-relevant stimuli. *Behaviour Research and Therapy, 92*, 1-10. doi:10.1016/j.brat.2017.01.017

Thompson, A., McEvoy, P. M., & Lipp, O. V. (2018). Enhancing extinction learning: Occasional presentations of the unconditioned stimulus during extinction eliminate spontaneous recovery, but not necessarily reacquisition of fear. *Behaviour Research and Therapy, 108*, 29-39. doi:10.1016/j.brat.2018.07.001

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

Alina Thompson

I, as Co-Author, endorse that this level of contribution by the candidate indicated above is appropriate.

Peter McEvoy

Ottmar Lipp

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Publication: Behaviour Research and Therapy

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References

Abend, R., Pine, D. S., Fox, N. A., & Bar-Haim, Y. (2014). Learning and memory consolidation processes of attention-bias modification in anxious and nonanxious individuals. *Clinical Psychological Science, 2*, 620-627. doi:10.1177/2167702614526571

Abramowitz, J. S., & Arch, J. J. (2014). Strategies for improving long-term outcomes in cognitive behavioral therapy for obsessive-compulsive disorder: Insights from learning theory. *Cognitive and Behavioral Practice, 21*, 20-31. doi:10.1016/j.cbpra.2013.06.004

Agren, T. (2014). Human reconsolidation: A reactivation and update. *Brain Research Bulletin, 105*, 70-82. doi:10.1016/j.brainresbull.2013.12.010

Agren, T., Björkstrand, J., & Fredrikson, M. (2017). Disruption of human fear reconsolidation using imaginal and in vivo extinction. *Behavioural Brain Research, 319*, 9-15. doi:10.1016/j.bbr.2016.11.014

Agren, T., Engman, J., Frick, A., Björkstrand, J., Larsson, E.-M., Furmark, T., & Fredrikson, M. (2012). Disruption of reconsolidation erases a fear memory trace in the human amygdala. *Science, 337*, 1550-1552. doi:10.1126/science.1223006

Agustina López, M., Jimena Santos, M., Cortasa, S., Fernández, R. S., Carbó Tano, M., & Pedreira, M. E. (2016). Different dimensions of the prediction error as a decisive factor for the triggering of the reconsolidation process. *Neurobiology of Learning and Memory, 136*, 210-219. doi:10.1016/j.nlm.2016.10.016

Åhs, F., Rosén, J., Kastrati, G., Fredrikson, M., Agren, T., & Lundström, J. N. (2018). Biological preparedness and resistance to extinction of skin conductance responses conditioned to fear relevant animal pictures: A systematic review. *Neuroscience & Biobehavioral Reviews, 95*, 430-437. doi:10.1016/j.neubiorev.2018.10.017

Alberini, C. M. (2011). The role of reconsolidation and the dynamic process of long-term memory formation and storage. *Frontiers in Behavioral Neuroscience, 5*(10), 1-10. doi:10.3389/fnbeh.2011.00012

Alfei, J. M., Ferrer Monti, R. I., Molina, V. A., Bueno, A. M., & Urcelay, G. P. (2015). Prediction error and trace dominance determine the fate of fear memories after post-training manipulations. *Learning & Memory, 22*, 385-400. doi:10.1101/lm.038513.115

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Washington, DC: Author.

Antony, M. M., Bieling, P. J., Cox, B. J., Enns, M. W., & Swinson, R. P. (1998). Psychometric properties of the 42-item and 21-item versions of the Depression Anxiety Stress Scales in clinical groups and a community sample. *Psychological Assessment, 10*, 176-181. doi:10.1037/1040-3590.10.2.176

Arnaudova, I., & Hagenaars, M. A. (2017). Lights … action: Comparison of trauma films for use in the trauma film paradigm. *Behaviour Research and Therapy, 93*, 67-77. doi:10.1016/j.brat.2017.02.007

Auber, A., Tedesco, V., Jones, C. E., Monfils, M.-H., & Chiamulera, C. (2013). Post-retrieval extinction as reconsolidation interference: Methodological issues or boundary conditions? *Psychopharmacology, 226*, 631-647. doi:10.1007/s00213-013-3004-1

Australian Bureau of Statistics. (2008). *National survey of mental health and wellbeing: Summary of results, 2007* (No. 4326.0). Retrieved from https://www.ausstats.abs.gov.au

Baeyens, F., Eelen, P., Crombez, G., & van den Bergh, O. (1992). Human evaluative conditioning: Acquisition trials, presentation schedule, evaluative style and contingency awareness. *Behaviour Research and Therapy, 30*, 133-142. doi:10.1016/0005-7967(92)90136-5

Baeyens, F., Eelen, P., Van den Bergh, O., & Crombez, G. (1992). The content of learning in human evaluative conditioning: Acquired valence is sensitive to US-revaluation. *Learning and Motivation, 23*, 200-224. doi:10.1016/0023-9690(92)90018-H

Baxter, A. J., Scott, K. M., Vos, T., & Whiteford, H. A. (2013). Global prevalence of anxiety disorders: A systematic review and meta-regression. *Psychological Medicine, 43*, 897-910. doi:10.1017/S003329171200147X

Beckers, T., & Kindt, M. (2017). Memory reconsolidation interference as an emerging treatment for emotional disorders: Strengths, limitations, challenges, and opportunities. *Annual Review of Clinical Psychology, 13*, 99-121. doi:10.1146/annurev-clinpsy-032816-045209

Bekinschtein, P., Cammarota, M., Katche, C., Slipczuk, L., Rossato, J. I., Goldin, A., . . . Medina, J. H. (2008). BDNF is essential to promote persistence of long-term memory storage. *Proceedings of the National Academy of Sciences, 105*, 2711. doi:10.1073/pnas.0711863105

Belova, M. A., Paton, J. J., Morrison, S. E., & Salzman, C. D. (2007). Expectation modulates neural responses to pleasant and aversive atimuli in primate amygdala. *Neuron, 55*, 970-984. doi:10.1016/j.neuron.2007.08.004

Bentz, D., & Schiller, D. (2015). Threat processing: Models and mechanisms. *Wiley Interdisciplinary Reviews-Cognitive Science, 6*, 427-439. doi:10.1002/wcs.1353

Besnard, A., Caboche, J., & Laroche, S. (2012). Reconsolidation of memory: A decade of debate. *Progress in Neurobiology, 99*, 61-80. doi:10.1016/j.pneurobio.2012.07.002

Björkstrand, J., Agren, T., Åhs, F., Frick, A., Larsson, E.-M., Hjorth, O., . . . Fredrikson, M. (2016). Disrupting reconsolidation attenuates long-term fear memory in the human amygdala and facilitates approach behavior. *Current Biology, 26*, 2690–2695. doi:10.1016/j.cub.2016.08.022

Björkstrand, J., Agren, T., Åhs, F., Frick, A., Larsson, E.-M., Hjorth, O., . . . Fredrikson, M. (2017). Think twice, it's all right: Long lasting effects of disrupted reconsolidation on brain and behavior in human long-term fear. *Behavioural Brain Research, 324*, 125-129. doi:10.1016/j.bbr.2017.02.016

Björkstrand, J., Agren, T., Frick, A., Engman, J., Larsson, E.-M., Furmark, T., & Fredrikson, M. (2015). Disruption of memory reconsolidation erases a fear memory trace in the human amygdala: An 18-month follow-up. *PLoS One, 10*, e0129393. doi:10.1371/journal.pone.0129393

Bos, M. G. N., Beckers, T., & Kindt, M. (2014). Noradrenergic blockade of memory reconsolidation: A failure to reduce conditioned fear responding. *Frontiers in Behavioral Neuroscience, 8*, 412-419. doi:10.3389/fnbeh.2014.00412

Boucsein, W. (2012). *Electrodermal activity* (2nd ed.). New York: Springer.

Bouton, M. E. (1993). Context, time, and memory retrieval in the interference paradigms of Pavlovian learning. *Psychological Bulletin, 114*, 80-99. doi:10.1037/0033-2909.114.1.80

Bouton, M. E. (2000). A learning theory perspective on lapse, relapse, and the maintenance of behavior change. *Health Psychology, 19(1S)*, 57-63. doi:10.1037/0278-6133.19.Suppl1.57

Bouton, M. E. (2002). Context, ambiguity, and unlearning: Sources of relapse after behavioral extinction. *Biological Psychiatry, 52*, 976-986. doi:10.1016/S0006-3223(02)01546-9

Bouton, M. E. (2004). Context and behavioral processes in extinction. *Learning & Memory, 11*, 485-494. doi:10.1101/lm.78804

Bouton, M. E., & Bolles, R. C. (1979). Contextual control of the extinction of conditioned fear. *Learning and Motivation, 10*, 445-466. doi:10.1016/0023-9690(79)90057-2

Bouton, M. E., Woods, A. M., & Pineño, O. (2004). Occasional reinforced trials during extinction can slow the rate of rapid reacquisition. *Learning and Motivation, 35*, 371-390. doi:10.1016/j.lmot.2004.05.001

Brown, L. A., LeBeau, R. T., Chat, K. Y., & Craske, M. G. (2017). Associative learning versus fear habituation as predictors of long-term extinction retention. *Cognition and Emotion, 31*, 687-698. doi:10.1080/02699931.2016.1158695

Brunet, A., Orr, S. P., Tremblay, J., Robertson, K., Nader, K., & Pitman, R. K. (2008). Effect of post-retrieval propranolol on psychophysiologic responding during subsequent script-driven traumatic imagery in post-traumatic stress disorder. *Journal of Psychiatric Research, 42*, 503-506. doi:10.1016/j.jpsychires.2007.05.006

Brunet, A., Poundja, J., Tremblay, J., Bui, É., Thomas, É., Orr, S. P., . . . Pitman, R. K. (2011). Trauma reactivation under the influence of propranolol decreases posttraumatic stress symptoms and disorder: 3 open-label trials. *Journal of Clinical Psychopharmacology, 31*, 547-550. doi:10.1097/JCP.0b013e318222f360

Brunet, A., Saumier, D., Liu, A. H., Streiner, D. L., Tremblay, J., & Pitman, R. K. (2018). Reduction of PTSD symptoms with pre-reactivation propranolol therapy: A randomized controlled trial. *American Journal of Psychiatry, 175*, 427-433. doi:10.1176/appi.ajp.2017.17050481

Buhr, K., & Dugas, M. J. (2002). The Intolerance of Uncertainty Scale: Psychometric properties of the English version. *Behaviour Research and Therapy, 40,* 931-945. doi:10.1016/S0005-7967(01)00092-4

Burhans, L. B., & Schreurs, B. G. (2019). Inactivation of the interpositus nucleus during unpaired extinction does not prevent extinction of conditioned eyeblink responses or conditioning-specific reflex modification. *Behavioral Neuroscience, 133,* 398-413. doi:10.1037/bne0000309

