**Influence of context and contingency awareness on fear conditioning –
an investigation in virtual reality**

*Der Einfluss von Kontext und Kontingenzbewusstsein auf Furchtkonditionierung –
eine Untersuchung in virtueller Realität*

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„We are more often frightened than hurt;
and we suffer more from imagination than from reality.“

Lucius Annaeus Seneca

Table of Contents

1. Abstract

Fear conditioning is an efficient model of associative learning, which has greatly improved our knowledge of processes underlying the development and maintenance of pathological fear and anxiety. In a differential fear conditioning paradigm, one initially neutral stimulus (NS) is paired with an aversive event (unconditioned stimulus, US), whereas another stimulus does not have any consequences. After a few pairings the NS is associated with the US and consequently becomes a conditioned stimulus (CS+), which elicits a conditioned response (CR).

The formation of explicit knowledge of the CS/US association during conditioning is referred to as contingency awareness. Findings about its role in fear conditioning are ambiguous. The development of a CR without contingency awareness has been shown in delay fear conditioning studies. One speaks of delay conditioning, when the US coterminates with or follows directly on the CS+. In trace conditioning, a temporal gap or "trace interval" lies between CS+ and US. According to existing evidence, trace conditioning is not possible on an implicit level and requires more cognitive resources than delay conditioning.

The associations formed during fear conditioning are not exclusively associations between specific cues and aversive events. Contextual cues form the background milieu of the learning process and play an important role in both acquisition and the extinction of conditioned fear and anxiety. A common limitation in human fear conditioning studies is the lack of ecological validity, especially regarding contextual information. The use of Virtual Reality (VR) is a promising approach for creating a more complex environment which is close to a real life situation.

I conducted three studies to examine cue and contextual fear conditioning with regard to the role of contingency awareness. For this purpose a VR paradigm was created, which allowed for exact manipulation of cues and contexts as well as timing of events. In all three experiments, participants were guided through one or more virtual rooms serving as contexts, in which two different lights served as CS and an electric stimulus as US. Fear potentiated startle (FPS) responses were measured as an indicator of implicit fear conditioning. To test whether participants had developed explicit awareness of the CS-US contingencies, subjective ratings were collected.

The first study was designed as a pilot study to test the VR paradigm as well as the conditioning protocol. Additionally, I was interested in the effect of contingency awareness. Results provided evidence, that eye blink conditioning is possible in the virtual environment and that it does not depend on contingency awareness. Evaluative conditioning, as measured by subjective ratings, was only present in the group of participants who explicitly learned the association between CS and US.

To examine acquisition and extinction of both fear associated cues and contexts, a novel cue-context generalization paradigm was applied in the second study. Besides the interplay of cues and contexts I was again interested in the effect of contingency awareness. Two different virtual offices served as fear and safety context, respectively. During acquisition, the CS+ was always followed by the US in the fear context. In the safety context, none of the lights had any consequences. During extinction, a additional (novel) context was introduced, no US was delivered in any of the contexts. Participants showed enhanced startle responses to the CS+ compared to the CS- in the fear context. Thus, discriminative learning took place regarding both cues and contexts during acquisition. This was confirmed by subjective ratings, although only for participants with

explicit contingency awareness. Generalization of fear to the novel context after conditioning did not depend on awareness and was observable only on trend level.

In a third experiment I looked at neuronal correlates involved in extinction of fear memory by means of functional magnetic resonance imaging (fMRI). Of particular interest were differences between extinction of delay and trace fear conditioning. I applied the paradigm tested in the pilot study and additionally manipulated timing of the stimuli: In the delay conditioning group (DCG) the US was administered with offset of one light (CS+), in the trace conditioning group (TCG) the US was presented 4s after CS+ offset. Most importantly, prefrontal activation differed between the two groups. In line with existing evidence, the ventromedial prefrontal cortex (vmPFC) was activated in the DCG. In the TCG I found activation of the dorsolateral prefrontal cortex (dlPFC), which might be associated with modulation of working memory processes necessary for bridging the trace interval and holding information in short term memory.

Taken together, virtual reality proved to be an elegant tool for examining human fear conditioning in complex environments, and especially for manipulating contextual information. Results indicate that explicit knowledge of contingencies is necessary for attitude formation in fear conditioning, but not for a CR on an implicit level as measured by FPS responses. They provide evidence for a two level account of fear conditioning. Discriminative learning was successful regarding both cues and contexts. Imaging results speak for different extinction processes in delay and trace conditioning, hinting that higher working memory contribution is required for trace than for delay conditioning.

2. Zusammenfassung

Furchtkonditionierung ist ein effizientes Modell für assoziatives Lernen und hat unser Wissen über Prozesse, die der Entstehung und Aufrechterhaltung von pathologischer Furcht und Angst zugrunde liegen, entscheidend vergrößert. In einem differentiellen Furchtkonditionierungparadigma wird ein zunächst neutraler Reiz (NS) gemeinsam mit einem aversiven Ereignis (unbedingter Reiz, US) dargeboten, während ein zweiter Stimulus nicht mit dem Ereignis gepaart wird. Nach mehrmaliger gemeinsamer Darbietung wird der NS mit dem US assoziiert. Dadurch wird er zu einem bedingten Reiz (CS+) und löst eine konditionierte Furchtreaktion (CR) aus.

