



# **Fear learning via verbal instructions and stimulus pairings**

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**CHAPTER** **1**

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**GENERAL INTRODUCTION**

# DEEP DARK FEARS



WHEN I WAS LITTLE,



MY AUNT TOLD ME  
THAT A HUNGRY WORM  
LIVED IN MY NOSE.



IF I KEPT PICKING MY  
NOSE, IT WOULD BITE MY  
FINGERTIP OFF.



IT TERRIFIED ME.

FRAN KRAUSE 11/21/2013

Image reprinted from the illustrated book "Deep Dark Fears" by Fran Krause, with permission from the author.



## INTRODUCTION

In the face of danger, mammals show an adaptive behavior pattern, called the 'fight-or-flight' response, that mobilizes the organism to take appropriate action. These behavior patterns are accompanied by physiological arousal and subjective feelings of anxiety and stress. Together, the action tendencies (attack or flight), physiological arousal, and negative subjective apprehension are summarized with the term fear (Frijda, 1986; Lang, Bradley, & Cuthbert, 1998).

It is conceivable that what we fear has been shaped by our evolutionary history (Seligman, 1971). Some fears, such as fear for heights, open spaces and snakes, are far more common than other fears, such as for cars and guns, even though the latter probably pose a greater threat for physical harm for modern humans. Nevertheless, what we fear is not limited to a fixed set of innately fearful stimuli. Humans and other mammals possess the adaptive ability to learn to fear stimuli that are predictive of harmful or unpleasant events. This has been famously demonstrated with an experiment by Watson and Rayner (1920). In their study, Watson and Rayner (1920) showed that a young boy could learn to fear a rat when experience with the rat was repeatedly paired with a loud and aversive sound. Many subsequent experiments have provided further evidence that all three components of fear (behavior tendencies, physiological arousal and negative subjective apprehensions) can be installed through learning experiences (for an extensive overview of laboratory fear conditioning studies, see Öhman & Mineka, 2001).

Many instances of fear acquisition can be conceptualized in terms of classical conditioning (Field, 2006; Mineka & Zinbarg, 2006). For instance, in the study of Watson and Rayner (1920), the rat can be seen as a conditioned stimulus (CS) that was repeatedly paired with an aversive unconditioned stimulus (US) (the loud noise), the latter of which tends to automatically elicit fearful unconditioned reactions (URs). After repeated pairings of the CS and the US, the CS started to elicit a conditioned reaction (CRs) (fear of the rat). Note that this conceptualization provides a functional explanation of the fear for the rat. It refers to the effects of observable events (e.g., stimulus pairings) on a specific

behavior (e.g., expressions of fear) but does not make any claims about mediating mental processes (De Houwer, 2011).

Whereas the conditioning approach to fear has been highly successful in generating a better understanding of fear and, importantly, in developing successful interventions to remedy pathological fear (McNally, 2007; Mineka & Zinbarg, 2006), it has been strongly criticized as well (e.g., Menzies & Clarke, 1995; Rachman, 1977). One important criticism is that stimulus pairings are not sufficient nor necessary for the acquisition of fear. That is, on the one hand, stimulus pairings are not sufficient because many people that have experienced some sort of trauma (e.g., being in a car accident) will not go on to develop an anxiety disorder (e.g., not all people that have been in a car accident will go on to develop fear for being in a car) (Beckers, Krypotos, Boddez, Eftting, & Kindt, 2013; Mineka & Zinbarg, 2006). On the other hand, stimulus pairings are not necessary because people may develop a fear for a certain stimulus without having experienced a pairing of this stimulus with a highly unpleasant or traumatic event (e.g., someone who develops fear for snakes in a Northern European country) (King, Eleonora, & Ollendick, 1998). Such observations have led theorists to propose that fear could also be acquired through other pathways, besides stimulus pairings, such as through verbal instructions and social observation (Field, 2006; Rachman, 1977, 1991).

Against this background, the aim of this PhD thesis was to investigate how fear is acquired via verbal instructions. As the opening cartoon of this chapter nicely demonstrates, verbal threat instructions can install intense fear that impacts on behavior (e.g., avoiding nose picking), cognitions (e.g., negative feelings about nose picking) and psychophysiological reactions (not in this illustration, but, see Cook & Harris, 1937; Grillon, Ameli, Woods, Merikangas, & Davis, 1991), that can persist for many years. Furthermore, in retrospective reports from phobic patients, verbal information is often reported as a cause for the onset of pathological fear (King et al., 1998; Merckelbach, de Jong, Muris, & van den Hout, 1996). However, despite these reports, fear acquisition via verbal instructions remains poorly understood (Muris & Field, 2010; Olsson & Phelps, 2007). So far, few research programs have been initiated to systematically study

the properties of fear acquisition via verbal instructions, probably because research on fear acquisition has been largely dominated by a focus on the effects of stimulus pairings (Field, 2006; Mitchell, De Houwer, & Lovibond, 2009; Rachman, 1991). Therefore, the aim of the research in this PhD project was to come to a better understanding of the properties of fear acquisition via verbal instructions. Specifically, we wanted to test a number of predictions that can be derived from mental process models of fear learning that allowed to come to a better understanding of how fear can be learned via verbal instructions. Furthermore, we wanted to investigate several functional properties of fear learning via verbal instructions that may explain how intense and persistent fears can be installed via verbal instructions.

To introduce the research in this PhD thesis, I will first present three mental process models that try to explain how fear learning takes place for both learning through the pairing of stimuli and learning via verbal instructions. After describing these models, I will introduce the different chapters of the thesis and clarify how they tried to increase the functional knowledge of fear learning via verbal instructions and how they attempted to test some of the hypotheses that can be raised on the basis of mental process models of fear learning.

## **MENTAL PROCESS MODELS OF FEAR LEARNING VIA VERBAL INSTRUCTIONS**

### **Fear learning is the result of the formation and strengthening of mental associations**

An important idea in psychology is that associative learning (i.e., learning through the pairing of stimuli) is the result of the formation and strengthening of associative connections in memory. An association can be thought of as an unqualified connection between representations that allows for the spreading of activation. That is, when one of the representations is activated, it will (automatically) tend to activate the other representation. According to this model, repeated pairings of a CS and an aversive US will result in the formation of a strong association between the representation of these two stimuli. Due to

this association, subsequent presentations of the CS will tend to activate the representation of the US, which in turn will tend to elicit a fearful CR.

According to Field (2006), the same mechanism also supports fear learning via verbal instructions (see also: Muris & Field, 2010). That is, the verbal information “stay away from this dog because it might bite” will allow for a strengthening of a mental association between the representation of the dog (the CS) and the representation of being bitten (the US). Thus, with this model, Field proposes that the process that allows for fear learning through verbal instructions is exactly the same as the process that allows for fear learning through the pairing of stimuli (and fear learning through social observation). However, it should be noted that many different models of association formation exist that differ in their predictions about when associations can be formed and expressed (e.g., Mackintosh, 1975; Rescorla & Wagner, 1972), as we will see later in this section.

### **Fear learning is the result of the formation of propositions**

A second model of (fear) learning is the single-process propositional learning model of Mitchell et al. (2009; see also De Houwer, 2009; Lovibond, 2011). According to this model, associative learning, and thus also fear conditioning, is the result of the non-automatic formation of propositions. Propositions are qualified beliefs about the relationship between events. These beliefs can be formed through stimulus pairings, verbal instructions, observation and reasoning alike. Importantly, these propositions or beliefs are formed through effortful reasoning processes (Mitchell et al., 2009, pp. 184). Hence, their formation requires time, attention, awareness and motivation. Nevertheless, once a belief or proposition is formed, it can later be automatically retrieved. Furthermore, such propositions can allow for the automatic elicitation of preparatory or anticipatory behavior such as (autonomic) fear reactions.

According to this model, fear learning via pairings of stimuli and fear learning via verbal instructions should strongly interact because they are both mediated by the same mental mechanism (Lovibond, 2003). Furthermore, on the

basis of this model, we can also predict that these two pathways of fear learning should have very similar properties.

It can be noted that the propositional account of fear learning is closely related to expectancy models of fear (Davey, 1997; Reiss, 1980), according to which fear is the result of the expectancy of encountering an aversive or harmful event in the presence of certain antecedent stimuli. However, the propositional account makes explicit that these expectancies are the result of the non-automatic formation of propositions (Lovibond, 2011), whereas other researchers could claim that these expectancies reflect the formation and activation of associations (e.g., Mineka & Öhman, 2002).

**Fear learning is the result of both the formation of propositions and the formation and strengthening of associations**

A third model of fear learning is the social fear learning model of Olsson and Phelps (2007). This model partly distinguishes between learning via the pairings of stimuli and learning through observation on the one hand, and learning through verbal instructions on the other hand. That is, all pathways of learning result in the formation of conscious propositional knowledge about the contingencies between events (in a similar fashion as described in the single-process propositional model). However, specifically learning through the pairing of stimuli and learning through social observation also allow for the formation of implicit associative memories in the amygdala (Olsson & Phelps, 2007, 2004) that are formed according to Hebbian learning principles (Blair, Schafe, Bauer, Rodrigues, & LeDoux, 2001). This latter associative learning process can be viewed as a relatively automatic and reflex-like process, as it can take place outside of conscious awareness, it is goal-independent and it cannot be controlled (LeDoux, 2014; Öhman & Mineka, 2001). Learning via verbal instructions, on the other hand, only results in the acquisition of conscious knowledge of the contingency between events, but does not allow for the formation of such associative fear memories in the amygdala (Olsson & Phelps, 2007, pp. 1100). Hence, according to this model, learning via the different pathways do not necessarily have to share similar properties. Other restricting

conditions may apply for the different pathways for when learning can take place and under which conditions learned information can be expressed. For instance, awareness of the CSs may be required to express learned fear that has been acquired via verbal instructions, but not for learned fear acquired through stimulus pairings or social observation (Olsson & Phelps, 2004).

### **Evaluation of the different models**

Simple single-process association formation models in which stimulus co-occurrence is a sufficient condition for associative learning have long been abandoned. For instance, demonstrations of the blocking effect (Kamin, 1969) have shown that the mere pairing of stimuli is not sufficient to support learning. More complex association formation models have been put forward, including models according to which association formation is affected by surprise (Rescorla & Wagner, 1972) and attention (Mackintosh, 1975). But even these models have difficulties accounting for the fact that associative learning is strongly affected by awareness (Dawson & Furedy, 1976) and reasoning (De Houwer, Vandorpe, & Beckers, 2005). Likewise, associative models are unlikely to hold for fear learning via verbal instructions as well. For instance, the instruction “this picture will be followed by an electric shock” does result in the establishment of autonomic fear reactions to the picture (e.g., Cook & Harris, 1937), whereas the instruction “this picture will NOT be followed by an electric shock” does not result in autonomic fear reactions (e.g., Sevenster, Beckers, & Kindt, 2012), even though the representations of the picture and the shock are probably being activated together while reading both sentences. Such a result is difficult to explain with a simple association formation account.

For these and other reasons, most contemporary learning researchers agree that controlled reasoning processes are involved in associative (fear) learning. A central question in contemporary learning research is whether there is a need to assume also other (i.e., associative) learning processes (McLaren et al., 2014; Shanks, 2010). As noted above, the social fear learning model of Olsson and Phelps (2007) argues that learning through controlled reasoning processes is supplemented with learning via simple association formation,

specifically for learning via stimulus pairings and learning through social observation (see also: LeDoux, 2014; Öhman & Mineka, 2001), but not for fear learning via verbal instructions. A key question for understanding fear learning via verbal instructions becomes then: Are there certain properties of fear learning through stimulus pairings and social observation that do not apply for fear learning through verbal instructions, and the other way around? And if so, when and how do the properties of fear learning from the different pathways differ? The answer to these questions will inform us whether the learning processes of the different pathways of fear learning are indeed partly independent and under which conditions the learning processes of the different pathways contribute to the acquisition of fear.

## INTRODUCTION OF THE CHAPTERS

### **Chapter 2: Fear expression and return of fear with or without actual contingency experience**

In this chapter, we investigated whether fear learning via verbal instructions and via stimulus pairings can have additive effects. Every model of fear learning would predict that the effects of verbal instructions and of stimulus pairings can be additive under certain conditions. That is, a single-process propositional model could predict that stimulus pairings can add to the effects of verbal instructions because it might add to the truth validity of the proposition that was installed through verbal instructions. Similarly, an associative model could predict that a verbal instruction installs a CS-US association that is further strengthened by subsequent CS-US pairings (see Raes, De Houwer, De Schryver, Brass, & Kalisch, 2014, for an extensive discussion). However, some measures, such as the startle reflex, are believed to reflect primarily the implicit fear memory represented in the amygdala (Hamm & Weike, 2005; Sevenster et al., 2012). According to the social fear learning model of Olsson and Phelps (2007), additive effects of CS-US pairings should therefore be primarily pronounced for these measures because only stimulus pairings are believed to install such

implicit fear memories. This hypothesis was investigated in this chapter using a paradigm developed by Raes et al. (2014).

### **Chapter 3: Does confirmation of verbal threat modulate early visual processing?**

In Chapter 3, we investigated whether fear learning via verbal instructions and via stimulus pairings can have additive effects on early sensory processing as measured by Event-Related Potentials (ERPs). Previous studies have shown that both threat information and fear conditioning through stimulus pairings can enhance components related to early sensory processing. So far, to our knowledge, no study had investigated whether the combination of these two pathways of fear acquisition can have an additive effects on the enhancement of early sensory processing. Because this enhanced sensory processing for fearful stimuli is believed to be due attention gain mechanisms afforded by the amygdala (Pourtois, Schettino, & Vuilleumier, 2013), dual process models of fear learning would predict that such enhancement of components related to early sensory processing should be particularly pronounced when threat information is combined with stimulus pairings.

### **Chapter 4: Can prepared fear conditioning result from verbal instructions?**

In this fourth chapter, we investigated whether verbal threat instructions can produce prepared learning effects. That is, for fear learning via stimulus pairings, previous research has demonstrated that fear acquisition is facilitated and extinction of conditioned fear is delayed for fear-relevant CSs (such as pictures of snakes and spiders) as compared to fear-irrelevant CSs (such as pictures of birds or butterflies) (Öhman & Mineka, 2001). These prepared learning effects are believed to be the result of the operation of a specifically evolved fear learning module centered on the amygdala (Mineka & Öhman, 2002; Öhman & Mineka, 2001). Because verbal instructions do not allow for the formation of fear memories in the amygdala according to dual process models of fear learning, we would predict that such prepared learning effects cannot be



obtained through verbal threat instructions. This prediction was tested in this Chapter 4.

### **Chapter 5: Potentiation of the startle reflex is in line with contingency reversal instructions rather than the conditioning history**

In Chapter 5 we investigated whether verbal instructions can overturn fear reactions installed through stimulus pairings and through verbal instructions. If stimulus pairings install an implicit fear memory, verbal instructions might not be sufficient to overturn this fear memory (Hugdahl, 1978). Furthermore, this should be particularly be pronounced for measures that reflect this implicit fear memory such as the startle reflex (Hamm & Weike, 2005; Sevenster et al., 2012). In contrast, verbal instructions are not assumed to install such an implicit fear memory. Rather, if verbal instructions install a conscious accessible proposition about the contingencies between events, convincing instructions could probably easily overturn these propositions and install new propositions of how events are related. These hypotheses were tested in Chapter 5.

### **Chapter 6: The impact of a context switch and context instructions on the return of verbally conditioned fear**

Finally, in Chapter 6 we investigated whether renewal of fear memories can be found when fear is established through verbal instructions. That is, it is well known that extinction (i.e., the presentation of a CS without reinforcement) does not result in the erasure of fear memory, but rather creates a competing inhibitory memory that suppresses the fear memory within a specific context. This has been demonstrated by fear renewal studies, in which fear is acquired within a certain context A and extinguished in another context B. When participants are subsequently tested in the acquisition context A, or a new context C, fear tends to return compared to when the context is not switched after the extinction phase. However, so far no study has investigated whether extinction of verbally established fear memories is equally context-dependent. If a single underlying process is responsible for both fear learning through stimulus pairings and through verbal instructions, we would expect that context

dependent extinction applies to both pathways of learning. This was investigated in this final chapter. Furthermore, we investigated whether verbal information about the relevance of the context for the presence of the electrical shock could strengthen the contextual control of fear memories. If the renewal effect is due to propositions participants form about when it is likely to expect a shock, such contextual information should be able to strengthen the renewal effect. This prediction was also investigated in this final empirical chapter.

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# CHAPTER 2

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## FEAR EXPRESSION AND RETURN OF FEAR FOLLOWING THREAT INSTRUCTION WITH OR WITHOUT DIRECT CONTINGENCY EXPERIENCE<sup>1</sup>

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

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<sup>1</sup>Based on Mertens, G., Kuhn, M., Raes, A. K., Kalisch, R., De Houwer, J., & Lonsdorf, T. B. (2015). Fear expression and return of fear following threat instruction with or without direct contingency experience. *Cognition and Emotion*, (ahead-of-print), 1–17. doi: 10.1080/02699931.2015.1038219





## INTRODUCTION

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

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

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

To date, however, few studies have looked at the joint effects of the actual experience of and instructions about CS-US contingencies on fear expression and ROF. This could not only shed light on the unique contribution of both pathways to fear but also on their interaction. An additive effect of experience and instruction is expected on the basis of conditioning theories for phobic fear. For instance, Mineka and Zinbarg (2006) proposed that a trauma (e.g. being bitten by a dog) should lead to stronger fear reactions when it matches with previous beliefs on the trauma inducing stimulus (e.g. dangerous dog vs. non-dangerous dog). Similarly, it is known for decades that fear conditioning is more pronounced and extinction is attenuated when “biologically prepared” stimuli (e.g. snakes) are used as CSs (Öhman & Mineka, 2001). In line with this, it has been shown that the combination of threat information about an animal and an actual negative encounter indeed produced more fear in children than either threat information or a negative encounter alone (Field & Storksen-Coulson, 2007). Such an additive effect was, however, not observed in a similar study in adults (Ugland, Dyson, & Field, 2013). In these two studies however, participants were provided with general threat information rather than specific contingency instructions. Moreover, possible differences in US expectancy between the threat and no-threat groups were not controlled for.

These issues were addressed in a recent study by Raes, De Houwer, De Schryver, Brass, and Kalisch (2014) that compared reactions to two CSs, both of

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

If this post-hoc explanation of the results of Raes et al. (2014) is correct, also other indices that tap primarily into affective components of fear should reveal an impact of actual CS-US pairings beyond the impact of contingency instructions. Fear potentiated startle (FPS) is a prime candidate for such an affective index of fear. The startle response is a defensive reflex, measured at the orbicularis oculi muscle that can be elicited by a sudden high intensity noise

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

Finally, in a second extension of the design of Raes et al. (2014), we implemented a reinstatement procedure to complement the previous studies on the return of fear following reinstatement. The majority of studies on reinstatement in humans have used instructed acquisition (i.e., CS-US contingency instructions in combination with actual CS-US pairings) while extinction was with few exceptions uninstructed (Haaker et al., 2014). In these studies, instructed extinction as compared to uninstructed extinction leads to resistance to return of fear for SCRs and US expectancy ratings but not FPS (Sevenster et al., 2012a). Similarly, observational extinction (i.e., observing a third person being exposed to unreinforced post-acquisition CS trials) following regular fear conditioning also attenuated ROF following reinstatement (Golkar et al., 2013). While instructed and observational extinction seems to prevent the

ROF, explicit tests of the effect of instructed vs. uninstructed fear acquisition are still awaited (Haaker et al., 2014). In an attempt to shed light on this question, our study for the first time directly compares the return of fear following reinstatement between two instructed CSs that differ in the presence or absence of a history of direct CS-US contingency experience. Given the assumption that both contingency instructions and CS-US pairings can contribute to the development of (pathological) fear, information about the impact of both pathways on ROF could shed new light on the long-term outcome of treatment of (pathological) fear.

## METHOD

### Participants

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

### Materials

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

#### Experimental stimuli

Stimulus presentation was controlled with Presentation software (NeuroBehavioral Systems, Albany California, USA). Three blue snow fractals (200 by 200 pixels) in a white square presented in the center of a black background served as CSs (duration 8 s, see Figure 1) and a white fixation cross on a black

background served as the ITI (duration 13, 15 or 17 s). The US was an electro-tactile stimulus administered to the back of the right hand with a 1 cm diameter surface electrode with a platinum pin (Specialty Developments, Bexley, UK). It consisted of three 2 ms rectangular pulses with an inter pulse interval of 40 ms. US administration was controlled via a Digitimer DS7A constant current stimulator (Hertfordshire, UK). In the training phase a picture of a lightning bolt (approximately 200 by 200 pixels) presented for 500 ms was used as the placeholder for the US.

### **Subjective ratings**

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

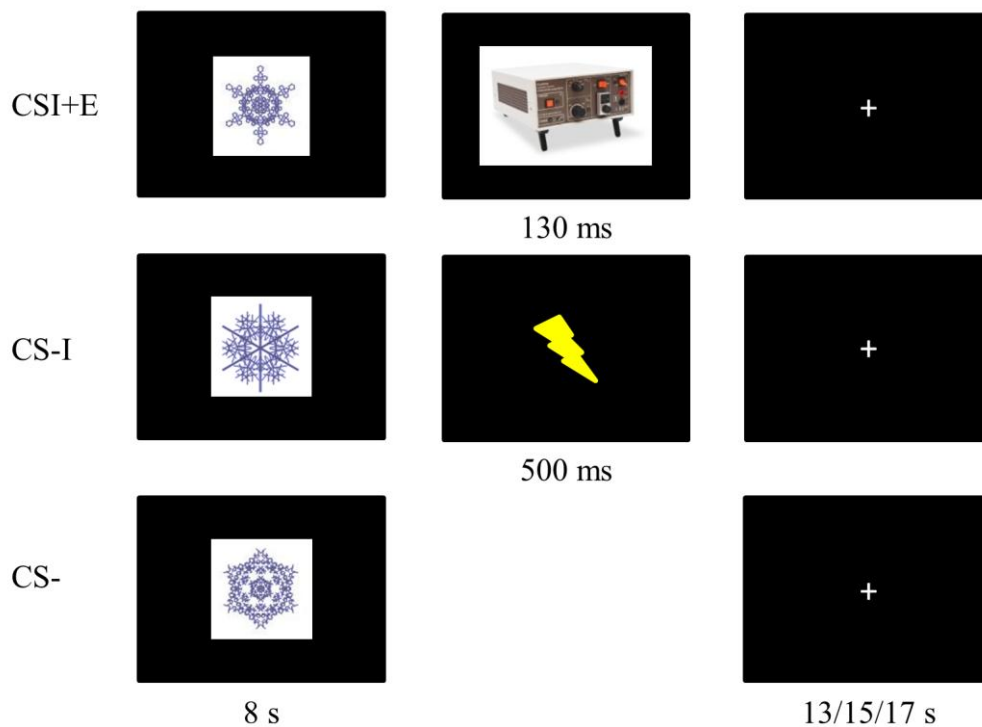
### **Manipulation checks**

After the experiment, pleasantness and pain ratings were collected for both the acquisition US and the reinstatement USs on 9-point Likert scales. Pleasantness ratings were accompanied by the caption “How pleasant/unpleasant did you find the electrical (unexpected/unsigned) stimulation?” and the anchors were: (1) very unpleasant, (5) uncertain, (9) very pleasant. Pain ratings were accompanied by the caption “How painful did you

find the (unexpected/unsigned) stimulation?" together with anchors: (1) totally not, (3) rather not, (5) uncertain, (7) rather much, (9) very much.

### Questionnaires

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



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

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

## Procedure

### Start-up

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

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

### Training phase

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



presentation was reinforced either for the CSI+E or CS-I in a counterbalanced fashion. Thus, in total two presentations of each CS type (CSI+E and CS-I) were reinforced.

### **Test phase**

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

### **Reinstatement and reinstatement test**

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

## **Psychophysiological recordings**

### **Skin conductance responses (SCR)**

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

normalize the data and range-corrected to account for individual differences in skin conductivity.

### **Fear potentiated startle**

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

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

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

Acquired data were scored offline with a custom-made program. Startle responses 20 - 120 ms post startle probe onset were scored (Blumenthal et al., 2005). Responses were treated as missing when confounded by recording artifacts or when spontaneous blinks occurred right before, during or right after the startle probe onset. Prior to analysis, FPS data were T-transformed. One

participant was excluded from FPS analyses because he had a large proportion of unusable trials for this measure (85.39 %).

### **Statistical analyses**

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

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

## **RESULTS**

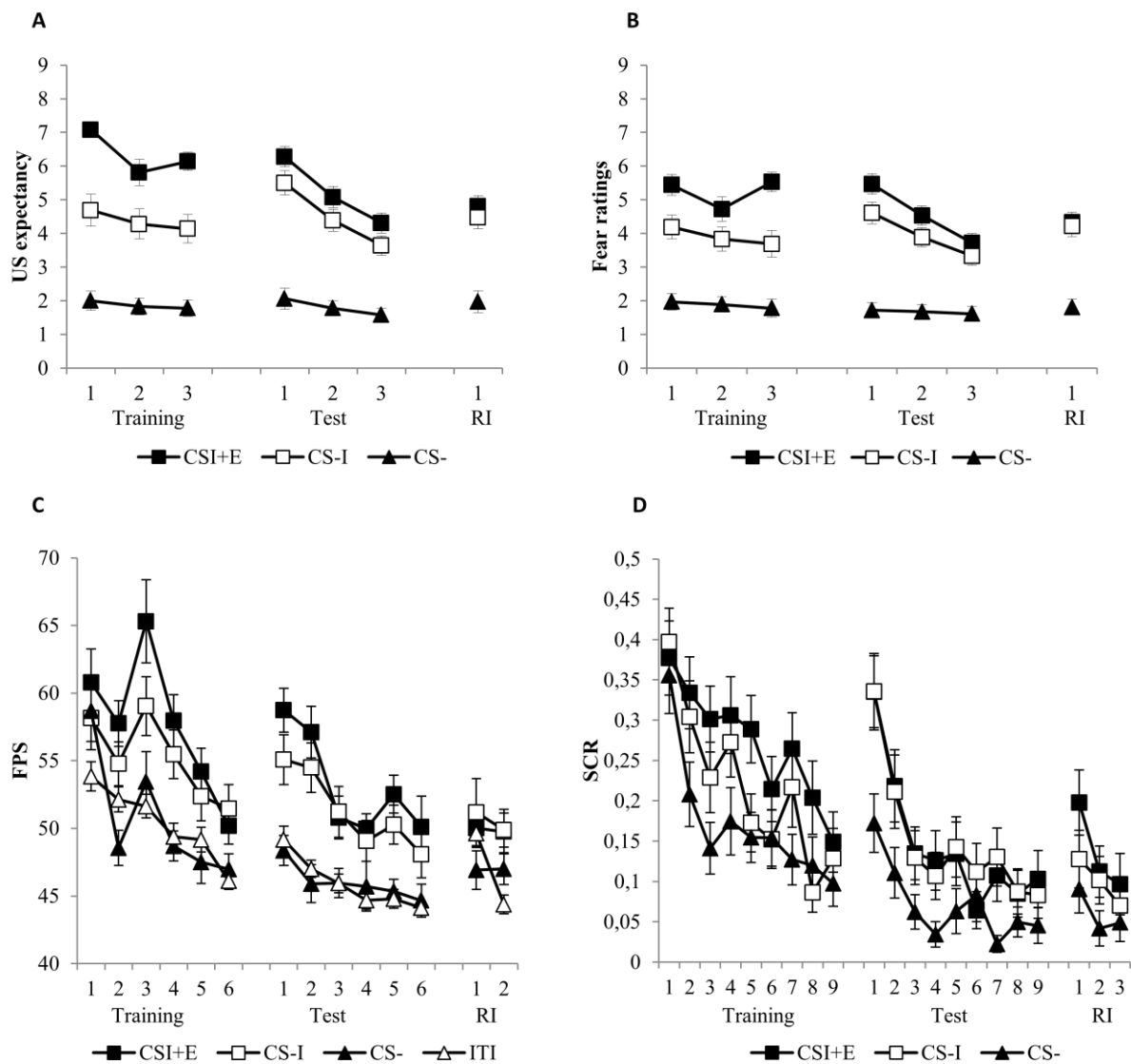
### **Manipulation checks**

Four participants who rated believability as assessed by the custom-made questionnaire as 5 or less and one participant who consistently rated the US as pleasant and not painful in the post-experiment manipulation check ratings were excluded from the analyses (see the Materials and Participants sections). The

remaining participants reported the instructions to be both clear (*mean* = 9.54, *SD* = 0.74) and believable (*mean* = 9.18, *SD* = 0.90). Furthermore, participants generally reported the US to be both rather unpleasant (*mean pleasantness rating* = 3.22, *SD* = 2.00) and moderately painful (*mean pain rating* = 6.33, *SD* = 1.17). Similar ratings were given for the reinstatement USs (*mean pleasantness rating* = 2.58, *SD* = 1.93; *mean pain rating* = 6.83, *SD* = 1.38).

### **Training phase**

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



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

## Test phase

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

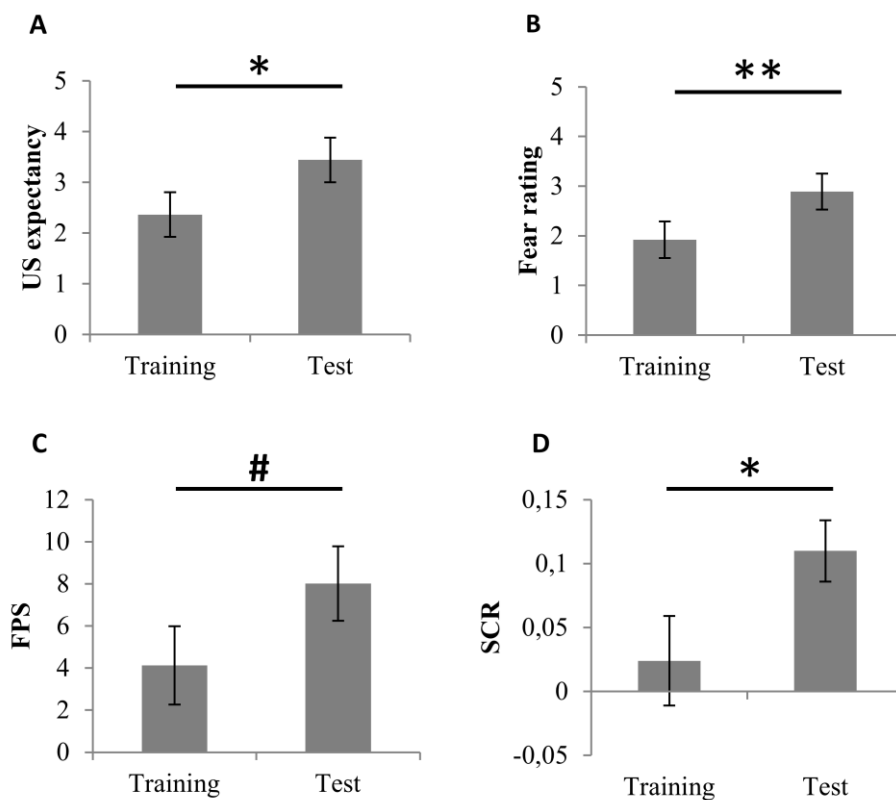
A significant main effect of block was also observed for all measures, all  $p$ -values  $< .001$ . Importantly, this main effect of block was qualified by an interaction between CS type and block for US expectancy, Fear ratings, and FPS, all  $p$ -values  $< .05$ . For US expectancy and Fear ratings,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

## Comparing training and test phase

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

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



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

## Reinstatement

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

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

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

DV	Df	F	Partial Eta <sup>2</sup>	p-value	Contrasts
US expectancy	1.75, 61.23	65.98	.65	< .001	a <sup>1</sup>
Fear ratings	2, 70	63.01	.64	< .001	a <sup>2</sup>
SCR	2, 70	9.03	.21	< .001	a <sup>3</sup>
FPS	3, 102	17.40	.34	< .001	b

DV: dependent variable

<sup>a</sup>All stimuli differ significantly or trend-wise ( $p < .1$ ) from each other:

- 1: CSI+E vs. CS-:  $F(1, 35) = 208.70$ ,  $p < .001$ ,  $Partial\ Eta^2 = .86$ ; CS-I vs. CS-:  $F(1, 35) = 32.80$ ,  $p < .001$ ,  $Partial\ Eta^2 = .48$ ; CSI+E vs. CS-I:  $F(1, 35) = 22.82$ ,  $p < .001$ ,  $Partial\ Eta^2 = .40$
- 2: CSI+E vs. CS-:  $F(1, 35) = 126.91$ ,  $p < .001$ ,  $Partial\ Eta^2 = .78$ ; CS-I vs. CS-:  $F(1, 35) = 45.24$ ,  $p < .001$ ,  $Partial\ Eta^2 = .56$ ; CSI+E vs. CS-I:  $F(1,35) = 19.06$ ,  $p < .001$ ,  $Partial\ Eta^2 = .35$
- 3: CSI+E vs. CS-:  $F(1, 35) = 14.62$ ,  $p = .001$ ,  $Partial\ Eta^2 = .30$ ; CS-I vs. CS-:  $F(1, 35) = 3.37$ ,  $p = .075$ ,  $Partial\ Eta^2 = .09$ ; CSI+E vs. CS-I:  $F(1, 35) = 7.57$ ,  $p = .009$ ,  $Partial\ Eta^2 = .18$

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

- CSI+E vs. CS-:  $F(1, 34) = 28.08$ ,  $p < .001$ ,  $Partial\ Eta^2 = .45$ ; CS-I vs. CS-:  $F(1, 34) = 11.62$ ,  $p = .002$ ,  $Partial\ Eta^2 = .26$ ; CSI+E vs. CS-I:  $F(1, 34) = 3.44$ ,  $p = .072$ ,  $Partial\ Eta^2 = .09$ ; ITI vs. CS-:  $F(1, 34) < 1$



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

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

DV: dependent variable

<sup>a</sup>All stimuli differ significantly from each other:

- 1: CSI+E vs. CS-:  $F(1, 35) = 104.17, p < .001, \text{Partial Eta}^2 = .75$ ; CS-I vs. CS-:  $F(1, 35) = 56.18, p < .001, \text{Partial Eta}^2 = .62$ ; CSI+E vs. CS-I:  $F(1, 35) = 18.94, p < .001, \text{Partial Eta}^2 = .35$
- 2: CSI+E vs. CS-:  $F(1, 35) = 95.55, p < .001, \text{Partial Eta}^2 = .73$ ; CS-I vs. CS-:  $F(1, 35) = 58.20, p < .001, \text{Partial Eta}^2 = .62$ ; CSI+E vs. CS-I:  $F(1, 35) = 18.77, p < .001, \text{Partial Eta}^2 = .35$

<sup>b</sup>For all measures, there is a significant linear decrease in responding over blocks:

- 1:  $F(1,35) = 32.82, p < .001, \text{Partial Eta}^2 = .48$
- 2:  $F(1,35) = 39.75, p < .001, \text{Partial Eta}^2 = .53$

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

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

<sup>c</sup>**The difference between both CSI+E and CS-I and CS- decreases over blocks, linear contrasts with block:**

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

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

<sup>d</sup>**CSI+E and CS-I both differ from CS-, but not from one another:**

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

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

CSI+E vs. CS-:  $F(1, 32) = 40.70, p < .001, \text{Partial } \eta^2 = .56$ ; CS-I vs. CS-:  $F(1, 32) = 21.27, p < .001, \text{Partial } \eta^2 = .40$ ; CSI+E vs. CS-I:  $F(1, 32) = 3.23, p = .082, \text{Partial } \eta^2 = .09$ ; ITI vs. CS-:  $F(1, 32) < 1$

<sup>f</sup>**The difference between CSI+E and CS- decreases over blocks, but not the difference between CS-I and CS- or CSI+E and CS-I, linear contrasts with block:**

CSI+E vs. CS- \* Block:  $F(1, 32) = 7.85, p = .009, \text{Partial } \eta^2 = .20$ ; CS-I vs. CS- \* Block:  $F(1, 32) = 2.20, p = .15, \text{Partial } \eta^2 = .06$ ; CS-I vs. CSI+E \* block:  $F(1, 32) = 1.45, p = .237, \text{Partial } \eta^2 = .04$

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

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

DV	Df	F	Partial Eta <sup>2</sup>	p-value
US expectancy	1, 35	6.04	.15	.019
Fear ratings	1, 35	8.96	.20	.005
SCR	1, 35	5.62	.14	.023
FPS	1, 31	2.93	.09	.097

**DV:** dependent variable

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

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

DV: dependent variable

<sup>a</sup>All stimuli differ significantly or trend-wise ( $p < 0.1$ ) from each other

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

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

<sup>b</sup>Post-reinstatement significantly stronger reactions than pre-reinstatement

<sup>c</sup>Response enhancement to CS-I differs significantly from CS- , but response enhancement

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to CSI+E and CS- and to CSI+E and CS-I does not differ:

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

<sup>d</sup>CSI+E differs significantly from CS- while all other stimuli do not differ

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

<sup>e</sup>CS-I differs significantly from CS- while all other stimuli do not differ

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

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

## DISCUSSION

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

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

First, results are in line with previous demonstrations that actual experience of CS-US contingencies enhances fear reactions beyond the effect of verbal instructions (Field & Storksen-Coulson, 2007; Raes et al., 2014). These and related results (e.g., Field & Storksen-Coulson, 2007) are in agreement with theories that highlight the role of conditioning in phobic fears which propose

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

Based on this evidence, it seems unlikely that experience only adds an effect beyond instructions for measures that tap the emotional but not a rather cognitive component of fear or for measures that capture subjective ratings as opposed to psychophysiological reactions. In fact, interesting differences between both psychophysiological measures emerged. While FPS reactions during the test phase showed only marginal effects of both CS-US contingency experience during the preceding phase (marginal CSI+E/CS-I difference during test) and situational threat instruction provided before the test phase (marginal change in CS-I/CS- difference from training to test), SCRs were not influenced by direct CS-US contingency experience during the preceding phase (no CSI+E/CS-I difference during test) but were very sensitive to situational threat information

(large change in CS-I/CS- difference from training to test). Thus, our results suggest that SCRs and FPS might be differentially sensitive to contingency instructions and direct experience respectively. This is in line with previous studies that demonstrated that FPS is less affected by verbal instructions and explicit contingency knowledge than SCR or subjective ratings (Sevenster et al., 2012a, 2012b). Alternatively, FPS might follow the initial threat information that both CSs will at a later time point be predictive of the US. This is reflected in a weak CSI+E/CS-I discrimination for FPS while both CSI+E and CS-I are potentiated against the CS- and the ITI. In sum, our data replicate previous findings that show an additive effect of experience and verbal threat information that, however, does not emerge in the same way in different dependent variables. Furthermore these inconsistencies between different dependent measures highlight the importance of multimodal assessment (e.g., different subjective measures and psychophysiological indicators of fear; see also: Mauss and Robinson, 2009).