Cahill, E. N., & Milton, A. L. (2019). Neurochemical and molecular mechanisms underlying the retrieval-extinction effect. *Psychopharmacology, 236,* 111-132. doi:10.1007/s00213-018-5121-3

Capaldi, E. J. (1966). Partial reinforcement: A hypothesis of sequential effects. *Psychological Review, 73,* 459-477. doi:10.1037/h0023684

Capaldi, E. J. (1994). The sequential view: From rapidly fading stimulus traces to the organization of memory and the abstract concept of number. *Psychonomic Bulletin & Review, 1,* 156-181. doi:10.3758/BF03200771

Carleton, R. N., Norton, M. A. P. J., & Asmundson, G. J. G. (2007). Fearing the unknown: A short version of the Intolerance of Uncertainty Scale. *Journal of Anxiety Disorders, 21,* 105-117. doi:10.1016/j.janxdis.2006.03.014

Carleton, R. N. (2012). The intolerance of uncertainty construct in the context of anxiety disorders: Theoretical and practical perspectives. *Expert Review of Neurotherapeutics, 12,* 937-947. doi:10.1586/ern.12.82

Carleton, R. N. (2016). Fear of the unknown: One fear to rule them all? *Journal of Anxiety Disorders, 41,* 5-21. doi:10.1016/j.janxdis.2016.03.011

Carpenter, J. K., Pinaire, M., & Hofmann, S. G. (2019). From extinction learning to anxiety treatment: Mind the gap. *Brain Sciences, 9*(164), 9-24. doi:10.3390/brainsci9070164

Chalkia, A., Weermeijer, J., Van Oudenhove, L., & Beckers, T. (2019). Acute but not permanent effects of propranolol on fear memory expression in humans. *Frontiers in Human Neuroscience, 13,* 51-64. doi:10.3389/fnhum.2019.00051

Chan, W. Y. M., Leung, H. T., Westbrook, R. F., & McNally, G. P. (2010). Effects of recent exposure to a conditioned stimulus on extinction of Pavlovian fear conditioning. *Learning & Memory, 17,* 512-521. doi:10.1101/lm.1912510

Cheung, J., Garber, B., & Bryant, R. A. (2015). The role of stress during memory reactivation on intrusive memories. *Neurobiology of Learning and Memory, 123,* 28-34. doi:10.1016/j.nlm.2015.04.004

Chin, B., Nelson, B. D., Jackson, F., & Hajcak, G. (2016). Intolerance of uncertainty and startle potentiation in relation to different threat reinforcement rates. *International Journal of Psychophysiology, 99,* 79-84. doi:10.1016/j.ijpsycho.2015.11.006

Clem, R. L., & Schiller, D. (2016). New learning and unlearning: Strangers or accomplices in threat memory attenuation? *Trends in Neurosciences, 39,* 340-351. doi:10.1016/j.tins.2016.03.003

Costanzi, M., Saraulli, D., Cannas, S., D'Alessandro, F., Florenzano, F., Rossi-Arnaud, C., & Cestari, V. (2014). Fear but not fright: Re-evaluating traumatic experience attenuates anxiety-like behaviors after fear conditioning. *Frontiers in Behavioral Neuroscience, 8,* 1-15. doi:10.3389/fnbeh.2014.00279

Craske, M. G. (2015). Optimizing exposure therapy for anxiety disorders: An inhibitory learning and inhibitory regulation approach. *Verhaltenstherapie, 25,* 134-143. doi:10.1159/000381574

Craske, M. G., Hermans, D., & Vervliet, B. (2018). State-of-the-art and future directions for extinction as a translational model for fear and anxiety. *Philosophical Transactions of the Royal Society B-Biological Sciences, 373,* 20170025. doi:10.1098/rstb.2017.0025

Craske, M. G., & Mystkowski, J. L. (2006). Exposure therapy and extinction: Clinical studies. In M. G. Craske, D. Hermans, & D. Vansteenwegen (Eds.), *Fear and learning: From basic processes to clinical implications* (pp. 37-52). Washington: American Psychological Association.

Craske, M. G., & Stein, M. B. (2016). Anxiety. *The Lancet, 388,* 3048-3059. doi:10.1016/S0140-6736(16)30381-6

Craske, M. G., Treanor, M., Conway, C. C., Zbozinek, T., & Vervliet, B. (2014). Maximizing exposure therapy: An inhibitory learning approach. *Behaviour Research and Therapy, 58*, 10-23. doi:10.1016/j.brat.2014.04.006

Crawford, J. R., Cayley, C., Lovibond, P. F., Wilson, P. H., & Hartley, C. (2011). Percentile norms and accompanying interval estimates from an Australian general adult population sample for self-report mood scales (BAI, BDI, CRSD, CES-D, DASS, DASS-21, STAI-X, STAI-Y, SRDS, and SRAS). *Australian Psychologist, 46*, 3-14. doi:10.1111/j.1742-9544.2010.00003.x

Crawford, J. R., & Henry, J. D. (2003). The Depression Anxiety Stress Scales (DASS): Normative data and latent structure in a large non-clinical sample. *British Journal of Clinical Psychology, 42*, 111-131. doi:10.1348/014466503321903544

Cristea, I. A., Naudet, F., Shanks, D. R., & Hardwicke, T. E. (2018). Post-retrieval Tetris should not be likened to a 'cognitive vaccine'. *Molecular Psychiatry, 23*, 1972-1973. doi:10.1038/mp.2017.222

Culver, N. C., Stevens, S., Fanselow, M. S., & Craske, M. G. (2018). Building physiological toughness: Some aversive events during extinction may attenuate return of fear. *Journal of Behavior Therapy and Experimental Psychiatry, 58*, 18-28. doi:10.1016/j.jbtep.2017.07.003

Das, R. K., Gale, G., Hennessy, V., & Kamboj, S. K. (2018). A prediction error-driven retrieval procedure for destabilizing and rewriting maladaptive reward memories in hazardous drinkers. *Journal of Visualized Experiments, 131*, e56097. doi:10.3791/56097

Das, R. K., Lawn, W., & Kamboj, S. K. (2015). Rewriting the valuation and salience of alcohol-related stimuli via memory reconsolidation. *Translational Psychiatry, 5*, e645. doi:10.1038/tp.2015.132

Davey, G. C. L. (1989). UCS revaluation and conditioning models of acquired fears. *Behaviour Research and Therapy, 27*, 521-528. doi:10.1016/0005-7967(89)90086-7

Davey, G. C. L. (1992). Classical conditioning and the acquisition of human fears and phobias: A review and synthesis of the literature. *Advances in Behaviour Research and Therapy, 14*, 29-66. doi:10.1016/0146-6402(92)90010-L

Davey, G. C. L., De Jong, P. J., & Tallis, F. (1993). UCS Inflation in the aetiology of a variety of anxiety disorders: Some case histories. *Behaviour Research and Therapy, 31*, 495-498. doi:10.1016/0005-7967(93)90130-M

Davey, G. C. L., & McKenna, I. (1983). The effects of postconditioning revaluation of CS1 and UCS following Pavlovian second-order electrodermal conditioning in humans. *The Quarterly Journal of Experimental Psychology Section B, 35:2*, 125-133. doi:10.1080/14640748308400899

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

De Houwer, J. (2007). A conceptual and theoretical analysis of evaluative conditioning. *Spanish Journal of Psychology, 10*, 230-241. doi:10.1017/s1138741600006491

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

de Jong, P. J., Merckelbach, H., Koertshuis, G., & Muris, P. (1994). UCS-inflation and acquired fear responses in human conditioning. *Advances in Behaviour Research and Therapy, 16*, 131-165. doi:10.1016/0146-6402(93)E0001-S

De Oliveira Alvares, L., Crestani, A. P., Cassini, L. F., Haubrich, J., Santana, F., & Quillfeldt, J. A. (2013). Reactivation enables memory updating, precision-keeping and strengthening: Exploring the possible biological roles of reconsolidation. *Neuroscience, 244*, 42-48. doi:10.1016/j.neuroscience.2013.04.005

Dębiec, J., Díaz-Mataix, L., Bush, D. E. A., Doyère, V., & LeDoux, J. E. (2010). The amygdala encodes specific sensory features of an aversive reinforcer. *Nature Neuroscience, 13*, 536-537. doi:10.1038/nn.2520

Den, M. L., Graham, B. M., Newall, C., & Richardson, R. (2015). Teens that fear screams: A comparison of fear conditioning, extinction, and reinstatement in adolescents and adults. *Developmental Psychobiology, 57*, 818-832. doi:10.1002/dev.21330

Díaz-Mataix, L., Ruiz Martinez, R. C., Schafe, G. E., LeDoux, J. E., & Doyère, V. (2013). Detection of a temporal error triggers reconsolidation of amygdala-dependent memories. *Current Biology, 23*, 467-472. doi:10.1016/j.cub.2013.01.053

Díaz-Mataix, L., Tallot, L., & Doyère, V. (2014). The amygdala: A potential player in timing CS–US intervals. *Behavioural Processes, 101*, 112-122. doi:10.1016/j.beproc.2013.08.007

Dibbets, P., Lemmens, A., & Voncken, M. (2018). Turning negative memories around: Contingency versus devaluation techniques. *Journal of Behavior Therapy and Experimental Psychiatry, 60*, 5-12. doi:10.1016/j.jbtep.2018.02.001

Dibbets, P., Poort, H., & Arntz, A. (2012). Adding imagery rescripting during extinction leads to less ABA renewal. *Journal of Behavior Therapy and Experimental Psychiatry, 43*, 614-624. doi:10.1016/j.jbtep.2011.08.006

Dudai, Y. (2004). The neurobiology of consolidations. Or, how stable is the engram? *Annual Review of Psychology, 55*, 51-86. doi:10.1146/annurev.psych.55.090902.142050

Dudai, Y. (2012). The restless engram: Consolidations never end. *Annual Review of Neuroscience, 35*, 227-247. doi:10.1146/annurev-neuro-062111-150500

Dudai, Y., Karni, A., & Born, J. (2015). The consolidation and transformation of memory. *Neuron, 88*, 20-32. doi:10.1016/j.neuron.2015.09.004

Dunbar, A. B., & Taylor, J. R. (2017). Reconsolidation and psychopathology: Moving towards reconsolidation-based treatments. *Neurobiology of Learning and Memory, 142*, 162-171. doi:10.1016/j.nlm.2016.11.005

Duncan, C. P. (1949). The retroactive effect of electroshock on learning. *Journal of Comparative & Physiological Psychology, 42*, 32-44. doi:10.1037/h0058173

Dunsmoor, J. E., Campese, V. D., Ceceli, A. O., LeDoux, J. E., & Phelps, E. A. (2015). Novelty-facilitated extinction: Providing a novel outcome in place of an expected threat diminishes recovery of defensive responses. *Biological Psychiatry, 78*, 203-209. doi:10.1016/j.biopsych.2014.12.008

Dunsmoor, J. E., & Paz, R. (2015). Fear generalization and anxiety: Behavioral and neural mechanisms. *Biological Psychiatry, 78*, 336-343. doi:10.1016/j.biopsych.2015.04.010