Die Bildung expliziten Wissens über die CS/US-Assoziation während der Konditionierung bezeichnet man als Kontingenzbewusstsein. Befunde über die Rolle dieses Bewusstseins in der Furchtkonditionieung sind uneinheitlich. In Delay-Furchtkonditionierungsstudien konnte die Entwicklung einer CR unabhängig von Kontingenzbewusstsein gezeigt werden. Man spricht von Delay-Konditionierung, wenn der US direkt auf den CS+ folgt. Bei der Trace-Konditionierung liegt zwischen dem CS und dem US ein kurzer zeitlicher Abstand (Trace-Interval). Für Trace-Konditionierung werden mehr kognitive Ressourcen benötigt als für Delay-Konditionierung. Auf einer impliziten Ebene ist Trace-Konditionierung nicht möglich.

Die Assoziationen, die während der Furchtkonditionierung gebildet werden, beschränken sich nicht auf Assoziationen zwischen spezifischen Reizen und aversiven Ereignissen. Kontextuelle Reize bilden den Hintergrund des Lernprozesses und spielen sowohl bei der Akquisition als auch bei der Extinktion von Furcht und Angst eine wichtige Rolle. Eine häufige Einschränkung in Furchtkonditionierungsstudien beim Menschen ist der Mangel an ökologischer Validität, besonders hinsichtlich der Kontextinformationen. Der Einsatz von

virtuellen Realtitäten (VR) stellt einen vielversprechenden Ansatz dar um komplexe Umgebungen nachzubilden, die nahe an Alltagssituationen sind.

Um Hinweisreiz- und Kontextkonditionierung unter Berücksichtigung des Kontingenzbewusstseins zu untersuchen habe ich drei Experimente durchgeführt. Dafür wurde ein Paradigma in virtueller Realität entwickelt, das es ermöglicht, Reize, Kontexte sowie zusätzlich das Timing der Ereignisse exakt zu manipulieren. In allen drei Studien wurden Versuchspersonen durch einen oder mehrere virtuelle Räume geführt, in denen zwei verschiedene Lichter als bedingte Reize und ein elektrischer Reiz als unbedingter Reiz dienten. Furchtpotenzierte Startlereaktionen wurden gemessen als Indikator für implizite Furchtkonditionierung. Um zu überprüfen, ob die Versuchspersonen auch explizites Kontingenzbewusstsein erwoben hatten, wurden subjektive Ratings erfasst.

Die erste Studie wurde als Pilotstudie konstruiert, um sowohl das VR Paradigma als auch das Konditionierungsprotokoll zu testen. Zusätzlich hat mich der Effekt des Kontingenzbewusstseins interessiert. Die Ergebnisse zeigten, dass Lidschlag-konditionierung im VR Paradigma möglich ist und dass sie nicht vom Kontingenz-bewusstsein abhängt. Allerdings war evaluative Konditionierung, gemessen durch subjektive Ratings, nur erkennbar bei Personen, die die Assoziation von CS und US explizit gelernt hatten.

Um Akquisition und Extinktion sowohl furchtassoziierter Reize als auch furchtassoziierter Kontexte zu untersuchen, wurde in der zweiten Studie ein neues Reiz-Kontext-Generalisierungsparadigma eingesetzt. Neben dem Zusammenspiel von Reizen und Kontexten war ich auch hier an der Rolle des Kontingenzbewusstseins interessiert. Zwei verschiedene virtuelle Büros dienten als Furcht- bzw. Sicherheitskontext. Während der Akquisition folgte auf den CS+ im Furchtkontext immer ein US. Im Sicherheitskontext

hatte keines der Lichter Konsequenzen. In der Extinktionsphase wurde zusätzlich ein neuer Kontext eingeführt. In keinem der Kontexte wurde ein US appliziert. Die Versuchspersonen reagierten nur im Furchtkontext mit erhöhter Startlereaktion auf den CS+ im Vergleich zum CS-. Diskriminatives Lernen hat sowohl hinsichtlich der Reize als auch hinsichtlich der Kontexte stattgefunden. Dies wurde bestätigt durch die subjektiven Ratings, allerdings nur bei Probanden mit Kontingenzbewusstsein. Eine Generalisierung der Angst vom Furchtkontext auf den neuen Kontext war nicht abhängig vom Kontingenzbewusstsein, konnte allerdings in der Gesamtgruppe nur tendenziell beobachtet werden.