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



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

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

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

conditions (Haaker et al., 2014). Nevertheless, our data illustrates how harmful and resistant verbally transmitted fears might be.

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

Our study might be extended in several ways. First, neutral stimuli were used as CSs in this experiment. It cannot be excluded that results would be different if fear-relevant or “biologically prepared” stimuli were used as CSs (Hugdahl & Öhman, 1977; Hugdahl, 1978; Lipp & Edwards, 2002), in particular as theories relying on conditioning models of phobic fears propose that a trauma should induce stronger effects when it matches previous beliefs (Mineka & Zinbarg, 2006). Hence, effects of actual CS-US pairings might be particularly pronounced when fear-relevant stimuli are used as CSs. Furthermore, it would also be interesting to investigate conditions in which the trauma does not match the beliefs, for instance, when a stimulus was previously experienced or instructed to be safe or has been predictive of a positive event (e.g. a reward). Previous studies with observational learning suggest that such prior positive information could be protective for later acquisition of fear (Egliston & Rapee, 2007; Mineka & Cook, 1986). Second, it would be interesting in future studies to not pair the CS-I during the training phase with the placeholder US. Such a procedure would allow us to strengthen the conclusion that CRs to CS-I during training are due to the threat instructions rather than to the pairings between CS-I and the placeholder. Related to this, it would be interesting to include a pretesting phase in which CRs to the CSs are measured before any instructions have been given. Including this phase would provide a baseline for each participant and each CS for the effect of the threat instructions. Finally, the fact that participants directly experienced the electro-tactile stimulus might have influenced how participants reacted to our threat instructions. Other studies investigating effects of threat instructions have often not exposed the participants to the US before the experiment (Olsson & Phelps, 2004; Soeter & Kindt, 2012). In future studies it would certainly be worthwhile to compare the effect of threat instructions between groups of participants that did or did not directly experience the US.

In sum, our data demonstrate that instructions represent a very powerful tool for the acquisition of fear and that verbal threat information can only partly be overridden by later situational safety information. We also demonstrate that

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direct experience can, at least for some dependent measures, have an effect beyond contingency instructions. Importantly, ROF as a model for clinical relapse, did not differ for fears that are acquired through instructions with or without compound CS-US experience. Taken together we provide evidence for the power and persistence of verbal threat information but also highlight the importance of considering different pathways to fear (direct experience, instructions) and stress the importance of multimodal assessment in experimental research.

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# CHAPTER 3

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## DOES CONFIRMATION OF VERBAL THREAT MODULATE EARLY VISUAL PROCESSING?<sup>1</sup>

*Fear conditioning procedures, that is, the pairing of a conditioned stimulus (CS) with an aversive unconditioned stimulus (US), can change the early sensory processing of the CS as evidenced by ERP. Similar modulations of early sensory processing have been obtained by merely providing CS-US contingency instructions. So far, however, no study has investigated whether direct experience of instructed contingencies adds to the effects of the instructions. To address this question, we instructed participants in a series of blocks about the contingency between two CS+s and an electric stimulus (US). A third CS was instructed to be safe. Novel CSs were selected in each block and only half of the instructed CS+s were actually paired with the US. We found a reduction of the P3 component due to the contingency instructions, but we did not find effects on earlier components (C1, P1 and N1). Furthermore, no additive effects of pairing the threatened CS with the US were found. We discuss these results in relation to the results of previous studies investigating the impact of fear conditioning and threat instructions on visual processing.*

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<sup>1</sup>Based on Mertens, G., Everaert, T., Rossi, V., & De Houwer, J. (Manuscript in preparation). Does confirmation of verbal threat modulate early visual processing?



## INTRODUCTION

Survival relies in part on the effective detection of threatening stimuli. Evidence for such facilitated processing of threatening stimuli comes from event-related potential (ERP) studies. These studies have found that early sensory responses are strengthened for threatening stimuli compared to neutral stimuli, suggesting that these stimuli are subject to preferential processing (Li, Howard, Parrish, & Gottfried, 2008; Phelps, Ling, & Carrasco, 2006; Pourtois, Grandjean, Sander, & Vuilleumier, 2004). It has been proposed that these effects are the result of attention gain control mechanisms mediated by the amygdala (Pourtois, Schettino, & Vuilleumier, 2013). Interestingly, this facilitated processing of threatening stimuli seems to be a flexible process. Initially neutral stimuli (or conditioned stimuli, CSs) that have been paired with an aversive event (or an unconditioned stimulus, US) also engage facilitated sensory processing compared to stimuli that were not paired with the US (for a review see: Miskovic & Keil, 2012). Hence, attention gain mechanisms can be flexibly deployed in response to newly acquired information. Furthermore, similar enhanced visual processing has even been obtained by merely threatening participants that a certain stimulus would be followed by an unpleasant event (Baas, Kenemans, Böcker, & Verbaten, 2002; Bublatzky & Schupp, 2012; Weymar, Bradley, Hamm, & Lang, 2013).

In the current study we were interested whether the latter two ways of learning about the threatening value of a stimulus can have additive effects with regard to the amplification of early sensory processing. More specifically, we were interested whether actual experience of CS-US pairings can add to the effect of contingency instructions. There are several reasons to suspect that such additive effects of instructions and actual experience could be obtained. First of all, research from our own laboratory has found that actually experiencing an instructed CS-US contingency can add to the effects of mere CS-US contingency instructions on subjective and physiological measures of fear (Mertens et al., 2015; Raes, De Houwer, De Schryver, Brass, & Kalisch, 2014; see also: Field &

Storksen-Coulson, 2007). These results fit with conditioning models of (maladaptive) fear acquisition which propose that prior negative beliefs can amplify the effects of a negative encounter (e.g., Davey, 1997; Field & Storksen-Coulson, 2007; Mineka & Zinbarg, 2006). Hence, both prior research and models of fear acquisition propose that threat information and negative experiences can have additive effects with regard to the experience of fear. In turn, based on these models and findings, it could be predicted that these two pathways of fear learning also have an additional impact on facilitating early stimulus processing. Another reason to expect that actual CS-US pairings might add to the effect of threat instructions is that actual CS-US pairing experience seem to recruit the amygdala into the learning process, whereas threatening instructions alone are believed to be insufficient for such amygdala mediated learning (Olsson & Phelps, 2004, 2007). This more affective way of learning due to CS-US pairing experience may also result in the facilitated processing of threatened CSs. Conversely, however, it could be argued that threat instructions result in a (near) asymptotic level of fear for a CS and that actual CS-US pairings might therefore not result in much additional fear. This, in turn, could suggest that CS-US pairings would not further modulate early processing of a threatened CS.

All in all, there are arguments for both the absence or presence of additive effects of CS-US pairings to the effects of threat instructions. Interestingly, the additive effects of these two pathways of fear acquisition could depend on which time point in the visual processing stream the additive effects are assessed. In a study of Bublatzky and Schupp (2012) such dynamic effects of two independent sources of threat were obtained. That is, early visually evoked event-related potentials (VEPs) were sensitive only to threat instructions but not to the inherent threatening properties of the pictures themselves, whereas later VEPs were sensitive to threat instructions and threatening picture contents in an additive fashion. Thus, continued stimulus processing resulted in more accurate representation of different sources of threat. In line with these results, it could be that ERPs related to early sensory processing are only sensitive to one source

of threat information, whereas later components may differentiate between our different sources of threat.

Hence, the study presented in this manuscript aimed at answering two related questions: (a) Whether additive effects of threat instructions and experience of CS-US pairings can be obtained with regard to the early processing of CSs and (b) whether the observation of these additive effects depends on continued stimulus processing. We have focused on several ERP components in the visual processing cascade that have been found to be affected by fear conditioning. A particularly striking example of modulation of early visual processing by fear conditioning is the amplification of the C1 response (Baas et al., 2002; Stolarova et al., 2006). The C1 component is usually observed around 60-90 ms post stimulus onset and its polarity depends on the retinotopic presentation of the stimulus. That is, stimuli presented in the upper half of the visual field elicit a negative C1, while stimuli presented in the lower half of the visual field elicit a positive C1. Given the early peak of this component, it is well suited to answer our research question on whether our two sources of threat can have additive effects on very early stimulus processing. Furthermore, we have looked at effects of threat instructions on two more components related to early visual processing that have previously been found to be affected by fear conditioning, the P1 and N1 (Liu, Keil, & Ding, 2012; Uglund, Dyson, & Field, 2013; Wong, Bernat, Bunce, & Shevrin, 1997). These two components are expressed later along the visual processing stream and may therefore be more sensitive to combined effects of our two sources of threat. Finally, a final component that we have considered is the P3 component. The P3 component is believed to reflect attention allocation (Böcker, Baas, Kenemans, & Verbaten, 2004; Polich, 2007) and has previously been found to be affected by fear conditioning (Baas et al., 2002; Weymar et al., 2013). Furthermore, given its relatively late latency, it should be particularly sensitive to additive effects of our two sources of threat (Bublitzky & Schupp, 2012). For all these different components, we expect amplitudes to be larger for the stimulus predicting an

aversive event (i.e., CS+) compared to the stimulus that does not predict the aversive event (i.e., CS-) (Ugland et al., 2013).

## METHOD

### Participants

Twenty-four students (6 men, 18 women) at Ghent University participated in the experiment in exchange for a monetary compensation. Age ranged between 20 and 34 years ( $M = 22.92$ ,  $SD = 2.76$ ). All participants filled in an informed consent and were instructed that they could discontinue the experiment at any point without any negative consequences. This study was approved by the ethics committee of the Faculty of Psychology and Educational Sciences of Ghent University. Two participants from this sample were excluded from the analyses because they were insufficiently attentive during the experiment.

### Material

#### Conditioned Stimuli

CSs were either Chinese, Mayan or Arabic symbols that were presented in arrays of six by fifteen stimuli that covered the whole upper half of a 19 inch Dell computer screen with a black background (resolution: 1024 by 768 pixels), above a fixation cross that was presented in the middle of the screen (see Figure 1). In each new block of the experiment (see further), new stimuli were randomly drawn from our stimuli set to ensure that the threatening properties of these stimuli were a function only of verbal instructions, rather than actual CS-US pairings.

#### Unconditioned Stimulus

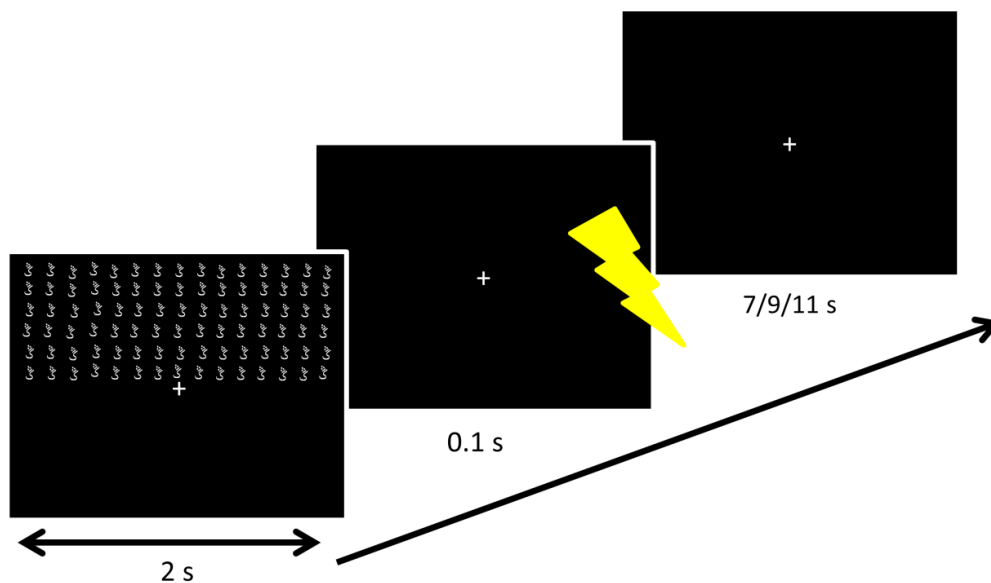
The US was an electrical stimulus that consisted of 10 rectangular pulses of 2 ms with and inter pulse interval of 8 ms, creating an electrical stimulus of 100 ms. This stimulus was administered by two lubricated Fukuda standard Ag/AgCl



electrodes (1-cm diameter; inter-electrode distance: ~2-cm) to the left leg over the retromalleolar course of the sural nerve. The stimulus was generated by a constant current stimulator (DS7A, Digitimer, Hertfordshire, UK). The intensity of the electrical stimulus was determined for each participant individually to be unpleasant but not painful using a stepwise work-up procedure (see the Procedure section).

### Questionnaires

Anxiety levels of the participants was assessed with the trait version of the State-Trait Anxiety Inventory (STAI-T; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983; Dutch translation: van der Ploeg, Defares, & Spielberger, 2000). STAI-T scores in our sample ranged between 24 and 54 ( $M = 34.5$ ;  $SD = 8.64$ ). Because STAI-T scores did not moderate any of the critical effects, we will not discuss this variable in the remainder of the manuscript.



**Figure 1.** Schematic overview of the trial procedure. Shock administration was limited to 50% of the trials on which a CS+s was presented.

## Procedure

### Work-up procedure

After filling in the informed consent and STAI-T, participants first went through a work-up procedure to determine the intensity level of the electrical stimulus. During this procedure, participants were exposed to gradually increasing stimulus intensity levels and were asked to report on their experience. Specifically, participants were asked after each intensity level to verbally rate the electric stimulus on a painfulness scale ranging from zero (not painful at all) to ten (maximally tolerable pain). A minimal painfulness threshold for the electric stimulus was set at seven. The procedure was stopped when participants indicated that they felt uncomfortable experiencing higher intensities of the electric stimulus. If a participant gave a rating of less than seven and indicated that he or she did not want to experience a more intense electric stimulus, the work-up was also stopped and the stimulus with the highest tolerated intensity was selected. The final selected electrical stimulus intensity levels ranged between 1.5 and 10 mA ( $M = 4.13$ ,  $SD = 1.84$ ) and pain ratings ranged between 6 and 9 ( $M = 7.5$ ,  $SD = 0.71$ ).

### Instructions

At the start of the experiment, participants were informed that they would see symbols appear on the computer screen. They were additionally informed that their task was to attend to the presentation of the stimuli while retaining their gaze fixated on a cross presented in the middle of the screen. Next, participants were informed that in each block of the experiment they would see three different symbols. They were further informed that two of these symbols could sometimes be followed by an electrical stimulus while the third stimulus would not be followed by the electrical stimulus. Furthermore, they were informed that the administration of the electrical stimulus after the symbols that could be followed by the electrical stimulus was determined completely at random and that this implied that the chance of getting an electric stimulus on those trials was completely independent of what happened on the previous

trials. These latter instructions were added because we wanted to avoid that participants would increase their expectancies after a non-reinforced CS (i.e., display the gambler's fallacy; e.g., McAndrew, Jones, McLaren, & McLaren, 2012). Results from a prior pilot study indicated that participants did not display this pattern, but rather slightly increased their expectancies after a reinforced CS (see also the expectancy ratings results in the Results section).

Before each block, participants were shown an exemplar of each of the three arrays of symbols (see Figure 1) that would be presented in that particular block together with information about which symbols would be predictive of an electrical stimulation and which symbol would be safe. The sentence "Can be followed by the electrical stimulation." was presented next to the two CS+s and the sentence "Can NOT be followed by the electrical stimulation." was presented next to the CS-. They were encouraged to remember this information well and reminded to keep their eyes fixated on the fixation cross. The three CSs were randomly drawn in each block from the three different families of symbols (Mayan, Arabic or Chinese symbols) and were hence easily distinguishable. There was no systematic relationship between the type of CS and the families of the symbols.

### **Stimulus presentation**

After the contingency instructions, stimuli were presented in short blocks of six trials (two presentations of each CS). They were presented in a pseudo-random order, implying that each stimulus had to be displayed once before a second presentation of any of the stimuli could occur. Importantly, this allowed us to distinguish between the first presentation of the stimuli, where learning could be established only on the basis of verbal instructions because no prior learning based on stimulus pairings could have occurred at this point, and the second presentation of the stimuli, where learning through verbal information and stimuli pairings is combined for those CSs that were paired with the US on the first presentation. Stimuli were presented above the fixation cross, occupying the whole upper half of the screen (see the Materials section and Figure 1). The fixation cross remained continuously on the screen throughout the blocks.

Stimuli were presented on the screen for 2 seconds and, in case of a CS+, could be followed by the electrical stimulus immediately at offset on half of the presentations (50% reinforcement rate). We refer to a CS+ that was paired with the electric stimulus as CS+T/P (threatened and paired) and a CS+ that was not paired with the electric stimulus a CS+T (threatened). The inter-trial interval was either 7, 9 or 11 seconds, randomly selected.

### **Expectancy ratings**

At the end of one third of the blocks (i.e., after 16 of the blocks), participants were asked to provide expectancy ratings about the CSs in that block to ensure that participants were able to remember the contingencies. On each trial, the CSs were presented in the upper half of the screen (as in the conditioning blocks) and participants were asked to rate to what extent they thought that these symbols would be followed by the electrical stimulus. They could indicate their answer by clicking one of the options on a 9-point Likert scale presented at the bottom of the screen. Anchor were “certainly not” (1), “unsure” (5) and “completely certain” (9). There was no response deadline for providing the expectancy ratings.

### **EEG acquisition, response definition and statistical comparisons**

An elastic cap was used to allow for the recording of the EEG through 64 Ag/AgCl electrodes that were distributed according to the extended 10/20 system. The signal was recorded with a Biosemi Active Two System (<http://www.biosemi.com>) and was referenced online to a CMS-DRL ground which drives the subject's average potential as close as possible to the reference voltage of the amplifier (i.e. the amplifier zero). Additionally, two external electrodes linking the mastoids were used to reference the data off line and 4 external electrodes served to monitor vertical and horizontal eye movements. EEGs were digitized at 512 Hz and were band-pass filtered off line between 0.016 and 70 Hz. An additional notch filter centered around 50 Hz reduced AC interference.

Off line computations were performed with Brain Vision Analyzer 2.0 (Brain Products, GmbH, Munich, Germany). Segmentation was performed relative to stimulus onset with an interval ranging from 100 ms before to 2000 ms after stimulus onset. We corrected for eye-blink artifacts using the standard algorithm of Gratton et al. (1983). Each segment was baseline corrected to the 100 ms pre-stimulus onset interval. Trials contaminated by residual artifacts were semi-automatically detected and rejected with a  $\pm 75 \mu\text{V}$  criterion relative to the baseline. Grand average waveforms were calculated separately for each CS (CS+T, CS+T/P, CS-).

Electrode sites for our components of interest were selected on the basis of previous reports and inspection of the scalp voltage distributions and are reported below. C1 peaks were identified in the 54-94 ms interval, P1 peaks in the 114-154 ms interval, N1 peaks in the 154-214 ms interval and P3 peaks in the 324-674 ms interval post stimulus onset (see also: Figure 3).

For the statistical comparisons, we have focused on the second presentation of the CSs only, because only at this point could stimuli that were previously paired (i.e., on the first trial) with the US be compared to stimuli that were not previously paired with the US (see the Stimulus presentation section). Voltages from our different components of interest were compared with repeated measures ANOVA's with the within-subjects factor CS (CS-, CS+T, CS+T/P). Violations of the sphericity assumption were corrected using Greenhouse-Geisser adjusted degrees of freedom.

## RESULTS

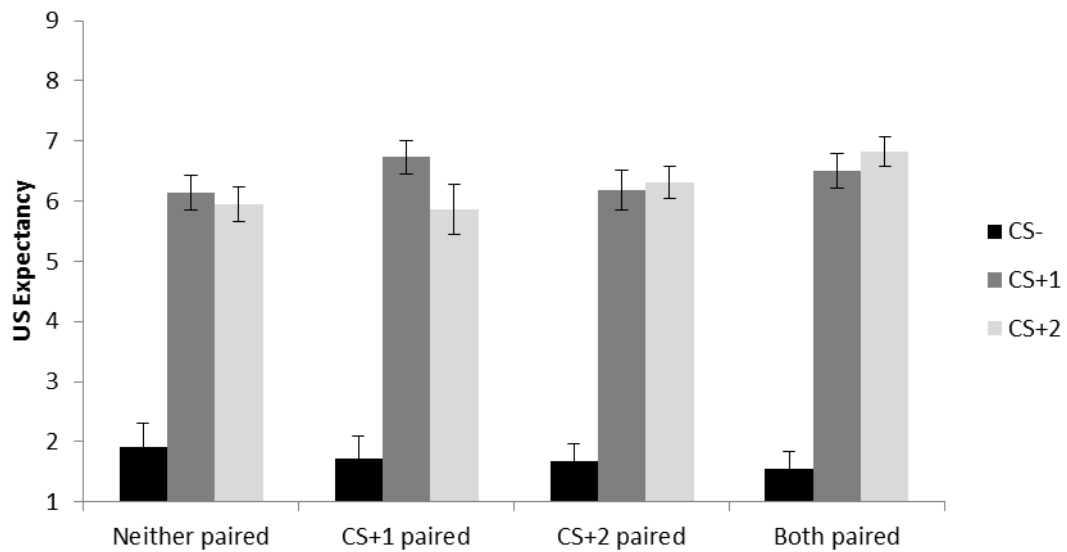
### US expectancy ratings

We selected four blocks after which participants provided US expectancy ratings in which either one of the CS+s was paired, both were paired or none were paired with the electric stimulus on the first presentation, and where none of the CSs were followed by the electric stimulus on the second presentation. Hence, with this selection of blocks, we could investigate how participants

adjusted their expectancies to the CS-US pairings on the first presentations, without interference of learning on the second presentations (because US expectancy ratings were provided at the end of the blocks and could therefore be influenced by learning on the second presentations of the stimuli as well). These results are summarized in Figure 2. These US expectancy ratings were analyzed with a repeated measures ANOVA with CS (CS-, CS+1, CS+2) and block (neither paired, CS+1 paired, CS+2 paired, both paired) as within-subject factors<sup>1</sup>. This analysis produced a significant effect of CS,  $F(1.37, 28.73) = 217.10, p < .001, \eta^2p = .91$ , but no significant effect of block,  $F < 1$ , and no significant interaction effect between CS and block,  $F(3.36, 70.51) = 1.55, p = .204, \eta^2p = .07$ . These results demonstrate that US expectancy ratings were very much in line with the threat instructions, illustrated by higher US expectancy ratings for CS+1 and CS+2 than for CS-, even in a block where these two CS+s were not actually paired with the US. We do not find, however, that US expectancy ratings were strongly modulated by CS-US pairings. That is, US expectancy were not specifically increased for the CS+ that was paired with the US, as illustrated by a lack of an interaction between CS and block. However, when we have a closer look at the US expectancy ratings, we do find some suggestive evidence that US expectancy ratings were slightly increased for a CS+ that was paired with the US in the block where CS+1 was paired with the US,  $t(21) = 1.93, p = .067, \text{Cohen's } d = 0.55$  (see Figure 2). A (near) significant difference between CS+1 and CS+2 was not observed in any of the other blocks,  $t$ 's  $< 1.6, p$ -values  $> .140$ .

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<sup>1</sup> Note that the analyses of the US expectancy ratings are more fine-grained than the analyses of the ERP components. This is due to the requirement of having a sufficient number of trials to detect differences on ERP components. Applying the analyses of the US expectancy ratings to the ERP components would entail only having 12 observations in each cell, which would render the signal-to-noise ratio too low for the analyses of the ERP components. Therefore, we collapsed cells according to whether CSs were safe, were threatened, or were threatened and paired with the US (see the EEG acquisition, response definition and statistical comparisons section), resulting in 48 observations in each cell.



**Figure 2.** US expectancy ratings for the three different CSs when either none of the CS+s were paired, CS+1 was paired, CS+2 was paired, or both were paired with the electric stimulus. Error bars reflect standard error.

### C1

No effects of the within-subject factor CS (CS-, CS+T/P, CS+T) for the amplitude of the C1 component were found for the Pz, Oz or POz electrode,  $F$ -values  $< 1$  (see Figures 3 and 4), suggesting that our threat instructions and the combination of the threat instructions with CS-US pairings were not successful to modulate this component.

### P1

No effects of the factor CS were found for the amplitude of the P1 component either on the same selection of electrodes (Pz, Oz or POz),  $F$ -values  $< 1$  (see Figures 3 and 4), again suggesting that our threat and CS-US pairing manipulations were unsuccessful to manipulate stimulus processing at this time-point.

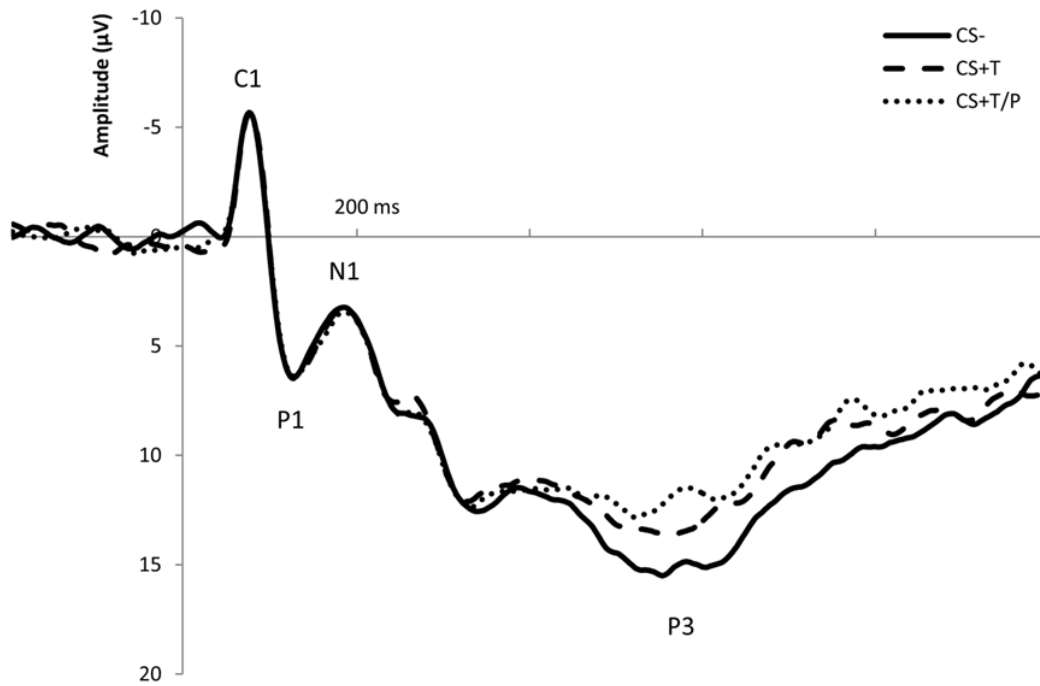
**N1**

No effects of the factor CS were found for the amplitude of the N1 component either on the Fz or Pz electrodes,  $F$ -values  $< 1$ , the FPz electrode,  $F(2, 42) = 1.22$ ,  $p = .306$ ,  $\eta^2p = .06$ , or the AFz electrode,  $F(1.63, 34.27) = 1.73$ ,  $p = .197$ ,  $\eta^2p = .08$  (see Figures 3 and 4). Again, these results suggest that our manipulations were unsuccessful to impact on stimulus processing at this time-point.

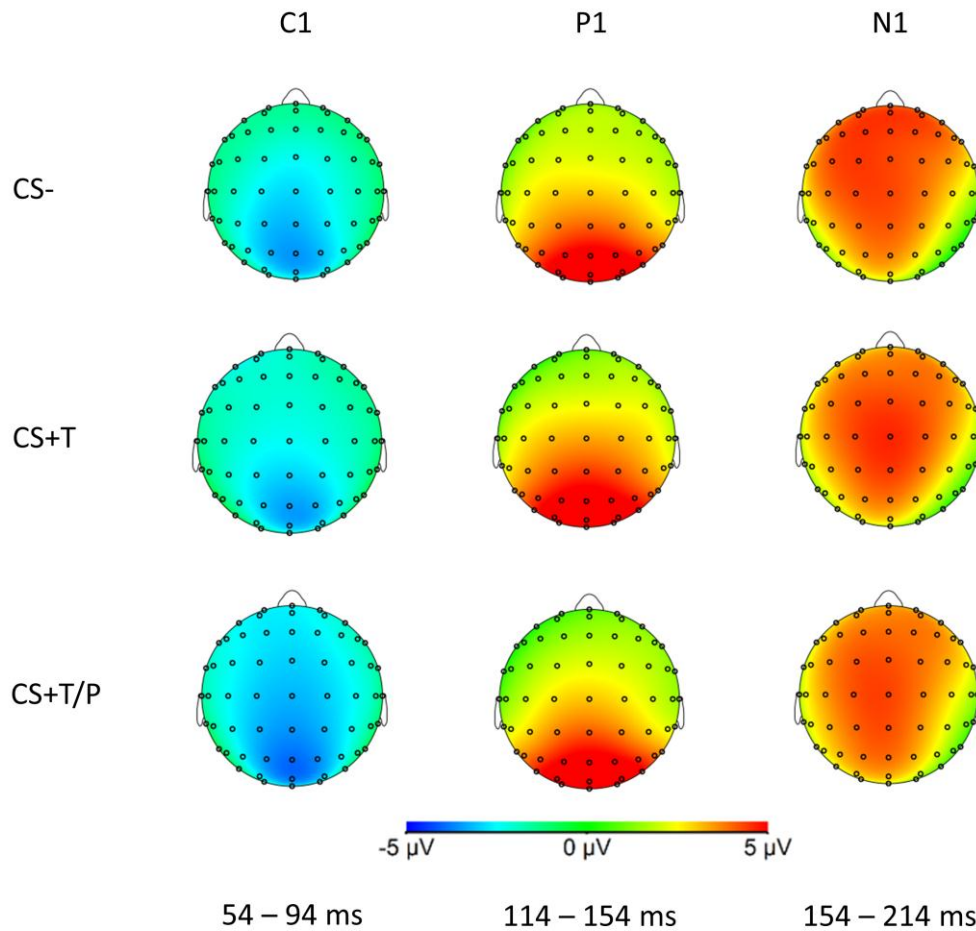
**P3**

A significant effect of the factor CS was found for the amplitude of the P3 component on the Pz electrode (no other electrodes were investigated),  $F(2, 42) = 4.38$ ,  $p = .019$ ,  $\eta^2p = .17$ . This effect was due to smaller P3 amplitudes for the CS+T ( $M = 12.29 \mu\text{V}$ ,  $SD = 9.88$ ),  $t(21) = 1.88$ ,  $p = .074$ , *Cohen's d* = 0.12, and the CS+T/P ( $M = 11.82 \mu\text{V}$ ,  $SD = 9.38$ ),  $t(21) = 2.86$ ,  $p = .009$ , *Cohen's d* = 0.18, compared to the CS- ( $M = 13.53 \mu\text{V}$ ,  $SD = 10.54$ ) (see Figure 3). The P3 amplitude for the CS+T and CS+T/P did not differ,  $t(21) < 1$ . Thus, these results demonstrate that threat instructions led to a reduction of the P3 component. Furthermore, our results suggest that actual experience of CS-US pairings did not add much to the effect of the threat instructions on the P3 amplitude.





**Figure 3.** Smoothed ERPs (20 Hz low-pass filter) relative to CS onset at the Pz electrode for the different CSs on their second presentation (see the Procedure and the EEG acquisition, response definition and statistical comparisons sections).



**Figure 4.** Scalp voltage distributions for the different CS types during the time intervals of the components of interest.

## DISCUSSION

In the current study we investigated whether actually experiencing instructed CS-US contingencies adds to the effects of these instructions. To this end, participants were on a block by block basis instructed about the contingency between certain symbols (i.e., CSs) and an electric stimulus (i.e., the US). New symbols were randomly selected in each block to ascertain that learning was only established on the basis of verbal instructions. Within the blocks, CSs that were instructed to be predictive of the US (i.e., CS+s) were reinforced with the US on 50% of the trials, allowing us to compare CS+s that were and were not paired with the US. Our results indicate that participants could remember which

symbols would be paired with the US, as evidenced by the higher US expectancy ratings for the CS+T and CS+T/P compared to the CS-. With regard to the ERP components, we did not find evidence that either the contingency instructions or the combination of contingency instructions and a CS-US pairing resulted in a modulation of components related to early visual processing. However, at later stages (on the P3b component) we did find differences between the different CSs. Specifically, the P3 amplitude was larger for the CS- compared to the CS+T and the CS+T/P. The P3 amplitude for these latter two CSs did not differ. We will discuss these results in more detail below.

First, contingency instructions resulted in higher US expectancy ratings for the instructed CS+ compared to the instructed CS-, even when in the block preceding the expectancy ratings no USs were delivered. This result replicates the well-known finding that verbal instructions can install conditioned fear (as measured by expectancy of the US in this case) (e.g., Cook & Harris, 1937; Hugdahl, 1978). Furthermore, experience of an instructed CS-US contingency seemed to further strengthen US expectancies, replicating previous results from our lab (Mertens & De Houwer, 2016; Mertens et al., 2015). However, it must be noted that the additional effect of a CS-US pairing on the US expectancies was quite modest, perhaps because the verbal contingency instructions installed a near asymptotic level of fear. This could in turn have limited the effects of a CS-US pairing on our other measures of conditioned fear.

Second, with regard to the strengthening of ERP components related to early sensory processing, we did not find any evidence that either contingency instructions or CS-US pairings were successful in modulating components related to early visual processing (i.e., C1, P1 and N1). This is a somewhat surprising result in light of many previous studies that did find modulations of these components due to fear conditioning (e.g., Baas et al., 2002; Bublatzky & Schupp, 2012; Liu et al., 2012; Stolarova et al., 2006; Ugland et al., 2013; Weymar et al., 2013; Wong, Shevrin, & Williams, 1994). However, this difference between these previous studies and our own study may be explained by our specific procedure. That is, new CSs were constantly selected throughout our experiment, whereas

in these earlier studies the same CSs or a certain shared feature of the CSs recurred throughout the experiment. This specific feature of our experiment may have rendered our participants insufficiently familiar with the CSs and therefore left participants unable to differentiate between the CSs very early after CS onset. It would be interesting in a future study to use stimuli with which participants are highly familiar or for which participants have a high expertise of discriminating (e.g., alphabetical letters, colors or human faces) and see whether modulations of early sensory processing on the basis of contingency instructions can be obtained for these stimuli. Alternatively, it could be that extended fear conditioning with the same stimuli (regardless of prior expertise) is necessary to obtain early modulations of sensory processing.

Finally, we did obtain an effect of contingency instructions on the P3 component. We did not find evidence, however, that this effect was further modulated by experience of a CS-US pairing. Furthermore, and surprisingly, the P3 amplitude was larger for the CS- rather than for the CS+T or the CS+T/P. This in contrast to our initial predictions, according to which we had expected that the P3 amplitude would be bigger for the CS+ and CS+T/P compared to the CS- (e.g., Baas et al., 2002; Weymar et al., 2013). A likely explanation for our finding could be that participants tended to pay more attention to the CS- because it was more infrequent (i.e., the CS- acted as an oddball) and/or because it was easier to remember the instructions by remembering the CS-, thereby making the CS- more relevant and resulting in a larger P3 for this stimulus. Furthermore, the CS- was the most reliable predictor of events in our experiment, predicting the absence of the US 100% accurately, again making this CS more relevant and thereby possibly increasing the P3 amplitude for this CS.