Dymond, S., Dunsmoor, J. E., Vervliet, B., Roche, B., & Hermans, D. (2015). Fear generalization in humans: Systematic review and implications for anxiety disorder research. *Behavior Therapy, 46*, 561-582. doi:10.1016/j.beth.2014.10.001

Exton-McGuinness, M. T. J., Lee, J. L. C., & Reichelt, A. C. (2015). Updating memories: The role of prediction errors in memory reconsolidation. *Behavioural Brain Research, 278*, 375-384. doi:10.1016/j.bbr.2014.10.011

Exton-McGuinness, M. T. J., & Milton, A. L. (2018). Reconsolidation blockade for the treatment of addiction: Challenges, new targets, and opportunities. *Learning & Memory, 25*, 492-500. doi:10.1101/lm.046771.117

Faliagkas, L., Rao-Ruiz, P., & Kindt, M. (2018). Emotional memory expression is misleading: Delineating transitions between memory processes. *Current Opinion in Behavioral Sciences, 19*, 116-122. doi:10.1016/j.cobeha.2017.12.018

Fernandez-Rey, J., Gonzalez-Gonzalez, D., & Redondo, J. (2018). Preventing the return of fear memories with postretrieval extinction: A human study using a burst of white noise as an aversive stimulus. *Behavioral Neuroscience, 132*, 23239. doi:10.1037/bne0000245

Fernández, R. S., Boccia, M. M., & Pedreira, M. E. (2016). The fate of memory: Reconsolidation and the case of prediction error. *Neuroscience & Biobehavioral Reviews, 68*, 423-441. doi:10.1016/j.neubiorev.2016.06.004

Fernández, R. S., Pedreira, M. E., & Boccia, M. M. (2017). Does reconsolidation occur in natural settings? Memory reconsolidation and anxiety disorders. *Clinical Psychology Review, 57*, 45-58. doi:10.1016/j.cpr.2017.08.004

Finnie, P. S. B., & Nader, K. (2012). The role of metaplasticity mechanisms in regulating memory destabilization and reconsolidation. *Neuroscience & Biobehavioral Reviews, 36*, 1667-1707. doi:10.1016/j.neubiorev.2012.03.008

Flores, A., Lopez, F. J., Vervliet, B., & Cobos, P. L. (2018). Intolerance of uncertainty as a vulnerability factor for excessive and inflexible avoidance behavior. *Behaviour Research and Therapy, 104*, 34-43. doi:10.1016/j.brat.2018.02.008

Foa, E. B., & McLean, C. P. (2016). The efficacy of exposure therapy for anxiety-related disorders and its underlying mechanisms: The case of OCD and PTSD. *Annual Review of Clinical Psychology, 12,* 1-28. doi:10.1146/annurev-clinpsy-021815-093533

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

Freeston, M. H., Rhéaume, J., Letarte, H., Dugas, M. J., & Ladouceur, R. (1994). Why do people worry? *Personality and Individual Differences, 17*, 791-802. doi:10.1016/0191-8869(94)90048-5

Frey, P. W., & Butler, C. S. (1977). Extinction after aversive conditioning: An associative or nonassociative process? *Learning and Motivation, 8,* 1-17. doi:10.1016/0023-9690(77)90063-7

Fricchione, J., Greenberg, M. S., Spring, J., Wood, N., Mueller-Pfeiffer, C., Milad, M. R., . . . Orr, S. P. (2016). Delayed extinction fails to reduce skin conductance reactivity to fear-conditioned stimuli. *Psychophysiology, 53,* 1343-1351. doi:10.1111/psyp.12687

Gawronski, B., Gast, A., & De Houwer, J. (2015). Is evaluative conditioning really resistant to extinction? Evidence for changes in evaluative judgements without changes in evaluative representations. *Cognition & Emotion, 29,* 816-830. doi:10.1080/02699931.2014.947919

Germeroth, L. J., Carpenter, M. J., Baker, N. L., Froeliger, B., LaRowe, S. D., & Saladin, M. E. (2017). Effect of a brief memory updating intervention on smoking behavior: A randomized clinical trial. *JAMA Psychiatry, 74*, 214-223. doi:10.1001/jamapsychiatry.2016.3148

Gershman, S. J., Jones, C. E., Norman, K. A., Monfils, M. H., & Niv, Y. (2013). Gradual extinction prevents the return of fear: Implications for the discovery of state. *Frontiers in Behavioral Neuroscience, 7*(164), 1-6. doi:10.3389/fnbeh.2013.00164

Golkar, A., Bellander, M., Olsson, A., & Öhman, A. (2012). Are fear memories erasable? Reconsolidation of learned fear with fear relevant and fear-irrelevant stimuli. *Frontiers in Behavioral Neuroscience, 6*(80), 1-10. doi:10.3389/fnbeh.2012.00080

Golkar, A., & Öhman, A. (2012). Fear extinction in humans: Effects of acquisition–extinction delay and masked stimulus presentations. *Biological Psychology, 91*, 292-301. doi:10.1016/j.biopsycho.2012.07.007

Golkar, A., Tjaden, C., & Kindt, M. (2017). Vicarious extinction learning during reconsolidation neutralizes fear memory. *Behaviour Research and Therapy, 92*, 87-93. doi:10.1016/j.brat.2017.02.004

Goode, T. D., Holloway-Erickson, C. M., & Maren, S. (2017). Extinction after fear memory reactivation fails to eliminate renewal in rats. *Neurobiology of Learning and Memory, 142*, 41-47. doi:10.1016/j.nlm.2017.03.001

Gray, R., Budden-Potts, D., & Bourke, F. (2019). Reconsolidation of traumatic memories for PTSD: A randomized controlled trial of 74 male veterans. *Psychotherapy Research, 29*, 621-639. doi:10.1080/10503307.2017.1408973

Gray, R., & Liotta, R. F. (2012). PTSD: Extinction, reconsolidation, and the visual-kinesthetic dissociation protocol. *Traumatology, 18*(2), 3-16. doi:10.1177/1534765611431835

Greco, V., & Roger, D. (2003). Uncertainty, stress, and health. *Personality and Individual Differences, 34*, 1057-1068. doi:10.1016/S0191-8869(02)00091-0

Grégoire, L., & Greening, S. G. (2019). Opening the reconsolidation window using the mind's eye: Extinction training during reconsolidation disrupts fear memory expression following mental imagery reactivation. *Cognition, 183*, 277-281. doi:10.1016/j.cognition.2018.12.001

Grillon, C. (2008). Models and mechanisms of anxiety: Evidence from startle studies. *Psychopharmacology, 199*, 421-437. doi:10.1007/s00213-007-1019-1

Groves, P. M., & Thompson, R. F. (1970). Habituation: A dual-process theory. *Psychological Review, 77*, 419-450. doi:10.1037/h0029810

Gruss, L. F., & Keil, A. (2019). Sympathetic responding to unconditioned stimuli predicts subsequent threat expectancy, orienting, and visuocortical bias in human aversive Pavlovian conditioning. *Biological Psychology, 140*, 64-74. doi:10.1016/j.biopsycho.2018.11.009

Haaker, J., Golkar, A., Hermans, D., & Lonsdorf, T. B. (2014). A review on human reinstatement studies: An overview and methodological challenges. *Learning & Memory, 21*, 424-440. doi:10.1101/lm.036053.114

Haesen, K., & Vervliet, B. (2015). Beyond extinction: Habituation eliminates conditioned skin conductance across contexts. *International Journal of Psychophysiology, 98*, 529-534. doi:10.1016/j.ijpsycho.2014.11.010

Hale, W., Richmond, M., Bennett, J., Berzins, T., Fields, A., Weber, D., . . . Osman, A. (2016). Resolving uncertainty about the Intolerance of Uncertainty Scale-12: Application of modern psychometric strategies. *Journal of Personality Assessment, 98*, 200-208. doi:10.1080/00223891.2015.1070355

Harnett, N. G., Shumen, J. R., Wagle, P. A., Wood, K. H., Wheelock, M. D., Baños, J. H., & Knight, D. C. (2016). Neural mechanisms of human temporal fear conditioning. *Neurobiology of Learning and Memory, 136*, 97-104. doi:10.1016/j.nlm.2016.09.019

Haubrich, J., Crestani, A. P., Cassini, L. F., Santana, F., Sierra, R. O., Alvares, L. D., & Quillfeldt, J. A. (2015). Reconsolidation allows fear memory to be updated to a less aversive level through the incorporation of appetitive information. *Neuropsychopharmacology, 40*, 315-326. doi:10.1038/npp.2014.174

Henry, J. D., & Crawford, J. R. (2005). The short-form version of the Depression Anxiety Stress Scales (DASS-21): Construct validity and normative data in a large non-clinical sample. *British Journal of Clinical Psychology, 44*, 227-239. doi:10.1348/014466505X29657

Hermans, D., Dirikx, T., Vansteenwegenin, D., Baeyens, F., Van den Bergh, O., & Eelen, P. (2005). Reinstatement of fear responses in human aversive conditioning. *Behaviour Research and Therapy, 43*, 533-551. doi:10.1016/j.brat.2004.03.013

Hermans, D., Vansteenwegen, D., Crombez, G., Baeyens, F., & Eelen, P. (2002). Expectancy-learning and evaluative learning in human classical conditioning: Affective priming as an indirect and unobtrusive measure of conditioned stimulus valence. *Behaviour Research and Therapy, 40*, 217-234. doi:10.1016/S0005-7967(01)00006-7

Ho, Y., & Lipp, O. V. (2014). Faster acquisition of conditioned fear to fear-relevant than to nonfear-relevant conditional stimuli. *Psychophysiology, 51*, 810-813. doi:10.1111/psyp.12223

Hofmann, W., De Houwer, J., Perugini, M., Baeyens, F., & Crombez, G. (2010). Evaluative conditioning in humans: A meta-analysis. *Psychological Bulletin, 136*, 390-421. doi:10.1037/a0018916

Högberg, G., & Hällström, T. (2018). Mood regulation focused CBT based on memory reconsolidation, reduced suicidal ideation and depression in youth in a randomised controlled study. *International Journal of Environmental Research and Public Health, 15*, 921. doi:10.3390/ijerph15050921

Holland, P. C. (2008). Cognitive versus stimulus-response theories of learning. *Learning & Behavior, 36*, 227-241. doi:10.3758/lb.36.3.227

Holland, P. C., & Rescorla, R. A. (1975). Second-order conditioning with food unconditioned stimulus. *Journal of Comparative & Physiological Psychology, 88*, 459-467. doi:10.1037/h0076219

Holland, P. C., & Schiffino, F. L. (2016). Mini-review: Prediction errors, attention and associative learning. *Neurobiology of Learning and Memory, 131*, 207-215. doi:10.1016/j.nlm.2016.02.014