Combined, in the current study we did not find evidence for additive effects of a CS-US pairing to the effects of instructions, neither at the early visual level, nor at later processing stages. These results may be explained by certain features of our procedure such as a near asymptotic level of fear due to the contingency instructions, the use of unfamiliar stimuli as CSs, the lack of extended training of the contingencies, the use of an infrequent CS- and the use

of a 50% reinforcement rate for the CS+s. Further research will be required to disentangle how contingency instructions and experience of CS-US pairings interact to shape early sensory processing of stimuli.

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# CHAPTER 4

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## CAN PREPARED FEAR CONDITIONING RESULT FROM VERBAL INSTRUCTIONS?<sup>1</sup>

*Evolutionary fear-relevant stimuli such as snakes or spiders are thought to be prepared to elicit fear reactions. This implies that the acquisition of conditioned fear responses is facilitated when these stimuli serve as conditioned stimuli (CSs). Moreover, extinction of conditioned fear responses is delayed when CSs are prepared stimuli. The research presented in this article addresses the question whether such selective learning effects can be obtained even when participants do not experience pairings of CSs and US but receive only instructions about those pairings. Two experiments were conducted in which participants were verbally informed about the relationship between fear-relevant and fear-irrelevant CSs and the presence of an electrical stimulus (US). However, CSs were never actually paired with the US. US expectancy ratings and skin conductance responses were recorded during multiple CS only trials. In the first experiment, we observed acquisition, extinction and reinstatement of fear on the basis of instructions, but these effects were not modulated by the fear-relevance of the CSs. In the second experiment, we manipulated whether participants actually experienced the CS-US contingencies or were merely instructed. We obtained facilitated acquisition for the merely instructed fear-relevant CS+. We discuss these results in relation to the evolutionary fear learning model of Öhman and Mineka (2001) and the expectancy bias model of Davey (1992).*

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<sup>1</sup>Based on Mertens, G., Raes, A. K., & De Houwer, J. (2016). Can prepared fear conditioning result from verbal instructions? *Learning and Motivation*, 53, 7–23. doi:10.1016/j.lmot.2015.11.001



## INTRODUCTION

Fear conditioning in the lab is commonly established by repeatedly pairing an initial neutral stimulus (the Conditioned Stimulus or CS) with an aversive stimulus (the Unconditioned Stimulus or US), resulting in fearful reactions (or Conditioned Responses, CRs) to the initial neutral CS. However, direct pairings of the CS and US are not necessary to establish fearful CRs. Fearful reactions can also be established by providing participants with verbal information about the contingency between the CS and US, in the absence of any actual CS-US pairings (Field, 2006; Rachman, 1977). Previous research has demonstrated that verbal instructions can be a very powerful tool for inducing fear reactions (e.g., Cameron, Roche, Schlund, & Dymond, 2016; King, Eleonora, & Ollendick, 1998; Merckelbach, de Jong, Muris, & van den Hout, 1996; Muris & Field, 2010). Despite its potency, however, fear conditioning through verbal instructions is still poorly understood (e.g., Olsson & Phelps, 2007).

According to Olsson and Phelps (2007), fear conditioning through verbal instructions can be partly dissociated from learning via direct experience and learning via social observation (see also: Olsson & Phelps, 2004). That is, verbal instructions primarily result in cognitive contingency learning, while learning via direct experience and via social observation result in both contingency learning and affective learning. Affective learning is the acquisition of defensive responses to potentially threatening stimuli. This type of learning is proposed to take place in an automatic way and is assumed to be independent of cognitive contingency learning (Hamm & Weike, 2005; Mineka & Öhman, 2002). Cognitive contingency learning, on the other hand, refers to the purely cognitive learning of contingencies between events.

Alternatively, according to single-process models of associative learning (De Houwer, 2009; Mitchell, De Houwer, & Lovibond, 2009), learning is the result of the non-automatic formation of propositions. According to this view, there should not be any qualitative differences between pathways of learning because learning via all the different pathways is mediated by the same underlying

processes. Thus, verbal instructions should be able to result in learning on measures that are believed to capture affective components of learning as well. This is supported by a number of studies that show that verbal instructions can result in the acquisition of defensive responses (Cameron et al., 2016; Costa, Bradley, & Lang, 2015; Grillon, Ameli, Woods, Merikangas, & Davis, 1991) and subjective feelings of fear and distress (Raes, De Houwer, De Schryver, Brass, & Kalisch, 2014; Soeter & Kindt, 2012), which are considered to be affective measures of fear (Hamm & Weike, 2005; Soeter & Kindt, 2012). Such results call into question whether distinctions should be made between the processes underlying learning via verbal instructions and other types of learning.

Nevertheless, it may be that there are certain instances of affective learning that cannot be obtained through verbal instructions, thus requiring a multi-process account for the different pathways of fear acquisition. The most prototypical example of affective learning is perhaps prepared learning (Öhman & Mineka, 2001). Selective or prepared learning refers to the finding that the pairing of a fear-relevant CS (e.g., pictures of snakes or spiders) with an aversive US (e.g., an electric shock) produces a stronger CR that is more easily established or more resistant to extinction than CRs to fear-irrelevant CSs (e.g., a picture of a flower or a bird). The idea for a varying capacity of stimuli to become associated with an aversive event was introduced by Seligman (1971) in his preparedness theory. According to this theory, stimuli that were potentially threatening for survival in our ancestral history are more easily learned to be feared. This auxiliary assumption to learning theory could explain why certain types of phobias, such as these for heights and spiders, are more prevalent than others (Rachman, 1977). A large set of experiments have provided evidence for this preparedness theory in the lab using fear conditioning (for a review see: Öhman & Mineka, 2001).

It has been argued that prepared learning is due to the operation of a specific fear learning module (Mineka & Öhman, 2002; Öhman & Mineka, 2001). Features of this proposed module include selective activation in the presence of aversive events, automatic activation with a minimal amount of stimulus processing, and encapsulation from higher cognitive influences. Because of the

selective and automatic nature of this learning module, we would not expect that prepared learning is a property of fear conditioning via verbal instructions because it seems unlikely that verbal instructions provide the conditions to recruit this module in the learning process (Olsson & Phelps, 2004, 2007).

However, several previous experiments have provided evidence that prepared learning can be obtained via verbal instructions. Öhman, Eriksson, Fredriksson, Hugdahl and Olofsson (1974) and Davey (1992) both reported that threatening participants that a shock will follow the CSs during the experiment, without actually pairing the CSs with the US, potentiated fear reactions more in the group that saw fear-relevant CSs than in the group that saw fear-irrelevant CSs. However, because no non-threatened CSs had been included in these experiments, it is impossible to determine whether threat instructions generated specific potentiation of fearful reactions to the threatened fear-relevant CS+s, or generated a general potentiation of fearful reactions to all fear-relevant stimuli. While the former would be an instance of selective learning, the latter is not. In two other studies by Hugdahl and Öhman (1977) and Hugdahl (1978), participants were given instructions that one CS would be followed by a shock but the other CS would not. These instructions led to stronger acquisition effects (Hugdahl & Öhman, 1977) and to more resistance to instructed extinction (i.e., the combination of verbal CS-no US instructions and removal of the shock electrodes; Hugdahl, 1978) in the group receiving these instructions about fear-relevant CSs compared to the group receiving these instructions about fear-irrelevant CSs, even though participants had never actually experienced the instructed contingencies. These studies clearly show that prepared learning can be obtained when conditioning is established through verbal instructions, and thus further show that learning via verbal instructions and learning through direct experience of contingencies may be very similar. However, the fact that instructed extinction was less strong with fear-relevant than with fear-irrelevant CSs does demonstrate that there are limits to what can be learned through verbal instructions (Hugdahl, 1978), and seems to contradict a single-process account of fear learning (De Houwer, 2009; Mitchell et al., 2009).

Nevertheless, there are several caveats that potentially limit the interpretability of these experiments. First, the resistance to instructed extinction effect (Hugdahl & Öhman, 1977; Hugdahl, 1978; Öhman, Erixon, & Lofberg, 1975) has been difficult to replicate. In subsequent studies, the combination of an extinction phase with explicit instructions that USs would no longer be presented, resulted in a complete reduction of the CR for both fear-relevant and fear-irrelevant CSs (Lovibond, 2004; McNally, 1987). Second, there is a methodological issue that might complicate the interpretation of the results from Hugdahl and Öhman (1977) and Hugdahl (1978). In both experiments, a between-subjects design was used in which one group was verbally conditioned with fear-relevant CSs and the other group was verbally conditioned with fear-irrelevant CSs. Such a design is not optimal because differences between groups, such as elevated state-anxiety due to repeated exposure to fear eliciting stimuli (pictures of snakes and spiders), are not controlled for. Such uncontrolled differences in state-anxiety between groups may lead to alterations in conditioning that do not reflect prepared learning (e.g., Vriends et al., 2011). Recent studies on prepared learning therefore usually make use of a within-subject design (Ho & Lipp, 2014; Olsson, Ebert, Banaji, & Phelps, 2005). Finally, it is possible that the prepared learning in the studies of Hugdahl might reflect expectancy biases, that is, a bias to expect the US in the presence of a fear-relevant CS, and thus do not reflect learning through a specialized module that is independent of higher-order cognitions (Davey, 1992). Demonstrating that prepared learning effects are due to expectancy biases would challenge the need for a separate fear learning system (Öhman & Mineka, 2001). The studies of Hugdahl and Öhman (1977) and Hugdahl (1978) lacked the inclusion of expectancy ratings to determine whether the observed prepared learning effects were due to such expectancy biases.

Hence, so far it remains unclear whether prepared learning can be obtained with verbal instructions, and whether these prepared learning effects are due to expectancy biases. We conducted two new experiments employing a within-subjects design to investigate these issues and to thus shed new light on the important theoretical debate about the need to supplement a cognitive

(propositional) learning system with an affective fear learning module. In both experiments, participants received instructions about the contingency between fear-relevant and fear-irrelevant CSs, on the one hand, and an aversive US (an electrical stimulation), on the other hand. Within each fear-relevance category, one CS was instructed to be followed by the US while the other was instructed never to be followed by the US. After the instructions, a short test was given to make sure that participants understood and remembered the instructions correctly. After the instructions and test, participants continued to an unannounced extinction phase. Evidence for selective learning could be established during this phase through stronger CRs to the fear-relevant CS+ (i.e., facilitated acquisition) or through resistance to extinction of CRs for the fear-relevant CS+.

We collected skin conductance responses (SCR) and US expectancy ratings as dependent measures in both experiments. SCRs are traditionally measured in studies that investigated prepared fear learning, and have been shown to be sensitive to prepared learning effects. Inclusion of US expectancy ratings allowed us to evaluate whether obtained preparedness effects were due to expectancy biases (Davey, 1992). Furthermore, these two measures were supplemented with fear ratings in the second experiment. Fear ratings are considered to be a more affective measure of fear learning (Mertens et al., 2015; Soeter & Kindt, 2012) and may therefore be more sensitive to preparedness effects.

Additionally, in the second experiment, we manipulated whether the instructed contingencies were actually experienced or merely instructed. This was accomplished by adopting a procedure introduced by Raes, De Houwer, De Schryver, Brass and Kalisch (2014) that allowed us to manipulate on a within-subjects basis whether verbally threatened CSs are actually paired with the US. On the basis of the fear learning module account of Öhman and Mineka (2001), we expected that actually experiencing the instructed contingencies would strengthen prepared learning effects because effects of actual CS-US pairings are assumed to be mediated in part by the fear learning module (Olsson & Phelps, 2004, 2007).

Finally, we included a reinstatement shock after the extinction phase. So far, preparedness effects on the reinstatement of extinguished fear have not been investigated before, although several interesting predictions can be made with regard to the reinstatement of fear with fear-relevant stimuli as CSs. That is, given that reinstatement is believed to reflect the facilitated retrieval of the excitatory CS-US relationship learned during acquisition instead of the competing inhibitory CS-noUS relationship learned during extinction (Bouton, 2004; Haaker, Golkar, Hermans, & Lonsdorf, 2014), reinstatement should be less pronounced for fear-relevant CSs because less inhibitory learning (extinction) takes place for fear-relevant CSs. Alternatively, more reinstatement could be expected for fear-relevant CSs because acquisition is more pronounced for these CSs (Fredrikson, Hugdahl, & Öhman, 1976; Öhman, Fredrikson, & Hugdahl, 1978) and therefore the excitatory CS-US memory is more easily retrieved after a reinstatement manipulation. Including a reinstatement shock after the extinction phase in our study allowed us to explore these different predictions regarding reinstatement of fear for fear-relevant CSs.

## EXPERIMENT 1

### Participants

Thirty-seven right-handed psychology students at Ghent University participated in exchange for €8. Of these, the data of eight participants were excluded from analyses because they either did not believe the instructions ( $N = 5$ ), did not find the US sufficiently unpleasant ( $N = 1$ ), or both ( $N = 2$ ) (see below for more details on how this was assessed).

### Material

#### Psychophysiology

SCRs were collected using a Coulbourn Lablinc V system (Coulbourn Instruments, Allentown, PA) and standard Ag/AgCl electrodes (0.8 cm diameter)



filled with K-Y jelly. The electrodes were attached to the thenar and hypothenar eminences of the non-dominant hand. The signal was measured using a constant voltage coupler (0.5 V) and digitized at 10 Hz. The collected data were analyzed with Psychophysiological Analysis (PSPHA) (De Clercq, Verschuere, De Vlieger, & Crombez, 2006). SCRs were calculated by subtracting the mean value of a baseline period (2 seconds before CS onset) from the highest amplitude within a 1 to 8 s interval after CS onset (Pineles, Orr, & Orr, 2009). In this scoring method, negative values were recoded to zero. Finally, collected SCRs were range corrected with the highest recorded amplitude for that participant to account for individual differences in responsivity (Lykken & Venables, 1971) and square root transformed to normalize the data (Dawson, Schell, Filion, & Berntson, 2007).

### **US expectancy ratings**

US expectancy ratings were collected on each trial using a 9-point Likert scale presented below the CSs with 5 anchor points: 1 = “not at all”, 3 = “rather not”, 5 = “uncertain”, 7 = “to some extent”, 9 = “certainly”. Above the CSs the question: “To what extent do you expect the shock?” was presented. Participants indicated their answer by clicking one of the response options of the Likert scale with the computer mouse using their dominant hand.

### **Stimuli**

Four pictures of a snake, spider, bird and butterfly, each 570 by 400 pixels in size (15 by 10.5 cm), were used as CSs in the experiment. These were the same pictures as used in the first experiment from Olsson et al. (2005) in which prepared learning was observed. Assignment of the pictures to the role of CS+ and CS- was orthogonally counterbalanced over participants within each fear-relevance category. For the practice phase, a white and a yellow square were used, also 570 by 400 pixels in size.

The US was an electrical stimulus that consisted of 38 rectangular pulses of 2 ms with and inter pulse interval of 6 ms, creating a stimulus of approximately 300 ms. This stimulus was administered by two lubricated Fukuda standard Ag/AgCl electrodes (1-cm diameter) to the left leg over the retromalleolar course

of the sural nerve. The stimulus was generated by a constant current stimulator (DS7A, Digitimer, Hertfordshire, UK). The intensity of this stimulus was determined for each participant individually to be unpleasant but not painful using a stepwise work-up procedure (*mean intensity* = 3.17 mA, *SD* = 1.13).

## **Procedure**

### **General information and work-up procedure**

Upon arrival at the laboratory, participants were seated and informed about the general characteristics of the experiment. They were informed that in the experiment an electrical stimulus would be presented to them but that this stimulus was not harmful. Furthermore, they were informed about the presence of a camera and an intercom system that allowed the experimenter to monitor for artifacts that might interfere with the skin conductance measures (such as sneezing, yawning, ...). After this information, the electrodes for measuring skin conductance and for administering the US were attached to the left hand and the left leg, respectively. The skin conductance signal was checked by having participants breathing deeply in and out. If the signal was clear, participants went through the work-up procedure. During this work-up, participants were exposed to gradually increasing intensities of the electrical stimulus. Participants could verbally report after each exposure how they had experienced the stimulus. When they indicated the stimulus to be unpleasant but not painful, the procedure was stopped and participants were informed that this was the electrical stimulus intensity level that would be used during the experiment. Finally, the experiment program was started and continued automatically without the presence of the experimenter.

### **Pre-training instructions and training phase**

Participants were told that they would see four pictures during the experiment and that two of these pictures would sometimes be followed by the shock while the other two pictures would never be followed by the shock. They were instructed that their task was to indicate during the experiment to what extent they expected the shock using a scale presented below the pictures.

Furthermore, they were told that before they would see the pictures they had to complete a training phase to become familiar with the setup of the experiment. In this training phase, they would see a white square which would sometimes be followed by the shock and a yellow square which would never be followed by the shock.

During the training phase, participants were shown white and yellow squares, each presented four times (8 trials in total), in the middle of the screen for 8 s. The squares were preceded by a fixation cross presented for 3 s, signaling the onset of the CSs. Three out of four presentations of the white square (CS+) were followed by the US, determined in a random fashion. CSs were presented in a random sequence. On each trial, SCRs were measured and participants provided expectancy ratings (see the Materials section). The inter-trial interval was 10, 12 or 16 s, randomly selected.

### **Verbal instructions and retention test**

After the training phase, participants were told that they would now see the different stimuli together with information about whether a stimulus can be followed by the shock. Participants were asked to remember this information well and were told that they would have to complete a test afterwards about the instructions. Subsequently, all the CSs were shown one by one in the middle of the screen in a random order. For CS+s the sentence “Will SOMETIMES be followed by the shock” was presented above the picture, while for CS-s the sentence “Will NEVER be followed by the shock” was presented. Participants could navigate through these instructions by pressing the space bar.

After these instructions, the participants completed a short test about the instructions. During this test, each stimulus was presented twice in the middle of the screen in a random sequence. Participants were asked to decide for each stimulus whether it could be followed by a shock by clicking one of two response buttons depicted on the screen below the stimulus. On one button (200 by 100 pixels) “sometimes shock” and on the other “never shock” was projected. If participants failed to select the correct answer for each picture, they were told that they had not selected the right option for all the pictures and that they will

have to redo the test. After that, they received the contingency instructions again, followed by the test. This continued until participants passed the test without making errors (*average number of tests until pass* = 1.28, *SD* = 0.53, *range* = 1-3).

### **Extinction phase and reinstatement**

After the retention test, participants continued to an unannounced extinction phase. During this phase, participants saw each stimulus six times (24 trials in total). On no occasion was a stimulus followed by the US. Stimulus order was randomized with the exception that the same CS could not be presented on more than two consecutive trials. Stimulus and ITI duration were identical to those used in the preceding training phase. Similarly, SCRs and US expectancy ratings were collected online throughout the extinction phase.

After the last extinction trial, an unannounced reinstatement US was presented to the participants. The US was administered 15, 17 or 21 s after the last extinction trial ended and was followed by the first post-reinstatement trial after 18, 20 or 24 s, randomly selected. The reinstatement US was administered while participants viewed a black screen. After this unannounced US, each stimulus was presented to the participants two more times using a trial procedure identical to that of the extinction trials.

### **Manipulation checks**

At the end of the experiment, participants were asked to rate their belief in the instructions by selecting one of four forced-choice options (“not believable”, “somewhat believable”, “very believable” and “fully believable”) and to indicate how unpleasant they found the shock, using a scale with seven forced-choice possibilities (“very pleasant”, “rather pleasant”, “somewhat pleasant”, “neutral”, “somewhat unpleasant”, “rather unpleasant”, “very unpleasant”). In both cases, options were presented using a dropdown list. The two questions were presented in a random order.

### **Data analysis**

US expectancy ratings and SCRs were analyzed separately with repeated measures ANOVAs. Two ANOVAs were run to assess the effects of our experimental manipulations. First, the extinction phase was analyzed using an ANOVA with CS (CS+ or CS-), Fear-relevance (fear-relevant or fear-irrelevant) and Trial (one to six) as within-subject factors. Second, the effect of the reinstatement US was assessed by comparing the last trial of the test phase with the first trial after the reinstatement US (factor Time) with factors CS and Fear-relevance. The crucial interactions for our research question are those between Fear-relevance and CS (facilitated acquisition), between Fear-relevance, CS and trial (resistance to extinction) and between Fear-relevance, CS and Time (reinstatement for the fear-relevant CS+).

An alpha-level of .05 was applied for statistical significance and Greenhouse-Geisser corrections are reported when the sphericity assumption was violated.

## **Results**

### **Manipulations checks**

The majority of the participants indicated that they found the instructions to be very believable (20 participants, 54%) or fully believable (10 participants, 27%). Six participants indicated that they found the instructions somewhat believable (16%) and one participant reported not to believe the instructions (3%). These latter seven participants were excluded from analyses.

Furthermore, most of the participants experienced the US as rather unpleasant (24 participants, 65%) or as somewhat unpleasant (10 participants, 27%). Three participants indicated the US to be neutral or pleasant (8%) and were excluded from analyses (see Participants section)<sup>1</sup>.

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<sup>1</sup> Exclusion of these participants did not alter our conclusions regarding the data. Similar results were obtained when all participants were included in the statistical analyses.

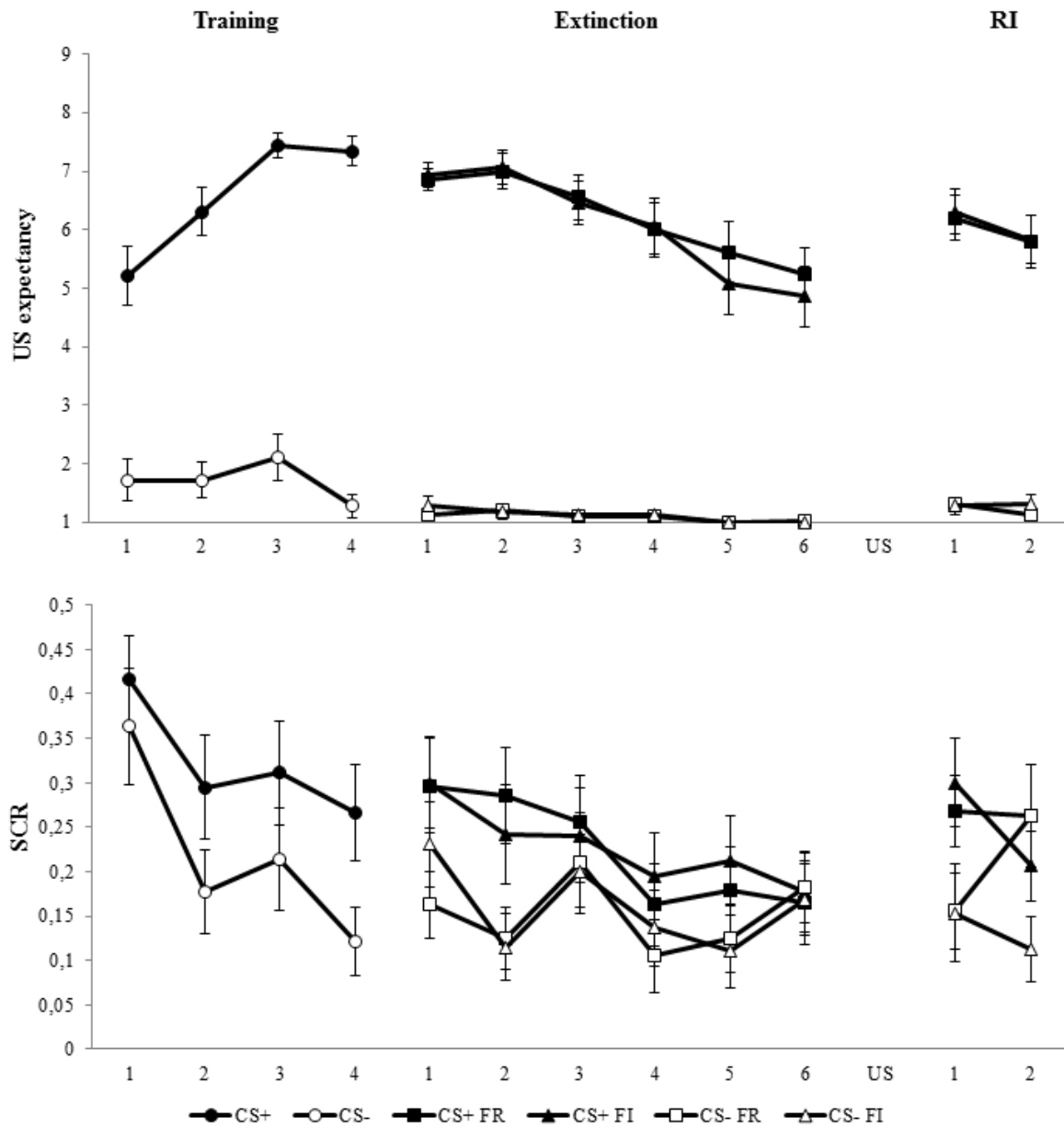
## US expectancy ratings

### *Extinction phase*

We obtained instruction-based fear conditioning as evidenced by higher US expectancy ratings for CS+s than for CS-s (see Figure 1), main effect of CS:  $F(1, 28) = 208.31, p < .001, \eta^2p = .88$ . However, this conditioning effect was not qualified by the nature of the CS, interaction between CS and Fear-relevance:  $F(1, 28) = 1.93, p = .176, \eta^2p = .06$ . Hence, no evidence for facilitated acquisition was obtained for US expectancy ratings. Furthermore, US expectancy ratings tended to decrease over trials, main effect of Trial:  $F(1.85, 51.91) = 18.52, p < .001, \eta^2p = .40$ , and this effect was qualified by a significant interaction between CS and Trial,  $F(1.75, 48.90) = 11.29, p < .001, \eta^2p = .29$ , indicating that US expectancy decreased more strongly for the CS+s (i.e., extinction; see Figure 1). However, this extinction effect was also not reliably modulated by the nature of the CS, three way interaction between CS, Trial and Fear-relevance:  $F(3.58, 100.17) = 1.91, p = .122, \eta^2p = .06$ . Hence, neither facilitated acquisition nor resistance to extinction was obtained for the US expectancy ratings during the extinction phase.

### *Reinstatement effect*

The reinstatement shock led to a general increase in US expectancy, main effect of Time:  $F(1, 28) = 21.98, p < .001, \eta^2p = .44$ . Importantly, the main effect of time was qualified by a significant interaction between Time and CS,  $F(1, 28) = 7.83, p = .009, \eta^2p = .22$ . This interaction was due to larger increases in US expectancy for CS+s after the reinstatement shocks than for CS-s (see Figure 1). Again, these results were not modulated by the factor Fear-relevance: main effect Relevance,  $F < 1$ ; interaction between Time and Relevance,  $F(1, 28) = 1.51, p = .229, \eta^2p = .05$ ; three way interaction between Time, CS and Relevance,  $F(1, 28) = 2.03, p = .165, \eta^2p = .07$ .



**Figure 1.** Mean US expectancy ratings and SCRs for all CSs throughout Experiment 1. Error bars represent Standard Error. RI = reinstatement; FR = fear-relevant; FI = fear-irrelevant.

## SCRs

### *Extinction phase*

SCRs to the CS+s were stronger than to the CS-s (see Figure 1), demonstrating acquisition of fearful reactions on an autonomous measure on the basis of verbal instructions, main effects of CS,  $F(1, 28) = 9.68, p = .004, \eta^2p = 0.26$ . Furthermore, SCRs decreased over trials throughout the extinction phase, main effect of Trial:  $F(3.16, 88.47) = 3.19; p = .026; \eta^2p = 0.10$ . This effect tended to be larger for CS+s than for CS-s (i.e., extinction; see Figure 1), interaction between CS and Trial number,  $F(5, 140) = 2.16; p = .062; \eta^2p = 0.07$ .

However, as for the US expectancy ratings, neither the acquisition effect nor the extinction effect on the SCRs were qualified by Fear-relevance, all  $F$ 's < 1.

### *Reinstatement effect*

Unlike the case for the US expectancy ratings, the reinstatement US did not lead to a general increase in SCRs, main effect of Time,  $F(1, 28) = 1.90; p = .179; \eta^2p = 0.06$ . However, it did lead to a specific increase of SCRs for CS+s (see Figure 1), interaction between Time and CS,  $F(1, 28) = 5.83; p = .023; \eta^2p = 0.17$ . The main effect of Fear-relevance and the interaction effects related to this factor were not significant, all  $F$ 's < 1.

## Discussion

In Experiment 1, we observed acquisition, extinction and reinstatement effects on the basis of verbal contingency instructions, both on US expectancy ratings and SCRs. To our knowledge, this is the first study to show reinstatement for US expectancy ratings and SCRs in conditioning through verbal instructions (Haaker et al., 2014).

Regarding our main research question, however, we did not find evidence for stronger acquisition or slower extinction for fear-relevant CSs. One reason for a lack of prepared learning effects in our experiment might be the fact that the CSs and the US were never actually paired. On the basis of the previously outlined evolutionary fear learning module, one could argue that giving mere



verbal contingency instructions might not be sufficient to recruit this module into the learning process (Mineka & Öhman, 2002; Olsson & Phelps, 2004, pp. 287). Previous experiments indeed suggest that verbal instructions mainly result in expectancy learning but to a smaller degree in affective learning (Lipp, Mallan, Libera, & Tan, 2010; Olsson & Phelps, 2004; Sevenster, Beckers, & Kindt, 2012). Therefore, direct experience of the CS-US contingency might be necessary for affective learning and consequently to obtain fear-relevance effects.

To test for this hypothesis, we adopted a procedure developed by Raes, De Houwer, De Schryver, Brass, and Kalisch (2014) which allowed a within-subject comparison of instructed fear conditioning with and without actual CS-US pairings. In this procedure, participants are instructed about two CSs that are said to be predictive of an electrical stimulus. Participants are also informed that in a first training phase only one of the CSs (i.e., CS+1) will be actually paired with the US while the other CS (i.e., CS+2) will only be followed by the electrical stimulus in a later test phase of the experiment. Before that test phase, participants are warned that now both CS+1 and CS+2 will be followed by the electrical stimulus, while in fact neither of them is followed by the stimulus during this phase. The crucial comparison in this procedure is the comparison during test between the CS whose relation to the US was both instructed and experienced as the result of actual CS-US pairings (i.e., CS+1) and the CS whose relation with the US was only instructed (i.e., CS+2). Any difference between CS+1 and CS+2 during test can be attributed to the presence of actual CS-US pairings. We extended this procedure by including both a fear-relevant and a fear-irrelevant CS+1 and CS+2 to assess whether actual experience strengthens selective learning effects.

Furthermore, in Experiment 2, we collected fear ratings in addition to US expectancy ratings and SCRs. Even though prepared learning effects have been observed on US expectancy ratings and SCRs (e.g., Davey, 1992; Lovibond, Siddle, & Bond, 1993; Öhman, Erixon, & Löfberg, 1975), fear ratings might be a better measure for capturing the emotional components of preparedness effects, that is, components that are driven by a fear learning module (Mertens et al., 2015; Soeter & Kindt, 2012).

## EXPERIMENT 2

### Participants

A sample of 41 right-handed psychology students at Ghent University completed the experiment in exchange for €12. Of these, five were removed from analyses because they reported not to believe the instructions ( $N = 2$ ) or because of lost data during recording ( $N = 3$ ).

### Material

#### Psychophysiology

SCRs were collected and pre-processed in the same manner as in Experiment 1. Additionally, SCRs were averaged per two trials to obtain an equal number of data points as for the subjective ratings (as in Raes et al., 2014).

#### US expectancy and fear ratings

US expectancy and fear ratings were collected in separate blocks, interspersed throughout the experiment (as in Raes et al., 2014). Right before this block, participants were asked to think back to their most recent encounter with the stimulus and were told that questions regarding the expectancy of the electrical stimulus referred only to the actual electrical stimulus and not the picture of the lightning bolt (which was used as a placeholder for CS+2 during a practice phase; see below). For each CS, fear and US expectancy ratings were collected, resulting in 12 trials in each ratings block. The order of the trials in the rating block was randomized.

During rating trials, a CS was presented in the middle of the screen with either the question “To what extent did you expect an electrical stimulus while viewing this photo?” (US expectancy) or “How much fear did you experience while viewing this photo?” (fear). Participants could provide an answer by clicking one of the response alternatives of a 9-point Likert scale presented below the CSs. For US expectancy, the anchors of the Likert scale were 1 = “certainly not”, 3 = “rather not”, 5 = “uncertain”, 7 = “rather certain”, 9 =

“certain”. For fear ratings, the anchors were 1 = “none at all”, 3 = “very little”, 5 = “uncertain”, 7 = “to some extent”, 9 = “very much”. There was no response deadline.

### **Stimuli**

Six stimuli were selected from the IAPS database. These were pictures of a spider, snake, rat, cow, deer and a rabbit (IAPS pictures 1200, 1080, 1280, 1670, 1620 and 1610, respectively)<sup>2</sup>. All pictures were 300 by 230 pixels in size (8 by 6 cm). Allocation of these pictures within each fear-relevance category to the role of CS+1, CS+2 and CS- was randomized for each participant.

The same electrical stimulus as in Experiment 1 served as the US in this experiment. Again, the intensity of this stimulus was determined for each participant individually via a work-up procedure (*mean intensity* = 5.36 mA, *SD* = 2.86).

Finally, a picture of a lightning bolt (approximately 200 by 200 pixels) presented for 500 ms was used as the placeholder US (as in Raes et al., 2014).

### **Questionnaires**

Prior to the experiment, participants completed a Dutch translation of the trait version of the State-Trait Anxiety Inventory (STAI-S; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983; van der Ploeg, Defares, & Spielberger, 2000). After the experiment, participants completed a custom-made questionnaire about credibility of the experimental instructions. In this questionnaire, participants indicated the clarity and believability of the instructions on a 10-point scale and could additionally provide general remarks about the experiment (as in Raes et al., 2014).

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<sup>2</sup> Virtually all fear conditioning studies investigating prepared learning in the laboratory have used pictures of either snakes or spiders as CSs. We considered rats to be a third fear-relevant CS based on a study from Hygge and Öhman (1978) who found preparedness effects with a rat picture as a fear-relevant stimulus and based on correlational studies that have found rats to be a universal fear-relevant animal similar to snakes and spiders (Davey et al., 1998; Ware, Jain, Burgess, & Davey, 1994).

## Procedure

### Pre-practice instructions

After arrival at the laboratory, participants were informed about the general characteristics of the study and went through a work-up procedure as in Experiment 1.

After all electrodes had been applied and checked, participants received instructions about the contingencies on the computer screen without the presence of the experimenter. Participants could move through the instructions in a self-paced way by pressing the spacebar. First, participants were shown the six CSs simultaneously on the screen for a minimum duration of 5 s. On the following instruction page, participants were informed that four of these photographs could sometimes be followed by an electrical stimulus, while the other two could never be followed by the stimulus. Next, participants saw the four CS+s, two fear-relevant and two fear-irrelevant, presented simultaneously on the screen with the sentence “+electrical stimulus!” beneath them. Thereafter, they saw the two CS-s simultaneously on the screen together with the sentence “NO electrical stimulus!”.

After these instructions, participants were warned that they would first go through a practice phase. In this phase, they were told, some of the electrical stimuli would be replaced by a picture of a lightning bolt in order to avoid administering too many electrical stimuli before the actual experiment starts. They were informed that on the next page they would see which photographs would be followed, during the practice phase, by the picture of the lightning bolt. On the following page, participants again saw all four CS+s. Two CS+s (CS+1s), one fear-relevant and one fear-irrelevant, were accompanied by the sentence “+electrical stimulus!” and the other two CSs (CS+2s), one fear-relevant and one fear-irrelevant, were accompanied by a plus sign and the placeholder US.

Finally, participants were warned that they would have to complete a short test about the instructions. In the first half of this test, participants were asked to indicate for each picture whether it could be followed by the electrical stimulus during the test phase. In the second half of the test, they were asked to indicate

whether a picture would be followed by either the electrical stimulus, a picture of a lightning bolt or whether it would not be followed by the electrical stimulus during the practice phase. On each trial of this test, participants were shown a CS in the middle of the screen together with the possible response buttons (100 by 100 pixels): “shock”, “no shock” and (only for trials related to the practice phase) “lightning bolt” depicted below it. Above the CSs, the sentence “Please select the correct option for the test(/practice) phase.” was shown. Participants could select their answer by clicking one of the response buttons. Each CS was presented twice, once for the questions regarding the test phase and once for the questions regarding the practice phase (12 trials in total). Participants were required to select the correct answer on each trial. If they failed to do so, they received the instructions again and had to redo the test (*average number of tests until pass* = 1.81, *SD* = 0.95, *range* = 1-5).

### **Practice phase**

During the practice phase, CSs were presented on the screen for 8 seconds. CS presentation was preceded by fixation cross depicted for 4 s. The ITI was either 12, 14 or 16 s, randomly selected. Each CS was shown six times (36 trials total). Trial order was randomized with the restriction that maximally two consecutive trials could contain the same CS. On the first, third and last occurrence of the fear-relevant and fear-irrelevant CS+1, stimulus offset was followed by the electrical stimulus. Similarly, on the first, second and fifth occurrence of the fear-relevant and fear-irrelevant CS+2, stimulus offset was followed by the placeholder US. The practice phase was interrupted three times, after every twelve trials, for a rating block (see the Materials section).

### **Pre-test instructions, test phase and reinstatement**

Before the start of the test phase, participants were warned that all CS+s would now be predictive of the electrical stimulus. The trial procedure was identical to that of the practice phase, apart from omitting all US and placeholder US administration. Similarly, the test phase was interrupted three times for a rating block.

After the last rating block of the test phase, an unannounced US was presented. Next, each CS was presented two more times using the same trial procedure as in the test phase. The experiment ended with a final rating block.

### Data analysis

US expectancy ratings, fear ratings and SCRs were analyzed separately with repeated measures ANOVAs. Both the practice and the test phase were analyzed using an ANOVA with the within-subject factors CS (CS+1, CS+2, CS-), Fear-relevance (relevant or irrelevant) and Block (1 to 3). Additionally, an extra ANOVA was run to examine the reinstatement effect. Responses from the last block of the test phase and the reinstatement phase (factor Phase) were subjected to a repeated measures ANOVA with CS, Fear-relevance and Time (before or after reinstatement US) as within-subjects variables. As in Experiment 1, evidence for prepared learning can be provided by significant interactions between Fear-relevance and CS (facilitated acquisition), between Fear-relevance, CS and Block (resistance to extinction), or between Fear-relevance, CS and Phase (facilitated or inhibited reinstatement). Such interactions were followed up with specific *t*-tests. Greenhouse-Geisser corrections are reported when the sphericity assumption was violated and an alpha-level of .05 was applied.

## Results

### Questionnaires

Trait STAI scores ranged between 21 and 59 with a mean of 36.83 (*SD* = 9.19). Because of the small sample, STAI scores were not included as a variable in the analyses. Participants indicated that the instructions were clear (*Range* = 5-10; *Mean* = 9.43; *SD* = 1.17) and believable (*Range* = 5-10; *Mean* = 8.77; *SD* = 1.42). Two participants who rated the believability as 5 were excluded from analyses<sup>3</sup>.

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<sup>3</sup> As in Experiment 1, exclusion of these participants did not alter our conclusions.

## US expectancy ratings

### *Practice phase*

The main effects of all three factors were significant: CS,  $F(1.39, 48.57) = 218.50$ ,  $p < .001$ ,  $\eta^2p = .86$ ; Fear-relevance,  $F(1, 35) = 9.07$ ,  $p = .005$ ,  $\eta^2p = .21$ ; Block,  $F(2, 70) = 6.21$ ,  $p = .003$ ,  $\eta^2p = .15$ . The main effect of CS reflects significantly higher US expectancy ratings for CS+1,  $F(1, 35) = 1291.37$ ,  $p < .001$ ,  $\eta^2p = .97$ , and CS+2,  $F(1, 35) = 60.26$ ,  $p < .001$ ,  $\eta^2p = .63$ , compared to CS- (see Figure 2). Furthermore, US expectancy ratings were also significantly higher for CS+1s than for CS+2s,  $F(1, 35) = 105.47$ ,  $p < .001$ ,  $\eta^2p = .75$ . The main effect of Fear-relevance was due to higher US expectancy ratings for fear-relevant CSs ( $M = 4.39$ ,  $SD = 0.91$ ) than for fear-irrelevant CSs ( $M = 4.09$ ,  $SD = 0.87$ ). The main effect of Block was due to gradually lower US expectancy ratings throughout the Practice phase (see Figure 2).

The main effects of CS and Block were partially qualified by an interaction between these two factors,  $F(2.67, 93.34) = 3.39$ ,  $p = .015$ ,  $\eta^2p = .09$ . This interaction reflects changes in the difference between CS+1 and CS+2 over blocks. While US expectancy for CS+1 dropped from Block 1 to Block 2 and then remained constant, US expectancy for CS+2 slightly increased in Block 2 and then dropped in Block 3. Furthermore, and crucially, the interaction between CS and Fear-relevance,  $F(1.69, 59.05) = 6.74$ ,  $p = .004$ ,  $\eta^2p = .16$ , was significant because of larger conditioning effects for the fear-relevant CS+2 ( $M = 2.92$ ,  $SD = 2.22$ ) than for the fear-irrelevant CS+2 ( $M = 2.48$ ,  $SD = 2.05$ ;  $t(35) = 2.79$ ,  $p = .008$ , *Cohen's d* = .47) (see Figure 2).

Finally, neither the interaction between Fear-relevance and Block,  $F < 1$ , nor the three-way interaction between CS, Fear-relevance and Block,  $F(3.29, 115.02) = 1.70$ ,  $p = .167$ ,  $\eta^2p = .05$ , was significant.

### *Test phase*

As in the Practice phase, all three main effects were significant: CS,  $F(2, 70) = 157.07$ ,  $p < .001$ ,  $\eta^2p = .82$ , Fear-relevance,  $F(1, 35) = 10.00$ ,  $p = .003$ ,  $\eta^2p = .22$ , Block,  $F(1.73, 60.38) = 56.67$ ,  $p < .001$ ,  $\eta^2p = .62$ . These effects are due to similar

patterns as in the Practice phase. Importantly, however, the difference between CS+1 and CS+2 was still significant,  $F(1, 35) = 30.51, p < .001, \eta^2p = .47$ , despite instructions that both would be equally predictive of the shock during this phase.

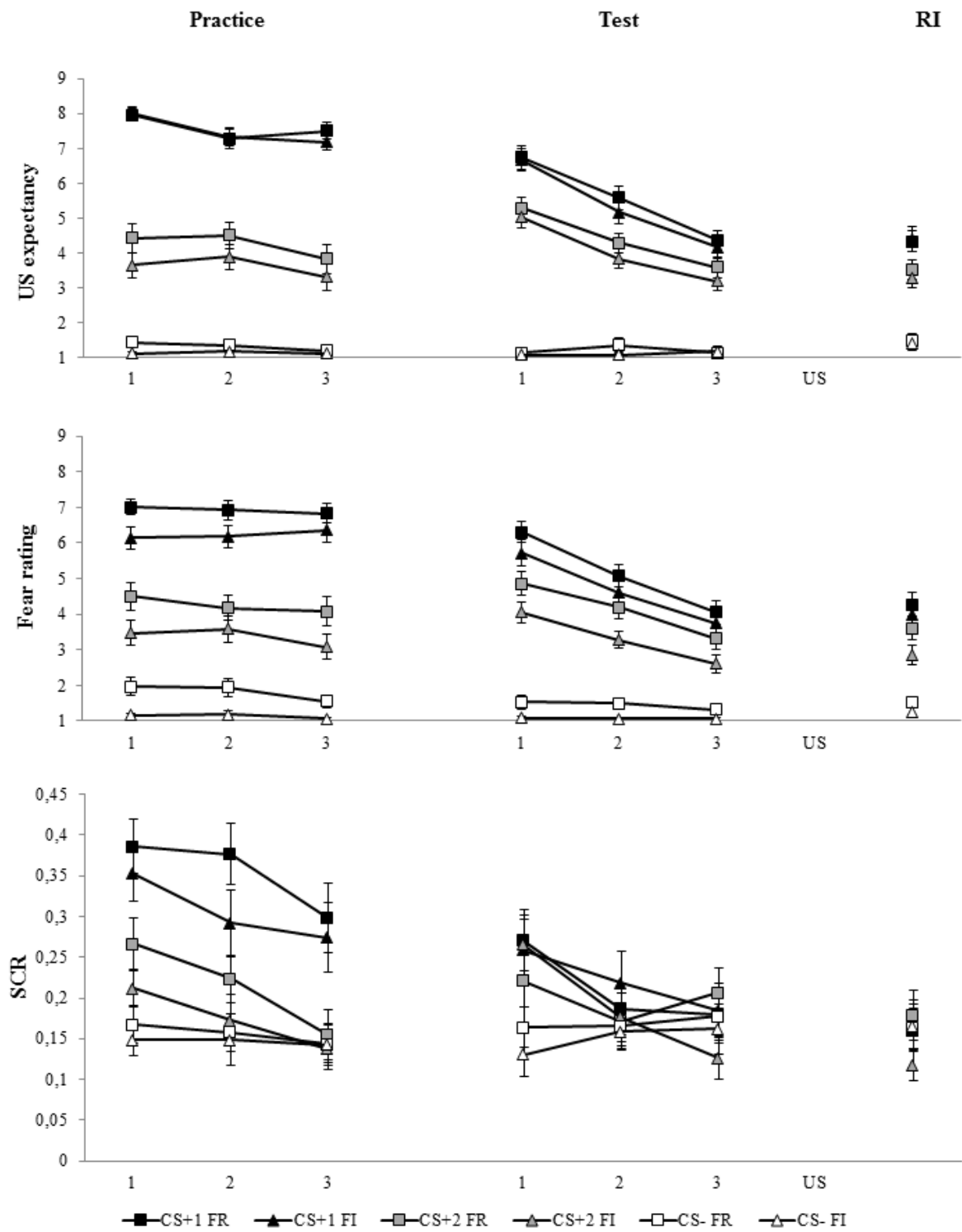
Furthermore, the interaction between CS and Block was significant,  $F(2.75, 96.16) = 30.14, p < .001, \eta^2p = .46$ . This interaction is due to decreasing US expectancy ratings for CS+1 and CS+2 throughout the test phase, while US expectancy remained similar for CS- (i.e., extinction; see Figure 2). The interaction between Fear-relevance and Block approached significance,  $F(2, 70) = 2.72, p = .073, \eta^2p = .07$ , due to a slightly slower decrease of US expectancy ratings throughout the Test phase for fear-relevant CSs (see Figure 2).

The crucial interactions between CS and Fear-relevance,  $F(1.65, 57.68) = 1.95, p = .159, \eta^2p = .05$ , and between CS, Fear-relevance and Block,  $F < 1$ , did not reach significance in the test phase.

### ***Reinstatement effect***

Only the main effect of CS was significant,  $F(1.68, 58.91) = 78.15, p < .001, \eta^2p = .69$ , again reflecting the same differences between CSs as in the practice phase. The effects of all other main and interaction effects did not reach significance threshold,  $F's < 2.1, p\text{-values} > .15$ .





**Figure 2.** Mean US expectancy ratings, fear ratings and SCRs for all CSs throughout Experiment 2. Error bars represent Standard Error. RI = reinstatement; FR = fear-relevant; FI = fear-irrelevant; CS+1 = instructed + paired with US; CS+2 = instructed.

## Fear ratings

### *Practice phase*

Both the main effects of CS,  $F(2, 70) = 135.16, p < .001, \eta^2p = .79$ , and of Fear-relevance,  $F(1, 35) = 24.79, p < .001, \eta^2p = .42$ , were significant. The main effects of CS reflects higher fear ratings for CS+1s,  $F(1, 35) = 349.61, p < .001, \eta^2p = .91$ , and CS+2s,  $F(1, 35) = 54.61, p < .001, \eta^2p = .61$ , than for CS-s. Fear ratings for CS+1s were in turn higher than for CS+2s,  $F(1, 35) = 66.43, p < .001, \eta^2p = .66$  (see Figure 2). The effect of Fear-relevance is explained by higher fear ratings for fear-relevant CSs than for fear-irrelevant CSs (see Figure 2). All other main and interaction effects were not significant,  $F$ 's  $< 1.7, p$ -values  $> .2$ .

### *Test phase*

The main effects of all three main factors were significant: CS,  $F(1.70, 59.54) = 113.69, p < .001, \eta^2p = .77$ , Fear-relevance,  $F(1, 35) = 13.39, p = .001, \eta^2p = .28$ , Block,  $F(1.39, 48.80) = 53.39, p < .001, \eta^2p = .60$ . The first two effects reflect similar patterns as in the Practice phase. Importantly, the difference between CS+1s and CS+2s remained significant in the Test phase,  $F(1, 35) = 31.15, p < .001, \eta^2p = .47$ , despite instructions that both would be equally predictive of the US during this phase.

Furthermore, the interaction between CS and Block was significant,  $F(2.26, 78.94) = 24.77, p < .001, \eta^2p = .41$ , due to decreasing fear ratings for the CS+1s and CS+2s throughout the Test phase while fear ratings for the CS-s remained constant (i.e., extinction; see Figure 2).

The interactions related to the factor Fear-relevance failed to reach significance: interaction between CS and Fear-relevance,  $F(1.64, 57.46) = 2.31, p = .118, \eta^2p = .06$ ; interaction between Block and Fear-relevance,  $F(2, 70) = 1.75, p = .182, \eta^2p = .05$ ; three way interaction between CS, Trial and Fear-relevance,  $F < 1$ .

### *Reinstatement effect*

Both the main effects of CS,  $F(1.68, 58.74) = 65.15, p < .001, \eta^2p = .65$ , and of Fear-relevance,  $F(1, 35) = 12.19, p = .001, \eta^2p = .26$ , were significant.

Importantly, the main effect of Time approached significance,  $F(1, 35) = 3.73$ ,  $p = .062$ ,  $\eta^2p = .10$ , due to higher fear ratings after the reinstatement US compared to before (see Figure 2).

The only interaction effect that approached significance was that between CS and Fear-relevance,  $F(1.76, 61.61) = 3.03$ ,  $p = .062$ ,  $\eta^2p = .08$ . This interaction was due to larger conditioning effects for the fear-relevant CS+2 ( $M = 2.01$ ,  $SD = 1.58$ ) than for the fear-irrelevant CS+2 ( $M = 1.58$ ,  $SD = 1.25$ ;  $t(35) = 2.39$ ,  $p = .023$ , *Cohen's d* = .40) (see Figure 2). The other interaction effects were not significant,  $F$ 's < 1.

### SCRs

#### *Practice phase*

The main effect of CS was significant,  $F(1.40, 49.09) = 33.36$ ,  $p < .001$ ,  $\eta^2p = .49$ . This was due to significantly stronger SCRs for CS+1s,  $F(1, 35) = 43.02$ ,  $p < .001$ ,  $\eta^2p = .55$ , and CS+2s,  $F(1, 35) = 9.88$ ,  $p = .003$ ,  $\eta^2p = .22$ , compared to CS-s (see Figure 2). Furthermore, SCRs to CS+1s were in turn significantly stronger than to CS+2s,  $F(1, 35) = 28.99$ ,  $p < .001$ ,  $\eta^2p = .45$ . The main effects for Fear-relevance,  $F(1, 35) = 6.71$ ,  $p = .014$ ,  $\eta^2p = .16$ , and for Block,  $F(1.70, 59.35) = 7.05$ ,  $p = .003$ ,  $\eta^2p = .17$ , were also significant. The former was due to stronger SCRs to fear-relevant CSs than to fear-irrelevant CSs, while the latter was the result of gradually lower SCRs for all CSs throughout the practice phase (see Figure 2). None of the interaction effects between these factors was significant, all  $F$ 's < 1.5.

#### *Test phase*

Both the main effects of CS,  $F(1.63, 56.97) = 4.24$ ,  $p = .026$ ,  $\eta^2p = .11$ , and of Block,  $F(1.61, 56.31) = 4.79$ ,  $p = .018$ ,  $\eta^2p = .12$ , were significant. The main effect of CS was due to stronger SCRs to CS+1s,  $F(1, 35) = 5.78$ ,  $p = .025$ ,  $\eta^2p = .14$ , and marginally stronger SCRs to CS+2s,  $F(1, 35) = 3.35$ ,  $p = .076$ ,  $\eta^2p = .09$  than to CS-s (see Figure 2). Importantly, the difference between CS+1s and CS+2s was not significant,  $F(1, 35) = 2.00$ ,  $p = .166$ ,  $\eta^2p = .05$ , which is in line with the

instructions that both CSs would be equally predictive of the US during the Test phase.

Furthermore, the interaction between CS and Block was also significant,  $F(4, 140) = 3.32, p = .012, \eta^2p = .09$ , which was due to gradually decreasing SCRs for CS+1s and CS+2s while SCRs to the CS-s remained constant (extinction, see Figure 2).

The main effect of Fear-relevance,  $F < 1$ , and the crucial interactions between CS and Fear-relevance,  $F < 1$ , Block and Fear-relevance,  $F < 1$ , and between CS, Block and Fear-relevance,  $F(4, 140) = 1.34, p = .257, \eta^2p = .04$ , did not reach significance.

### ***Reinstatement effect***

The only significant effect observed was the interaction between factors CS and Fear-relevance,  $F(2, 70) = 3.73, p\text{-value} = .029, \eta^2p = .10$ . This interaction was due to larger differential SCRs to the fear-relevant CS+2 ( $M = 0.02, SD = 0.16$ ) than to the fear-irrelevant CS+2 ( $M = -0.04, SD = 0.14; t(35) = 2.08, p = .045, Cohen's d = .35$ ; see Figure 2). The main effects of and interaction effects of the other factors did not reach significance: main effect of Fear-relevance,  $F(1, 35) = 2.26, p = .142, \eta^2p = .06$ ; all other  $F$ 's  $< 1$ .

### **Discussion**

In Experiment 2, we again obtained acquisition and extinction of fearful CRs on the basis of verbal instructions on all collected measures. A reinstatement manipulation only generated a trend for increased fear ratings for all CSs, but did not generate effects for US expectancy and SCRs. Furthermore, in line with the results of Raes et al. (2014), we observed additional effects of previous CS-US pairings for both fear ratings and US expectancy ratings in the test phase, while such an effect was not significant for SCRs.

Regarding our main research question, however, no evidence was obtained for the hypothesis that selective learning effects are especially pronounced for the fear-relevant CS+ that had actually been paired with the US as compared to the fear-relevant CS+ that was merely involved in verbal instructions. On the

contrary, during the practice phase, conditioning effects were especially pronounced for the fear-relevant verbally conditioned CS+. Specifically, conditioning effects for the fear-relevant CS+2 were stronger than those for the fear-irrelevant CS+2 on US expectancy ratings during the practice phase and for fear ratings and SCRs at the time of the reinstatement manipulation.

## **BAYESIAN ANALYSIS OF EXPERIMENT 1 AND 2**

We performed Bayesian analyses to complement the traditional analyses of Experiment 1 and 2. There are a number of advantages of using Bayesian statistics over classical null hypothesis significance testing, which we will not all reiterate here (arguments for using Bayesian analyses can be found in, Dienes, 2011, and Rouder, Speckman, Sun, Morey, & Iverson, 2009). With regard to our own research questions, performing Bayesian analyses allowed us to (a) evaluate to what extent non-significant effects actually provided evidence for the null hypothesis (i.e., the absence of preparedness effects) (Dienes, 2014); and (b) interpret the evidence for preparedness effects from the different tests we have conducted, without being confronted with the multiple testing problem (Dienes, 2011).

The primary tool for statistical inference in a Bayesian framework is the Bayes Factor (BF). The BF expresses the likelihood of the alternative hypothesis relative to the null hypothesis. For instance, a BF of 2 would indicate that the likelihood of the alternative hypothesis being true based on the data is two times larger than the null hypothesis being true. In line with Jeffreys (1961; see also: Andraszewicz et al., 2015), we consider BFs larger than 3 (moderate evidence) or larger than 10 (strong evidence) as providing evidence for the alternative hypothesis. Likewise, BFs smaller than 0.33 (moderate evidence) or smaller than 0.10 (strong evidence) were considered as evidence for the null hypothesis. BFs between three and 0.33 are considered to provide only anecdotal evidence for either the null or alternative hypothesis. BFs for our different tests of preparedness in the two experiments were calculated using Bayesian repeated

measures (with default priors; see: Rouder, Morey, Speckman, & Province, 2012) in JASP (version 6.0; Love et al., 2014) and are summarized in Tables 1 to 3.

As can be seen in Tables 1 to 3, most tests of preparedness in both experiments provided moderate ( $BF < 0.33$ ) to strong ( $BF < 0.1$ ) evidence for an absence of a preparedness effect. However, in a few instances, the overall test for preparedness effects was inconclusive (i.e., a  $BF > 0.33$  and  $< 3$ ). Specifically, this was the case for the interaction between fear-relevance and CS in the practice phase for US expectancy ratings and the interactions between fear-relevance and CS in the test phase and at the time of the reinstatement manipulation for fear ratings (see Tables 1 and 3). When these interactions were analyzed further using Bayesian paired samples t-test (Cauchy prior width = 0.707), evidence for preparedness effects (i.e., larger differential conditioning for the fear-relevant CS+ than for the fear-irrelevant CS-) were obtained for CS+2. That is, moderate evidence for preparedness effects for the fear-relevant CS+2 was obtained on US expectancy ratings in the practice phase ( $BF = 4.89$ ) and on fear ratings in the test phase ( $BF = 3.11$ ). At the time of the reinstatement manipulation, there was anecdotal evidence for larger differential fear ratings for the fear-relevant CS+2 ( $BF = 2.13$ ). In contrast, corresponding tests for the CS+1s resulted in moderate evidence for an absence of preparedness effects ( $BFs < 0.3$ ; see also Figure 2).

Taken together, these results demonstrate that our data provided moderate to strong evidence for an absence of most of the potential preparedness effects in our experiments. However, in cases of uncertainty with regard to the presence of preparedness effects, our data favored the conclusion that preparedness effects were present for the fear-relevant CS+2.

**Table 1. F-values, p-values and BF's for the different tests for preparedness effects for US expectancy ratings.**

	F-value	p-value	BF
<b>Experiment 1</b>			
Extinction phase			
Fear-relevance*CS	$F(1, 28) = 1.93$	.176	0.032
Fear-relevance*CS*Trial	$F(3.58, 100.17) = 1.91$	.122	< 0.001
Reinstatement			
Fear-relevance*CS*Time	$F(1, 28) = 2.03$	.165	0.023
<b>Experiment 2</b>			
Practice phase			
Fear-relevance*CS	$F(1.69, 59.05) = 6.74$	.004	0.509
Fear-relevance*CS*Trial	$F(3.29, 115.02) = 1.70$	.167	< 0.001
Test phase			
Fear-relevance*CS	$F(1.65, 57.68) = 1.95$	.159	0.082
Fear-relevance*CS*Trial	$F < 1$	.821	< 0.001
Reinstatement			
Fear-relevance*CS	$F(2, 70) = 1.20$	.308	0.033
Fear-relevance*CS*Time	$F(2, 70) = 1.23$	.300	< 0.001

**Table 2. F-values, p-values and BF's for the different tests for preparedness effects for SCRs.**

	F-value	p-value	BF
<b>Experiment 1</b>			
Extinction phase			
Fear-relevance*CS	$F < 1$	.867	0.021
Fear-relevance*CS*Trial	$F < 1$	.815	< 0.001
Reinstatement			
Fear-relevance*CS*Time	$F < 1$	.942	0.008
<b>Experiment 2</b>			
Practice phase			
Fear-relevance*CS	$F(2, 70) = 1.02$	.368	0.107
Fear-relevance*CS*Trial	$F < 1$	.868	< 0.001
Test phase			
Fear-relevance*CS	$F < 1$	.592	0.010
Fear-relevance*CS*Trial	$F(4, 140) = 1.34$	.257	< 0.001
Reinstatement			
Fear-relevance*CS	$F(2, 70) = 3.73$	.029	0.041
Fear-relevance*CS*Time	$F < 1$	.954	< 0.001



**Table 3. F-values, p-values and BF's for the different tests for preparedness effects for fear ratings. Note that fear ratings were only collected in Experiment 2.**

	<i>F</i> -value	<i>p</i> -value	BF
Practice phase			
Fear-relevance*CS	$F < 1$	.683	0.091
Fear-relevance*CS*Trial	$F(3.20, 112.06) = 1.42$	.238	< 0.001
Test phase			
Fear-relevance*CS	$F(1.64, 57.46) = 2.31$	.118	0.350
Fear-relevance*CS*Trial	$F < 1$	.872	0.002
Reinstatement			
Fear-relevance*CS	$F(1.76, 61.61) = 3.03$	.062	0.384
Fear-relevance*CS*Time	$F < 1$	.962	< 0.001

## GENERAL DISCUSSION

Two experiments were conducted to assess whether selective learning effects can be obtained when conditioning is established via verbal instructions. In the first experiment, participants were verbally instructed about the contingency between fear-relevant and fear-irrelevant CSs and an electrical stimulus. In the second experiment, we additionally manipulated whether the contingency described in the instructions was actually experienced by the participants, using a design developed by Raes et al. (2014). This allowed us to test whether selective learning depends on actual experience of the fear-relevant CS-US pairing. As a secondary aim, we also examined reinstatement of fear induced by verbal instructions. Below we will first summarize and discuss

the findings related to selective learning. Afterwards, we focus on the reinstatement results.

In both experiments, participants reacted more fearfully towards instructed CS+s than to CS-s. These fearful reactions tended to extinguish throughout the test phase. These results replicate the well-known finding that fear conditioning can be observed on the basis of verbal instructions (Cook & Harris, 1937; Grings, 1973; Lovibond, 2003). However, the extinction of fear reactions was not modulated by the fear-relevance of the CSs in either experiment. Hence, we did not find evidence for resistance to extinction when learning took place via verbal instructions, even when participants had experienced the instructed CS-US pairings in Experiment 2. This result is in contrast with those from Hugdahl (1978), who did find strong resistance to extinction for fear-relevant CS+s on the basis of verbal instructions, but is reminiscent of the results of several subsequent studies that failed to replicate this effect (see: Lovibond, 2004; McNally, 1981; 1987).

In Experiment 2, however, stronger acquisition effects were observed for fear-relevant verbally conditioned CSs compared to fear-irrelevant verbally conditioned CSs. This result can be regarded as an instance of selective learning (i.e., stronger acquisition) and is in line with the results of the only relevant study (i.e., Hugdahl & Öhman, 1977). Specifically, this effect was observed during the practice phase for US expectancy ratings and at the time of the reinstatement US for fear ratings and SCRs. Surprisingly, this selective learning effect was specifically observed for the CS that was merely instructed to be contingent with the US (CS+2), but not for the CS that was instructed and actually paired with the US (CS+1). Our Bayesian analyses confirmed that there was substantial evidence for this preparedness effect on the US expectancy ratings and fear ratings for the CS+2s. Conversely, there was substantial evidence for an absence of preparedness effects on all different measures of conditioned fear for the CS+1s.

Hence, some aspects of our results suggest that selective learning effects can be obtained when conditioning is established on the basis of verbal

instructions. Furthermore, our results provide no indication that actual CS-US pairings strengthen selective learning effects. On the contrary, we obtained selective learning effects specifically for the CS that was not actually paired with the US in Experiment 2.

These results do not fit well with the proposal that an evolved fear learning module explains selective learning effects (Mineka & Öhman, 2002; Öhman & Mineka, 2001). As mentioned before, this module is thought to be specifically and automatically activated by fear-relevant stimuli and to be encapsulated from conscious cognitive control. Based on this model, we would predict that selective learning would not be observed when conditioning is established through verbal instructions, unless the CSs are actually paired with the US (Olsson & Phelps, 2004, 2007). However, we obtained the opposite pattern of results in Experiment 2: selective learning was observed on the basis of verbal instructed conditioning and only for the CSs that were not paired with the US. Furthermore, if prepared learning is due to affective learning, prepared learning effects should have been particularly pronounced on measures that are thought to be particularly sensitive to capture this type of learning (i.e., fear ratings; Mertens et al., 2015; Soeter & Kindt, 2012). This was not the case in our results. If anything, according to our Bayesian analyses, preparedness effects seemed to be especially pronounced on US expectancy ratings, which is considered to be a cognitive measure of learning (e.g., Kindt, Soeter, & Vervliet, 2009).

We believe that our results fit better with the expectancy bias model of Davey (Davey, 1992; Honeybourne, Matchett, & Davey, 1993). According to this model, fear-relevant CSs are accompanied by a bias to expect aversive events before any conditioning has taken place. This bias rapidly disappears when the fear-relevant CSs are not reinforced but is maintained when fear-relevant CSs are reinforced, resulting in an expectancy bias specifically for the fear-relevant CS+. This bias is translated into stronger conditioned reactions on other measures, such as SCRs (Davey, 1992; Lovibond, Siddle, & Bond, 1993). In our Experiment 2, selective learning effects were obtained on US expectancy ratings, replicating the US expectancy bias effect of Davey (1992) and Lovibond et al. (1993). Arguably,

the no-reinforcement instructions rapidly abolished the expectancy bias for the fear-relevant CS- whereas the threat instructions retained the expectancy bias for the fear-relevant CS+2, resulting in a selective learning effect on US expectancy ratings. However, in our experiment, the US expectancy bias is not accompanied by selective learning effects on other measures during the practice phase. Only later in the experiment, at the time of the reinstatement shock, are selective learning effects obtained for SCRs and fear ratings (but not US expectancy, see Figure 2). These inconsistencies between US expectancy ratings and other measures do not fit with the expectancy bias model of Davey (1992) and may be accounted for by subtle differences in the measures. However, taken together, selective learning effects are most pronounced on US expectancy ratings, and therefore we believe that our data are best accounted for by the model of Davey (1992).

It is still not clear, however, why selective learning effects are obtained for the fear-relevant CS+2, but not for the fear-relevant CS+1 or for the fear-relevant CS+ in Experiment 1. We propose that expectancy bias for fear-relevant threatened CSs is especially pronounced under conditions of uncertainty. While uncertain, there is opportunity for expectancy biases to shift conditioned responses. In our own experiment, there is most uncertainty for the CS+2s, as indicated by the US expectancy ratings for these CSs that are situated in the middle of the scale. For the CS+1s the training phase of Experiment 2 and for CS+s in Experiment 1, US expectancy ratings are at an extreme end of the scale. These differences in uncertainty might explain why we observe selective learning for CS+2s but not for CS+1s. Indeed, research on expectancy biases of anxious populations has shown that these biases are especially pronounced under conditions of uncertainty (Calvo & Dolores Castillo, 2001; Chan & Lovibond, 1996; Ly & Roelofs, 2009). However, while there is some suggestive correlational evidence showing that the spatiotemporal uncertainty of aversive events (US) related to the CS makes this CS fearful (Harald Merckelbach, van den Hout, Jansen, & van der Molen, 1988), our suggestion that expectancy biases for fear-

relevant CSs are especially pronounced under uncertainty remains to be explicitly tested (for instance by manipulating the CS-US contingency).

Whereas our data are generally in correspondence with the expectancy bias model of Davey (1992), our experiments did not include a replication of the resistance to instructed extinction effect (Hugdahl & Öhman, 1977; Hugdahl, 1978). This effect strongly favors the theory of a fear learning module because it shows that fear for fear-relevant CSs seems to be insensitive to cognitive interventions once it has been installed (Öhman & Mineka, 2001). Interestingly, Hugdahl (1978) and Öhman and Hugdahl (1977) show that verbal threat instructions can install prepared learning, but safety instructions do not disrupt this learning. Thus, the limitations of learning via verbal instructions may be primarily pronounced during safety (i.e., extinction) learning (see also: Luck & Lipp, 2015 and Sevenster et al., 2012 for related results). However, as mentioned previously, this effect has proven difficult to reproduce in subsequent experiment (see: Lovibond, 2004; McNally, 1981; 1987). It would certainly be worthwhile for future studies to further explore this intriguing finding. This could help us to pinpoint the conditions under which the effect occurs and, in doing so, map the possible limitations of learning through instructions. Furthermore, we want to stress that the systematic inclusion of expectancy ratings in these studies is necessary to evaluate whether these effects could be explained by expectancy biases, or are independent of higher-order cognitions as proposed by Öhman and Mineka (2001).

Finally, we turn to the issue of reinstatement. We observe a selective return of US expectancy ratings and SCRs to the CS+s in the first experiment, and marginally increased fear ratings for all CSs in the second experiment after the reinstatement US. This is the first study, to our knowledge, to demonstrate differential reinstatement on US expectancy ratings and SCRs when conditioning was established on the basis of verbal instructions (Haaker et al., 2014). This is an interesting finding because it suggests that similar mechanisms of return of fear may exist for fear acquired through verbal instructions and fear acquired through direct CS-US pairings (see also: Mertens et al., 2015). Unfortunately, due to the

absence of (strong) preparedness effects in our experiments, it is difficult to evaluate the (lack of) impact of fear-relevance on the return of fear through reinstatement. As we noted in the final paragraph of the introduction, stronger acquisition and/or less extinction for fear-relevant CSs are probably necessary conditions to observe an effect of fear-relevance on subsequent reinstatement of fear. Hence, the fact that reinstatement effects in our studies were similar for fear-relevant and fear-irrelevant CSs could simply be due to the limited effect of fear-relevance on acquisition and extinction. It would certainly be interesting for future studies to further investigate the impact of fear-relevance on the return of fear through reinstatement or other manipulations in situations where there are strong preparedness effects in the preceding acquisition or extinction phase.

In summary, our data suggests that selective learning can be obtained when conditioning is established through verbal instructions, without requiring direct CS-US pairings. Our results further suggest that these selective learning effects are due to US expectancy biases for fear-relevant CSs, which are proposed to be especially pronounced under conditions of uncertainty. Finally, our results revealed reinstatement of fear that was induced by verbal instructions, but reinstatement was not modulated by fear-relevance.

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# CHAPTER 5

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## POTENTIATION OF THE STARTLE REFLEX IS IN LINE WITH CONTINGENCY REVERSAL INSTRUCTIONS RATHER THAN THE CONDITIONING HISTORY<sup>1</sup>

*In the context of fear conditioning, different psychophysiological measures have been related to different learning processes. Specifically, skin conductance responses (SCRs) have been related to cognitive expectancy learning, while fear potentiated startle (FPS) has been proposed to reflect affective learning that operates according to simple associative learning principles. On the basis of this two level account of fear conditioning we predicted that FPS should be less affected by verbal instructions and more affected by direct experience than SCRs. We tested this hypothesis by informing participants that contingencies would be reversed after a differential conditioning phase. Our results indicate that contingency reversal instructions led to an immediate and complete reversal of FPS regardless of the previous conditioning history. This change was accompanied by similar changes on US expectancy ratings and SCRs. These results conform with an expectancy model of fear conditioning but argue against a two level account of fear conditioning.*

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<sup>1</sup>Based on 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





## INTRODUCTION

Fear conditioning is an adaptive process through which organisms learn to fear and avoid a conditioned stimulus (CS) that has been paired with an aversive event (unconditioned stimulus, US). This is usually modeled in the lab by pairing neutral stimuli (lights, geometric shapes) with an unpleasant but harmless electric stimulus. For humans, fear conditioning is often believed to be mediated by the generation of cognitive expectancies about the occurrence of the US in the presence of the CS (e.g., Lovibond & Shanks, 2002; Mitchell et al., 2009; Reiss, 1980). However, according to the two level account of human fear conditioning (e.g., Hamm & Weike, 2005; Öhman & Mineka, 2001; Sevenster, Beckers, & Kindt, 2012a), this cognitive contingency learning between the CS and the US is supplemented with affective learning. Affective learning is proposed to be a highly automatic process, taking place independent of cognitive contingency learning (Baeyens, Eelen, & Crombez, 1995; Hamm & Weike, 2005; Mineka & Öhman, 2002; Öhman & Mineka, 2001) and mediated by a specifically dedicated neural system centered on the amygdala (Mineka & Öhman, 2002; Öhman & Mineka, 2001).

These two forms of learning have been mapped onto different physiological responses (Hamm & Weike, 2005). Conditioned skin conductance responses (SCRs) are usually considered to reflect cognitive contingency learning about the occurrence of the aversive US in the presence of the CS (e.g., Dawson & Furedy, 1976; Lovibond & Shanks, 2002). This hypothesis is supported by studies showing that conditioning of the SCRs only occurs when participants are aware of the CS-US contingencies (e.g., Dawson, 1970; Dawson & Furedy, 1976; Sevenster et al., 2014; Singh et al., 2013) and that conditioned SCRs are very sensitive to verbal instructions (Hugdahl, 1978; Luck & Lipp, 2015b; Sevenster et al., 2012a). Conditioned potentiation of the startle reflex (or fear potentiated startle, FPS), on the other hand, is believed to primarily reflect affective learning. Evidence for this idea was provided by studies suggesting that conditioning of

the startle reflex does not require awareness of the CS-US contingency (Hamm & Weike, 2005; Hamm & Vaitl, 1996; Sevenster et al., 2014) and that FPS is less sensitive to verbal instructions (Dawson, Rissling, Schell, & Wilcox, 2007; Sevenster et al., 2012). Furthermore, in a number of recent psychopharmacological studies, FPS was abolished by the administration of propranolol during a reconsolidation period while leaving expectancy of the US and SCRs intact, demonstrating a strong dissociation between FPS and cognitive measures of conditioned fear (Kindt, Soeter, & Vervliet, 2009; Soeter & Kindt, 2010). However, the evidence is not unequivocal. For instance, in a number of other studies, conditioning of the startle reflex was obtained only for participants who became aware of the CS-US contingencies (Dawson, Rissling, et al., 2007; Grillon, 2002; Jovanovic et al., 2006; Purkis & Lipp, 2001).