Hon, T., Das, R. K., & Kamboj, S. K. (2016). The effects of cognitive reappraisal following retrieval-procedures designed to destabilize alcohol memories in high-risk drinkers. *Psychopharmacology, 233*, 851-861. doi:10.1007/s00213-015-4164-y

Hosoba, T., Iwanaga, M., & Seiwa, H. (2001). The effect of UCS inflation and deflation procedures on 'fear' conditioning. *Behaviour Research and Therapy, 39*, 465-475. doi:10.1016/S0005-7967(00)00025-5

Hu, J., Wang, W., Homan, P., Wang, P., Zheng, X., & Schiller, D. (2018). Reminder duration determines threat memory modification in humans. *Scientific Reports, 8*(8848), 1-10. doi:10.1038/s41598-018-27252-0

Huang, B., Zhu, H. W., Zhou, Y. M., Liu, X., & Ma, L. (2017). Unconditioned- and conditioned-stimuli induce differential memory reconsolidation and beta-AR-dependent CREB activation. *Frontiers in Neural Circuits, 11*, 53-62. doi:10.3389/fncir.2017.00053

Huff, N. C., Hernandez, J. A., Blanding, N. Q., & LaBar, K. S. (2009). Delayed extinction attenuates conditioned fear renewal and spontaneous recovery in humans. *Behavioral Neuroscience, 123*, 834-843. doi:10.1037/a0016511

Ishii, D., Matsuzawa, D., Matsuda, S., Tomizawa, H., Sutoh, C., & Shimizu, E. (2015). An isolated retrieval trial before extinction session does not prevent the return of fear. *Behavioural Brain Research, 287*, 139-145. doi:10.1016/j.bbr.2015.03.052

Iyadurai, L., Blackwell, S. E., Meiser-Stedman, R., Watson, P. C., Bonsall, M. B., Geddes, J. R., . . . Holmes, E. A. (2018). Preventing intrusive memories after trauma via a brief intervention involving Tetris computer game play in the emergency department: A proof-of-concept randomized controlled trial. *Molecular Psychiatry, 23*, 674-682. doi:10.1038/mp.2017.23

Izquierdo, L. A., Barros, D. M., Medina, J. H., & Izquierdo, I. (2000). Novelty enhances retrieval of one-trial avoidance learning in rats 1 or 31 days after training unless the hippocampus is inactivated by different receptor antagonists and enzyme inhibitors. *Behavioural Brain Research, 117*, 215-220. doi:10.1016/S0166-4328(00)00286-2

Jacoby, R. J., Fabricant, L. E., Leonard, R. C., Riemann, B. C., & Abramowitz, J. S. (2013). Just to be certain: Confirming the factor structure of the Intolerance of Uncertainty Scale in patients with obsessive-compulsive disorder. *Journal of Anxiety Disorders, 27*, 535-542. doi:10.1016/j.janxdis.2013.07.008

James, E. L., Bonsall, M. B., Hoppitt, L., Tunbridge, E. M., Geddes, J. R., Milton, A. L., & Holmes, E. A. (2015). Computer game play reduces intrusive memories of experimental trauma via reconsolidation-update mechanisms. *Psychological Science, 26*, 1201-1215. doi:10.1177/0956797615583071

Jensen-Fielding, H., Luck, C. C., & Lipp, O. V. (2017). Is the devil in the detail? Evidence for S-S learning after unconditional stimulus revaluation in human evaluative conditioning under a

broader set of experimental conditions. *Cognition and Emotion, 32*, 1275-1290. doi:10.1080/02699931.2017.1408573

Johnson, D. C., & Casey, B. J. (2015). Extinction during memory reconsolidation blocks recovery of fear in adolescents. *Scientific Reports, 5*(8863), 1-5. doi:10.1038/srep08863

Kamin, L. J. (1969). Predictability, surprise, attention, and conditioning. In B. A. Campbell & R. M. Church (Eds.), *Punishment and aversive behavior* (pp. 279-296). New York: Appleton-Century-Crofts.

Kang, S., Vervliet, B., Engelhard, I. M., van Dis, E. A. M., & Hagenaars, M. A. (2018). Reduced return of threat expectancy after counterconditioning versus extinction. *Behaviour Research and Therapy, 108*, 78-84. doi:10.1016/j.brat.2018.06.009

Khawaja, N. G., & Yu, L. N. H. (2010). A comparison of the 27-item and 12-item intolerance of uncertainty scales. *Clinical Psychologist, 14*, 97-106. doi:10.1080/13284207.2010.502542

Kida, S. (2018). Reconsolidation/destabilization, extinction and forgetting of fear memory as therapeutic targets for PTSD. *Psychopharmacology, 236*, 49-57. doi:10.1007/s00213-018-5086-2

Kindt, M. (2018). The surprising subtleties of changing fear memory: A challenge for translational science. *Philosophical Transactions of the Royal Society B, 373*, 20170033. doi:10.1098/rstb.2017.0033

Kindt, M., & Soeter, M. (2013). Reconsolidation in a human fear conditioning study: A test of extinction as updating mechanism. *Biological Psychology, 92*, 43-50. doi:10.1016/j.biopsycho.2011.09.016

Kindt, M., & Soeter, M. (2018). Pharmacologically induced amnesia for learned fear is time and sleep dependent. *Nature Communications, 9*(1316), 1-10. doi:10.1038/s41467-018-03659-1

Kindt, M., Soeter, M., & Vervliet, B. (2009). Beyond extinction: Erasing human fear responses and preventing the return of fear. *Nature Neuroscience, 12*, 256-258. doi:10.1038/nn.2271

Kindt, M., & van Emmerik, A. (2016). New avenues for treating emotional memory disorders: Towards a reconsolidation intervention for posttraumatic stress disorder. *Therapeutic Advances in Psychopharmacology, 6*, 283-295. doi:10.1177/2045125316644541

Klorman, R., Weerts, T. C., Hastings, J. E., Melamed, B. G., & Lang, P. J. (1974). Psychometric description of some specific-fear questionnaires. *Behavior Therapy, 5*, 401-409. doi:10.1016/S0005-7894(74)80008-0

Klucken, T., Kruse, O., Schweckendiek, J., Kuepper, Y., Mueller, E. M., Hennig, J., & Stark, R. (2016). No evidence for blocking the return of fear by disrupting reconsolidation prior to extinction learning. *Cortex, 79*, 112-122. doi:10.1016/j.cortex.2016.03.015

Konorski, J., & Szwejkowska, G. (1950). Chronic extinction and restoration of conditioned reflexes. I. Extinction against the excitatory background. *Acta Biologiae Experimentalis, 15*, 155-170. Retrieved from http://konorski.nencki.gov.pl

Kredlow, M. A., Orr, S. P., & Otto, M. W. (2018a). Exploring the boundaries of post-retrieval extinction in healthy and anxious individuals. *Behaviour Research and Therapy, 108*, 45-57. doi:10.1016/j.brat.2018.06.010

Kredlow, M. A., Orr, S. P., & Otto, M. W. (2018b). Who is studied in de novo fear conditioning paradigms? An examination of demographic and stimulus characteristics predicting fear learning. *International Journal of Psychophysiology, 130*, 21-28. doi:10.1016/j.ijpsycho.2018.05.008

Kredlow, M. A., & Otto, M. W. (2015). Interference with the reconsolidation of trauma-related memories in adults. *Depression and Anxiety, 32*, 32-37. doi:10.1002/da.22343

Kredlow, M. A., Unger, L. D., & Otto, M. W. (2016). Harnessing reconsolidation to weaken fear and appetitive memories: A meta-analysis of post-retrieval extinction effects. *Psychological Bulletin, 142*, 314-336. doi:10.1037/bul0000034

Kreibig, S. D. (2010). Autonomic nervous system activity in emotion: A review. *Biological Psychology, 84*, 394-421. doi:10.1016/j.biopsycho.2010.03.010

Krompinger, J. W., Van Kirk, N. P., Garner, L. E., Potluri, S. I., & Elias, J. A. (2018). Hope for the worst: Occasional reinforced extinction and expectancy violation in the treatment of OCD. *Cognitive and Behavioral Practicee, 26*, 143-153. doi:10.1016/j.cbpra.2017.12.002

Krypotos, A.-M., Vervliet, B., & Engelhard, I. M. (2018). The validity of human avoidance paradigms. *Behaviour Research and Therapy, 111*, 99-105. doi:10.1016/j.brat.2018.10.011

Kunze, A. E., Arntz, A., & Kindt, M. (2015). Fear conditioning with film clips: A complex associative learning paradigm. *Journal of Behavior Therapy and Experimental Psychiatry, 47*, 42-50. doi:10.1016/j.jbtep.2014.11.007

LaBar, K. S., & Cabeza, R. (2006). Cognitive neuroscience of emotional memory. *Nature Reviews Neuroscience, 7*, 54-64. doi:10.1038/nrn1825

Laborda, M. A., & Miller, R. R. (2011). S-R associations, their extinction, and recovery in an animal model of anxiety: A new associative account of phobias without recall of original trauma. *Behavior Therapy, 42*, 153-169. doi:10.1016/j.beth.2010.06.002

Lang, P. J. (1985). The cognitive psychophysiology of emotion: Fear and anxiety. In A. H. Tuma & J. D. Maser (Eds.), *Anxiety and the anxiety disorders*. Hillsdale, NJ: Erlbaum.

Lang, P. J., & Bradley, M. M. (2010). Emotion and the motivational brain. *Biological Psychology, 84*, 437-450. doi:10.1016/j.biopsycho.2009.10.007

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1998). Emotion, motivation, and anxiety: Brain mechanisms and psychophysiology. *Biological Psychiatry, 44*, 1248-1263. doi:10.1016/S0006-3223(98)00275-3

Lavazza, A. (2019). Moral bioenhancement through memory-editing: A risk for identity and authenticity? *Topoi, 38*, 15-27. doi:10.1007/s11245-017-9465-9

LeDoux, J. E. (2000). Emotion circuits in the brain. *Annual Review of Neuroscience, 23*, 155-184. doi:10.1146/annurev.neuro.23.1.155

LeDoux, J. E. (2014). Coming to terms with fear. *Proceedings of the National Academy of Sciences, 111*, 2871-2878. doi:10.1073/pnas.1400335111

Lee, J. L. C. (2008). Memory reconsolidation mediates the strengthening of memories by additional learning. *Nature Neuroscience, 11*, 1264-1266. doi:10.1038/nn.2205

Lee, J. L. C. (2009). Reconsolidation: Maintaining memory relevance. *Trends in Neurosciences, 32*, 413-420. doi:10.1016/j.tins.2009.05.002

Lee, J. L. C., Everitt, B. J., & Thomas, K. L. (2004). Independent cellular processes for hippocampal memory consolidation and reconsolidation. *Science, 304*, 839-843. doi:10.1126/science.1095760

Lee, J. L. C., Nader, K., & Schiller, D. (2017). An update on memory reconsolidation updating. *Trends in Cognitive Sciences, 21*, 531-545. doi:10.1016/j.tics.2017.04.006