In the current study, we tested a different prediction that follows from the proposal that FPS reflect automatic affective learning. That is, if FPS primarily reflects simple associative learning, it should primarily be a function of the past stimulus pairings (i.e., conditioning history; Lipp & Purkis, 2005) and should be relatively insensitive to verbal instructions about future stimulus pairings (Mineka & Öhman, 2002; Sevenster et al., 2012a). To test this hypothesis, we made use of the contingency reversal procedure (Grings, Schell, & Carey, 1973; McNally, 1981; Wilson, 1968). In this procedure, participants are informed after a differential conditioning phase that the contingencies of the first phase will be reversed in a second phase. Consequently, in this second phase, cognitive contingency information as provided by the verbal instructions is directly opposed to what has been learned through CS-US pairings in the first phase. If learning is a function of experienced stimulus pairings, conditioned responses in the second phase should be in line with the conditioning history of the first phase. However, if learning is the result of cognitive beliefs regarding the CS-US contingency, conditioned responses should be in line with the verbal instructions, regardless of the conditioning history. In previous studies employing this procedure with SCRs as the measure of conditioning (Grings et al., 1973; McNally, 1981; Wilson, 1968), conditioning in the second phase of the

experiment was in line with the verbal instructions and no evidence for effects of past stimulus pairings was obtained.

In a recent study by Costa, Bradley, and Lang (2015), fear was installed in a first phase by providing threat information to participants. In a second phase, one of the threatened CSs was instructed to be safe, while the other threatened CS remained a threatening stimulus. Similarly, for the initially safe CSs, one of these was threatened, while the other CS remained safe. This adapted reversal procedure allowed them to compare reversed and non-reversed CSs after the reversal instructions and thus controlled for time-related changes (e.g., habituation, sensitization) that could explain the reversal effect. Costa et al. (2015) found that fear reactions, including FPS, completely reversed on the basis of verbal contingency instructions, which demonstrates that FPS is very sensitive to cognitive information (see also: Grillon, Ameli, Woods, Merikangas, & Davis, 1991). However, conditioned responses in their study were instantiated only via verbal threat instructions and not by direct conditioning. Therefore, pairings of the CS in close proximity to the US were absent in the study of Costa et al. (2015), possibly excluding simple associative learning as the result of actual stimulus pairings (Blair, Schafe, Bauer, Rodrigues, & LeDoux, 2001). Hence, it is possible that affective learning did not take place in the study of Costa et al. (2015) due to the absence of CS-US pairings (see also: Olsson & Phelps, 2007, 2004). Therefore, in the current study, we set out to investigate whether FPS to a threatened CS can be reversed on the basis of verbal instructions, even when this CS has actually been paired with the US. Furthermore, we included threatened, but not actually conditioned CSs in our experiment to conceptually replicate the results of Costa et al. (2015) and to compare reversal on these CSs to reversal of threatened CSs that have been actually paired with the US. In line with the hypothesis that FPS reflects affective learning, we predicted that reversal of conditioned responses would be less pronounced for FPS than for SCR and ratings of US expectancy when threat instructions are combined with direct CS-US pairings.

## METHOD

### Participants

Thirty-six right-handed students (11 men, 25 women) at Ghent University participated in the experiment in exchange for €8. Age ranged between 18 and 32 years ( $M = 21.44$ ,  $SD = 2.66$ ). Psychophysiological data from one participant was lost due to a recording error. All participants completed an informed consent form and were instructed that they could discontinue the experiment at any point without any negative consequences. This study was approved by the ethics committee of the Faculty of Psychology and Educational Sciences of Ghent University.

### Materials

#### Conditioned Stimuli

CSs were six white geometric shapes (circle, square, triangle, pentagon, trapezium and diamond) with a maximal radius, longitude and/or latitude of 300 pixels presented in the middle of a 17 inch Dell computer screen (resolution: 1024 by 768 pixels) with a black background. Assignment of these shapes to the different CS types (see Table 1) was randomized over participants.

**Table 1. Overview of the Different CS Types.**

CS	Relationship with the US	Contingency reversal?
CS+T/P	Threatened + paired	No
R-CS+T/P	Threatened + paired	Yes
CS+T	Threatened	No
R-CS+T	Threatened	Yes
CS-	Safe	No
R-CS-	Safe	Yes

### **Unconditioned Stimulus**

The US was an electric stimulus that consisted of 10 rectangular pulses of 2 ms with an inter pulse interval of 8 ms, creating an electric stimulus of 100 ms. This stimulus was administered by two lubricated Fukuda standard Ag/AgCl electrodes (1-cm diameter; inter-electrode distance: ~2-cm) to the left leg over the retromalleolar course of the sural nerve. The stimulus was generated by a constant current stimulator (DS7A, Digitimer, Hertfordshire, UK). The intensity of the electric stimulus was determined for each participant individually to be unpleasant but not painful using a stepwise work-up procedure (see the Procedure section for details concerning this work-up procedure).

### **Psychophysiology**

#### **Skin Conductance Responses (SCRs)**

SCRs were collected using a Coulbourn V71-23 skin conductance coupler (Coulbourn Instruments, Allentown, PA) and disposable Ag/AgCl electrodes (3M Red Dot 2259-50, 17 mm diameter) attached to the thenar and hypothenar eminences of the non-dominant hand. The signal was measured using a constant voltage coupler (0.5 V) and digitized at 10 Hz. The collected data were smoothed and further analyzed offline with Psychophysiological Analysis (PSPHA) (De Clercq, Verschuere, De Vlieger, & Crombez, 2006). SCRs were calculated by subtracting the mean value of a baseline period (2 seconds before CS onset) from the highest amplitude within a 1 to 7 seconds interval after CS onset (Milad, Orr, Pitman, & Rauch, 2005; Pineles, Orr, & Orr, 2009; Raes, De Houwer, De Schryver, Brass, & Kalisch, 2014; Soeter & Kindt, 2012). In this scoring method, negative values and values smaller than  $0.02\mu\text{S}$  were recoded to zero. Finally, collected SCRs were range corrected with the highest recorded amplitude for that participant to account for individual differences in responsivity (Lykken & Venables, 1971) and square root transformed to normalize the data (Dawson, Schell, Filion, & Berntson, 2007).

### **Fear Potentiated Startle (FPS)**

FPS was measured using two miniature Ag/AgCl electrodes (0.5 cm diameter) filled with conductive gel. One electrode was placed just below the pupil of the left eye and the other electrode was placed approximately 1 cm laterally. A ground electrode was placed in the middle of the forehead (Blumenthal et al., 2005). Electrode sites were first gently cleaned with scrub gel and water. The raw electromyographic signal was amplified 50,000 times, filtered online (band pass: 13 – 1000 Hz) and digitally stored at 1000 Hz using a Coulbourn V75-01 bioamplifier (Coulbourn Instruments, Allentown, PA). The acquired data were rectified and smoothed in the area of interest (0 – 150 ms after probe onset) with a FIR filter (Nitschke, Miller, & Cook, 1998) using PSPHA. The startle probe was a 50 ms white noise burst (104 dB) generated using a V85-05C Coulbourn audio module and administered via Sennheiser headphones.

The acquired signal was scored semi-automatically using PSPHA by subtracting the mean baseline value (0 - 20 ms after probe onset) from the peak value in the 21 - 150 ms window after probe onset. All startle responses were visually inspected and scored as missing values if a voluntary blink occurred just before, during or after probe onset, or if there were any other artifacts obscuring the startle response. On average, 4.25% of the trials were scored as missing for each participant ( $SD = 3.38$ ;  $Range = 0\% - 11.11\%$ ). The scores were subsequently T-transformed to control for inter-individual differences in responsivity.

### **US expectancy ratings**

US expectancy ratings were collected on each trial using a 9-point Likert scale presented below the CSs with 5 anchor points: 1 = “not at all”, 3 = “probably not”, 5 = “uncertain”, 7 = “to some extent”, 9 = “certainly”. Above the CSs, the question “To what extent do you expect the electric stimulus?” was presented. Participants indicated their answer by clicking one of the response options of the Likert scale with the computer mouse using their dominant hand.

## Questionnaire

The trait version of the State-Trait Anxiety Inventory (STAI-T; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983; Dutch translation: van der Ploeg, Defares, & Spielberger, 2000) was used to determine the general anxiety level of the participants.

## Procedure

### Work-up procedure

After filling in the informed consent form and the STAI-T questionnaire (Spielberger et al., 1983; van der Ploeg et al., 2000), participants first went through a work-up procedure to determine the intensity level of the electric stimulus. During this procedure, participants were exposed to gradually increasing stimulus intensity levels and were asked to report on their experience. Specifically, participants were asked after each intensity level to verbally rate the electric stimulus on a painfulness scale ranging from zero (not painful at all) to ten (maximally tolerable pain). A minimal painfulness threshold for the electric stimulus was set at seven. The procedure was stopped when participants indicated that they felt uncomfortable experiencing higher intensities of the electric stimulus. If a participant gave a rating of less than seven and indicated that he or she did not want to experience a more intense electric stimulus, the work-up was also stopped and the stimulus with the highest tolerated intensity was selected<sup>1</sup>. The final selected electric stimulus intensity levels ranged between 1.6 and 14 mA ( $M = 5.00$ ,  $SD = 2.62$ ) and pain ratings ranged between 6 and 9.5 ( $M = 7.88$ ,  $SD = 0.81$ ). After the work-up procedure, psychophysiology recording electrodes were applied as described above. Finally, headphones for the startle probe administration were put on. Participants were verbally informed that these headphones served to present loud but harmless noises to them throughout the experiment.

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<sup>1</sup> The results remained similar when the data of four participants who did not reach the painfulness threshold were excluded.

### Contingency instructions and memory test

After the work-up procedure, further instructions regarding the experiment were provided on the computer screen in the absence of the experimenter. Participants were asked to read the instructions carefully. The instructions started with an overview of the different geometric shapes together with the names of these shapes to make sure participants would understand the instructions regarding these shapes. Next, participants were told that some of the shapes would be followed by the electric stimulus and that their task was to indicate to what extent they expect that stimulus after the shape by clicking one of the options on the scale below the shape. Additionally, participants were told that even CSs that could be followed by an electric stimulus would often not be followed by an electric stimulus. This instruction was added to keep the instructions about CS-US relations credible for the instructed CS+s that were never actually paired with a US (see Table 1; see the supplementary materials for a translation of the instructions in the experiment).

Next, participants were instructed about which four geometrical shapes would be predictive of the electric stimulus and which other two shapes would not be followed by the electric stimulus. Participants were told to remember these instructions well because they would afterwards be tested to ascertain that they had memorized the instructions. During this test, participants were shown all the different geometrical shapes twice in a random order. They were asked to indicate for each shape whether it could be followed by the electric stimulus by clicking one of three response buttons projected on the computer screen below the shapes (yes, no, uncertain). There was no response deadline during the test. After responding, participants received feedback for 400 ms indicating whether they were correct. If they made an error on one of the twelve trials or indicated that they were unsure about the correct response, they received the contingency instructions again and had to redo the test, until they passed it (*average number of memory tests until pass* = 1.31, *SD* = 0.58, *Range* = 1-3).



### **Conditioning phase**

Following the contingency instructions, participants continued to the first phase of the experiment. This phase started with six startle probe habituation trials (ITI: 7 s). After these habituation trials, all six different CSs were presented six times (36 trials total). CSs were presented on the screen for eight seconds and were preceded by a fixation cross presented for one second. Startle probes were administered on each trial seven seconds after CS onset (Sevenster et al., 2012a). The ITI was 13, 15 or 17 seconds, randomly selected. Trial order was randomized in small blocks containing two presentations of each CS (limiting the number of consecutive identical trials to maximally four). Two of the four CSs that had been instructed to be contingent with the electric stimulus were followed by the stimulus immediately at offset on three trials (50% reinforcement rate; CS+T/P's). The other two threatened shapes were never reinforced (CS+T's, see Table 1). The reinforcement rate of the CS+T/P's was chosen to be 50% because this is low enough to maintain the credibility of the threat instructions for the threatened but not paired CS+T's, but also high enough to allow for a sufficient number of CS-US pairings for the CS+T/P's. Reinforcement of the CS+T/P's with the US was distributed equally over the course of the conditioning phase (one reinforcement on the first or the second trial, the third or the fourth trial and the fifth or the sixth trial, randomly determined). SCRs, FPS and US expectancy ratings were collected on every trial as described above.

### **Reversal instructions and memory test**

Following the conditioning phase, participants received new instructions that informed them that in the next phase of the experiment, other shapes would predict the electric stimulus. Three shapes were instructed to be predictive of the electric stimulus during this second phase, of which one was previously reinforced (CS+T/P), one was previously threatened (CS+T) and one was previously safe (R-CS-; see Table 1). Furthermore, three shapes were instructed to not be followed by electric stimulus during the second phase, of which one was previously paired with the stimulus (R-CS+T/P), one was previously threatened (R-CS+T) and one was previously safe (CS-; see Table 1). As

for the previous contingency instructions, participants again had to complete a short test to make sure that they remembered these new instructions. The procedure of this memory test was the same as for the previous test. Participants could again continue to the next part of the experiment only if they completed the test without making errors (*average number of memory tests until pass* = 1.39, *SD* = 0.64, *Range* = 1-3).

### **Reversal phase and believability rating**

The procedure of the reversal phase was identical to the procedure of the conditioning phase except that none of the CSs were reinforced during this phase. The reversal phase was followed by a final question that asked participants to indicate to what extent they found the instructions of the experiment believable at the moment they received them. They could select one option of a dropdown list: “not believable”, “not very believable”, “believable” and “very believable”.

### **Data analysis**

In order to present the data of this relatively complex experiment in a concise and clear way, we averaged our collected measures for each of the CSs over trials within the two phases, thus ignoring the factor trial. Exclusion of this factor did not alter our conclusions because the results for the different CSs were consistent over trials. Results and graphs including the factor trial are provided in the supplementary materials. The averaged data were first analyzed with the within-subject factors CS (CS+T/P, CS+T, CS-), reversal (yes, no) and phase (conditioning, reversal). The crucial effect in this analysis is, for our purposes, the three-way interaction which indicates whether reversal instructions resulted in a reduction of conditioned fear for the reversed relative to the consistent CS+T/P and CS+T in the reversal phase, but an increase in conditioned fear for the reversed relative to the consistent CS-. Furthermore, results from the reversal phase were analyzed separately employing omnibus ANOVAs. Specifically, in a first ANOVA we compared the three CSs that, according to the instructions for the second phase, could be followed by the US (i.e., CS+T/P, CS+T, R-CS-; see

Table 1). If the prior conditioning history carried over to the reversal phase, conditioned fear reactions in this phase to the CS+T/P should be higher than to the R-CS- and CS+T. A similar ANOVA was carried out to compare the different CSs that, according to the instructions for the second phase, would not be followed by a US (CS-, R-CS+T/P, R-CS+T; see Table 1). Again, if the conditioning history of the conditioning phase carried over to the reversal phase, conditioned fear reactions to the R-CS+T/P should be higher than to the R-CS+T and the CS-. Degrees of freedom of these ANOVAs were corrected with Greenhouse-Geisser corrections when the sphericity assumption was violated. Finally, we calculated Bayes Factors (BF) using JASP (version 0.6; Love et al., 2015) for our different ANOVAs to complement the results of these traditional analyses. As discussed extensively elsewhere, there are several important limitations to classical null hypothesis significance testing (NHST) (e.g., Wagenmakers, 2007). A problem that is of particular relevance for our own research question is that a non-significant result in NHST does not provide evidence for the null hypothesis (and hence, does not provide evidence for the absence of an effect). Thus, the absence of a significant effect in the traditional ANOVAs does not inform us whether there was a genuine absence of an effect of the prior conditioning phase or of verbal instructions, or whether our data was inconclusive in this regard. However, Bayesian hypothesis testing does allow to quantify the evidence in favor of the null hypothesis (reflected by the BF) and thus allows to evaluate whether effects were genuinely absent or whether our data was inconclusive (e.g., Dienes, 2014; Rouder, Speckman, Sun, Morey, & Iverson, 2009). In line with Jeffreys (1961; see also: Andraszewicz et al., 2015) we considered BFs between  $1/3$  and 1 as anecdotal evidence for the absence of an effect. BFs smaller than  $1/3$  or smaller than  $1/10$  were considered substantial and strong evidence, respectively, for the absence of an effect. Similarly, BFs between 1 and 3 were considered anecdotal evidence for the presence of an effect, while BFs greater than 3 or 10 were considered to be substantial and strong evidence for the presence of an effect, respectively.

## RESULTS

### Believability of the instructions.

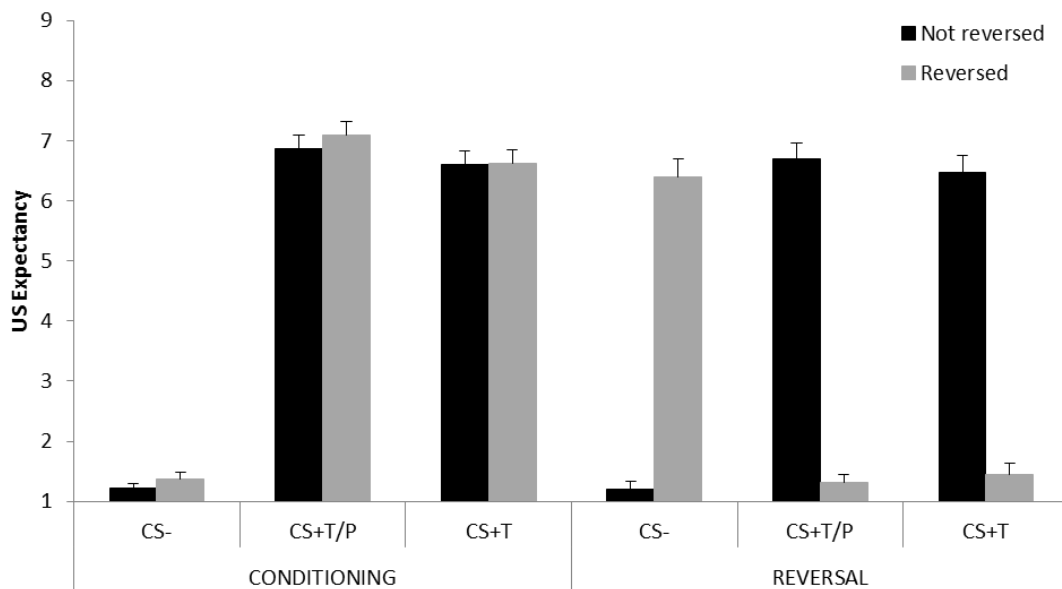
Thirty-one of the participants indicated that the instructions in the experiment were believable or very believable. Three participants indicated that the instructions were not very believable. Ratings from two participants were missing. Overall, these results indicate that our instructions were considered believable by the participants. The results remained similar regardless of whether we included or excluded participants who indicated that the instructions were not very believable. Below, we report only the results for the full sample.

### US expectancy ratings.

The crucial three-way interaction between CS, reversal and phase reached significance for the US expectancy ratings,  $F(1.27, 44.36) = 339.08, p < .001, \eta^2p = .91$ . This interaction was driven by a significant increase in US expectancy ratings for the R-CS- from the conditioning phase to the reversal phase, while US expectancy ratings for the R-CS+T/P and R-CS+T decreased (see Figure 1, all  $t$ -values  $> 18$ ,  $p$ -values  $< .001$ , *Cohen's d's*  $> 3.8$ ). US expectancy ratings for the consistent CS-, CS+T/P and CS+T did not differ significantly across the two phases (all  $t$ -values  $< 1$ ,  $p$ -values  $> .3$ , *Cohen's d's*  $< 0.14$ ; see Figure 1). Hence, US expectancy ratings were very sensitive to the contingency reversal instructions. The Bayesian analysis confirmed that this three-way interaction was a very robust result ( $BF = \sim\infty$ ).

The omnibus ANOVA comparing the CS-, the R-CS+T/P and the R-CS+T, did not reach significance,  $F(1.69, 59.17) = 2.06, p = .143, \eta^2p = .06$ . This results suggests that there is little difference in US expectancy between a consistent CS- and a previously conditioned CS+ or threatened CS+ after contingency reversal instructions (see Figure 1). However, the result of the Bayesian analysis shows that there is only anecdotal evidence for an absence of a difference between these CSs ( $BF = .445$ ). The omnibus ANOVA comparing CS+T/P, CS+T and R-CS-

did reach significance,  $F(1.73, 60.61) = 3.63$ ,  $p = .039$ ,  $\eta^2p = .09$ . This overall effect was due to significantly higher US expectancy ratings for CS+T/P than for R-CS-,  $t(35) = 2.24$ ,  $p = .031$ , *Cohen's d* = 0.17, and CS+T,  $t(35) = 1.93$ ,  $p = .062$ , *Cohen's d* = 0.13 (see Figure 1). There was no significant difference between R-CS- and CS+T,  $t(35) < 1$ ,  $p = .449$ , *Cohen's d* = 0.04. Hence, this result demonstrates that US expectancy was slightly elevated for a consistent CS+ that was previously paired with the electric stimulus compared to a previously threatened CS+ or a reversed CS-. However, there is only anecdotal evidence for this effect according to the corresponding Bayesian analysis (BF = 1.49).



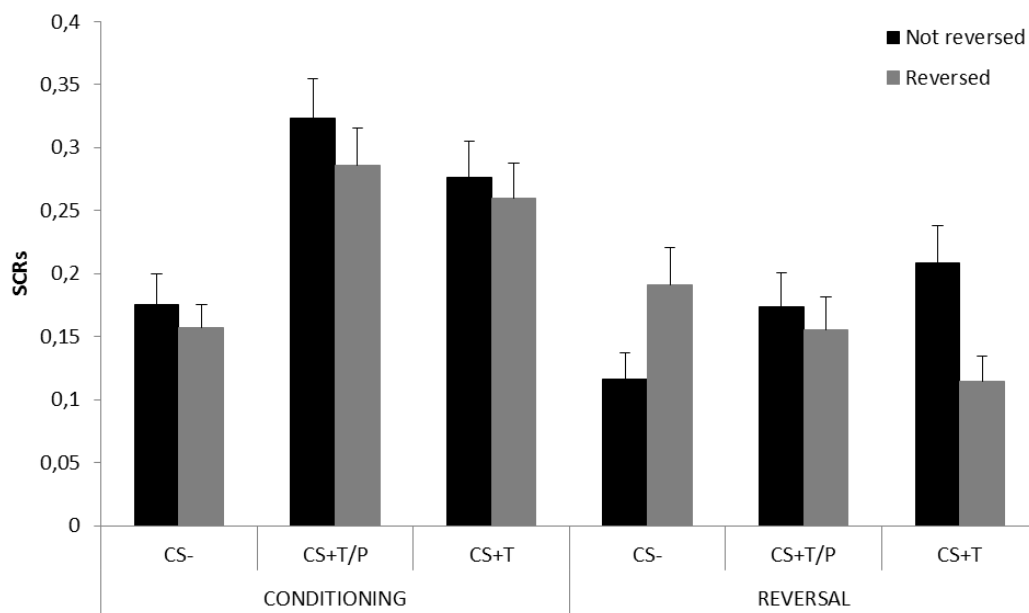
**Figure 1.** Mean US expectancy rating for the different types of CSs in the two phases of the experiment. Error bars represent standard errors.

### SCR

The crucial interaction between CS, reversal and phase reached significance for SCRs as well,  $F(1.74, 59.20) = 5.29$ ,  $p = .010$ ,  $\eta^2p = .10$ . SCRs were lower for all CSs in the reversal phase compared to the conditioning phase ( $t$ -values  $> 1.9$ ,  $p$ -values  $< .07$ , *Cohen's d's*  $> 0.39$ ; see Figure 2), except for R-CS-,  $t(34) = -1.17$ ,  $p = .250$ , *Cohen's d* = -0.24. More importantly, SCRs were larger for

R-CS- than for CS- in the reversal phase, while SCRs for these two CSs were comparable in the conditioning phase (see Figure 2), interaction between reversal and phase for the two types of CS-'s,  $F(1, 34) = 5.65, p = .023, \eta^2p = .14$ . The reversed pattern was found for CS+T. That is, smaller SCRs were found for the R-CS+T compared to the CS+T in the reversal phase, again despite these two CSs being comparable in the conditioning phase (see Figure 2), interaction between reversal and phase for the CS+T's:  $F(1, 34) = 4.50, p = .041, \eta^2p = .12$ . However, there was no indication for an effect of verbal instructions on the CS+T/P's, interaction between reversal and phase for CS+T/P's,  $F(1, 34) < 1$ . That is, SCRs were comparable for CS+T/P and R-CS+T/P both in the reversal and conditioning phase (see Figure 2). These results demonstrate that our reversal instructions successfully induced larger SCRs for a reversed CS- and reduced SCRs for a reversed threatened CS+. Interestingly, however, our reversal instructions did not significantly influence conditioned SCRs to a threatened CS+ that has actually been paired with the US (i.e., CS+T/P). In fact, a Bayesian analysis showed that there was substantial evidence for an absence of an effect of verbal instructions on the CS+T/P's (BF interaction reversal and phase = 0.254). Furthermore, the Bayesian analysis of the three-way interaction between CS, reversal and phase showed that there was only anecdotal evidence for an effect of verbal instructions on SCRs (BF= 2.527).

The results from the reversal phase were again further explored using an ANOVA that compared responses to CS-, R-CS+T and R-CS+T/P. This ANOVA did not reveal a significant effect,  $F(2, 68) = 2.05, p = .136, \eta^2p = .06$ , thus failing to provide evidence for transfer effects of the conditioning phase to the reversal phase for these CSs. The corresponding Bayesian analysis showed that there was only anecdotal evidence for an absence of difference between these CSs (BF = 0.454). Likewise, the ANOVA comparing R-CS-, CS+T and CS+T/P did not reach significance,  $F(2, 68) = 1.17, p = .318, \eta^2p = .03$ , thus also failing to provide evidence for transfer effects of the conditioning history to the verbally established CS+s in the reversal phase on SCRs. In fact, the corresponding Bayesian analysis showed that our data provided substantial evidence for an absence of transfer effects (BF = 0.221).



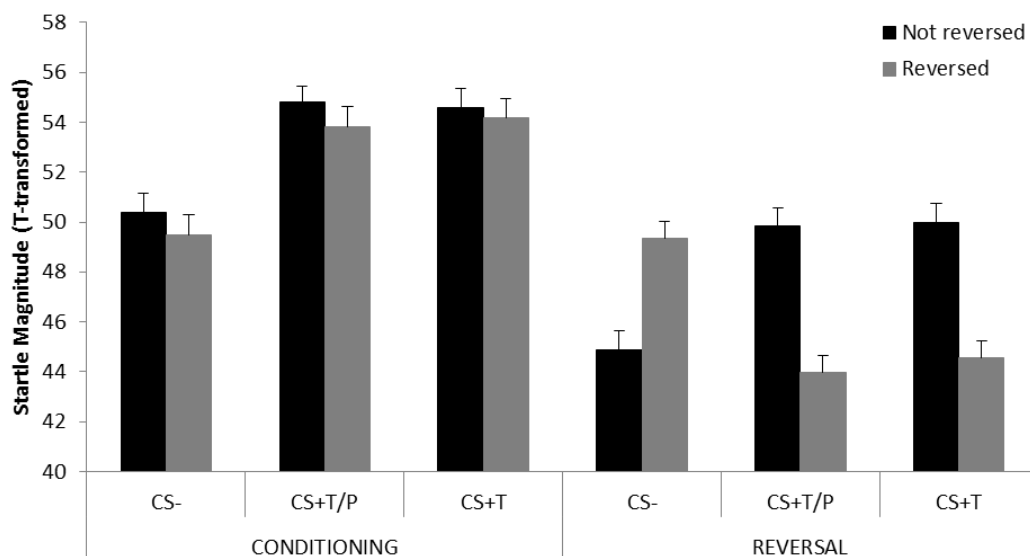
**Figure 2.** Mean range corrected and square root transformed SCRs (measured in  $\mu\text{S}$ ) for the different types of CSs in the two phases of the experiment. Error bars represent standard errors.

### FPS

The crucial three-way interaction between CS, reversal and phase was significant for FPS as well,  $F(1.64, 55.91) = 13.35$ ,  $p < .001$ ,  $\eta^2 p = .28$ . As for SCRs, a general reduction of startle magnitude was observed in the reversal phase for all CSs (see Figure 3;  $t$ -values  $> 3.9$ ,  $p$ -values  $< .001$ , *Cohen's d's*  $> 1.00$ ) except for R-CS-,  $t(34) < 1$ ,  $p = .925$ , *Cohen's d* = 0.03. Importantly, reversal instructions resulted in larger FPS for R-CS- than for CS- in the reversal phase, while FPS was comparable for both these CSs in the conditioning phase (see Figure 3), interaction between phase and reversal for CS-'s:  $F(1, 34) = 11.83$ ,  $p = .002$ ,  $\eta^2 p = .26$ . This pattern was reversed for the CS+T's, with smaller FPS for R-CS+T than for CS+T in the reversal phase, even though FPS was comparable for these two CSs in the conditioning phase (see Figure 3), interaction between phase and reversal for CS+T's:  $F(1, 34) = 11.86$ ,  $p = .002$ ,  $\eta^2 p = .26$ . A similar pattern was

obtained for CS+T/P and R-CS+T/P. That is, FPS was also smaller for R-CS+T/P than for CS+T/P in the reversal phase, while it was comparable for these two CSs in the conditioning phase (see Figure 3), interaction between phase and reversal for CS+T/P's,  $F(1, 34) = 9.26$ ,  $p = .004$ ,  $\eta^2p = .21$ . Combined, these results demonstrate that our verbal instructions were successful in influencing FPS both for previously safe and threatened CSs, including CSs that were actually followed by US. That is, reversal instructions resulted in an increase of FPS for the reversed CS- while it decreased FPS for a reversed threatened CS+, regardless of whether this CS+ was actually paired with the US. The Bayesian analysis confirmed the robustness of this effect of verbal instructions (BF three-way interaction CS, reversal and phase = 567 304).

The ANOVAs comparing the different CS-'s (CS-, R-CS+T/P, R-CS+T) and CS+'s (R-CS-, CS+T/P, CS+T) in the reversal phase did not reach significance,  $F$ -values  $< 1$ . The corresponding BFs for these respective ANOVAs were 0.160 and 0.111. Hence, our data provide substantial evidence for an absence of transfer effects of the previous conditioning history to the reversal phase for FPS.



**Figure 3.** Mean T-transformed startle response (measured in  $\mu\text{V}$ ) for the different types of CSs in the two phases of the experiment. Error bars represent standard errors.



## DISCUSSION

In the current study, we investigated whether verbal instructions can reverse conditioned fear responses. In the two level account of human fear conditioning (Hamm & Weike, 2005; Sevenster et al., 2012), FPS is considered to be a measure of automatic affective learning that operates according to simple associative learning principles whereas SCR is assumed to capture cognitive expectancies. We therefore predicted that the effect of reversal instructions on conditioned fear reactions would be less pronounced for FPS than for SCR, especially when CSs have been repeatedly paired with the US. Our results demonstrated that all measures of conditioned fear were sensitive to the contingency reversal instructions. Interestingly, we also obtained suggestive evidence for effects of CS-US pairings for US expectancy ratings and SCRs, but not FPS.

FPS reactions in the second phase of the experiment were completely in line with the verbal instructions, and no evidence for effects of the prior CS-US pairings were obtained for FPS. These results extend the findings of Costa et al. (2015) by showing that reversal of FPS can take place even when threat instructions are combined with actual CS-US pairings. Hence, even though there was opportunity for simple associative learning to take place in the current study (Olsson & Phelps, 2004), verbal instructions still primarily determined FPS. This finding is even more striking in light of the significant impact of actual CS-US pairings on other measures that are typically considered to be more cognitive in nature (i.e., SCRs, US expectancy ratings). Hence, our results do not fit well with the two level account of fear conditioning that propose that FPS is a measure of automatic affective learning that operates according to the principles of simple associative learning (Blair et al., 2001; Lipp & Purkis, 2005) and that is independent from cognitive contingency learning as measured by SCRs and expectancy ratings (Hamm & Weike, 2005; Hamm & Vaitl, 1996; Sevenster et al., 2012a). Rather, the results in the current study suggest that FPS is very sensitive to verbal instructions and is not influenced by previous CS-US pairings.

Less surprisingly, US expectancy ratings were also very sensitive to verbal reversal instructions as illustrated by an increase in US expectancy ratings for R-CS- and a decrease for the R-CS+T/P and R-CS+T after the contingency reversal instructions (see Figure 1). Furthermore, also a small but reliable effect of the previous conditioning history was obtained for US expectancy ratings. That is, US expectancy ratings were slightly higher in the second phase for a threatened CS that was previously paired with the US (CS+T/P), compared to a threatened CS that was not previously paired with the US (CS+T) and a threatened CS that was previously safe (R-CS-). This latter result is in line with a prior study from our lab showing that US expectancy ratings were slightly elevated for a threatened CS when it was previously paired with the US (Mertens et al., 2015). However, the results of our Bayesian analysis showed that there was only anecdotal evidence for this effect in our data. Combined, these results show that participants adapted their expectancies about receiving an electric stimulus in accordance with the instructions, demonstrating that the instructions were clear. Interestingly, our data suggests that participants also took previous CS-US pairings into account when providing US expectancy ratings.

Finally, results obtained for SCRs were also in line with the verbal instructions, except for the CSs that had been actually paired with the US (CS+T/P's). For these latter CSs, SCRs were comparable in the second phase of the experiment, regardless of the reversal instructions (see Figure 2). However, results in the reversal phase for the CS+T/P's were not completely in line with the prior conditioning history either. That is, SCRs to R-CS+T/P were not significantly higher than to CS- in the reversal phase. Hence, SCRs seem to have been influenced by two opposing influences, that is, instructions on the one hand and actual CS-US pairings on the other hand. This result is in contrast with previous studies employing the contingency reversal procedure that found that SCRs were completely in line with the reversal instructions (Grings et al., 1973; McNally, 1981; Wilson, 1968). One potential reason for this discrepancy between our own results and the results from these earlier studies is the inclusion of threatened, but not actually conditioned, CSs. The fact that participants noticed

that there were threatened and actually conditioned CS+s, as illustrated by the US expectancy ratings, may have prompted them to be more cautious about the instructions concerning the actually conditioned CS+s. However, this interpretation does not explain why we did not obtain a similar pattern for FPS. Alternatively, this result could suggest that SCRs reflect both simple associative learning and cognitive contingency learning. This interpretation is not in line with the findings of the studies outlined in the introduction, but does fit with the results of other studies that have found that SCRs can be dissociated under certain conditions from cognitive contingency learning (Bechara et al., 1995; McAndrew, Jones, McLaren, & McLaren, 2012). Regardless of the exact interpretation of the SCRs results, our results illustrate that SCRs were insensitive to verbal instructions when a CS had been paired with the USs, while such an effect was not observed for FPS. This finding demonstrates that the classification of FPS and SCRs as affective and cognitive measures of fear conditioning, respectively, does not correspond with our data.

Our conclusion runs counter the results of a number of studies that we mentioned in the introduction. We will discuss these studies in more detail here. First, some studies have found that conditioning of the startle reflex can occur in the absence of CS-US contingency awareness while such unaware conditioning was not obtained for SCRs (Hamm & Weike, 2005; Hamm & Vaitl, 1996; Sevenster et al., 2014). However, as mentioned before, a number of other studies have found conditioning of the startle reflex only for participants that became aware of the CS-US contingencies (Dawson, Rissling, et al., 2007; Grillon, 2002; Jovanovic et al., 2006; Purkis & Lipp, 2001). Whether fear conditioning, or learning in general, can occur without contingency awareness is a question that has been proven to be difficult to answer and that requires appropriate measurement of contingency awareness (e.g., Shanks & St. John, 1994) and careful experimental control of other contingencies that could explain learning (e.g., Singh et al., 2013). A recent study by Sevenster et al. (2014) seems to meet these two criteria and nevertheless provide evidence for unaware conditioning of FPS but not of SCRs. While these results are certainly promising, it seems

premature to us to conclude that conditioning of the startle reflex can occur in the absence of awareness given the conflicting evidence. Further replication of the result of Sevenster et al. (2014) will clarify whether this claim can be upheld.

Second, two studies have shown that verbal instructions that the US will no longer be applied results in a complete reduction of SCRs but not of FPS, suggesting a dissociation between SCRs and FPS in their sensitivity to verbal instructions (Dawson, Rissling, et al., 2007; Sevenster et al., 2012a). However, in a recent study by Luck and Lipp (2015a), in which instructed extinction was combined with removal of the shock electrodes, a complete reduction of both SCRs and FPS was observed. As argued by Luck and Lipp (2015b), the incomplete reduction of FPS in the study of Sevenster et al. (2012a) can perhaps be explained by a subset of participants in their experiment that did not find the extinction instructions believable (because the shock electrodes remained attached in the study of Sevenster et al., 2012a). Furthermore, this incomplete reduction was perhaps not observed for SCRs due to increased SCRs to the CS- in the extinction phase for the instructed extinction group in the study of Sevenster et al. (2012a). A similar reasoning can also be applied to the results of Dawson, Rissling, et al. (2007) because their experiment employed a picture-picture evaluative conditioning procedure. Hence, participants could not be sure that the USs would no longer be applied (because the computer screen was not removed), resulting in unconvincing extinction instructions. However, it remains unclear from the data of Dawson, Rissling, et al. (2007) why such an effect was not obtained for SCRs because pre and post extinction SCR data are not provided in their article. Hence, taken together, the studies investigating effects of instructed extinction on SCRs and FPS do not provide definitive evidence for a dissociation between SCRs and FPS either.

Finally, a number of recent studies have demonstrated that behavioral or pharmacological manipulations during a reconsolidation phase specifically reduce FPS but leave expectancy ratings and conditioned SCRs intact (Kindt et al., 2009; Sevenster, Beckers, & Kindt, 2012b, 2013; Soeter & Kindt, 2010, 2012). These studies provide persuasive evidence that FPS can be dissociated from

cognitive measures of conditioned fear. However, other studies have found a reduction of conditioned SCRs after disruption of reconsolidation as well (Oyarzún et al., 2012; Schiller et al., 2010), while still others did not find the disruption of reconsolidation effect either for FPS or SCRs (Bos, Beckers, & Kindt, 2014; Golkar, Bellander, Olsson, & Ohman, 2012). Furthermore, erasure of fear memories through reconsolidation has been shown to depend on prediction error as captured by US expectancy ratings (Sevenster et al., 2013) and some evidence could even suggest that these disruption of reconsolidation effects are more pronounced with concurrent US expectancy ratings (Warren et al., 2014). Thus, reduction of FPS through disruption of reconsolidation may not be as independent of expectancies and SCRs as some studies suggest. Hence, considering all these different studies, currently the data available with regard to unaware learning, instructed extinction and disruption of reconsolidation do not provide definitive evidence that FPS reflects automatic affective learning. The results of the current study provide further evidence that FPS may reflect cognitive contingency learning rather than automatic affective learning.

However, there are several limitations to this study that should be acknowledged. First, as described by Öhman and Mineka (2001), the affective learning module is only selectively activated by biologically relevant or highly aversive stimuli. Therefore, mild electric stimuli as USs and geometric shapes as CSs might not be sufficient to recruit this affective learning module in the learning process. It would be interesting to conduct a follow-up study looking at whether similar results would be obtained with more ecologically valid CSs and USs. A second limitation is that the CS+T/P's and the US were paired on only three occasions throughout the experiment, which might not be a sufficient number of pairings for simple associative learning to take place. On the other hand, if affective learning is an evolutionary adaptive process, it is unlikely that a high number of CS-US pairings is required for simple associative learning to take place. Third, we gave explicit verbal instructions about all the contingencies and asked participants on every trial to provide ratings about the extent to which they expected the US. There is evidence that including online US expectancy

ratings maintains fear conditioning in patients with damage in the amygdala (Coppens, Spruyt, Vandenbulcke, Van Paesschen, & Vansteenwegen, 2009). Furthermore, some studies have compared participants who were instructed about the stimulus contingencies with participants who were either unaware of the contingencies or who learned the contingencies spontaneously. These studies revealed increased activation in brain areas that have been related to decision making and cognitive control in the instructed group (e.g., rostral dmPFC, lateral OFC; Mechias, Etkin, & Kalisch, 2010; Tabbert et al., 2011). Combined, these studies suggest that online US expectancy ratings and contingency instructions may induce a more cognitive way of learning about the CS-US pairings and consequently limited the contribution of affective learning (Coppens et al., 2009). Therefore, it is possible that if CS-US contingencies are learned in a spontaneous manner, stronger effects of the previous conditioning history could be obtained.

Taking into account these reservations, we conclude that FPS should not by default be regarded as a measure of affective learning that is independent of SCRs and expectancy ratings. The results of our experiment demonstrate that conditioning of the startle reflex can depend on verbal instructions and expectancies about the occurrence of the US and does not necessarily follow simple associative learning rules.

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# CHAPTER 6

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## THE IMPACT OF A CONTEXT SWITCH AND CONTEXT INSTRUCTIONS ON THE RETURN OF VERBALLY CONDITIONED FEAR <sup>1</sup>

*Background and Objectives:* Repeated exposure to a conditioned stimulus can lead to a reduction of conditioned fear responses towards this stimulus (i.e., extinction). However, this reduction is often fragile and sensitive to contextual changes. In the current study, we investigated whether extinction of fear responses established through verbal threat instructions is also sensitive to contextual changes. We additionally examined whether verbal instructions can strengthen the effects of a context change.

*Methods:* Fifty-two participants were informed that one colored rectangle would be predictive of an electrocutaneous stimulus, while another colored rectangle was instructed to be safe. Half of these participants were additionally informed that this contingency would only hold when the background of the computer screen had a particular color but not when it had another color. After these instructions, the participants went through an unannounced extinction phase that was followed by a context switch.

*Results:* Results indicate that extinguished verbally conditioned fear responses can return after a context switch, although only as indexed by self-reported expectancy ratings. This effect was stronger when participants were told that CS-US contingency would depend on the background color, in which case a return of fear was also observed on physiological measures of fear.

*Limitations:* Extinction was not very pronounced in this study, possibly limiting the extent to which return of fear could be observed on physiological measures.

*Conclusions:* Contextual cues can impact the return of fear established via verbal instructions. Verbal instructions can further strengthen the contextual control of fear.

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<sup>1</sup>Based on Mertens, G., & De Houwer, J. (2016). The impact of a context switch and context instructions on the return of verbally conditioned fear. *Journal of Behavior Therapy and Experimental Psychiatry*, 51, 10–18. doi:10.1016/j.jbtep.2015.11.001





## INTRODUCTION

Fear conditioning and extinction are considered to provide laboratory analogues for the acquisition of fear and phobias and the subsequent reduction of fear via exposure-based therapy (Field, 2006; Mineka & Zinbarg, 2006). Whereas fear conditioning refers to the acquisition of fear for a Conditioned Stimulus (CS) due to the pairing of the CS with an aversive Unconditioned Stimulus (US), extinction refers to the reduction of conditioned fear through the repeated unreinforced presentation of a CS after the CS-US pairings. Both phenomena have attracted widespread research interest because they allow to investigate complex phenomena such as anxiety disorders and therapeutic interventions in a safe and well controlled laboratory environment.

Despite being an extremely useful framework for understanding the pathogenesis of anxiety disorders and the development of therapeutic interventions, fear conditioning as a model of the development of anxiety disorders has attracted strong criticism as well (e.g., Beckers, Krykotos, Boddez, Eftting, & Kindt, 2013; Field, 2006; Rachman, 1977). One important point of criticism is that fear conditioning in the lab nearly always relies on directly pairing a CS with an aversive US. In contrast to this standard practice in lab studies, retrospective studies with patients have found that it is often not possible to identify direct experience with a traumatic event as the etiology of anxiety disorders (for example, most people in Western countries will in general not have any experience with snakes, but may nevertheless develop phobias for them; e.g., Fredrikson, Annas, Fischer, & Wik, 1996; Oosterink, de Jongh, & Hoogstraten, 2009). Rachman (1977) and Field (2006) argue that, besides directly experiencing a traumatic event, acquisition of (maladaptive) fear can also be based on verbal instructions and social observation. This suggestion is supported by both laboratory research in which fear and avoidance responses have been established on the basis of verbal instructions and observation (Cameron, Roche, Schlund, & Dymond, 2016; Lovibond, 2003; Muris & Field, 2010; Olsson & Phelps, 2007) and retrospective reports of anxiety patients who identified verbal threats and social observation as the starting point of their psychopathology (e.g., King et

al., 1998; Merckelbach, de Jong, Muris, & van den Hout, 1996). However, fear acquisition via verbal instructions and via observation remain relatively understudied phenomena compared to the large amount of research available on fear conditioning through direct CS-US pairings. Arguably, such a lack of research concerning two of the major pathways of fear acquisition hampers a full understanding of the development and treatment of fear and phobias. Therefore, the primary goal of our research was to further investigate the properties of fear acquired through verbal instructions.

Specifically, we wanted to investigate whether extinction of fear established through verbal instructions is similarly sensitive to contextual cues as fear established through direct experience of CS-US pairings. That is, research on extinction of fear (established through direct experience) has shown that extinction often results in a fragile reduction of the conditioned fearful reactions that can easily be overturned by a change in contextual cues. Based on laboratory research it has been suggested that extinction does not lead to unlearning of previously learned contingencies, but rather results in context-dependent inhibitory learning that suppresses the expression of previously learned contingencies within a certain context (Bouton, 2004). This context specificity of extinction is an important phenomenon to understand why relapse can occur after successful therapy (Bouton, 2002). That is, because extinction memory is more context specific than the original acquisition memory, confrontation with a fear-eliciting stimulus in a new context tends to preferentially activate the original acquisition memory rather than the extinction memory, resulting in a return of fear. So far, however, no study has investigated whether extinction of fear established through verbal information is similarly context-specific. Given that verbal instructions can be regarded as a major pathway to the development of maladaptive fears and phobias, it is important to investigate whether return of verbally acquired fear can occur under similar circumstances as for fear acquired through direct experience.

The context-specificity of extinction is most convincingly demonstrated by the renewal effect. In a typical renewal experiment, conditioned fear is established by pairing a CS with an aversive US during an acquisition phase in a

certain context A. This phase is then followed by an extinction phase in a new context B, in which the CS is repeatedly presented without reinforcement. The renewal effect refers to a rapid return of the previously extinguished fear response that occurs when subjects are exposed to the CS in the original acquisition context A (ABA renewal) or in a new context C (ABC renewal), compared to a control condition where the context is not changed (ABB). This basic effect has been obtained both in animal studies (for a review see: Bouton, 2002) and more recently in human studies as well (e.g., Alvarez, Johnson, & Grillon, 2007; Milad, Orr, Pitman, & Rauch, 2005; Vansteenwegen et al., 2005).

In the current study we investigated whether renewal effects can be obtained for verbally conditioned fear (see Dieussaert, Vansteenwegen, & Van Assche, 2005, 2006, for related studies in the context of human contingency learning). We therefore told participants that a certain CS (CS+) would be predictive of an electrocutaneous stimulus (the US) while another CS was said to be safe (CS-). Subsequently, these participants underwent an unannounced extinction phase that was followed by a context switch by changing the background color of the computer screen (e.g., Dibbets, Havermans, & Arntz, 2008; Haesen & Vervliet, 2014)<sup>1</sup>. We expected that the context switch would lead to a return of conditioned fear reactions similar to what has been observed in fear conditioning studies with direct CS-US pairings, even though the CS-US contingency was never directly experienced but merely instructed. We assessed conditioned fear reactions by collecting US expectancy ratings, fear potentiated startle reactions and skin conductance responses on every trial.

A second aim of our study was to investigate whether verbal instructions could modulate the renewal effect. Several models of human associative learning

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<sup>1</sup> To control for time related changes which may explain the renewal effect (i.e., spontaneous recovery) usually a second group is included in which the extinction context is not changed (ABB group). However, in the current study the extinction phase was immediately followed by the context switch which reduces the likelihood that time related changes cause context switch effects. Previous studies with a short delay between the extinction and the renewal phase did not find evidence for time related changes that could explain the renewal effect (Alvarez et al., 2007; Vansteenwegen et al., 2005).

argue that the acquisition and expression of fear is a function of cognitive expectancies about the occurrence of harmful events (Lovibond, 2004; Mitchell, De Houwer, & Lovibond, 2009; Reiss, 1980). These expectancies can be strongly influenced by verbal instructions (e.g., Lovibond, 2003; McNally, 1981). Furthermore, verbal instructions not only allow to communicate whether two stimuli are related, but also allow to specify how they are related and under which conditions the relationship is valid (De Houwer, 2014). Hence, based on these models and studies, we expect that verbal instructions about the relevance of the context for the CS-US relationship could strongly impact the contextual expectancies of encountering an aversive event and hence strongly influence the magnitude of the renewal effect. So far, only one study has addressed the impact of verbal instructions on the renewal effect. In four studies, Neumann (2007) found that verbal instructions that informed the participant that the context was irrelevant for the CS-US contingency was ineffective in attenuating the renewal effect. However, while instructing participants that the context is irrelevant for the CS-US contingency seems to be ineffective in influencing the renewal effect, it cannot be excluded that making the context explicitly relevant for the CS-US contingency via verbal instructions could potentially strengthen the renewal effect. To test for this possibility, we included a second group of participants (context instructions, CI, group) who were informed that the previously instructed CS-US contingency would be instantiated only when the background of the computer screen had a particular color but not when the background of the computer screen had another color. We expected that the effect of the context switch would be particularly pronounced for this group compared to the group that did not receive these context instructions (no context instructions, NCI, group).

Finally, we measured startle reactions during noise alone trials to determine whether the obtained renewal effects could be explained by context conditioning (Alvarez et al., 2007). Specifically, while the renewal effect is usually explained by the context gated expression of a learned inhibitory CS-noUS relationship (Bouton, 2004), an alternative explanation is that participants learn that the context itself is a cue for the presence or absence of the US (context

conditioning; see: Vervliet, Baeyens, Van den Bergh, & Hermans, 2013 for an overview of explanations for the renewal effect)<sup>2</sup>. If renewal in our study is explained by context conditioning, startle reactions should be potentiated in the context predicting the US (or in any other context not predicting the absence of a US), even in the absence of a CS (Alvarez et al., 2007; Vansteenwegen, Iberico, Vervliet, Marescau, & Hermans, 2008). Hence, including startle probes in the absence of CSs allowed us to test whether the renewal effect could be explained by context conditioning, both in the CI and the NCI group.

## METHOD

### Participants

Fifty-two right-handed students at Ghent University participated in the experiment in exchange for €8. Eight of these participants were excluded from analyses because they did not remember the instructions correctly after the experiment (5), because they reported not to believe the instructions (2), or because of a technical failure (headphones were unplugged; 1). Half of the participants were assigned to the CI condition and the other half to the NCI condition. Detailed information about each group is provided in Table 1. Even though there was an imbalance between the two groups in the sex distribution, the results remained similar when the analyses were restricted to include only female participants. We therefore report the results for the full sample.

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<sup>2</sup> Because we established conditioned fear via verbal instructions (and hence, the US was never presented), little or no excitatory conditioning will take place between the context and the US. Nevertheless, inhibitory context conditioning can still take place in our design during the unannounced extinction phase. In fact, inhibitory context conditioning is a more likely alternative account to the Bouton (2004) retrieval model than excitatory context conditioning because it can account for all the different types of renewal (e.g., ABA, ABC and AAB renewal; see Vervliet et al., 2013).

**Table 1. Detailed information for the two experimental groups (standard deviation between brackets).**

	Context instructions group ( <i>N</i> = 22)	No context instruction group ( <i>N</i> = 22)	Difference between groups
Age	23.18 (5.43)	21.27 (2.00)	$t(42) = 1.55$
Sex	6 male	1 male	$\chi^2(1) = 4.25^*$
Final US intensity (mA)	17.30 (12.29)	17.93 (14.46)	$t(42) < 1$
Final US painfulness rating	7.84 (0.66)	7.57 (1.81)	$t(42) < 1$
STAI-T	36.59 (7.18)	36.72 (7.37)	$t(42) < 1$

\* $p < .05$

Note: US = Unconditioned Stimulus (an electrocutaneous stimulation); STAI-T = State-Trait Anxiety Inventory – Trait version (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983).

## Materials

### Psychophysiology

Recording and scoring of the psychophysiological measures was done in accordance with standard procedures in our lab that have been published before. For the sake of brevity, we refer readers to Raes, De Houwer, De Schryver, Brass and Kalisch (2014) regarding the collection and scoring of skin conductance responses (SCR) and to Verschuere, Crombez, Koster, Van Bockstaele and De Clercq (2007) regarding the collection of the startle response.

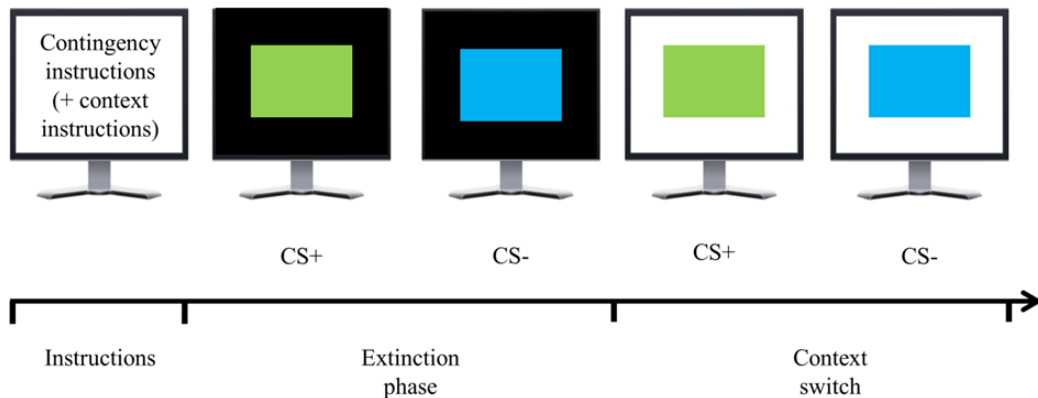
### **US expectancy ratings**

US expectancy ratings were collected on each trial using a 9-point Likert scale presented below the CSs with 5 anchor points: 1 = “not at all”, 3 = “rather not”, 5 = “uncertain”, 7 = “to some extent”, 9 = “certainly”. Above the CSs the question: “To what extent do you expect the shock?” was presented. Participants indicated their answer by clicking one of the response options of the Likert scale with the computer mouse using their dominant hand.

### **Stimuli**

CSs were a blue and a green rectangle (500 by 400 pixels). Assignment of these rectangles as the CS+ and the CS- was counterbalanced over participants.

The US was an electrocutaneous stimulus of 300 ms administered by two lubricated Fukuda standard Ag/AgCl electrodes (1-cm diameter) to the left leg over the retromalleolar course of the sural nerve. The stimulus was generated by a constant current stimulator (DS7A, Digitimer, Hertfordshire, UK). The intensity of this stimulus was determined for each participant individually to be unpleasant but not painful using a stepwise work-up procedure. Note however that this stimulus was never administered during the experiment, but only during the work-up procedure.



**Figure 1.** Schematic overview of the experiment. Note that the assignment of background color and CS colors were counterbalanced over participants. Only the CI group received additional context instructions.

### Procedure

At the start of the experiment, participants had to complete an informed consent that instructed them about the presence of an unpleasant but unharmed electrical stimulus and informed them that they could interrupt the experiment at any time without negative consequences.

Next, participants went through a work-up procedure to determine an appropriate intensity level of the electrocutaneous stimulus. Participants were exposed to gradually increasing levels of intensity of the electrocutaneous stimulus and were asked to select an unpleasant but not painful stimulus. After a final intensity level had been determined (see Table 1), participants were told that this would be the stimulus intensity that they could expect during the experiment. Participants were asked to give a verbal rating between zero and ten of the experienced painfulness of the stimulation (see Table 1). Subsequently, physiology recording electrodes were applied and the experiment was launched on the test computer.

In the first part of the experiment, participants received on the computer screen instructions about the contingencies between the colored rectangle and the electrocutaneous stimulus. This can be considered to be the “acquisition” phase because participants learned the contingencies at this point but were



never directly exposed to these instructed contingencies at any point during the experiment (see Figure 1 for a schematic overview of the experiment). The instructions (in Dutch) informed participants that they would see two colored rectangles during the experiment. One colored rectangle (green or blue, counterbalanced) was instructed to sometimes be followed by the electrocutaneous stimulus while the other colored rectangle was instructed to never be followed by the electrocutaneous stimulus. One half of the participants (CI group) was also informed that this rule would be valid only when the background of the screen is white (or black, counterbalanced) and that when the background of the screen is black (or white) no electrocutaneous stimuli would be applied. The other half of the participants did not receive these additional instructions (NCI, group; see Appendix A for a translation of the instructions). Participants were further informed that their task was to indicate to what extent they expect the electrocutaneous stimulus each time a rectangle appears by clicking one of the response options on the scale below the rectangle. They were asked to provide their ratings quickly after the appearance of the rectangle. The background color of the computer screen during the instructions was either black or white (counterbalanced) and was always the same as the background color of the renewal phase, but different from the background color in the extinction phase. Hence, the procedure resembled an ABA renewal design, with this difference that the first phase involved only instructions that referred to events on the second phase or third phase. Because we did not have an acquisition phase in which CS-US pairings were presented, it was not possible to return to the original acquisition context (and hence have a standard ABA renewal design).

After these instructions, the extinction phase started. The background of the computer screen during the extinction phase changed to either black or white (counterbalanced; see Figure 1) and was in the color that was instructed to be safe for the CI group. The extinction phase started with 5 habituation startle probes with an interval of 7 seconds. Next, both colored rectangles were each presented 10 times during 8 seconds without reinforcement, preceded by a fixation cross during 2 seconds. On each trial, a startle probe occurred 7 seconds

after CS onset (Sevenster, Beckers, & Kindt, 2012). The inter-trial interval between the stimuli was either 6, 8 or 12 seconds, randomly determined. Furthermore, 10 startle probes were presented throughout the extinction phase in the absence of a CS (noise alone, NA, trials) with the same inter-trial interval. The sequence of trials was randomly determined with the exception that no more than two identical trials could occur in sequence.

The extinction phase was immediately followed by an unannounced change in the background color of the screen (context switch; from black to white or vice versa). A short 5 seconds interval followed the context switch to avoid that SCRs on the first trial in the new context would be influenced by the orienting response. This new background color was always the instructed threatened background color for the CI group. Three unreinforced presentations of each colored rectangle and three additional NA trials were presented within this new context with the same trial procedure as during the extinction phase. The first trial after the context switch was experimentally controlled: half of the participants saw the CS+ first while the other half saw the CS- first.

At the end of the experiment, participants were asked to retrospectively rate the believability of the instructions at the moment they received them by selecting one of four options from a dropdown list: “not believable”, “not very believable”, “very believable” and “completely believable”. Two participants who selected “not believable” were excluded from the analyses. Finally, participants were asked to indicate which of the two colored rectangles would be followed by the electrocutaneous stimulation according to the instructions by selecting one of three response options from a dropdown list: “the green rectangle”, “the blue rectangle” or “I don’t know”. Five participants who did not correctly identify the CS+ were excluded from the analyses.

## RESULTS

The different measures of conditioned fear (US expectancy, SCR and startle) were analyzed separately. For each measure, two mixed ANOVAs were

conducted. First, the data obtained during the extinction phase were analyzed using an ANOVA with the within-subjects factors CS (US expectancy ratings and SCR: CS+, CS-; startle: CS+, CS-, NA) and Trial (1 to 10), and the between-subjects factor Group (CI or NCI group). Second, the effect of the context switch was assessed by comparing the responses from the last trial of the extinction phase with the responses from the first trial of the switch phase (factor Phase). Additional factors in this second analyses were the factors CS (CS+, CS-) and Group (CI or NCI group).

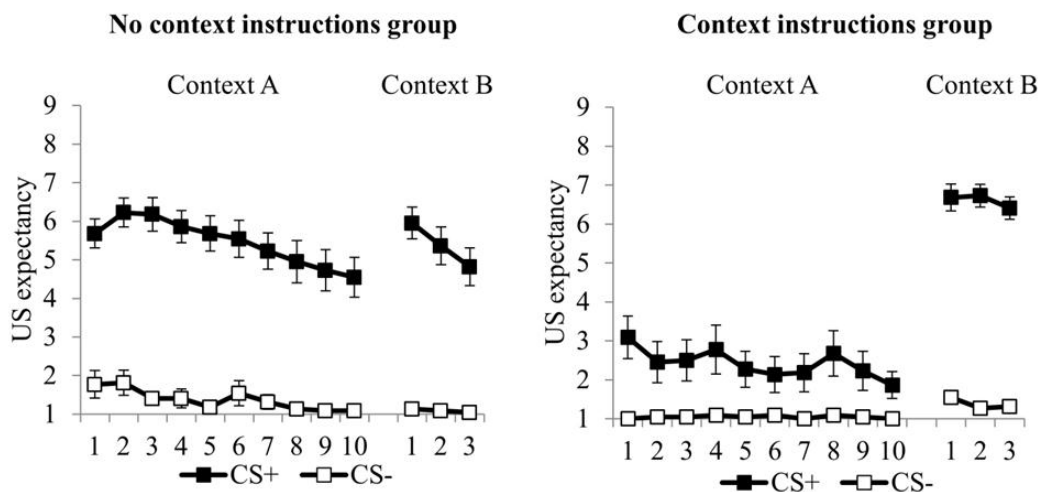
Greenhouse-Geisser corrections were applied when the sphericity assumption was violated and  $p$ -values below .05 were considered significant.

### **US expectancy ratings**

Successful conditioning was obtained on the basis of verbal instructions as evidenced by significantly higher US expectancy ratings for the CS+ ( $M = 3.94$ ,  $SE = 0.30$ ) than for the CS- ( $M = 1.21$ ,  $SE = 0.07$ ) during the extinction phase, main effect of CS:  $F(1, 42) = 80.24$ ,  $p < .001$ ,  $\eta^2p = .66$ . Conditioning during the extinction phase was more pronounced for the NCI group ( $M = 4.09$ ,  $SD = 1.93$ ,  $t(21) = 9.94$ ,  $p < .001$ ,  $d = 2.12$ ) than for the CI group ( $M = 1.37$ ,  $SD = 2.11$ ,  $t(21) = 3.05$ ,  $p = .006$ ,  $d = 0.65$ ; see Figure 2), interaction between CS and group:  $F(1, 42) = 19.83$ ,  $p < .001$ ,  $\eta^2p = .32$ . This result demonstrates that our context instructions (i.e., no USs would be applied during the extinction context for the CI group) successfully reduced US expectancy ratings. Furthermore, US expectancy ratings tended to decrease throughout the extinction phase (see Figure 2), main effect of trial:  $F(3.81, 160.21) = 6.46$ ,  $p < .001$ ,  $\eta^2p = .32$ . There was a marginally significant interaction between CS and trial, indicating that US expectancies tended to decrease more for the CS+ than for the CS- during the extinction phase,  $F(3.68, 154.61) = 2.42$ ,  $p = .056$ ,  $\eta^2p = .05$ . The three way interaction between CS, trial and group was not significant,  $F(3.68, 154.61) = 1.32$ ,  $p = .267$ ,  $\eta^2p = .03$ , suggesting that the extinction tendency was comparable for the CI and NCI group.

The context switch led to a significant increase in US expectancy ratings after the context switch ( $M = 3.83$ ,  $SE = 0.14$ ) compared to before the context

switch ( $M = 2.13$ ,  $SE = 0.16$ ), main effect of phase:  $F(1, 42) = 237.20$ ,  $p < .001$ ,  $\eta^2p = .85$ . This effect was more pronounced for the CS+ (see Figure 2), interaction between CS and phase:  $F(1, 42) = 87.36$ ,  $p < .001$ ,  $\eta^2p = .65$ . Importantly, this specific renewal effect was especially pronounced for the CI group (*increase in differential conditioning* = 4.27,  $SD = 2.39$ ,  $t(21) = 8.37$ ,  $p < .001$ ,  $d = 1.79$ ) compared to the NCI group (*increase in differential conditioning* = 1.36,  $SD = 1.84$ ,  $t(21) = 3.48$ ,  $p = .002$ ,  $d = 0.74$ ; see Figure 2), three-way interaction between CS, phase and group:  $F(1, 42) = 20.42$ ,  $p < .001$ ,  $\eta^2p = .33$ . This result demonstrates that the context instructions strengthened the renewal effect. Note that the difference in the renewal effect was driven mainly by differences between the two groups in the extinction phase. That is, an interaction between CS and group was observed only at the end of the extinction phase,  $F(1, 42) = 17.23$ ,  $p < .001$ ,  $\eta^2p = .29$ , but not at the first trial of the renewal phase,  $F(1, 42) < 1$ . Thus, the greater renewal effect of the CI group was mainly due to reduced differential US expectancy ratings in the extinction phase, rather than larger differential US expectancy ratings in the renewal phase (see Figure 2)<sup>3</sup>.



**Figure 2.** US expectancy ratings throughout the experiment for the two experimental groups. Error bars represent standard error.

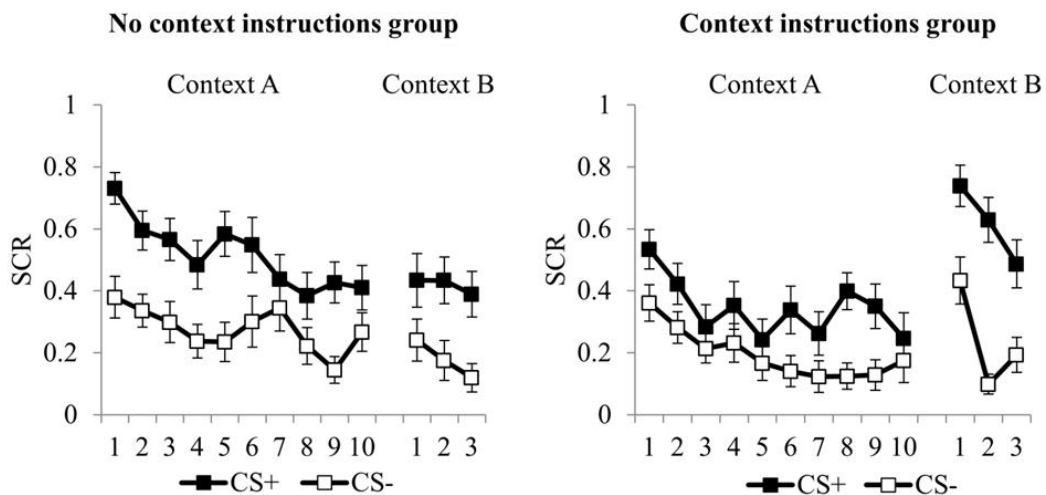
<sup>3</sup> However, note that when we included all three trial of the renewal phase to compare the CI and NCI group, we did obtain a trend for larger differential US expectancy ratings for the CI group,  $F(1, 42) = 3.24$ ,  $p = .079$ ,  $\eta^2p = .07$ .

**SCR**

Similar to the US expectancy ratings, there was a significant difference in SCR between the CS+ ( $M = 0.43$ ,  $SE = 0.03$ ) compared to the CS- ( $M = 0.24$ ,  $SE = 0.02$ ), demonstrating conditioning on the basis of verbal instructions, main effect of CS:  $F(1, 42) = 66.70$ ,  $p < .001$ ,  $\eta^2p = .61$ . Furthermore, there was a significant effect of the factor trial,  $F(9, 378) = 6.72$ ,  $p < .001$ ,  $\eta^2p = .14$ , but this did not interact with CS,  $F < 1$ , indicating that the extinction procedure led to a reduction of the SCRs, but not differently so for the two CSs (see Figure 3). Finally, the interaction effect between CS and group was marginally significant,  $F(1, 42) = 3.70$ ,  $p = .061$ ,  $\eta^2p = .08$ , due to less differential conditioning in the CI group ( $M = 0.15$ ,  $SD = 0.16$ ,  $t(21) = 4.24$ ,  $p < .001$ ,  $d = .94$ ) than in the NCI group ( $M = 0.24$ ,  $SD = 0.15$ ,  $t(21) = 7.45$ ,  $p < .001$ ,  $d = 1.60$ ; see Figure 3), suggesting that our instructions were successful to reduce conditioned reactions for the CI group during the extinction phase on SCRs as well. The other interaction effects did not reach significance,  $F$ -values  $< 1$ .

The context switch led to a significant increase in SCRs (see Figure 3), main effect of phase:  $F(1, 42) = 14.86$ ,  $p < .001$ ,  $\eta^2p = .26$ . However, there was no significant difference in the context switch effect between the CS+ and the CS-, interaction between phase and CS:  $F(1, 42) = 1.92$ ,  $p = .173$ ,  $\eta^2p = .04$ . This indicates that the context switch led to a general increase of fear for both the CS+ and CS-, rather than a specific increase of fear for the CS+. This is a commonly observed effect in studies on return of fear (Vervliet et al., 2013). Importantly, the interaction effect between the factor phase and group was significant,  $F(1, 42) = 15.06$ ,  $p < .001$ ,  $\eta^2p = .26$ , demonstrating that the effect of the context switch (a general increase in SCRs) was larger for the CI group ( $M = 0.38$ ,  $SD = 0.35$ ,  $t(21) = 4.97$ ,  $p < .001$ ,  $d = 1.09$ ) than for the NCI group ( $M = -0.001$ ,  $SD = 0.29$ ,  $t(21) = -0.02$ ,  $p = .984$ ,  $d \approx 0$ ; see Figure 3). This greater non-specific renewal effect for the CI group was mainly explained by larger SCRs in the CI group at the first trial of the renewal phase,  $F(1, 42) = 13.15$ ,  $p = .001$ ,  $\eta^2p = .24$ , rather than smaller SCRs in the CI group at the last trial of the extinction phase,  $F(1, 42) = 2.45$ ,  $p = .125$ ,  $\eta^2p = .06$ . Thus, the verbal instructions increased

SCRs to both the CS- and CS+ after a context switch, rather than reduced SCRs in the instructed safe context (see Figure 3). However, note that this does not apply for the whole extinction phase. When all trials of the extinction phase were considered, we did see evidence for a reduction of differential SCRs for the CI group (see the results of the extinction phase). Finally, there was no significant three-way interaction between phase, CS and group,  $F(1, 42) < 1$ .



**Figure 3.** Skin conductance responses throughout the experiment for the two experimental groups. Error bars represent standard error.

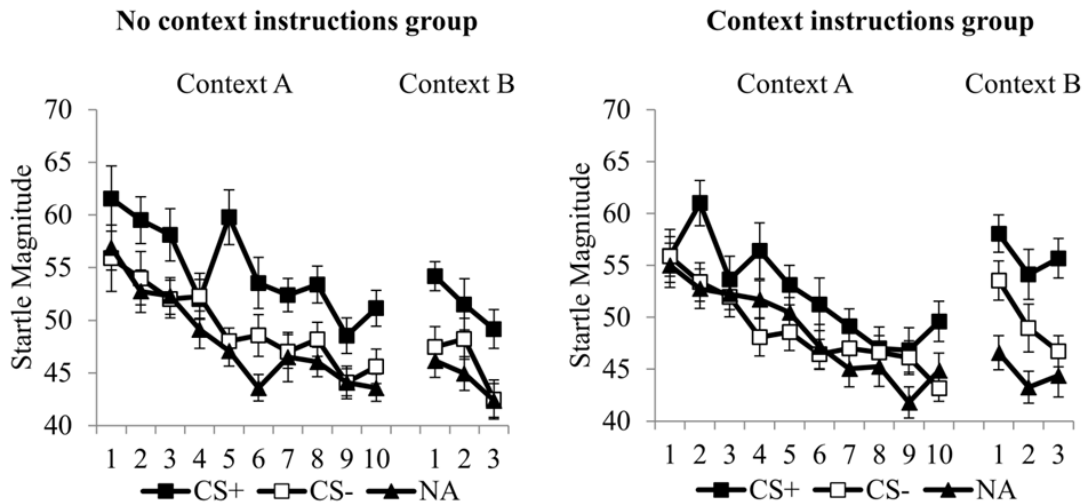
### Startle

For the analysis of the extinction phase, startle responses were averaged per two trials to reduce the impact of missing observations on the results. Startle responses were stronger to the CS+ probes ( $M = 53.75$ ,  $SE = 0.56$ ) than towards the CS- ( $M = 49.19$ ,  $SE = 0.47$ ) or the NA probes ( $M = 48.42$ ,  $SE = 0.46$ ), main effect of CS:  $F(2, 82) = 25.09$ ,  $p < .001$ ,  $\eta^2p = .38$ , demonstrating conditioning on the basis of verbal instructions for startle as well. Startle magnitude tended to decrease throughout the extinction phase, main effect of trial,  $F(3.33, 136.47) = 48.11$ ,  $p < .001$ ,  $\eta^2p = .54$ , but this effect did not interact with CS,  $F < 1$ . Descriptively, conditioning effects were larger in the NCI ( $M = 5.56$ ,  $SD = 5.08$ ,  $t(21) = 5.14$ ,  $p < .001$ ,  $d = 1.09$ ) than in the CI group ( $M = 3.64$ ,  $SD = 5.83$ ,  $t(21) =$

2.93,  $p = .008$ ,  $d = 0.62$ ; see Figure 4), which would be expected on the basis of our instructions, but this effect failed to reach significance; interaction between CS and group:  $F(2, 82) = 1.87$ ,  $p = .161$ ,  $\eta^2p = .04$ . All the other interaction effects were not significant,  $F$ -values  $< 1$ .

The context switch led to stronger startle responses after the context switch ( $M = 50.95$ ,  $SE = 0.67$ ) compared to before ( $M = 46.31$ ,  $SE = 0.68$ ); main effect of phase:  $F(1, 39) = 20.44$ ,  $p < .001$ ,  $\eta^2p = .34$ . This effect of phase did not interact with CS,  $F < 1$ , indicating that the context switch led to a general increase in startle responses (see Figure 4). Importantly, the interaction effect between phase and group approached significance,  $F(1, 39) = 4.01$ ,  $p = .052$ ,  $\eta^2p = .09$ , due to larger effects of the context switch (a general increase in startle responses) for the CI group ( $M = 6.99$ ,  $SD = 6.28$ ,  $t(21) = 5.22$ ,  $p < .001$ ,  $d = 1.11$ ) than for the NCI group ( $M = 2.60$ ,  $SD = 6.56$ ,  $t(21) = 1.86$ ,  $p = .077$ ,  $d = 0.40$ ; see Figure 4). As for SCRs, this non-specific renewal effect was mainly due to stronger startle responses in the CI group at the first trial of the renewal phase,  $F(1, 41) = 7.01$ ,  $p = .011$ ,  $\eta^2p = .15$ , rather than weaker startle responses in the CI group at the end of the extinction phase,  $F(1, 40) < 1$  (see Figure 4). The three way interaction between phase, CS and group did not reach significance,  $F(2, 78) = 2.13$ ,  $p = .126$ ,  $\eta^2p = .05$ .