Leer, A., & Engelhard, I. M. (2015). Countering fear renewal: Changes in the UCS representation generalize across contexts. *Behavior Therapy, 46*, 272-282. doi:10.1016/j.beth.2014.09.012

Leer, A., Engelhard, I. M., Dibbets, P., & van den Hout, M. A. (2013). Dual-tasking attenuates the return of fear after extinction. *Journal of Experimental Psychopathology, 4*, 325-340. doi:10.5127/jep.029412

Leer, A., Haesen, K., & Vervliet, B. (2018). Beyond extinction: Prolonged conditioning and repeated threat exposure abolish contextual renewal of fear-potentiated startle discrimination but leave expectancy ratings intact. *Frontiers in Psychiatry, 9*, 117-128. doi:10.3389/fpsyt.2018.00117

Lewis, D. J. (1979). Psychobiology of active and inactive memory. *Psychological Bulletin, 86*, 1054-1083. doi:10.1037/0033-2909.86.5.1054

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

Lipp, O. V. (2006b). Of snakes and flowers: Does preferential detection of pictures of fear-relevant animals in visual search reflect on fear-relevance? *Emotion, 6*, 296-308. doi:10.1037/1528-3542.6.2.296

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*, n/a-n/a. doi:10.1111/psyp.12513

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

Lipp, O. V., Oughton, N., & LeLievre, J. (2003). Evaluative learning in human Pavlovian conditioning: Extinct, but still there? *Learning and Motivation, 34*, 219-239. doi:10.1016/S0023-9690(03)00011-0

Lipp, O. V., & Waters, A. M. (2007). When danger lurks in the background: Attentional capture by animal fear-relevant distractors is specific and selectively enhanced by animal fear. *Emotion, 7*, 192-200. doi:10.1037/1528-3542.7.1.192

Lissek, S. (2012). Toward an account of clinical anxiety predicated on basic, neurally mapped mechanisms of Pavlovian fear-learning: The case for conditioned overgeneralization. *Depression and Anxiety, 29*, 257-263. doi:10.1002/da.21922

Lissek, S., Pine, D. S., & Grillon, C. (2006). The strong situation: A potential impediment to studying the psychobiology and pharmacology of anxiety disorders. *Biological Psychology, 72*, 265-270. doi:10.1016/j.biopsycho.2005.11.004

Lissek, S., Powers, A. S., McClure, E. B., Phelps, E. A., Woldehawariat, G., Grillon, C., & Pine, D. S. (2005). Classical fear conditioning in the anxiety disorders: A meta-analysis. *Behaviour Research and Therapy, 43*, 1391-1424. doi:10.1016/j.brat.2004.10.007

Liu, J. F., Zhao, L. Y., Xue, Y. X., Shi, J., Suo, L., Luo, Y. X., . . . Lu, L. (2014). An unconditioned stimulus retrieval extinction procedure to prevent the return of fear memory. *Biological Psychiatry, 76*, 895-901. doi:10.1016/j.biopsych.2014.03.027

Lonergan, M., Saumier, D., Tremblay, J., Kieffer, B., Brown, T. G., & Brunet, A. (2016). Reactivating addiction-related memories under propranolol to reduce craving: A pilot randomized controlled trial. *Journal of Behavior Therapy and Experimental Psychiatry, 50*, 245-249. doi:10.1016/j.jbtep.2015.09.012

Lonsdorf, T. B., Menz, M. M., Andreatta, M., Fullana, M. A., Golkar, A., Haaker, J., . . . Merz, C. J. (2017). Don't fear 'fear conditioning': Methodological considerations for the design and analysis of studies on human fear acquisition, extinction, and return of fear. *Neuroscience & Biobehavioral Reviews, 77*, 247-285. doi:10.1016/j.neubiorev.2017.02.026

Lonsdorf, T. B., & Merz, C. J. (2017). More than just noise: Inter-individual differences in fear acquisition, extinction and return of fear in humans - Biological, experiential, temperamental factors, and methodological pitfalls. *Neuroscience & Biobehavioral Reviews, 80*, 703-728. doi:10.1016/j.neubiorev.2017.07.007

Lovibond, P. F. (1998). Long-term stability of depression, anxiety, and stress syndromes. *Journal of Abnormal Psychology, 107*, 520-526. doi:10.1037//0021-843x.107.3.520

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

Lovibond, P. F. (2006). Fear and avoidance: An integrated expectancy model. In M. G. Craske, D. Hermans, & D. Vansteenwegen (Eds.), *Fear and learning: From basic processes to clinical implications* (pp. 117-132). Washington: American Psychological Association.

Lovibond, P. F., & Lovibond, S. H. (1995). The structure of negative emotional states: Comparison of the Depression Anxiety Stress Scales (DASS) with the Beck Depression and Anxiety Inventories. *Behaviour Research and Therapy, 33*, 335-343. doi:10.1016/0005-7967(94)00075-U

Lovibond, P. F., & Rapee, R. M. (1993). The presentation of feared outcomes. *Behaviour Research and Therapy, 31*, 595–608. doi:10.1016/0005-7967(93)90111-7

Lovibond, P. F., Siddle, D. A. T., & Bond, N. W. (1993). Resistance to extinction of fear-relevant stimuli: Preparedness or selective sensitization? *Journal of Experimental Psychology: General, 122*, 449-461. doi:10.1037/0096-3445.122.4.449

Lovibond, P. F., & Shanks, D. R. (2002). The role of awareness in Pavlovian conditioning: Empirical evidence and theoretical implications. *Journal of Experimental Psychology: Animal Behavior Processes, 28*, 3-26. doi:10.1037/0097-7403.28.1.3

Lovibond, S. H., & Lovibond, P. F. (1995). *Manual for the Depression Anxiety Stress Scales* (2nd ed.). Sydney: Psychology Foundation.

Lucas, K., Luck, C. C., & Lipp, O. V. (2018). Novelty-facilitated extinction and the reinstatement of conditional human fear. *Behaviour Research and Therapy, 109*, 68-74. doi:10.1016/j.brat.2018.08.002

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. (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

Luo, Y. X., Xue, Y. X., Liu, J. F., Shi, H. S., Jian, M., Han, Y., . . . Lu, L. (2015). A novel UCS memory retrieval-extinction procedure to inhibit relapse to drug seeking. *Nature Communications, 6*(7675), 1-14. doi:10.1038/ncomms8675

Luyten, L., & Beckers, T. (2017). A preregistered, direct replication attempt of the retrieval-extinction effect in cued fear conditioning in rats. *Neurobiology of Learning and Memory, 144*, 208-215. doi:10.1016/j.nlm.2017.07.014

Lykken, D. T. (1972). Range correction applied to heart rate and to GSR data. *Psychophysiology, 9*, 373-379. doi:10.1111/j.1469-8986.1972.tb03222.x

Mallan, K. M., & Lipp, O. V. (2011). The relationship between self-reported animal fear and ERP modulation: Evidence for enhanced processing and fear of harmless invertebrates in snake- and spider-fearful individuals. *Motivation and Emotion, 35*, 474-483. doi:10.1007/s11031-011-9218-9

Manfield, P., Lovett, J., Engel, L., & Manfield, D. (2017). Use of the flash technique in EMDR therapy: Four case examples. *Journal of EMDR Practice and Research, 11*, 195-205. doi:10.1891/1933-3196.11.4.195

Maples-Keller, J. L., Price, M., Jovanovic, T., Norrholm, S. D., Odenat, L., Post, L., . . . Rothbaum, B. O. (2017). Targeting memory reconsolidation to prevent the return of fear in patients with fear of flying. *Depression and Anxiety, 34*, 610-620. doi:10.1002/da.22626

Maren, S. (2014). Nature and causes of the immediate extinction deficit: A brief review. *Neurobiology of Learning and Memory, 113*, 19-24. doi:10.1016/j.nlm.2013.10.012

McConnell, B. L., & Miller, R. R. (2014). Associative accounts of recovery-from-extinction effects. *Learning and Motivation, 46*, 1-15. doi:10.1016/j.lmot.2014.01.003

McEvoy, P. M., & Erceg-Hurn, D. M. (2016). The search for universal transdiagnostic and trans-therapy change processes: Evidence for intolerance of uncertainty. *Journal of Anxiety Disorders, 41*, 96-107. doi:10.1016/j.janxdis.2016.02.002

McEvoy, P. M., Erceg-Hurn, D. M., Saulsman, L. M., & Thibodeau, M. A. (2015). Imagery enhancements increase the effectiveness of cognitive behavioural group therapy for social anxiety disorder: A benchmarking study. *Behaviour Research and Therapy, 65*, 42-51. doi:10.1016/j.brat.2014.12.011

McEvoy, P. M., & Mahoney, A. E. J. (2011). Achieving certainty about the structure of intolerance of uncertainty in a treatment-seeking sample with anxiety and depression. *Journal of Anxiety Disorders, 25*, 112-122. doi:10.1016/j.janxdis.2010.08.010

McEvoy, P. M., & Mahoney, A. E. J. (2012). To be sure, to be sure: Intolerance of uncertainty mediates symptoms of various anxiety disorders and depression. *Behavior Therapy, 43*, 533-545. doi:10.1016/j.beth.2011.02.007

McGaugh, J. L. (2000). Memory—A century of consolidation. *Science, 287*, 248-251. doi:10.1126/science.287.5451.248

McNally, G. P., Johansen, J. P., & Blair, H. T. (2011). Placing prediction into the fear circuit. *Trends in Neurosciences, 34*, 283-292. doi:10.1016/j.tins.2011.03.005

Meir Drexler, S., Merz, C. J., Hamacher-Dang, T. C., Marquardt, V., Fritsch, Otto, T., & Wolf, O. T. (2014). Effects of postretrieval-extinction learning on return of contextually controlled cued fear. *Behavioral Neuroscience, 128*, 474-481. doi:10.1037/a0036688

Meir Drexler, S., & Wolf, O. T. (2017). Stress disrupts the reconsolidation of fear memories in men. *Psychoneuroendocrinology, 77*, 95-104. doi:10.1016/j.psyneuen.2016.11.027

Merlo, E., Milton, A. L., Goozée, Z. Y., Theobald, D. E., & Everitt, B. J. (2014). Reconsolidation and extinction are dissociable and mutually exclusive processes: Behavioral and molecular evidence. *The Journal of Neuroscience, 34*, 2422-2431. doi:10.1523/jneurosci.4001-13.2014

Mertens, G., Boddez, Y., Sevenster, D., Engelhard, I. M., & De Houwer, J. (2018). A review on the effects of verbal instructions in human fear conditioning: Empirical findings, theoretical considerations, and future directions. *Biological Psychology, 137*, 49-64. doi:10.1016/j.biopsycho.2018.07.002