Finally, we did not observe a significant increase in NA startle reaction after compared to before the context switch for the NCI group,  $t(21) = 1.34$ ,  $p = .194$ , *Cohen's d* = .29, or the CI group,  $t(20) < 1$ ,  $p = .370$ , *Cohen's d* = .20. Also, there was no difference between the two groups in the increase in context conditioning as measured by NA startle reactions going from the extinction phase to the renewal phase,  $F(1, 41) < 1$ , suggesting that differences in context switch effects between the two groups cannot be explained by differences in context conditioning.



**Figure 4.** Startle responses throughout the experiment for the two experimental groups. Error bars represent standard error.

## DISCUSSION

The main aim of the current study was to investigate whether extinguished fear reactions that were initially established on the basis of verbal instructions are sensitive to a change in context. To this end, participants were verbally informed about the contingencies between two CSs and a US. After these instructions, participants were subjected to an unannounced extinction phase which was immediately followed by a context switch. US expectancy ratings, skin conductance responses and startle responses were measured throughout the experiment. In addition, a second group of participants was included who were explicitly informed about the two contexts in the experiment (i.e., a safe context and a second context in which CS-US pairings would occur). We report three main findings: First, a context switch after an unannounced extinction phase led to a selective return of conditioned responding (i.e., stronger for CS+ than for CS-) as measured by US expectancy ratings. However, this was not accompanied by a comparable selective return of fear on SCRs or startle responses. Instead, for startle (but not for SCR) we observed a trend for a general return of fear (i.e., not different for CS+ than for CS-). Second, verbal instructions about the relevance of the context for the CS-US contingency resulted in stronger context switch effects



on all measures. Third, no evidence was obtained that the context switch effects could be explained by context conditioning as measured by NA startle reactions. These three findings will be discussed in greater detail below.

First, evidence for a selective renewal effect was obtained on US expectancy ratings even though conditioning was established on the basis of verbal instructions (see left panel Figure 2). That is, US expectancy ratings, especially for the CS+, increased after the context switch relative to the last trial of the extinction phase. Furthermore, a trend for a general increase of fear (both for the CS+ and the CS-) was observed for startle responses after the context switch, which can also be considered to be an indication of renewal (Vervliet et al., 2013). However, the fact that we found specific renewal only for the US expectancy ratings but not for the psychophysiological measures calls for caution when interpreting the results. Previous studies in which conditioning was established through direct experience revealed specific renewal on psychophysiological measures as well (e.g., Alvarez et al., 2007; Milad et al., 2005; Vansteenwegen et al., 2005). One explanation for the absence of specific renewal effects on the psychophysiological measures in the current experiment might be because extinction, despite 10 extinction trials, was quite limited in magnitude. This weak extinction, in turn, limits the likelihood of finding strong renewal effects both statistically (less room for a return of conditioned fear reactions) and mechanistically (less inhibition learning). Alternatively, it is possible that uninstructed fear conditioning, relative to instructed fear conditioning, leads to either more context-independent acquisition learning or more context-dependent inhibitory learning, therefore resulting in stronger renewal effects that are also observed on psychophysiological measures. It would be interesting for future studies to directly study the impact of including contingency instructions on the renewal effect. Nevertheless, the specific renewal effect on US expectancy ratings and the general return of fear on startle in the current study provide reasonable evidence that contextual cues are important for the return of fear, also when this fear was initially established on the basis of verbal instructions.

Our results have both clinically and theoretically interesting implications. Clinically, they suggest that fears acquired on the basis of verbal instructions can quickly return when the context changes. This is an important finding because previous research has demonstrated that verbal instructions can be an important pathway through which fear and phobias are acquired (e.g., Muris & Field, 2010). Given that the renewal effect can be a source of relapse after successful therapy (Bouton, 2002), our results suggest that fear acquired through verbal instructions might pose similar challenges for successful therapy as fears established through direct conditioning. Therefore, it would be interesting for future studies to test whether procedures that seem to be effective in preventing or reducing renewal, such as extinction training in multiple context (e.g., Gunther, Denniston, & Miller, 1998) or including extra extinction cues in the extinction training and the renewal phase (e.g., Dibbets et al., 2008), are effective to reduce the renewal effect after verbally instructed fear conditioning as well. Theoretically, our results suggest that conditioning via verbal instructions also competes for expression with contingencies subsequently learned in an extinction phase and that the context can gate this expression of learned information (Bouton, 2004). More generally, our findings once again highlight the similarities between learning via instructions and via direct experience (Grings, 1973; Lovibond, 2003). Hence, they advocate a model of fear conditioning and learning that allows for strong similarities between learning via instructions and learning via direct experience.

Second, informing a group of participants about the presence of two contexts and its relevance for the CS-US contingencies strengthened the context switch effects on all collected measures for this group. This finding demonstrates that verbal information about the context can strongly modulate the impact of a context switch on the expression of fear. However, the effect of the context instructions was differently expressed on the different measures of fear. For US expectancy, the context instructions primarily reduced US expectancy ratings for the CS+ in the extinction phase, but did not reliably increase differential US expectancy ratings after the context switch. For the physiological measures, on the other hand, the context instructions resulted in larger fear responses to both

the CS+ and the CS- after the context switch, but did not reduce psychophysiological responses before the context switch (see Figures 2, 3 and 4). These results may suggest that our instructions differently affected self-report ratings and psychophysiological measures of conditioned fear. However, when all the trials of the extinction and renewal phase were considered, similar trends were observed for both types of measures. That is, increased differential and non-differential US expectancy ratings were obtained when all trials of the renewal phase were considered, and differential fear reactions tended to be reduced for SCRs and startle when all trials of the extinction phase were taken into account. Hence, there was some evidence in our data for similar effects of verbal instructions on psychophysiological and self-report measures. This may suggest that our study may have lacked sufficient power to reliably detect certain, more subtle, effects of the verbal instructions on self-report ratings and psychophysiological measures. Context-specific inflation and reduction of differential fear responses on both psychophysiological and self-report measures through verbal instructions may be obtained when a sufficient amount of observations are considered. Nevertheless, regardless of these considerations about the differences between the psychophysiological measures and the US expectancy ratings, our study does provide an important proof-of-principle that verbal instructions about the relevance of the context for the CS-US contingency can strengthen contextual control of fear, on both self-report and psychophysiological measures.

Our results are complementary to the only other study that has investigated the impact of verbal instructions on the renewal effect. Whereas we found that instructions can strengthen the renewal effect, Neumann (2007) observed that verbal instructions cannot attenuate the renewal effect. The combination of our own results with these of Neumann (2007) may suggest that verbal instructions are successful in strengthening the renewal effect, but not in attenuating it. However, the way conditioned reactions were measured differed between our own study (physiological and self-report measures) and the studies of Neumann (withholding a response in a videogame). Furthermore, conditioning was established by directly experiencing the contingencies in the studies by

Neumann (2007) rather than via verbal instructions in our own study. Further research will need to clarify exactly under which conditions verbal instructions can impact the contextual expression of fear.

More generally, the effects of context instructions that we observed in our study are in correspondence with the aforementioned expectancy models of associative learning and fear conditioning (De Houwer, 2009; Lovibond, 2004; Mitchell et al., 2009; Reiss, 1980) by showing that the expression of learned fear reactions is strongly influenced by verbal instructions about when the CS-US contingency applies. That is, our CS-US contingency instructions strengthened fear reaction within a certain context in which they were instructed to apply, and reduced fear reactions in a context in which they were instructed not to apply. Such a result cannot easily be explained by the formation of simple associations when receiving instructions (e.g., Field, 2006; Uglan, Dyson, & Field, 2013) because these associations do not offer a way to encode validity information. Rather, it shows that our instructions are encoded and expressed in a conditional format, which seems to fit better with the idea of learning through the formation of propositions about the relationship between the CS and the US as proposed by propositional models of associative learning (De Houwer, 2009; Mitchell et al., 2009).

Finally, we did not find evidence for context conditioning as measured by startle reactions during NA trials, neither for the CI group nor the NCI group. This result suggests that the effect of the context switch cannot be explained by context conditioning in either group. This result fits with the results from Alvarez et al. (2007) who did not find any evidence either that context conditioning, as measured by NA startle reactions, could explain the renewal effect.

In conclusion, our study demonstrates that extinction after conditioning via verbal instructions is sensitive to contextual cues. In addition, we provide evidence that verbal instructions can strengthen the contextual control of fear.

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**CHAPTER** **7**

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**GENERAL DISCUSSION**



## INTRODUCTION

The aim of the research presented in this thesis was to provide a contribution to the functional knowledge about fear learning via verbal instructions and stimulus pairings and to further test mental process models of fear learning. As we have seen in the introduction, currently fear learning via verbal instructions is not so well understood (Olsson & Phelps, 2007), despite that it probably constitutes a major pathway of how fears are acquired (Field, 2006; Rachman, 1977, 1991). Furthermore, mental process models disagree on the processes that allow for the acquisition of fear through verbal instructions and stimulus pairings, and under which conditions these pathways can produce learned fear responses. Therefore, the goal of the research presented in this thesis was to test a number of predictions of the different mental process models of fear learning and, more generally, to provide a contribution to the functional knowledge (i.e., which events in the environment determine behavior) of fear learning via verbal instructions and stimulus pairings.

In this General Discussion, I will first briefly recapitulate the results and conclusions from the different empirical chapters. Then, I will discuss how the results of the different experiments in this thesis add to our knowledge of the functional properties of fear learning via verbal instructions, and the implications they have for mental process models of fear learning. Furthermore, I will briefly discuss how the results of the different studies can help to come to a better understanding of the etiology of anxiety disorders and what possible implications could be drawn for the prevention and treatment of anxiety disorders. Finally, I will conclude this thesis by outlining a number of avenues for future research.

## SUMMARY OF THE EMPIRICAL CHAPTERS

In Chapter 2, the effect of experiencing CS-US pairings in combination with verbal threat instructions was investigated for several measures. Participants

were informed that two CSs are predictive of an electric shock. Participants were further told that in a first phase the shocks that would follow one of the CSs would be replaced by a picture of a lightning bolt in order to not present too many shocks before the actual experimental phase started. This cover story allowed us to present two instructed CS+ stimuli of which only one was actually paired with the US. When, in a second phase, participants were told that both CSs would now be followed by electric shocks, we found additive effects of the previous CS-US pairings for fear ratings and US expectancy ratings. Participants gave slightly higher ratings for the CS that had in the previous phase been paired with the shock. A trend for such an additive effect was also observed for fear potentiated startle. However, for skin conductance responses, no evidence for such additive effects were obtained. This latter finding replicated the results of Raes, De Houwer, De Schryver, Brass and Kalisch (2014) who did not find evidence for an additive effect of CS-US pairings for skin conductance responses either. Finally, in Chapter 2, we investigated the impact of an unpaired presentation of the shock on the different measures of fear after the testing phase. We found that this unpaired shock presentation led to an increase of fear on all the different measures (i.e., generalized reinstatement). Furthermore, for fear ratings, the unpaired shock presentation resulted in a specific increase for the instructed CS+ that had not been paired with the shock (i.e., differential reinstatement for the instructed but not paired CS+).

In Chapter 3, we investigated the additive impact of experiencing an instructed CS-US contingency on early visual processing of the CS. Previous research demonstrated that both verbal threat instructions and stimulus pairings can enhance ERP components related to early visual processing. However, no previous study investigated the effects of combining these two pathways of fear. In our experiment, participants were told that two CSs could be predictive of an electric shock, while another CS was instructed to be safe. New CSs were selected throughout the experiment to ensure that the observed effects could not be due to overtraining of the CS-US relation (i.e., a large number of CS-US pairings). Half of the instructed CS+s were followed by the electric shock. Our

results showed that our threat instructions resulted in a reduction of the P3 amplitude. However, no effects of threat instructions or CS-US pairings were found for any of the early visual components we investigated (i.e., C1, P1 and N1). Furthermore, no additive effects of adding stimulus pairings were obtained for any of the investigated components.

In Chapter 4, we investigated whether prepared fear learning effects could be obtained when fear is established via verbal instructions. Prepared fear learning refers to the observation that the acquisition of fear responses is facilitated, and extinction of these fear responses is delayed for fear-relevant stimuli such as pictures of snakes and spiders compared to fear-irrelevant stimuli such as pictures of flowers and birds. Therefore, in two experiments participants received contingency instructions about the relation between fear-relevant and fear-irrelevant CSs and the presence of an electric shock (the US). In the second experiment, we used the procedure from Chapter 2 to also manipulate whether participants actually experienced pairings of the fear-relevant and fear-irrelevant CSs with the US, or whether these pairings were merely instructed. This second experiment was included to test whether prepared learning effects might depend on stimulus pairings, as could be predicted from dual-process models of fear learning (Olsson & Phelps, 2007). We did not find any evidence for preparedness effects when fear was established via verbal instructions in the first experiment. However, in the second experiment, we found that fear acquisition was more pronounced for instructed fear-relevant CS+s compared to instructed fear-irrelevant CS+s. This effect was particularly pronounced on US expectancy ratings. Furthermore, we did not find evidence that preparedness effects depended on CS-US pairings, given that facilitated acquisition for fear-relevant CS+s was only found for the instructed but not paired CS+, whereas preparedness effects were absent for the fear-relevant CS+ that was both instructed and paired with the US.

In Chapter 5, we investigated whether fear reactions that were established by verbal threat instructions or the combination of threat instructions and stimulus pairings could be reversed with subsequent instructions. We predicted

that if stimulus pairings allow for the formation of implicit fear memories, as argued by the social fear learning model of Olsson and Phelps (2007), reversal instructions should be less pronounced for CSs that have been paired with the US compared to CSs that have not been paired with the US. Furthermore, given that the potentiation of the startle reflex is believed to reflect the implicit fear memory installed through stimulus pairings (Hamm & Weike, 2005; Sevenster, Beckers, & Kindt, 2012), the reduced instructed reversal effect for paired CSs should be primarily pronounced for this measure. In contrast to these predictions, we found a complete reversal of fear potentiated startle responses, regardless of whether the threatened CS had been paired with the US in a first phase or not. Similar patterns were found for US expectancy ratings and skin conductance responses.

Finally, in Chapter 6, we investigated the impact of a context change and context instructions on extinguished fear responses that were initially established via verbal instructions. Previous research had shown that such a contextual change after an extinction phase can allow for the return of conditioned fear (Bouton, 2004). Furthermore, we wanted to investigate the effect of instructing participants that the presence of the electrical shock depends on the context. In a first part of the experiment, participants were informed that one colored square could be followed by an electric shock, whereas another colored square would not be followed by the shock. Half of the participants were additionally informed that electric shocks would only be presented if the background of the computer screen had a particular color, but not when it had another color. The other half of the participants did not receive these context instructions. After these instructions, participants continued to an unannounced extinction phase by presenting the colored squares without administering any electric shocks. We found that a change in context after this extinction phase (i.e., a change of the background color of the computer screen) allowed for a return of verbally established fear. However, this effect was only reliable for US expectancy ratings, while the psychophysiological measures of fear (skin conductance responses and fear potentiated startle) were not very

affected by this manipulation. Furthermore, we found that the group of participants that were informed that the presence of the electrical shock would depend on the background color of the computer screen showed a larger return of fear after the context change. For this group, effects of a background change were both observed on US expectancy ratings and psychophysiological measures of fear.

### **NOVEL FUNCTIONAL KNOWLEDGE ABOUT FEAR LEARNING VIA VERBAL INSTRUCTIONS**

A first functional property of learning via verbal instructions that comes forward from our different studies is that effects of threat instructions can be stronger when they are combined with a pairing between the instructed CS+s and the US. We obtained evidence for such an additive effect of stimulus pairings in Chapters 2, 3 and 4. However, this additive effect of stimulus pairings may be moderated by the measure of fear that is investigated. That is, relatively clear added effects of stimulus pairings were observed for US expectancy ratings in Chapters 2, 3 and 4. Similarly, for fear ratings, we found clear evidence for additive effects in the studies that had included this measure (i.e., Chapters 2 and 4), and we found a trend for additive effects for fear potentiated startle in Chapter 2. However, on skin conductance responses in Chapters 2 and 4, and the P3 component in Chapter 3, such additive effects of threat instructions and stimulus pairings were absent.

A second newly discovered property of fear learning via verbal instructions is that an unpaired presentation of the US can allow for a return of extinguished fear that was initially installed via verbal instructions (i.e., reinstatement of verbally installed fear). Hence, much like extinguished fear that was initially established via stimulus pairings can return through an unpaired presentation of the US (e.g., Hermans et al., 2005), an unpaired US presentation can also allow for the return of extinguished fear that was established via verbal instructions (see Chapters 2 and 4). Furthermore, this return of fear was not always only limited to the CS that was threatened (i.e., differential reinstatement), but often

also generalized to the CS that was not threatened (i.e., generalized reinstatement). The same pattern is often observed for reinstatement of fear that was established via stimulus pairings (for a review see Haaker, Golkar, Hermans, & Lonsdorf, 2014). Currently, it is unclear which conditions determine whether differential or generalized reinstatement is observed.

A third property of fear learning via verbal instructions that was found in Chapters 2 and 6 is that the effects of threat information cannot be completely overturned by subsequent safety information. That is, threatened CSs that were told not to be followed by an electric shock in Chapter 2 and threatened CSs presented in an instructed safe context in Chapter 6 continued to elicit fearful reactions both as indicated by self-reported and psychophysiological measures of fear. Similar effects have previously been found for fear learning via stimulus pairings, where safety instructions and the removal of the shock electrodes were unsuccessful to completely abolish conditioned fear reactions (Hugdahl, 1978; Sevenster et al., 2012).

A fourth property was observed in Chapter 3, in which we demonstrated that verbal instructions can impact on the perceptual processing of stimuli as evidenced by ERPs. That is, we found that verbal instructions impacted processing of stimuli from about 300 ms after stimulus onset on. Although we did not find evidence for the idea that verbal instructions impacted on early, bottom-up perceptual processing of stimuli, other studies have found that verbal instructions may impact visual processing much earlier (Bublitzky & Schupp, 2012; Weymar, Bradley, Hamm, & Lang, 2013), and may even modulate visual processing as early as 60 to 100 ms past stimulus onset (Baas, Kenemans, Böcker, & Verbaten, 2002). This property of fear learning via verbal instructions can again be argued to be very similar as for fear learning via stimulus pairings. Fear conditioning via stimulus pairings seems to alter the early sensory processing of the CSs as well (for a review see Miskovic & Keil, 2012).

A fifth property was investigated in Chapter 4. In that chapter, the effect of verbal threat instructions was shown to depend on properties of the stimulus



that becomes threatening. That is, in Chapter 4, we found suggestive evidence that some effects of threat instructions are more outspoken for fear-relevant stimuli such as snakes, spiders and rats, compared to fear-irrelevant stimuli such as cow, deer and rabbits. Again, this is a property that is very similar for learning via the pairing of stimuli. Many previous experiments have demonstrated that fear acquisition through stimulus pairings is facilitated for fear-relevant stimuli compared to fear-irrelevant stimuli (e.g., Ho & Lipp, 2014; Öhman & Mineka, 2001).

A sixth property is that verbal threat instructions can override a previously established fear memory that had been learned on the basis of verbal instructions or on the basis of the combination of verbal instructions and stimulus pairings. While we had initially suspected that this effect might be strongly modulated by the specific fear measure under investigation, we found this effect to be present for all the measures of fear that we had investigated (US expectancy ratings, skin conductance responses, fear potentiated startle), although the evidence for a reversal effect was somewhat less pronounced for skin conductance responses than for the other measures.

A seventh property is that contextual cues are important for extinguished fear responses that were established on the basis of verbal instructions. That is, in our study we found that fear responses that were established via verbal instructions and that were gradually extinguished through presenting unreinforced CSs tended to return when the background color of the computer screen was changed. However, this effect was only reliably observed for self-reported expectancy of the US. Psychophysiological measures (skin conductance responses and fear potentiated startle) were not very affected by this context change. One exception was fear potentiated startle, for which we found a tendency for a general return of fear after the context switch. Again, this effect mirrors results that have been found for fear learning through the pairing of stimuli (renewal of conditioned fear; e.g., Alvarez, Johnson, & Grillon, 2007; Vansteenwegen et al., 2005).

Finally, an eighth property of verbally established fear that can be derived from the research in this thesis is that the contextual control of extinguished fear is influenced by verbal instructions about the context. That is, when participants were informed that the presence of the electric shock would depend on the background color of the computer screen, context switch effects were more outspoken and also observed on psychophysiological measures of fear.

### **THE IMPLICATIONS FOR MENTAL PROCESS MODELS**

Our summary of the known functional properties of fear learning via verbal instructions shows that some key characteristics of learning via stimulus pairings are also evident for learning via verbal instructions. Furthermore, we found that these two pathways of fear acquisition strongly interact as shown by the fact that verbal instructions can counter fear established through stimulus pairings (Chapter 5) and the observation that unreinforced CS presentations can reduce verbally established fear (Chapter 6). Such results argue strongly for a single process view in which both learning via direct experience and via instructions interact to create a common representation that drives fear (Lovibond, 2003). That is, it seems unlikely and unnecessary to assume partly independent learning processes for these two pathways of learning given that they share very similar characteristics. Rather, if different processes underlie learning via these two pathways it would have been more likely to observe different characteristics for these two pathways of fear learning. Furthermore, the fact that learning via one of the pathways can impact fear responses acquired through another pathway requires that at least a part of the representation that drives these fear responses is affected by both these two pathways. It seems most parsimonious then to assume that a shared representation is affected by both pathways of fear learning.

As we have seen in the introduction, this common representation can be thought of as the formation of mental associations that takes place for both learning via verbal instructions and learning via stimulus pairings (Field, 2006).

Alternatively, it can be argued that both pathways allow for the formation of propositional beliefs about the relationships between events in the environment (Lovibond, 2011; Mitchell, De Houwer, & Lovibond, 2009). There are some results in this thesis that would argue for the latter mental mechanism that is common for both learning via verbal instructions and learning via stimulus pairings. First, as argued in the introduction, the instruction “this stimulus will NOT be followed by an electrical shock” does not result in conditioned fear reactions for the instructed stimulus (see Chapter 5 and Sevenster et al., 2012), despite that the representation of the stimulus and the representation of the shock are being activated together, and thus there is opportunity for association formation. Thus, effects of verbal instructions are determined by relational qualifiers embedded in the sentence, which is a result that is hard to accommodate with association formation models. Furthermore, in Chapter 6 we showed that the expression of (extinguished) fear was made more context dependent via verbal instructions. Specifically, conditioned fear was reduced in a context in which the shock was instructed not to be presented and fear was more pronounced in a context in which the shock was told to be possibly presented. This result strongly supports the conclusion that what was learned via the verbal instructions in Chapter 6 was represented in a conditional format. That is, both the instructed relationship as well as the conditions under which this relationship applies must have been encoded. It is difficult to see how an association formation model that relies on the incremental strengthening of associative connections can allow for such a quick establishment of conditional information.

However, these results can also be accounted for by dual-process models of fear learning in which both verbal instructions and stimulus pairings allow for the formation of propositional representations. Dual-process models differ from single-process propositional models in that only the former propose that under certain circumstances (e.g., when stimuli are paired) fear learning is due to association formation (Olsson & Phelps, 2007). Therefore, we tested a number of predictions of dual-process models of fear learning to investigate whether under

these conditions simple association formation processes do indeed add to fear learning. Specifically, first, we investigated whether prepared fear learning is a property of fear learning via stimulus pairings only. As prepared learning is argued to be due to the formation of associative fear memories in the amygdala (Mineka & Öhman, 2002; Öhman & Mineka, 2001), it could be argued that such prepared learning can only be obtained through stimulus pairings, because only this procedure is argued to allow for amygdala-mediated fear learning (Olsson & Phelps, 2007). In contrast to this prediction, we have found suggestive evidence that prepared learning effects may also be obtained via verbal instructions, in line with the results of earlier studies (Hugdahl & Öhman, 1977; Hugdahl, 1978). This result argues against the idea that a separate association formation process needs to be assumed to account for prepared learning effects. Second, in Chapter 5, we tested whether fear reactions established through the combination of threat instructions and stimulus pairings can be reversed via verbal instructions. If stimulus pairings create implicit fear memories that are automatic and encapsulated from verbal instructions (LeDoux, 2014; Öhman & Mineka, 2001), verbal instructions should be unsuccessful to reverse these responses (e.g., McNally, 1981), especially for fear reactions that are believed to reflect this implicit associative memory such as the startle reflex (Hamm & Weike, 2005; Sevenster et al., 2012). In contrast to this prediction, we found that verbal instructions were highly successful to reverse conditioned fear reactions established through the combination of threat instructions and stimulus pairings. Furthermore, as we reviewed in Chapter 5, doubts were raised about a number of other claims that argue for dual-process models, such as that conditioned fear can be acquired outside of conscious awareness (Sevenster, Beckers, & Kindt, 2014) and that fear memories can be resistant to extinction instructions (Dawson, Rissling, Schell, & Wilcox, 2007; Sevenster et al., 2012).

Taken together, the results of the different chapters in this thesis seem to be best accounted for by a model that argues that learning is the result of the non-automatic formation of propositional beliefs (De Houwer, 2009; Lovibond, 2011; Mitchell et al., 2009). Nevertheless, it may well be that some necessary

conditions to obtain evidence for association formation processes were not fulfilled in our studies. Moreover, other studies have previously offered persuasive evidence that association formation processes do play a role in the acquisition of conditioned fear (e.g., McAndrew, Jones, McLaren, & McLaren, 2012). The debate whether one or multiple processes need to be assumed to account for (fear) learning is a very lively one that has been going on for decades (Brewer, 1974; McLaren et al., 2014; Mitchell et al., 2009; Shanks, 2010). It is unlikely that the debate will be resolved with one or even a series of studies, which may indicate that the different models are (currently) insufficiently specified to render them falsifiable. The position in this thesis is that, regardless of whether and how the debate will ultimately be settled with these studies, the continued research efforts that are being directed at resolving this debate have contributed tremendously to the understanding of (fear) learning and is therefore worthwhile. Mental process models are continuously being refined to make increasingly specific predictions about which factors contribute to fear learning. For instance, the fear learning models of Öhman and Mineka (2001), Hamm and Weike (2005) and Olsson and Phelps (2007) have guided the research in this thesis extensively and have allowed for specific predictions for when verbal instructions could or could not have an effect on fear reactions. Testing these predictions has provided new functional knowledge about fear learning via verbal instructions that can drive the development of new models and to generate a better understanding of how fear is acquired. Thus, despite not being able to provide a final answer to which sort of model should be preferred, the research in this thesis has provided new knowledge to further constrain mental process models, to generate new testable predictions and to come to a better understanding of how fear is acquired.

### **IMPLICATIONS FOR CLINICAL PRACTICE**

The functional knowledge that we gained about fear learning via verbal threat instructions and stimulus pairings not only provides an empirical basis to

test mental process models of fear conditioning against (De Houwer, 2011), but also provides a possibility to better understand anxiety disorders. Here we shortly discuss some of the results of the different chapters that allow for a better understanding of the acquisition, prevention and treatment of anxiety disorders.

A first important finding throughout the chapters is that verbal instructions can install strong and persistent fear that is both evident on self-report measures of fear and on psychophysiological measures. Hence, our results add to the validity of the idea that verbal instructions may be a pathway for the acquisition of pathological fear. Furthermore, in Chapters 2 and 6 we found that fear that was established via verbal instructions was resistant to safety instructions. That is, for the instructed CS+ during the training phase of Chapter 2 and during the extinction phase for the context instructions group in Chapter 6 fear reactions were still observed, despite that participants were explicitly told that no shocks would be provided during these phases. Thus, these results show that verbal instructions can install very persistent fear that may not be completely overridden by subsequent safety information. A clinical implication of these findings might then be that care should be taken when verbal warnings or threats are issued (such as when parents warn their children) because this verbal information may install lasting fear that is resistant to subsequent safety information.

Second, the studies of Chapter 4 illustrate that verbal instructions may even establish “irrational” fear for fear-relevant stimuli, in the absence of stimulus pairings. That is, the effects of threat instructions were more outspoken for threatened fear-relevant stimuli than for threatened fear-irrelevant stimuli in the second experiment of Chapter 4. A clinical implication of this finding is that it may be possible that irrational or pathological fears for fear-relevant stimuli could be established via verbal instructions, in the absence of any traumatic experience, as retrospective reports with phobic patients also seem to suggest (King, Eleonora, & Ollendick, 1998; Schindler, Vriends, Margraf, & Stieglitz, 2016). Again, the clinical implication is that care should be taken when providing verbal

threat instructions (such as, for instance, when parents warn their children). Note, however, that our evidence for prepared learning via instructions was limited only and should thus be interpreted with care. Nevertheless, the same qualification might apply for prepared fear learning via stimulus pairings (McNally, 1987). As we suggest in Chapter 4, preparedness effects might depend on certain moderating conditions such as uncertainty about the CS-US contingency. Future research will have to determine whether strong evidence for prepared learning could be obtained for fear learning via verbal instructions and stimulus pairings under these conditions.

Finally, Chapters 2, 4 and 6 suggest that the mechanisms of return of fear are similar for fear established via verbal instructions and fear established via stimulus pairings. That is, an unpaired presentation of the US (i.e., reinstatement) or a change in context (i.e., renewal) allowed for a return of fear established through verbal instructions in these chapters. As these phenomena are often regarded as the laboratory analogues of how fear can relapse after exposure-based therapy (Bouton, 2002; Haaker et al., 2014), these studies suggest that pathological fear that was acquired through verbal instructions may pose similar challenges for relapse after therapy as fear established via the pairing of stimuli. The potential clinical implication is that the same precautions for the prevention of relapse after therapy could be taken for fears acquired both via stimulus pairings and via verbal instructions.

These different findings provide a starting point for understanding how verbal instructions can add to the development of anxiety symptoms. However, much more research is obviously needed to come to a better understanding of how and to what extent verbal instructions contribute to the development of pathological fear (see also Muris & Field, 2010). As verbal instructions may constitute a major pathway of how fears are acquired (Field, 2006; Rachman, 1991; Schindler et al., 2016), such continued research efforts may have far-reaching implications for the understanding, treatment and prevention of anxiety disorders.

## FUTURE DIRECTIONS

One important objective of future research is to continue to refine and test models of fear acquisition. This practice has helped in gaining a better understanding of the etiology of anxiety symptoms and in developing more successful ways of treating anxiety disorders (Bouton, 2004; McNally, 2007; Mineka & Zinbarg, 2006). As we discussed previously, one crucial question is whether and when evidence can be obtained for the contribution of simple (associative) learning processes to the acquisition of fear. The contingency reversal procedure (Grings, Schell, & Carey, 1973; McNally, 1981; Wilson, 1968) could be very useful to study this question because it allows to pit against each other the effect of a conditioning history and the effect of verbal instructions. In our own study, we did not find evidence for fear responses that were solely a function of the prior conditioning history and that were not affected by the contingency reversal instructions. However, as we have argued in Chapter 6, some aspects of our procedure may have contributed to these results. Most importantly, if learning would be uninstructed in the first conditioning phase, if the conditioning phase would be more extended, and if other CSs and USs are used (e.g., fear-relevant pictures as CSs or interoceptive pain as a US), effects that reflect simple association formation may be obtained. Hence, one interesting future avenue for research would be to continue to use the contingency reversal procedure for studying the role of simple associative learning processes in fear learning.

Another interesting avenue for future research could be to investigate how verbal instructions can impact subsequent learning via the pairing of stimuli. A previous study of Field and Storksen-Coulson (2007) has shown that previous negative verbal information about unknown stimuli may strengthen the effect of subsequent conditioning experiences. It could be argued that verbal instructions install expectancy biases (Muris & Field, 2010) and thereby strengthen the effects of subsequent conditioning experience (Davey, 1997). Furthermore, it has been argued that prepared fear learning effects, as the ones in Chapter 4, are the



result of expectancy biases (Davey, 1992). Currently, it is not so clear where these expectancy biases for fear-relevant stimuli come from. Evolutionary selection may be offered as an explanation (Seligman, 1971), but it is also likely that cultural factors contribute to the development of these expectancy biases (Blanchette, 2006). Therefore it is likely that also verbal instructions add to the development of expectancy biases for fear-relevant stimuli. If this is indeed the case, initially neutral stimuli could be biased to become fear-relevant stimuli. Hence, it is possible that prepared learning effects could be obtained for initially neutral stimuli that have been biased through verbal instructions. For instance, unknown animals (e.g., Australian marsupials as in the study of Field and Storksen-Coulson, 2007) could be biased to become fear-relevant by telling participants that these animals carry diseases and are aggressive towards humans. Several properties of prepared learning for these biased stimuli could then be investigated such as facilitated fear acquisition, delayed fear extinction and facilitated return of fear. Such studies may elucidate the dynamic interplay between learning via verbal instructions and learning via stimulus pairings and help explain how stimuli can become “prepared” to elicit fear reactions.

Another interesting avenue of future research could be to look at the effects of positive instructions. If verbal instructions could strengthen the effects of subsequent conditioning experiences through expectancy biases, they could possibly also weaken the effects. Prior research has already shown that positive verbal instructions can have fear reducing effects (Muris & Field, 2010). However, it is not clear whether positive information can reduce the effects of subsequent conditioning experience. Particularly interesting would be to investigate whether positive verbal information could counteract expectancy biases for fear-relevant stimuli and thereby counteract prepared learning effects for fear-relevant stimuli. For instance, positive information about spiders (e.g., they control the insect population, they are afraid of humans) and snakes (e.g., they can be trained and can be kept as a pet, their venom may be used as a treatment for cancer) could be provided to participants. If these instructions were successful to counteract the expectancy biases for these stimuli, perhaps

prepared learning effects could be abolished for these participants compared to a control group that did not receive this positive information. Such studies could point to promising new ways for treatment and prevention of pathological fear for fear-relevant stimuli.

Finally, another potentially interesting avenue for future research is to investigate whether the bio-chemical processes that contribute to the (re)consolidation of fear conditioning via pairings also underlie (re)consolidation of verbally established fear memories. Research in recent years has booked remarkable successes in understanding the (re)consolidation of conditioned fear memories (e.g., Kindt, Soeter, & Vervliet, 2009; Soeter & Kindt, 2011). However, most of this research has focused on studying fear that was established via stimulus pairings, with some exceptions (Soeter & Kindt, 2012). Research on the (re)consolidation of verbally established fear memories provide further information about parallels or dissociations between different pathways of fear learning. Moreover, because verbal instructions probably constitute a major pathway of fear acquisition, it would be clinically relevant to investigate whether a promising new method for the treatment of anxiety disorders (i.e., disruption of fear memory reconsolidation; Soeter & Kindt, 2015) could be applied to treat anxiety symptoms acquired through verbal threat instructions as well.

These are just a few avenues for future research that follow up on some of the findings of the experiments in this thesis and from other researchers. Obviously, many more other interesting directions for future research could be pursued such as investigating the impact of personality traits, neurotransmitters and genetic variations on fear learning via verbal instructions, as has been done for fear learning via stimulus pairings (e.g., Davis, 2006; Duits et al., 2015; Lonsdorf et al., 2009). Hence, it is clear from this section that only the very first steps have been taken to come to a full understanding of how verbal instructions contribute to fear acquisition and the development of anxiety symptoms.

## CONCLUSION

The goal of the research presented in this thesis was to come to a better understanding of how fear is acquired through verbal instructions and stimulus pairings. This goal was justified by the claim that verbal instructions provide an important pathway of how pathological fears are acquired (Field, 2006; Rachman, 1977, 1991). However, so far, few studies had systematically investigated fear acquisition via verbal instructions. Moreover, different models of fear learning differed in their predictions under which conditions verbal instructions contribute to the acquisition of (pathological) fear (Mitchell et al., 2009; Olsson & Phelps, 2007). As we have seen in the different chapters of this thesis, the way in which fear is acquired via verbal instructions may be very similar to the way in which fear is acquired via stimulus pairings. Both pathways of fear acquisition share many functional properties. Furthermore, the studies in the different chapters of this thesis provide evidence that verbal instructions can install strong and persistent fear that may even override previously conditioned fear. The results of these studies put new constraints on mental process models of fear learning, help to understand how pathological fear may be acquired in the absence of traumatic experience and point to new opportunities for the prevention and treatment of pathological fear. However, the conducted research only provides a starting point to understand the contribution of verbal instructions and stimulus pairings to fear learning. A number of avenues for future research have been discussed in this final chapter, and it is undoubtedly so that many more other avenues for future research that we did not discuss also exist. To conclude this thesis then, we hope that the presented research can serve as an impetus for a durable interest in studying the contribution of verbal instructions and stimulus pairings to fear learning.