Mertens, G., Braem, S., Kuhn, M., Lonsdorf, T. B., van den Hout, M. A., & Engelhard, I. M. (2018). Does US expectancy mediate the additive effects of CS-US pairings on contingency

instructions? Results from subjective, psychophysiological and neural measures. *Behaviour Research and Therapy, 110,* 41-46. doi:10.1016/j.brat.2018.09.003

Mertens, G., & De Houwer, J. (2016). Potentiation of the startle reflex is in line with contingency reversal instructions rather than the conditioning history. *Biological Psychology, 113,* 91-99. doi:10.1016/j.biopsycho.2015.11.014

Mertens, G., Kuhn, M., Raes, A. K., Kalisch, R., De Houwer, J., & Lonsdorf, T. B. (2016). Fear expression and return of fear following threat instruction with or without direct contingency experience. *Cognition and Emotion, 30,* 968-984. doi:10.1080/02699931.2015.1038219

Merz, C. J., Hamacher-Dang, T. C., & Wolf, O. T. (2016). Immediate extinction promotes the return of fear. *Neurobiology of Learning and Memory, 131,* 109-116. doi:10.1016/j.nlm.2016.03.013

Mickley, G. A., DiSorbo, A., Wilson, G. N., Huffman, J., Bacik, S., Hoxha, Z., . . . Kim, Y.-H. (2009). Explicit disassociation of a conditioned stimulus and unconditioned stimulus during extinction training reduces both time to asymptotic extinction and spontaneous recovery of a conditioned taste aversion. *Learning and Motivation, 40,* 209-220. doi:10.1016/j.lmot.2009.01.001

Miller, G. A., & Chapman, J. P. (2001). Misunderstanding analysis of covariance. *Journal of Abnormal Psychology, 110,* 40-48. doi:10.1037/0021-843X.110.1.40

Mineka, S., & Öhman, A. (2002). Phobias and preparedness: The selective, automatic, and encapsulated nature of fear. *Biological Psychiatry, 52,* 927-937. doi:10.1016/S0006-3223(02)01669-4

Mineka, S., & Zinbarg, R. (2006). A contemporary learning theory perspective on the etiology of anxiety disorders: It's not what you thought it was. *American Psychologist, 61,* 10-26. doi:10.1037/0003-066X.61.1.10

Misanin, J. R., Miller, R. R., & Lewis, D. J. (1968). Retrograde amnesia produced by electro-convulsive shock after reactivation of a consolidated memory trace. *Science, 160,* 554-555. doi:10.1126/science.160.3827.554

Monfils, M.-H., Cowansage, K. K., Klann, E., & LeDoux, J. E. (2009). Extinction-reconsolidation boundaries: Key to persistent attenuation of fear memories. *Science, 324*, 951-955. doi:10.1126/science.1167975

Monti, R. I. F., Alfei, J. M., Mugnaini, M., Bueno, A. M., Beckers, T., Urcelay, G. P., & Molina, V. A. (2017). A comparison of behavioral and pharmacological interventions to attenuate reactivated fear memories. *Learning & Memory, 24*, 369-374. doi:10.1101/lm.045385.117

Morriss, J., Christakou, A., & van Reekum, C. M. (2016). Nothing is safe: Intolerance of uncertainty is associated with compromised fear extinction learning. *Biological Psychology, 121*, 187-193. doi:10.1016/j.biopsycho.2016.05.001

Morriss, J., Macdonald, B., & van Reekum, C. (2016). What is going on around here? Intolerance of uncertainty predicts threat generalization. *PLoS One, 11*, e0154494. doi:10.1371/journal.pone.0154494

Muris, P., & Merckelbach, H. (1996). A comparison of two spider fear questionnaires. *Journal of Behavior Therapy and Experimental Psychiatry, 27*, 241-244. doi:10.1016/S0005-7916(96)00022-5

Myers, K. M., Ressler, K. J., & Davis, M. (2006). Different mechanisms of fear extinction dependent on length of time since fear acquisition. *Learning and Memory, 13*, 216-223. doi:10.1101/lm.119806

Nader, K. (2013). The discovery of memory reconsolidation. In C. M. Alberini (Ed.), *Memory reconsolidation* (pp. 1-13). San Diego: Academic Press.

Nader, K. (2015). Reconsolidation and the dynamic nature of memory. *Cold Spring Harbor Perspectives in Biology, 7*, a021782. doi:10.1101/cshperspect.a021782

Nader, K., & Hardt, O. (2009). A single standard for memory: The case for reconsolidation. *Nature Reviews Neuroscience, 10*, 224-234. doi:10.1038/nrn2590

Nader, K., Schafe, G. E., & LeDoux, J. E. (2000a). Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature, 406*, 722-726. doi:10.1038/35021052

Nader, K., Schafe, G. E., & LeDoux, J. E. (2000b). The labile nature of consolidation theory. *Nature Reviews Neuroscience, 1*, 216-219. doi:10.1038/35044580

Napier, R. M., Macrae, M., & Kehoe, E. J. (1992). Rapid reacquisition in conditioning of the rabbit's nictitating membrane response. *Journal of Experimental Psychology: Animal Behavior Processes, 18*, 182-192. doi:10.1037/0097-7403.18.2.182

National Institute for Health and Care Excellence. (2018). *Post-traumatic stress disorder* (NG116). Retrieved from https://www.nice.org.uk/guidance/ng116

Norrholm, S. D., Vervliet, B., Jovanovic, T., Boshoven, W., Myers, K. M., Davis, M., . . . Duncan, E. J. (2008). Timing of extinction relative to acquisition: A parametric analysis of fear extinction in humans. *Behavioral Neuroscience, 122*, 1016-1030. doi:10.1037/a0012604

Oglesby, M. E., Raines, A. M., Short, N. A., Capron, D. W., & Schmidt, N. B. (2016). Interpretation bias for uncertain threat: A replication and extension. *Journal of Behavior Therapy and Experimental Psychiatry, 51*, 35-42. doi:10.1016/j.jbtep.2015.12.006

Oglesby, M. E., & Schmidt, N. B. (2017). The role of threat level and intolerance of uncertainty (IU) in anxiety: An experimental test of IU theory. *Behavior Therapy, 48*, 427-434. doi:10.1016/j.beth.2017.01.005

Öhman, A. (2009). Of snakes and faces: An evolutionary perspective on the psychology of fear. *Scandinavian Journal of Psychology, 50*, 543-552. doi:10.1111/j.1467-9450.2009.00784.x

Öhman, A., Eriksson, A., & Olofsson, C. (1975). One-trial learning and superior resistance to extinction of autonomic responses conditioned to potentially phobic stimuli. *Journal of Comparative & Physiological Psychology, 88*, 619-627. doi:10.1016/j.biopsycho.2016.05.001

Öhman, A., & Mineka, S. (2001). Fears, phobias, and preparedness: Toward an evolved module of fear and fear learning. *Psychological Review, 108*, 483-522. doi:10.1037//0033-295x.108.3.483

Olsson, A., Ebert, J. P., Banaji, M. R., & Phelps, E. A. (2005). The role of social groups in the persistence of learned fear. *Science, 309*, 785-787. doi:10.1126/science.1113551

Orr, S. P., Metzger, L. J., Lasko, N. B., Macklin, M. L., Peri, T., & Pitman, R. K. (2000). De novo conditioning in trauma-exposed individuals with and without posttraumatic stress disorder. *Journal of Abnormal Psychology, 109*, 290-298. doi:10.1037/0021-843x.109.2.290

Oyarzún, J. P., Lopez-Barroso, D., Fuentemilla, L., Cucurell, D., Pedraza, C., Rodriguez-Fornells, A., & de Diego-Balaguer, R. (2012). Updating fearful memories with extinction training during reconsolidation: A human study using auditory aversive stimuli. *PLoS One, 7*, e38849. doi:10.1371/journal.pone.0038849

Pace-Schott, E. F., Verga, P. W., Bennett, T. S., & Spencer, R. M. C. (2012). Sleep promotes consolidation and generalization of extinction learning in simulated exposure therapy for spider fear. *Journal of Psychiatric Research, 46*, 1036-1044. doi:10.1016/j.jpsychires.2012.04.015

Paulus, D. J., Kamboj, S. K., Das, R. K., & Saladin, M. E. (2019). Prospects for reconsolidation-focused treatments of substance use and anxiety-related disorders. *Current Opinion in Psychology, 30*, 80-86. doi:10.1016/j.copsyc.2019.03.001

Pavlov, I. P. (1927). *Conditioned reflexes* (G. V. Anrep, Trans.). London: Oxford University Press.

Payne, A. F. H., Schell, A. M., & Dawson, M. E. (2016). Lapses in skin conductance responding across anatomical sites: Comparison of fingers, feet, forehead, and wrist. *Psychophysiology, 53*, 1084-1092. doi:10.1111/psyp.12643

Pearce, J. M., & Hall, G. (1980). A model for Pavlovian learning: Variations in the effectiveness of conditioned but not of unconditioned stimuli. *Psychological Review, 87*, 532-552. doi:10.1037/0033-295X.87.6.532

Pedraza, L. K., Sierra, R. O., Boos, F. Z., Haubrich, J., Quillfeldt, J. A., & de Oliveira Alvares, L. (2016). The dynamic nature of systems consolidation: Stress during learning as a switch guiding the rate of the hippocampal dependency and memory quality. *Hippocampus, 26*, 362-371. doi:10.1002/hipo.22527

Phelps, E. A., & Schiller, D. (2013). Reconsolidation in humans. In C. M. Alberini (Ed.), *Memory reconsolidation* (pp. 185-211). San Diego: Academic Press.

Pile, V., Barnhofer, T., & Wild, J. (2015). Updating versus exposure to prevent consolidation of conditioned fear. *PLoS One, 10*, e0122971. doi:10.1371/journal.pone.0122971

Pine, A., Mendelsohn, A., & Dudai, Y. (2014). Unconscious learning of likes and dislikes is persistent, resilient, and reconsolidates. *Frontiers in Psychology, 5*(1051), 1-13. doi:10.3389/fpsyg.2014.01051

Pineles, S. L., Orr, M. R., & Orr, S. P. (2009). An alternative scoring method for skin conductance responding in a differential fear conditioning paradigm with a long-duration conditioned stimulus. *Psychophysiology, 46*, 984-995. doi:10.1111/j.1469-8986.2009.00852.x

Pineyro, M. E., Monti, R. I. F., Alfei, J. M., Bueno, A. M., & Urcelay, G. P. (2014). Memory destabilization is critical for the success of the reactivation-extinction procedure. *Learning & Memory, 21*, 46-54. doi:10.1101/lm.032714.113

Pittig, A., & Dehler, J. (2018). Same fear responses, less avoidance: Rewards competing with aversive outcomes do not buffer fear acquisition, but attenuate avoidance to accelerate subsequent fear extinction. *Behaviour Research and Therapy, 112*, 1-11. doi:10.1016/j.brat.2018.11.003

Pittig, A., Treanor, M., LeBeau, R. T., & Craske, M. G. (2018). The role of associative fear and avoidance learning in anxiety disorders: Gaps and directions for future research. *Neuroscience & Biobehavioral Reviews, 88*, 117-140. doi:10.1016/j.neubiorev.2018.03.015

Plendl, W., & Wotjak, C. T. (2010). Dissociation of within- and between-session extinction of conditioned fear. *Journal of Neuroscience, 30*, 4990-4998. doi:10.1523/jneurosci.6038-09.2010

Post, R. M., & Kegan, R. (2017). Prevention of recurrent affective episodes using extinction training in the reconsolidation window: A testable psychotherapeutic strategy. *Psychiatry Research, 249*, 327-336. doi:10.1016/j.psychres.2017.01.034

Poulos, C., Furedy, J., & Heslegrave, R. (1979). Effects of US habituation following skin-conductance response conditioning: Support for a Pavlovian S-S position and a habituation account of nonmonotonic acquisition functions. *Physiological Psychology, 7*, 278-282. doi:10.3758/BF03326640

Prado-Alcalá, R. A., Medina, A. C., Bello-Medina, P. C., & Quirarte, G. L. (2017). Inhibition of transcription and translation in the striatum after memory reactivation: Lack of evidence of

reconsolidation. *Neurobiology of Learning and Memory, 142*, 21-29. doi:10.1016/j.nlm.2016.12.018

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

Przybyslawski, J., & Sara, S. J. (1997). Reconsolidation of memory after its reactivation. *Behavioural Brain Research, 84*, 241-246. doi:10.1016/S0166-4328(96)00153-2

Rauhut, A. S., Thomas, B. L., & Ayres, J. J. B. (2001). Treatments that weaken Pavlovian conditioned fear and thwart its renewal in rats: Implications for treating human phobias. *Journal of Experimental Psychology: Animal Behavior Processes, 27*, 99-114. doi:10.1037/0097-7403.27.2.99

Rescorla, R. A. (1967). Pavlovian conditioning and its proper control procedures. *Psychological Review, 74*, 71-80. doi:10.1037/h0024109

Rescorla, R. A. (1973). Effects of US habituation following conditioning. *Journal of Comparative and Physiological Psychology, 82*, 137-143. doi:10.1037/h0033815

Rescorla, R. A. (1974). Effect of inflation of the unconditioned stimulus value following conditioning. *Journal of Comparative & Physiological Psychology, 86*, 101-106. doi:10.1037/h0035964

Rescorla, R. A., & Heth, C. D. (1975). Reinstatement of fear to an extinguished conditioned stimulus. *Journal of Experimental Psychology: Animal Behavior Processes, 1*, 88-96. doi:10.1037/0097-7403.1.1.88

Rescorla, R. A., & Skucy, J. C. (1969). Effect of response-independent reinforcers during extinction. *Journal of Comparative & Physiological Psychology, 67*, 381-389. doi:10.1037/h0026793

Rescorla, R. A., & Wagner, A. W. (1972). A theory of Pavlovian conditioning: Variations in the effectiveness of reinforcement and nonreinforcement. In A. H. Black & W. F. Prokasy (Eds.), *Classical conditioning II: Current research and theory* (pp. 64-99). New York: Appleton-Century-Crofts.

Reynolds, G., Field, A. P., & Askew, C. (2015). Preventing the development of observationally learnt fears in children by devaluing the model's negative response. *Journal of Abnormal Child Psychology, 43*, 1355-1367. doi:10.1007/s10802-015-0004-0

Ricker, S. T., & Bouton, M. E. (1996). Reacquisition following extinction in appetitive conditioning. *Animal Learning & Behavior, 24*, 423-436. doi:10.3758/bf03199014

Rodriguez-Ortiz, C. J., & Bermúdez-Rattoni, F. (2017). Determinants to trigger memory reconsolidation: The role of retrieval and updating information. *Neurobiology of Learning and Memory, 142*, 4-12. doi:10.1016/j.nlm.2016.12.005

Rubin, R. D. (1976). Clinical use of retrograde amnesia produced by electroconvulsive shock: A conditioning hypothesis. *Canadian Psychiatric Association Journal, 21*, 87-90. doi:10.1177/070674377602100205

Runyan, J. D., Moore, A. N., & Dash, P. K. (2019). Coordinating what we've learned about memory consolidation: Revisiting a unified theory. *Neuroscience & Biobehavioral Reviews, 100*, 77-84. doi:10.1016/j.neubiorev.2019.02.010

Sara, S. J. (2000). Retrieval and reconsolidation: Toward a neurobiology of remembering. *Learning & Memory, 7*, 73-84. doi:10.1101/lm.7.2.73

Schafe, G. E., & LeDoux, J. E. (2000). Memory consolidation of auditory pavlovian fear conditioning requires protein synthesis and protein kinase A in the amygdala. *Journal of Neuroscience, 20*(18), RC96-RC96. doi:10.1523/jneurosci.20-18-j0003.2000

Schafe, G. E., Nader, K., Blair, H. T., & LeDoux, J. E. (2001). Memory consolidation of Pavlovian fear conditioning: A cellular and molecular perspective. *Trends in Neurosciences, 24*, 540-546. doi:10.1016/S0166-2236(00)01969-X

Scheveneels, S., Boddez, Y., Vervliet, B., & Hermans, D. (2016). The validity of laboratory-based treatment research: Bridging the gap between fear extinction and exposure treatment. *Behaviour Research and Therapy, 86*, 87-94. doi:10.1016/j.brat.2016.08.015

Schiller, D. (2014). A lighter shade of trauma. *Biological Psychiatry, 76*, 838-839. doi:10.1016/j.biopsych.2014.09.016

Schiller, D., Cain, C. K., Curley, N. G., Schwartz, J. S., Stern, S. A., LeDoux, J. E., & Phelps, E. A. (2008). Evidence for recovery of fear following immediate extinction in rats and humans. *Learning & Memory, 15*, 394-402. doi:10.1101/lm.909208

Schiller, D., Kanen, J. W., LeDoux, J. E., Monfils, M.-H., & Phelps, E. A. (2013). Extinction during reconsolidation of threat memory diminishes prefrontal cortex involvement. *Proceedings of the National Academy of Sciences, 110*, 20040-20045. doi:10.1073/pnas.1320322110

Schiller, D., Monfils, M.-H., Raio, C. M., Johnson, D. C., LeDoux, J. E., & Phelps, E. A. (2010). Preventing the return of fear in humans using reconsolidation update mechanisms. *Nature, 463*, 49-53. doi:10.1038/nature08637

Schiller, D., Monfils, M.-H., Raio, C. M., Johnson, D. C., Ledoux, J. E., & Phelps, E. A. (2018). Addendum: Preventing the return of fear in humans using reconsolidation update mechanisms. *Nature, 562*(7727), E21. doi:10.1038/s41586-018-0405-7

Schiller, D., & Phelps, E. A. (2011). Does reconsolidation occur in humans? *Frontiers in Behavioral Neuroscience, 5*(24), 1-12. doi:10.3389/fnbeh.2011.00024

Schroyens, N., Beckers, T., & Kindt, M. (2017). In search for boundary conditions of reconsolidation: A failure of fear memory interference. *Frontiers in Behavioral Neuroscience, 11*(65), 1-13. doi:10.3389/fnbeh.2017.00065

Schultz, D. H., Balderston, N. L., Geiger, J. A., & Helmstetter, F. J. (2013). Dissociation between implicit and explicit responses in postconditioning UCS revaluation after fear conditioning in humans. *Behavioral Neuroscience, 127*, 357-368. doi:10.1037/a0032742

Schultz, W., & Dickinson, A. (2000). Neuronal coding of prediction errors. *Annual Review of Neuroscience, 23*, 473-500. doi:10.1146/annurev.neuro.23.1.473

Seligman, M. E. P. (1971). Phobias and preparedness. *Behavior Therapy, 2*, 307-320. doi:10.1016/S0005-7894(71)80064-3

Sevenster, D., Beckers, T., & Kindt, M. (2012). Retrieval per se is not sufficient to trigger reconsolidation of human fear memory. *Neurobiology of Learning and Memory, 97*, 338-345. doi:10.1016/j.nlm.2012.01.009

Sevenster, D., Beckers, T., & Kindt, M. (2013). Prediction error governs pharmacologically induced amnesia for learned fear. *Science, 339*, 830-833. doi:10.1126/science.1231357

Sevenster, D., Beckers, T., & Kindt, M. (2014). Prediction error demarcates the transition from retrieval, to reconsolidation, to new learning. *Learning & Memory, 21*, 580-584. doi:10.1101/lm.035493.114

Shiban, Y., Wittmann, J., Weissinger, M., & Mühlberger, A. (2015). Gradual extinction reduces reinstatement. *Frontiers in Behavioral Neuroscience, 9*(254), 1-11. doi:10.3389/fnbeh.2015.00254

Shihata, S., McEvoy, P. M., & Mullan, B. A. (2018). A bifactor model of intolerance of uncertainty in undergraduate and clinical samples: Do we need to reconsider the two-factor model? *Psychological Assessment, 30*, 893-903. doi:10.1037/pas0000540

Shihata, S., McEvoy, P. M., Mullan, B. A., & Carleton, R. N. (2016). Intolerance of uncertainty in emotional disorders: What uncertainties remain? *Journal of Anxiety Disorders, 41*, 115-124. doi:10.1016/j.janxdis.2016.05.001

Shin, K. E., & Newman, M. G. (2018). Using retrieval cues to attenuate return of fear in individuals with public speaking anxiety. *Behavior Therapy, 49*, 212-224. doi:10.1016/j.beth.2017.07.011

Siddle, D. A. T., Power, K., Bond, N., & Lovibond, P. F. (1988). Effects of stimulus content and postacquisition devaluation of the unconditioned stimulus on the retention of human electrodermal conditioning and relational-learning. *Australian Journal of Psychology, 40*, 179-193. doi:10.1080/00049538808259081

Sjouwerman, R., Niehaus, J., Kuhn, M., & Lonsdorf, T. B. (2016). Don't startle me—Interference of startle probe presentations and intermittent ratings with fear acquisition. *Psychophysiology, 53*, 1889-1899. doi:10.1111/psyp.12761

Soeter, M., & Kindt, M. (2010). Dissociating response systems: Erasing fear from memory. *Neurobiology of Learning and Memory, 94*, 30-41. doi:10.1016/j.nlm.2010.03.004

Soeter, M., & Kindt, M. (2011). Disrupting reconsolidation: Pharmacological and behavioral manipulations. *Learning & Memory, 18*, 357-366. doi:10.1101/lm.2148511

Soeter, M., & Kindt, M. (2015a). An abrupt transformation of phobic behavior after a post-retrieval amnesic agent. *Biological Psychiatry, 78*, 880-886. doi:10.1016/j.biopsych.2015.04.006

Soeter, M., & Kindt, M. (2015b). Retrieval cues that trigger reconsolidation of associative fear memory are not necessarily an exact replica of the original learning experience. *Frontiers in Behavioral Neuroscience, 9*(122), 1-10. doi:10.3389/fnbeh.2015.00122