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## NEDERLANDSTALIGE SAMENVATTING

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Mensen en andere dieren vertonen een specifiek gedragspatroon wanneer zij angstig zijn. De hartslag gaat omhoog, er wordt een 'vecht-of-vlucht' gedragspatroon geobserveerd en er worden negatieve subjectieve ervaringen gerapporteerd (Frijda, 1986; Lang, Bradley, & Cuthbert, 1998). Wat mensen en andere dieren vrezen is deels door hun evolutionaire geschiedenis bepaald (Seligman, 1971). Mensen hebben bijvoorbeeld veel vaker angst voor bepaalde stimuli zoals slangen, spinnen en open ruimtes, die allicht een bedreiging vormen voor de pre-technologische mens, dan voor moderne voorwerpen zoals auto's en wapens, hoewel deze laatste voorwerpen momenteel een grotere direct gevaar vormen voor overleving dan de eerste soort van stimuli. Niettegenstaande, wat mensen en andere dieren vrezen is niet beperkt tot een beperkt aantal intrinsiek angstaanjagende stimuli, maar mensen en andere dieren bezitten ook de capaciteit om nieuwe stimuli te leren vrezen die een onaangename gebeurtenis voorspellen. Dit is bijvoorbeeld aangetoond met het 'Kleine Albert experiment' door Watson en Rayner (1920). In hun experiment toonden deze onderzoekers dat een jong kind (Albert) kon worden aangeleerd om angstreacties te tonen in de nabijheid van een witte rat wanneer interactie met deze rat verschillende keren gepaard was geweest met een heel onaangenaam geluid. Dus, dit experiment toont dat angst kan worden aangeleerd omdat Albert geen angst vertoonde voor ratten alvorens de paringen tussen de rat en het onaangename geluid. Dit experiment wordt vaak verklaard in conditioneringstermen, waarbij de rat de geconditioneerde stimulus is (of conditioned stimulus, CS), het geluid de ongeconditioneerde stimulus (unconditioned stimulus, US) en de angstreactie voor de rat de geconditioneerde reactie (conditioned reaction, CR).

Dit inzicht dat angst kan aangeleerd worden via de principes van klassieke conditionering is belangrijk geweest voor het beter begrijpen van angst en het ontwikkelen van interventies om pathologische angst te behandelen (Field, 2006; Mineka & Zinbarg, 2006). Bijvoorbeeld, deze benadering kan verklaren waarom mensen die zijn betrokken geweest bij een scheepsramp vaker angst hebben

voor water of om op een schip te zijn (Field, 2006) of waarom mensen die bij een auto-ongeluk betrokken zijn geweest vaker angst hebben om met de auto te rijden. Ondanks deze successen heeft de conditioneringsverklaring van angst ook al veel kritiek gekregen (Menzies & Clarke, 1995; Rachman, 1977, 1991). Zo is het bijvoorbeeld niet duidelijk waarom mensen die een traumatische ervaring hebben ondergaan niet steeds angst ontwikkelen (Beckers, Kryptos, Boddez, Effting, & Kindt, 2013; Mineka & Zinbarg, 2006) en waarom mensen soms angst ontwikkelen zonder ooit negatieve ervaring te hebben gehad met de stimulus waarvoor ze angst hebben (bijvoorbeeld, angst voor slangen in Westerse landen) (King, Eleonora, & Ollendick, 1998). Het paren van stimuli is dus noch een voldoende noch een noodzakelijk voorwaarde voor het ontwikkelen van angst. Omwille van deze redenen hebben verschillende onderzoekers voorgesteld dat angst ook kan worden aangeleerd via andere wegen zoals via verbale instructies en sociale observatie (Field, 2006; Rachman, 1977).

Er is goede evidentie dat angst inderdaad kan worden aangeleerd via deze alternatieve wegen. Bijvoorbeeld, Olsson en Phelps (2004) hebben aangetoond dat als proefpersonen worden verteld dat een bepaalde stimulus door een schok zal gevolgd worden, of wanneer zij een andere persoon een schok zien krijgen in het bijzijn van deze stimulus, deze proefpersonen angstreactions zullen vertonen voor deze stimulus. Ook in retrospectieve studies bij patiënten met angststoornissen worden verbale instructies en sociale observatie vaak als een oorzaak gerapporteerd voor het ontstaan van de angststoornis (King et al., 1998; Schindler, Vriends, Margraf, & Stieglitz, 2016). Ondanks deze bevindingen is angstleren via verbale instructies en via sociale observatie nog niet zo goed begrepen (Olsson & Phelps, 2007). Verschillende psychologische modellen van angstleren maken andere voorspellingen over wat kan aangeleerd worden via deze verschillende wegen van angstleren. Tot nu toe zijn nog maar weinig van deze voorspellingen onderzocht geweest. De bedoeling van dit doctoraatsproefwerk was om een aantal van deze voorspellingen te onderzoeken en zodanig bij te dragen aan een beter begrip van angstleren via verbale instructies. Om het onderzoek in dit proefwerk samen te vatten zal ik eerst kort de verschillende psychologische modellen van angstleren voorstellen.

Daarna zal ik de bevindingen van de verschillende empirische hoofdstukken in dit proefwerk samenvatten en tenslotte eindigen met de implicaties samen te vatten dat het onderzoek in dit proefwerk heeft voor ons begrip van angstleren.

## PSYCHOLOGISCHE MODELLEN VAN ANGSTLEREN

### Angstleren via het vormen en versterken van mentale associaties

Een belangrijk idee binnen de psychologie is dat associatief leren (dit is: leren via het paren van stimuli, zoals bij klassieke conditionering) het gevolg is van het vormen van mentale associaties in het geheugen. Een associatie is een ongekwalficeerde connectie die toelaat om activatie te verspreiden. Volgens dit idee zal het herhaaldelijk paren van een CS en een US leiden tot het vormen van een associatie tussen de mentale representatie van deze twee stimuli. Daaropvolgende presentaties van de CS zullen hierdoor niet enkel de representatie van de CS activeren, maar ook de representatie van de US doordat deze twee representatie zijn gelinkt door een associatieve connectie. Deze activatie van de US lokt op zijn beurt de CR uit (bv. angstig gedrag). Deze theorie kan dus verklaren hoe leren plaatsvindt en waarom de CS na herhaalde paringen met de US een CR zal uitlokken.

Volgens Field (2006) is hetzelfde mechanisme verantwoordelijk voor het leren van angst via verbale instructies. Dus, volgens deze theorie, zal een verbale instructie “deze hond bijt” toelaten om een associatieve connectie te vormen tussen de CS (hond) en de US (gebeten worden). Op dezelfde manier kan dus latere activatie van de mentale representatie van de CS (door een hond te zien) een mentale representatie van de US oproepen (gebeten worden), waardoor een CR wordt uitgelokt (angst voor de hond). Belangrijk bij deze theorie is dat er ook vaak wordt aangenomen dat het vormen van associaties op een automatische manier gebeurt. Dat betekent dat mensen niet een intentie of motivatie moeten hebben om deze associaties te vormen en dat deze associaties snel en eventueel buiten bewustzijn kunnen gevormd worden.

### **Angstleren via het vormen van propositionele kennis**

Een andere theorie van hoe mensen associatief (angst)leren is de propositionele leertheorie van Mitchell, De Houwer en Lovibond (2009). Volgens deze theorie vormen mensen geen associaties tussen representaties in het geheugen, maar vormen ze propositionele ideeën over hoe evenementen of stimuli samenhangen. Een persoon die is gebeten geweest kan dus de propositie vormen dat honden hem/haar zullen bijten. Deze propositie of verwachting zal dan angstreactions ontlokken (Lovibond, 2011; Reiss, 1980). Het belangrijke verschil tussen deze theorie en de associatieve theorie is dat relaties in het geheugen gekwalificeerd kunnen zijn. Zo kan je bijvoorbeeld de propositie vormen dat een hond je kan bijten, maar dat dit enkel opgaat voor bepaalde hondenrassen en wanneer de hond niet aan een ketting hangt. Bepaalde conditionele voorwaarden kunnen dus opgenomen worden in een propositie. Zulke conditionele informatie kan doorgaans niet, of slechts rudimentair, opgeslagen worden via associaties. Verder voorspelt deze theorie ook dat proposities op een niet-automatische manier gevormd worden. Dit wil zeggen dat er tijd, motivatie en bewustzijn nodig is voor het vormen van proposities. Tenslotte voorspelt deze theorie ook dat het vormen van proposities het mechanisme vormt voor zowel leren via stimulusparingen als leren via verbale instructies. Dit houdt in dat deze twee manieren van angstleren sterke gelijkenissen zouden moeten vertonen en dat zij elkaar sterk kunnen beïnvloeden.

### **Angstleren via het vormen van propositionele kennis én mentale associaties**

Een derde theorie over angstleren is dat zowel het vormen van propositionele kennis als het vormen van associaties toedragen aan het leren van angst. Volgens het leermodel van Olsson en Phelps (2007) worden er zowel proposities als associaties gevormd wanneer mensen stimulusparingen ervaren. Deze processen vinden op een gelijkaardige manier plaats als beschreven in de vorige twee secties. Echter, wanneer leren plaatsvindt via verbale instructies worden er enkel proposities gevormd en geen associaties volgens dit model.

Meer specifiek beargumenteren Olsson en Phelps (2007) dat enkel paringen tussen neutrale stimuli (CS'en) en intrinsiek aversieve stimuli (US'en, dit kunnen ook negatieve gelaatsuitdrukkingen zijn) toelaat om impliciete associatieve connecties met de amygdala te vormen. De amygdala is een hersenstructuur waarvan geloofd wordt dat deze instaat voor het genereren van defensieve reacties en die beargumenteerd wordt automatisch te worden geactiveerd in situaties van dreiging (Mineka & Öhman, 2002; Öhman & Mineka, 2001). Deze theorie maakt dus een onderscheid tussen angstleren via verbale instructies en angstleren via stimulusparingen. Enkel de laatste manier van leren laat toe om op een onbewust en automatische manier defensieve reacties aan te leren die worden gemedieerd worden door associaties met de amygdala. Dit houdt in dat deze twee manieren van angstleren onder bepaalde condities andere eigenschappen kunnen vertonen en dat deze twee niet noodzakelijk elkaar zullen beïnvloeden onder bepaalde condities.

### **Evaluatie van de modellen**

Veel onderzoekers zijn momenteel overtuigd dat leren voor een groot deel het gevolg is van het vormen van propositionele kennis. Leertheorieën waarbij leren exclusief het gevolg is van het vormen van associaties zijn onwaarschijnlijk omdat deze theorieën moeilijk kunnen verklaren waarom leren vaak wordt beïnvloed door bewustzijn (Dawson & Furedy, 1976) en redeneerprocessen (De Houwer, Vandorpe, & Beckers, 2005). De belangrijkste hedendaagse discussie vindt dus plaats tussen de laatste twee theorieën (McLaren et al., 2014). Een belangrijke vraag bij het kiezen tussen deze twee theorieën is of er evidentie kan worden gevonden voor leren dat het gevolg is van het vormen van associaties. Zo een bevinding zou aantonen dat een tweede leer-mechanisme moet verondersteld worden om al het (angst)leren te kunnen verklaren. Er bestaat veel discussie of er reeds dergelijke evidentie is gevonden (Lovibond, 2011; Mitchell et al., 2009). Het onderzoek in dit doctoraatsproefwerk wil een empirische bijdrage leveren aan deze discussie door te onderzoeken of angstleren via verbale instructies en stimulusparingen gelijkaardige eigenschappen vertonen en of er evidentie kan worden gevonden voor het

bestaan van een associatief leerproces dat onafhankelijk opereert van propositioneel redeneren.

### **SAMENVATTING VAN DE EMPIRISCHE HOOFDSTUKKEN**

In hoofdstuk 2 onderzochten we het effect van het combineren van contingentie instructies en stimulusparingen. Voorgaand onderzoek heeft aangetoond dat instructies over de samenhang tussen een CS en een US angstreacties kunnen uitlokken. Weinig is echter gekend over de mate waarin ervaring van CS-US paringen iets kan bijdragen aan het effect van CS-US contingentie instructies. Om dit te onderzoeken werden proefpersonen verteld dat twee CS'en mogelijks gevolgd kunnen worden door een elektrische schok (CS+'en). Van een derde CS werd verteld dat deze niet kon gevolgd worden door de schok. Proefpersonen werden verder verteld dat één van de twee CS+'en voorlopig niet door een schok zou worden gevolgd als voorzorg om hen nog niet te veel schokken aan te bieden alvorens het eigenlijke experiment van start zou gaan. In de daaropvolgende fase werden proefpersonen de CS'en herhaaldelijk getoond. Gedurende deze fase werd slechts één van de twee CS+'en gevolgd door de schok. Na deze fase werden proefpersonen verteld dat nu beide CS+'en gevolgd zouden worden door de schok. In de volgende cruciale fase van het experiment observeerden we dat angstreacties iets meer uitgesproken waren voor de CS+ die in de voorgaande fase gepaard was geweest met de schok dan voor de CS+ die niet gepaard was geweest met de schok. Specifiek werd dit effect geobserveerd voor zelf-gerapporteerde angst en verwachting van de schok. Eenzelfde tendens werd geobserveerd voor de schrikreflex, maar dit effect was niet statistisch significant. Tenslotte was dit effect afwezig voor huidgeleidingsreacties. We concluderen dat ervaring van CS-US paringen kan bijdragen aan het effect van verbale instructies, maar dat dit effect mogelijks kan afhangen van de specifieke angstreactie die onderzocht wordt.

In hoofdstuk 3 onderzochten we of leren via verbale instructies en via het paren van stimuli additieve kunnen hebben op vroege sensorische verwerking



zoals gemeten door evenement-gerelateerde potentialen (ERPs). Eerdere studies hebben reeds aangetoond dat zowel verbale instructies als stimulusparingen ERP componenten kunnen versterken die gerelateerd zijn aan vroege sensorische verwerking. Tot nu toe heeft echter nog geen enkele studie onderzocht of deze twee wegen van angstleren additieve effecten kunnen hebben op vroege sensorische verwerking. Om dit te onderzoeken hebben we proefpersonen geïnstrueerd over een reeks blokken over de contingentie tussen twee CS+'en en een elektrische schok (de US). Een derde CS (CS-) werd geïnstrueerd om niet gevolgd te worden door de US. Nieuwe CS'en werden geselecteerd in ieder van deze blokken en slechts de helft van de CS+'en werden daadwerkelijk gevolgd door de US. Met deze procedure vonden we een grotere P3 component (een component die gerelateerd is aan aandachtstoewijding) voor de CS-. We vonden echter geen effect van stimulusparingen voor deze component. Op de andere componenten die gerelateerd zijn aan vroege sensorische verwerking (C1, P1 en N1) vonden we geen evidentie voor een effect van de contingentie instructies of van de stimulusparingen. We besluiten dat de CS- waarschijnlijk meer aandacht trok omdat deze een belangrijke stimulus was voor de proefpersonen doordat deze stimulus de afwezigheid van een schok perfect kon voorspellen.

In hoofdstuk 4 hebben we onderzocht of we evidentie konden vinden voor het bestaan van voorbereid angstleren via verbale instructies. Voorbereid angstleren verwijst naar de observaties dat angstleren soms makkelijker verloopt voor angst-relevante stimuli zoals foto's van spinnen en slangen dan voor angst-irrelevante stimuli zoals foto's van vogels en vlinders (Öhman & Mineka, 2001). Om dit te onderzoeken werden proefpersonen in een eerste experiment geïnstrueerd over de contingentie tussen angst-relevante en angst-irrelevante CS'en en een elektrische schok (US). In dit eerste experiment vonden we geen evidentie dat angstleren via verbale instructies gemoduleerd werd door de angst-relevantie van de CS'en. Angstleren via verbale instructies voor angst-relevante en angst-irrelevante CS'en in dit experiment. In een tweede experiment manipuleerde we of de geïnstrueerde contingenties tussen angst-relevant en irrelevant stimuli daadwerkelijk ervaren werden. In dit tweede experiment vonden we suggestieve evidentie dat angstleren via verbale

instructies kan gemoduleerd worden door de angst-relevantie van de CS'en. Dit effect hing echter niet af van het feit of deze CS'en daadwerkelijk werden gepaard met de US aangezien dit voorbereid leren effect enkel werd geobserveerd voor de CS die niet gepaard was geweest met de US. Een mogelijke verklaring voor deze resultaten die we voorstellen in dit hoofdstuk is dat voorbereid angstleren afhangt van onzekerheid, met meer voorbereid leren wanneer er veel onzekerheid is over de exacte contingentie tussen de CS'en en de US.

In hoofdstuk 5 onderzochten we of verbale instructies geconditioneerde angstreacties kunnen beïnvloeden. Als stimulusparingen angstreacties installeren via impliciete associatieve connecties kan er voorspeld worden dat verbale instructies niet voldoende kunnen zijn om deze angstreacties te beïnvloeden. Dit zou specifiek het geval moeten zijn voor maten van angst die deze impliciet associatieve connecties reflecteren, zoals de schrikreflex (Hamm & Weike, 2005). Om dit te onderzoeken gingen proefpersonen door een eerste angstleerfase waarbij angstreacties voor sommige CS'en geïnstalleerd werden via verbale instructies en voor andere CS'en via de combinatie van verbale instructies en stimulusparingen. Na deze fase werden proefpersonen verteld dat de contingenties omgedraaid zouden worden. Deze instructies beïnvloedde de angstreacties sterk, ook voor angstmaten waarvan geloofd worden dat deze de impliciete associatieve connecties met de amygdala reflecteren. Deze resultaten van deze studie zijn meer in lijn met een propositionele leertheorie dan een leertheorie met verschillende leerprocessen.

Tenslotte, in hoofdstuk 6 hebben we onderzocht of uitgedoofde angstreacties geïnstalleerd via verbale instructies gevoelig zijn aan context veranderingen. Voor uitgedoofde angstreacties die zijn geïnstalleerd via stimulusparingen zorgt een dergelijke contextverandering er vaak voor dat de originele angst-herinnering terug wordt opgeroepen en dus dat de angstreacties terugkeren (Alvarez, Johnson, & Grillon, 2007; Vansteenwegen et al., 2005). Om te onderzoeken of dit ook het geval is voor angstreacties geïnstalleerd via verbale instructies werden proefpersonen eerst verteld dat een CS gevolgd zou worden door een elektrische schok en een andere CS niet gevolgd zou worden

door een elektrische schok. Hierna werden proefpersonen de twee CS'en herhaaldelijk getoond zonder dat een elektrische schok werd aangeboden. Deze procedure leidde tot een graduele uitdoving van de verbaal geïnstalleerde angstreacties. Na deze fase werd de achtergrondkleur van het computerscherm veranderd en werden de CS'en opnieuw aangeboden. Deze manipulatie leidde ertoe dat er een terugkeer was van angstreacties, voornamelijk zoals gemeten door gerapporteerde verwachting van de elektrische schok.

Verder werden de helft van de proefpersonen ook verteld dat de aanwezigheid van de elektrische prikkel zou afhangen van de achtergrondkleur van het computerscherm. Deze groep toonde veel zwakkere angstreacties in de eerste fase en een grotere terugkeer van angstreacties in de tweede fase van het experiment. Dit laatste resultaat toont dat de contextuele controle van uitgedoofde angstreacties versterkt kan worden via verbale instructies.

### **IMPLICATIES VAN HET ONDERZOEK EN CONCLUSIES**

Samengenomen toont het onderzoek in dit doctoraatsproefwerk dat angstleren via verbale instructies en via stimulusparingen een aantal belangrijke kenmerken delen. Zo zullen angstreacties uitgedoofd worden wanneer CS'en herhaaldelijk aangeboden zonder de US en kunnen bepaalde manipulaties ervoor zorgen dat deze uitgedoofde angstreacties snel terugkeren zoals het aanbieden van een ongepaarde US of het veranderen van de context. Ook lijkt het zo dat zowel angstleren via verbale instructies en angstleren via stimulusparingen beïnvloed worden door de angst-relevantie van de CS'en. Zulke gemeenschappelijke kenmerken suggereren dat eenzelfde proces aan de grondslag ligt van deze twee verschillende manieren van angstleren. Verder werd er ook in hoofdstuk 5 aangetoond dat angstreacties die geïnstalleerd zijn via de combinatie van stimulusparingen en instructies sterk beïnvloed kunnen worden door instructies. Deze bevinding toont aan dat wat geleerd wordt via stimulusparingen en via verbale instructies elkaar sterk kunnen beïnvloeden.

Samengenomen tonen deze resultaten dus dat angstleren via stimulusparingen en angstleren via instructies sterke gelijkaardige kenmerken vertonen en dat het leren via de twee wegen elkaar sterk beïnvloed. Deze resultaten sluiten beter aan bij een leertheorie waarbij beide manieren van leren een gemeenschappelijke representatie creëren dan bij een leertheorie waarbij de manieren van leren worden onderscheiden. Indien angstleren via deze twee wegen inderdaad deels onafhankelijk zou zijn zou onderzoek moeten kunnen tonen dat deze twee manieren van angstleren verschillende kenmerken hebben en dat zij onafhankelijke effecten hebben op angst. Het is echter zo dat deze resultaten geen finaal antwoord kunnen bieden op de vraag of één of meerdere leerprocessen nodig zijn om angstleren te verklaren. Het is steeds mogelijk dat een experiment onder bepaalde condities evidentie kan bieden voor de onafhankelijkheid van deze twee verschillende manieren van leren (Olsson & Phelps, 2004). Onze resultaten dragen wel bij aan de empirische bevindingen over angstleren via stimulusparingen en via verbale instructies die moeten kunnen verklaard worden door theorieën van angstleren. Dus, onze bevindingen zullen toelaten om theorieën van angstleren verder te verfijnen en te testen.

Onze resultaten in dit doctoraatsproefwerk hebben ook een aantal implicaties voor het begrijpen en behandelen van pathologische angst. We hebben bijvoorbeeld gevonden dat angstleren via verbale instructies meer uitgesproken kan zijn voor angst-relevante stimuli. Dit kan betekenen dat schijnbaar irrationele of pathologische angst kan geïnstalleerd worden via verbale instructies (Hugdahl & Öhman, 1977; Hugdahl, 1978). Deze bevinding dient echter nog verder te worden onderzocht omdat het momenteel niet helemaal duidelijk is onder welke condities deze effecten gevonden kunnen worden. Verder hebben we ook gezien dat bepaalde manipulaties uitgedoofde angst geïnstalleerd via verbale instructies opnieuw kunnen installeren. Deze manipulaties worden vaak gezien als de laboratorium-analogen van hoe herval kan plaatsvinden na therapie (Bouton, 2002; Haaker, Golkar, Hermans, & Lonsdorf, 2014). Dit kan betekenen dat gelijkaardige voorzorgsmaatregelen om herval te voorkomen voor angst geïnstalleerd via stimulusparingen ook van

toepassing kunnen zijn om herval te voorkomen van angst aangeleerd via verbale instructies.

Een aantal interessante vervolgonderzoeken gebaseerd op het onderzoek in dit doctoraatsproefwerk kunnen zijn om de contingentie-omkering studie uit hoofdstuk 5 verder te gebruiken om evidentie te vinden voor associatieve leerprocessen, om te onderzoeken hoe verbale instructies effecten van conditioneringservaringen kunnen versterken of verzwakken en om meer onderzoek te doen naar de geheugenprocessen die betrokken zijn bij het opslagen en terug ophalen van angstrepresentaties geïnstalleerd via verbale instructies. Dit zijn slechts enkele van de mogelijke vervolgonderzoeken. Veel onderzoek is duidelijk nog mogelijk en nodig om tot een beter begrip te komen over hoe verbale instructies bijdragen aan het aanleren van angst.

Ter conclusie dan, het onderzoek in dit doctoraatsproefwerk heeft een aantal principes van angstleren via verbale instructies aangetoond. Het onderzoek in dit proefwerk biedt een eerste stap voor een volledig begrip van angstleren via verbale instructies en stimulusparingen. Verder onderzoek is echter nodig om bestaande theorieën over angstleren te blijven verder verfijnen en specificeren.

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Data Storage Fact Sheet **CHAPTER 2**

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

% Author: Gaëtan Mertens, Manuel Kuhn, An K. Raes, Raffael Kalisch, Jan De Houwer, Tina B. Lonsdorf

% Date: 10/05/2015

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**1c. Lab data management responsible**  
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**1d. Co-author of the paper**  
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**2. Information about the datasets to which this sheet applies**  
=====

\* Reference of the publication in which the datasets are reported:

Mertens, G., Kuhn, M., Raes, A. K., Kalisch, R., De Houwer, J., & Lonsdorf, T. B. (2015). Fear expression and return of fear following threat instruction with or without direct contingency experience. *Cognition and Emotion*, (May), 1–17. doi:10.1080/02699931.2015.1038219

\* Which datasets in that publication does this sheet apply to?:

All (one experiment)

### 3. Information about the files that have been stored

=====

#### 3a. Raw data

-----

\* Have the raw data been stored by the main researcher?  YES /  NO

If NO, please justify:

\* On which platform are the raw data stored?

- researcher PC
- research group file server: Z:\GaetanMertens\GM\_2013\_5 Startle project Hamburg\02RawData\instrfear.zip
- other (specify): ...

\* Who has direct access to the raw data (i.e., without intervention of another person)?

- main researcher
- responsible ZAP
- all members of the research group
- all members of UGent
- other (specify): Responsible for data management from our research group (Maarten De Schryver; see above); Co-author of the study: Manuel Kuhn (see above)

#### 3b. Other files

-----

\* Which other files have been stored?

- file(s) describing the transition from raw data to reported results. Specify: ...
- file(s) containing processed data. Specify: FPS2\_RESCORED.sav, expectancy.sav, FearRatings.sav, SCR.sav (Z:\GaetanMertens\GM\_2013\_5 Startle project Hamburg\03Analysis\Data)
- file(s) containing analyses. Specify: Z:\GaetanMertens\GM\_2013\_5 Startle project Hamburg\ReadMe\_GM\_2013\_5 startle Hamburg.txt
- file(s) containing information about informed consent
- a file specifying legal and ethical provisions
- file(s) that describe the content of the stored files and how this content should be interpreted. Specify: Z:\GaetanMertens\GM\_2013\_5 Startle project Hamburg\ReadMe\_GM\_2013\_5 startle Hamburg.txt
- other files. Specify: ...

\* On which platform are these other files stored?

- individual PC
- research group file server: Z:\GaetanMertens\GM\_2013\_5 Startle project Hamburg
- other: ...

\* Who has direct access to these other files (i.e., without intervention of another person)?

- main researcher
- responsible ZAP
- all members of the research group
- all members of UGent

- [X] other (specify): Responsible for data management from our research group (Maarten De Schryver; see above)

#### 4. Reproduction

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\* Have the results been reproduced independently?: [X] YES / [ ] NO

\* If yes, by whom (add if multiple):

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- affiliation: Department of Systems Neuroscience, University Medical Center Hamburg-Eppendorf
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% Data Storage Fact Sheet **CHAPTER 3**

% Name/identifier study: Does confirmation of verbal threat modulate early visual processing?

% Author: Gaëtan Mertens, Tom Everaert, Valentina Rossi, &amp; Jan De Houwer

% Date: 25/02/2015

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1c. Lab data management responsible  
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2. Information about the datasets to which this sheet applies  
=====

\* Reference of the publication in which the datasets are reported:

Mertens, G., Everaert, T., Rossi, V., & De Houwer, J. (Unpublished thesis chapter). Does confirmation of verbal threat modulate early visual processing?

\* Which datasets in that publication does this sheet apply to?:

All data

3. Information about the files that have been stored  
=====3a. Raw data  
-----

\* Have the raw data been stored by the main researcher?  YES /  NO

If NO, please justify:

\* On which platform are the raw data stored?

- researcher PC
- research group file server: Z:\GaetanMertens\GM\_2015\_1 EEG\_FC
- other (specify): ...

\* Who has direct access to the raw data (i.e., without intervention of another person)?

- main researcher
- responsible ZAP
- all members of the research group
- all members of UGent
- other (specify): Responsible for data management from our research group (Maarten De Schryver; see above)

### 3b. Other files

-----

\* Which other files have been stored?

- file(s) describing the transition from raw data to reported results.
- file(s) containing processed data. Specify: C1\_ST\_80\_120.xlsx, N1\_ST\_180\_240.xlsx, P1\_ST\_140\_180.xlsx, P3\_ST\_350\_700.xlsx, USexpectancy\_ratings.sav
- file(s) containing analyses. Specify:
- files(s) containing information about informed consent: Z:\GaetanMertens\GM\_2015\_1 EEG\_FC\01Background\IC\_FC.docx
- a file specifying legal and ethical provisions
- file(s) that describe the content of the stored files and how this content should be interpreted. Specify: Z:\GaetanMertens\GM\_2015\_1 EEG\_FC\ReadMe\_GM\_2015\_1\_EEG\_FC.txt
- other files. Specify: ...

\* On which platform are these other files stored?

- individual PC
- research group file server: Z:\GaetanMertens\GM\_2014\_2 Reversal
- other: ...

\* Who has direct access to these other files (i.e., without intervention of another person)?

- main researcher
- responsible ZAP
- all members of the research group
- all members of UGent
- other (specify): Responsible for data management from our research group (Maarten De Schryver; see above)

### 4. Reproduction

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\* Have the results been reproduced independently?:  YES /  NO

\* If yes, by whom (add if multiple):

- name:
- address:
- affiliation:

- e-mail:

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% Data Storage Fact Sheet **CHAPTER 4**

% Name/identifier study: Can prepared fear conditioning result from verbal instructions?

% Author: Gaëtan Mertens

% Date: 02/11/2015

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1c. Lab data management responsible  
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2. Information about the datasets to which this sheet applies  
=====

\* Reference of the publication in which the datasets are reported: Mertens, G., Raes, A. K., & De Houwer, J. (In press). Can prepared fear conditioning result from verbal instructions? Learning and Motivation. doi:10.1016/j.lmot.2015.11.001

\* Which datasets in that publication does this sheet apply to?: All experiments (Experiment 1 and 2)

3. Information about the files that have been stored  
=====3a. Raw data  
-----

\* Have the raw data been stored by the main researcher?  YES /  NO

If NO, please justify:

\* On which platform are the raw data stored?

- researcher PC
- research group file server: Z:\GaetanMertens\GM\_2013\_2 Prepared Learning

-  other (specify): ...

\* Who has direct access to the raw data (i.e., without intervention of another person)?

- main researcher
- responsible ZAP
- all members of the research group
- all members of UGent
- other (specify): Responsible for data management from our research group (see above)

### 3b. Other files

-----

\* Which other files have been stored?

- file(s) describing the transition from raw data to reported results. Specify: SCR\_rescored\_syntax.sps (Experiment 1) and SyntaxFysio.sps (Experiment 2)
- file(s) containing processed data. Specify: ExpectancySPSS.sav and SCR\_rescored.sav (Experiment 1) and RatingdataSPSS.sav and SelecCond\_AR\_SCR\_1.sav (Experiment 2)
- file(s) containing analyses. Specify: ...
- files(s) containing information about informed consent
- a file specifying legal and ethical provisions
- file(s) that describe the content of the stored files and how this content should be interpreted. Specify: Z:\GaetanMertens\GM\_2013\_2 Prepared Learning\README.txt
- other files. Specify: ...

\* On which platform are these other files stored?

- individual PC
- research group file server: Z:\GaetanMertens\GM\_2013\_2 Prepared Learning
- other: ...

\* Who has direct access to these other files (i.e., without intervention of another person)?

- main researcher
- responsible ZAP
- all members of the research group
- all members of UGent
- other (specify): Responsible for data management from our research group (see above)

### 4. Reproduction

-----

\* Have the results been reproduced independently?:  YES /  NO

\* If yes, by whom (add if multiple):

- name:
- address:
- affiliation:
- e-mail:



% Data Storage Fact Sheet **CHAPTER 5**

% Name/identifier study: Potentiation of the startle reflex is in line with contingency reversal instructions rather than the conditioning history

% Author: Gaëtan Mertens, Jan De Houwer

% Date: 30/11/2015

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2. Information about the datasets to which this sheet applies  
=====

\* Reference of the publication in which the datasets are reported:

Mertens, G., & De Houwer, J. (In press). Potentiation of the startle reflex is in line with contingency reversal instructions rather than the conditioning history. *Biological Psychology*. doi:10.1016/j.biopsycho.2015.11.014

\* Which datasets in that publication does this sheet apply to?:

All datasets (only one experiment)

3. Information about the files that have been stored  
=====

## 3a. Raw data

-----

\* Have the raw data been stored by the main researcher?  YES /  NO

If NO, please justify:

\* On which platform are the raw data stored?

- researcher PC
- research group file server: Z:\GaetanMertens\GM\_2014\_2 Reversal
- other (specify): ...

\* Who has direct access to the raw data (i.e., without intervention of another person)?

- main researcher
- responsible ZAP
- all members of the research group
- all members of UGent
- other (specify): Responsible for data management from our research group (Maarten De Schryver; see above)

## 3b. Other files

-----

\* Which other files have been stored?

- file(s) describing the transition from raw data to reported results. Specify: syntax\_EXPECTANCY\_ContingencyReversal.sps, syntax\_FPS\_ContingencyReversal.sps, syntax\_SCR\_ContingencyReversal.sps
- file(s) containing processed data. Specify: Expectancy\_ContingencyReversal.sav, FPS\_ContingencyReversal.sav, SCR\_ContingencyReversal.sav
- file(s) containing analyses. Specify: Z:\GaetanMertens\GM\_2014\_2 Reversal\ReadMe\_GM\_2014\_2\_Reversal.txt
- files(s) containing information about informed consent: Z:\GaetanMertens\GM\_2014\_2 Reversal\01Background\IC\_FC.docx
- a file specifying legal and ethical provisions
- file(s) that describe the content of the stored files and how this content should be interpreted. Specify: Z:\GaetanMertens\GM\_2014\_2 Reversal\ReadMe\_GM\_2014\_2\_Reversal.txt
- other files. Specify: ...

\* On which platform are these other files stored?

- individual PC
- research group file server: Z:\GaetanMertens\GM\_2014\_2 Reversal
- other: ...

\* Who has direct access to these other files (i.e., without intervention of another person)?

- main researcher
- responsible ZAP
- all members of the research group
- all members of UGent
- other (specify): Responsible for data management from our research group (Maarten De Schryver; see above)

## 4. Reproduction

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\* Have the results been reproduced independently?:  YES /  NO

\* If yes, by whom (add if multiple):

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% Data Storage Fact Sheet **CHAPTER 6**

% The impact of a context switch and context instructions on the return of verbally conditioned fear

% Author: Gaëtan Mertens, Jan De Houwer

% Date: 12/11/2015

## 1. Contact details

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- e-mail: jan.dehouwer@ugent.be

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- e-mail: Maarten.DeSchryver@UGent.be

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## 2. Information about the datasets to which this sheet applies

=====

\* Reference of the publication in which the datasets are reported:

Mertens, G. and De Houwer, J. (In press). The impact of a context switch and context instructions on the return of verbally conditioned fear. *Journal of Behavior Therapy and Experimental Psychiatry*. doi: 10.1016/j.jbtep.2015.11.001

\* Which datasets in that publication does this sheet apply to?:

All datasets.

## 3. Information about the files that have been stored

=====

## 3a. Raw data

-----

\* Have the raw data been stored by the main researcher?  YES /  NO

If NO, please justify:

\* On which platform are the raw data stored?

- researcher PC
- research group file server: Z:\GaetanMertens\GM\_2014\_1 Renewal
- other (specify): ...

\* Who has direct access to the raw data (i.e., without intervention of another person)?

- main researcher
- responsible ZAP
- all members of the research group
- all members of UGent
- other (specify): Responsible for data management from our research group (Maarten De Schryver; see above)

### 3b. Other files

-----

\* Which other files have been stored?

- file(s) describing the transition from raw data to reported results. Specify: syntax\_FPS\_renewal.sps; syntax\_SCR\_renewal.sps
- file(s) containing processed data. Specify: Renewal\_FPS.sav, Renewal\_SCR.sav, Renewal\_USexpectancy.sav
- file(s) containing analyses. Specify: Information about the analyses steps taken in SPSS are provided in Z:\GaetanMertens\GM\_2014\_1 Renewal\ReadMe\_GM\_2014\_1 Renewal.txt
- files(s) containing information about informed consent: Z:\GaetanMertens\GM\_2014\_1 Renewal\01Background\Materials\Questionnaires\_renewal\IC\_FC.docx
- a file specifying legal and ethical provisions: Z:\GaetanMertens\GM\_2014\_1 Renewal\01Background\Materials\Questionnaires\_renewal\EthicalApplication\_RenewalStudy.pdf
- file(s) that describe the content of the stored files and how this content should be interpreted. Specify: Z:\GaetanMertens\GM\_2014\_1 Renewal\ReadMe\_GM\_2014\_1 Renewal.txt
- other files. Specify: ...

\* On which platform are these other files stored?

- individual PC
- research group file server: Z:\GaetanMertens\GM\_2014\_1 Renewal
- other: ...

\* Who has direct access to these other files (i.e., without intervention of another person)?

- main researcher
- responsible ZAP
- all members of the research group
- all members of UGent
- other (specify): Responsible for data management from our research group (Maarten De Schryver; see above)

### 4. Reproduction

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\* Have the results been reproduced independently?:  YES /  NO

\* If yes, by whom (add if multiple):

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- address:
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