Squire, L. R., Slater, P. C., & Chace, P. M. (1976). Reactivation of recent or remote memory before electroconvulsive therapy does not produce retrograde amnesia. *Behavioral Biology, 18*, 335-343. doi:10.1016/S0091-6773(76)92295-1

Steinfurth, E. C. K., Kanen, J. W., Raio, C. M., Clem, R. L., Huganir, R. L., & Phelps, E. A. (2014). Young and old Pavlovian fear memories can be modified with extinction training during reconsolidation in humans. *Learning & Memory, 21*, 338-341. doi:10.1101/lm.033589.113

Storsve, A. B., McNally, G. P., & Richardson, R. (2010). US habituation, like CS extinction, produces a decrement in conditioned fear responding that is NMDA dependent and subject to renewal and reinstatement. *Neurobiology of Learning and Memory, 93*, 463-471. doi:10.1016/j.nlm.2009.12.011

Storsve, A. B., McNally, G. P., & Richardson, R. (2012). Renewal and reinstatement of the conditioned but not the unconditioned response following habituation of the unconditioned stimulus. *Behavioural Processes, 90*, 58-65. doi:10.1016/j.beproc.2012.03.007

Tanovic, E., Gee, D. G., & Joormann, J. (2018). Intolerance of uncertainty: Neural and psychophysiological correlates of the perception of uncertainty as threatening. *Clinical Psychology Review, 60*, 87-99. doi:10.1016/j.cpr.2018.01.001

Tay, K. R., Flavell, C. R., Cassini, L., Wimber, M., & Lee, J. L. C. (2019). Postretrieval relearning strengthens hippocampal memories via destabilization and reconsolidation. *Journal of Neuroscience, 39*, 1109-1118. doi:10.1523/jneurosci.2618-18.2018

Telch, M. J., York, J., Lancaster, C. L., & Monfils, M. H. (2017). Use of a brief fear memory reactivation procedure for enhancing exposure therapy. *Clinical Psychological Science, 5*, 367-378. doi:10.1177/2167702617690151

Thomas, B. L., Cutler, M., & Novak, C. (2012). A modified counterconditioning procedure prevents the renewal of conditioned fear in rats. *Learning and Motivation, 43*, 24-34. doi:10.1016/j.lmot.2012.01.001

Thomas, B. L., Longo, C. L., & Ayres, J. J. B. (2005). Thwarting the renewal (relapse) of conditioned fear with the explicitly unpaired procedure: Possible interpretations and implications for treating human fears and phobias. *Learning and Motivation, 36*, 374-407. doi:10.1016/j.lmot.2004.11.005

Thomas, É., Saumier, D., Pitman, R. K., Tremblay, J., & Brunet, A. (2017). Consolidation and reconsolidation are impaired by oral propranolol administered before but not after memory (re)activation in humans. *Neurobiology of Learning and Memory, 142*, 118-125. doi:10.1016/j.nlm.2016.12.010

Thome, J., Koppe, G., Hauschild, S., Liebke, L., Schmahl, C., Lis, S., & Bohus, M. (2016). Modification of fear memory by pharmacological and behavioural interventions during reconsolidation. *PLoS One, 11*, e0161044. doi:10.1371/journal.pone.0161044

Thompson, A., & Lipp, O. V. (2017). Extinction during reconsolidation eliminates recovery of fear conditioned to fear-irrelevant and fear-relevant stimuli. *Behaviour Research and Therapy, 92*, 1-10. doi:10.1016/j.brat.2017.01.017

Thompson, A., McEvoy, P. M., & Lipp, O. V. (2018). Enhancing extinction learning: Occasional presentations of the unconditioned stimulus during extinction eliminate spontaneous recovery, but not necessarily reacquisition of fear. *Behaviour Research and Therapy, 108*, 29-39. doi:10.1016/j.brat.2018.07.001

Todd, T. P., Vurbic, D., & Bouton, M. E. (2014). Behavioral and neurobiological mechanisms of extinction in Pavlovian and instrumental learning. *Neurobiology of Learning and Memory, 108*, 52-64. doi:10.1016/j.nlm.2013.08.012

Treanor, M., Brown, L. A., Rissman, J., & Craske, M. G. (2017). Can memories of traumatic experiences or addiction be erased or modified? A critical review of research on the disruption of memory reconsolidation and its applications. *Perspectives on Psychological Science, 12*, 290-305. doi:10.1177/1745691616664725

Tully, P. J., Zajac, I. T., & Venning, A. J. (2009). The structure of anxiety and depression in a normative sample of younger and older Australian adolescents. *Journal of Abnormal Child Psychology, 37*, 717-726. doi:10.1007/s10802-009-9306-4

Tylee, D. S., Gray, R., Glatt, S. J., & Bourke, F. (2017). Evaluation of the reconsolidation of traumatic memories protocol for the treatment of PTSD: A randomized, wait-list-controlled trial. *Journal of Military, Veteran and Family Health, 3*, 21-33. doi:10.3138/jmvfh.4120

van den Akker, K., Havermans, R. C., & Jansen, A. (2015). Effects of occasional reinforced trials during extinction on the reacquisition of conditioned responses to food cues. *Journal of Behavior Therapy and Experimental Psychiatry, 48*, 50-58. doi:10.1016/j.jbtep.2015.02.001

Vervliet, B., Craske, M. G., & Hermans, D. (2013). Fear extinction and relapse: State of the art. *Annual Review of Clinical Psychology, 9*, 215-248. doi:10.1146/annurev-clinpsy-050212-185542

Vervliet, B., Vansteenwegen, D., & Hermans, D. (2010). Unpaired shocks during extinction weaken the contextual renewal of a conditioned discrimination. *Learning and Motivation, 41*, 22-31. doi:10.1016/j.lmot.2009.08.001

Vigil, F. A., & Giese, K. P. (2018). Calcium/calmodulin-dependent kinase II and memory destabilization: A new role in memory maintenance. *Journal of Neurochemistry, 147*, 12-23. doi:10.1111/jnc.14454

Visser, R. M., Lau-Zhu, A., Henson, R. N., & Holmes, E. A. (2018). Multiple memory systems, multiple time points: How science can inform treatment to control the expression of unwanted emotional memories. *Philosophical Transactions of the Royal Society B: Biological Sciences, 373*(1742), 20170209. doi:10.1098/rstb.2017.0209

Vurbic, D., & Bouton, M. E. (2014). A contemporary behavioral perspective on extinction. In F. K. McSweeney & E. S. Murphy (Eds.), *The Wiley Blackwell handbook of operant and classical conditioning* (pp. 53-76). Oxford, UK: John Wiley & Sons, Ltd.

Walsh, K. H., Das, R. K., Saladin, M. E., & Kamboj, S. K. (2018). Modulation of naturalistic maladaptive memories using behavioural and pharmacological reconsolidation-interfering

strategies: A systematic review and meta-analysis of clinical and 'sub-clinical' studies. *Psychopharmacology, 235*, 2507-2527. doi:10.1007/s00213-018-4983-8

Walther, E., Gawronski, B., Blank, H., & Langer, T. (2009). Changing likes and dislikes through the back door: The US-revaluation effect. *Cognition & Emotion, 23,* 889-917. doi:10.1080/02699930802212423

Warren, V. T., Anderson, K. M., Kwon, C., Bosshardt, L., Jovanovic, T., Bradley, B., & Norrholm, S. D. (2014). Human fear extinction and return of fear using reconsolidation update mechanisms: The contribution of on-line expectancy ratings. *Neurobiology of Learning and Memory, 113*, 165-173. doi:10.1016/j.nlm.2013.10.014

Watson, J. B., & Rayner, R. (1920). Conditioned emotional reactions. *Journal of Experimental Psychology, 3,* 1-14. doi:10.1037/h0069608

Weisman, J. S., & Rodebaugh, T. L. (2018). Exposure therapy augmentation: A review and extension of techniques informed by an inhibitory learning approach. *Clinical Psychology Review, 59,* 41-51. doi:10.1016/j.cpr.2017.10.010

Wideman, C. E., Jardine, K. H., & Winters, B. D. (2018). Involvement of classical neurotransmitter systems in memory reconsolidation: Focus on destabilization. *Neurobiology of Learning and Memory, 156,* 68-79. doi:10.1016/j.nlm.2018.11.001

White, K., & Davey, G. C. L. (1989). Sensory preconditioning and UCS inflation in human 'fear' conditioning. *Behaviour Research and Therapy, 27,* 161-166. doi:10.1016/0005-7967(89)90074-0

Wood, N. E., Rosasco, M. L., Suris, A. M., Spring, J. D., Marin, M.-F., Lasko, N. B., . . . Pitman, R. K. (2015). Pharmacological blockade of memory reconsolidation in posttraumatic stress disorder: Three negative psychophysiological studies. *Psychiatry Research, 225,* 31-39. doi:10.1016/j.psychres.2014.09.005

Woods, A. M., & Bouton, M. E. (2007). Occasional reinforced responses during extinction can slow the rate of reacquisition of an operant response. *Learning and Motivation, 38,* 56-74. doi:10.1016/j.lmot.2006.07.003

Xue, Y.-X., Luo, Y.-X., Wu, P., Shi, H.-S., Xue, L.-F., Chen, C., . . . Lu, L. (2012). A memory retrieval-extinction procedure to prevent drug craving and relapse. *Science, 336*, 241-245. doi:10.1126/science.1215070

Zbozinek, T. D., Hermans, D., Prenoveau, J. M., Liao, B., & Craske, M. G. (2015). Post-extinction conditional stimulus valence predicts reinstatement fear: Relevance for long-term outcomes of exposure therapy. *Cognition & Emotion, 29*, 654-667. doi:10.1080/02699931.2014.930421

Zhang, J. J., Haubrich, J., Bernabo, M., Finnie, P. S. B., & Nader, K. (2018). Limits on lability: Boundaries of reconsolidation and the relationship to metaplasticity. *Neurobiology of Learning and Memory, 154*, 78-86. doi:10.1016/j.nlm.2018.02.018

Zhu, H. W., Zhou, Y. M., Liu, Z. Y., Chen, X., Li, Y. Q., Liu, X., & Ma, L. (2018). Beta 1-adrenoceptor in the central amygdala is required for unconditioned stimulus-induced drug memory reconsolidation. *International Journal of Neuropsychopharmacology, 21*, 267-280. doi:10.1093/ijnp/pyx104

Zoellner, L. A., & Foa, E. B. (2016). Applying Research Domain Criteria (RDoC) to the study of fear and anxiety: A critical comment. *Psychophysiology, 53*, 332-335. doi:10.1111/psyp.12588

Zuccolo, P. F., & Hunziker, M. H. L. (2019). A review of boundary conditions and variables involved in the prevention of return of fear after post-retrieval extinction. *Behavioural Processes, 162*, 39-54. doi:10.1016/j.beproc.2019.01.011