In der dritten Studie betrachtete ich neuronale Korrelate der Extinktion von Furchtgedächtnis mit Hilfe von funktioneller Magnetresonanztomographie (fMRI). Von besonderem Interesse waren dabei die Unterschiede zwischen der Extinktion von Delay- und Trace-Konditionierung. Ich habe das Paradigma aus der Pilotstudie angewendet und zusätzlich das Timing der Reize manipuliert. In der Delay-Konditionierungsgruppe (DCG) wurde der US zeitgleich mit dem Ende des CS+ appliziert, in der Trace-Konditionierungsgruppe (TCG) vier Sekunden nach Ende des CS+. Interessanterweise unterschieden sich die beiden Gruppen in ihrer präfrontalen Aktivierung. In Übereinstimmung mit der Literatur war der ventromediale Präfrontalkortex (vmPFC) in der DCG aktiviert. In der TCG konnte man Aktivierung des dorsolateralen Präfrontalkortex (dlPFC) beobachten. Dies könnte mit erhöhter Beteiligung des Arbeitsgedächtnisses zusammenhängen, die notwendig ist, um das Trace-Interval zu überbrücken und die Informationen im Kurzzeitgedächtnis zu halten.

Zusammengefasst hat sich virtuelle Realität als ein elegantes Instrument zur Fuchtkonditionierung beim Menschen herausgestellt, besonders zur Manipulation von

Kontextinformation. Die Ergebnisse deuten darauf hin, dass explizites Kontingenzwissen notwendig ist für evaluative Furchtkonditionierung, nicht jedoch für eine implizite CR gemessen an FPS Reaktionen. Außerdem liefern sie Evidenz für den "two level account of fear conditioning". Die Ergebnisse der Bildgebung sprechen für zwei unterschiedliche Extinktionsprozesse bei Delay- und Trace-Konditionierung und weisen darauf hin, dass für Trace-Konditionierung eine höhere Beteiligung des Arbeitsgedächtnisses notwendig ist als für Delay-Konditionierung.

3. General Introduction

Fear conditioning and the extinction of conditioned fear have considerably advanced the understanding of anxiety disorders in the last decades. The characteristics of anxiety disorders like specific phobias, post traumatic stress disorder (PTSD), panic disorder (PD) or generalized anxiety disorder (GAD) are increased levels of anxiety, fear, or both. Both deficient fear learning as well as impaired extinction of fear may lead to maladaptive fear responses and by this means contribute to the development and maintenance of anxiety disorders. Although plenty is already known about the neural and behavioral mechanisms underlying both fear learning and extinction, we are still far away from understanding the big picture. There are for example different theories about what exactly is learned during acquisition and extinction and about the role of the context of fear conditioning. Additionally, the debate about the role of contingency awareness which is defined as the explicit knowledge of the association between CS and US has not been fully resolved yet. Individual risk factors for anxiety disorders have been identified such as for example trait anxiety, but evidence regarding these factors is not always unambiguous. In almost a century of research after Pavlovs discovery in 1927, classical conditioning in general and fear conditioning in particular turned out to be much more complex as they might look like at first glance.

3.1. An evolutionary perspective on fear

Emotions have long been subject of research in psychology and neuroscience. From an evolutionary perspective, they can be described as states of readiness within a simple two-factor model of motivation (Lang, Bradley & Cuthbert, 1998). According to this

theory, affects developed from reflexive reactions elicited by appetitive or aversive stimuli that are essential for survival: When an organism is confronted with a threatening stimulus, e.g. a predator, the defensive system is activated and triggers an appropriate defensive reaction like fight or flight. Correspondingly, stimuli like food or a mating partner activate the appetitive system, which triggers an approach reaction.

Hence, from an evolutionary point of view, fear is the aversive emotional state that motivates a defensive reaction in response to an external threat. Reflexive escape responses for example are self protection mechanisms that allow for coping with imminent dangers like predators or other physical threats. Therefore, fear is central in evolution, since the associated defensive reactions are often essential for the survival of an organism. Öhmann and Mineka (2001) proposed a fear system shaped by natural selection, which contains four characteristics: selectively with regard to input, automaticity, encapsulation and a specialized neural circuitry.

By evolution, some stimuli, for example snakes, spiders or height, are innate sources of fear for us because they have been associated with threat in our evolutionary past. However, we live in a rapidly changing environment. For example, more and more people move to big cities: In 2010, already 50.5 per cent of the word population lived in cities ("Globalisierung", 2010). In modern city life, threats like cars or weapons appear to be much more salient than innate sources of fear shaped by evolution. Still, fear of snakes, spiders or heights is much more common than fear of todays everyday life threats like cars or weapons. The fear system can be activated by an innate fear-relevant stimulus much easier than by an initially neutral stimulus like a flower or a neutral face or also a car (e.g. Tomarken, Mineka & Cook, 1989; Hughdahl & Öhmann, 1980).We are biologically prepared to associate innate fear-relevant stimuli with a defensive response much faster

and also much more sustainably than stimuli which are, from an evolutionary perspective, fear-irrelevant (Seligman, 1971). However, in a changing environment, the ability to form associations between initially neutral stimuli and possible threats is also essential for survival of an animal or a human being (Mineka & Öhman, 2002). The fear system is provided with a high plasticity, which allows not only for biologically prepared fear learning facilitated by evolutionary history and natural selection. Additionally, the fear system can be elicited by random, initially fear-irrelevant stimuli that have been associated with threatening or painful events in the personal history of an individual (Öhman, 2009). The less fear-relevant a stimulus is to begin with, the more environmental input is necessary to associate it with threat.

The second feature of the fear system proposed by Öhman and Mineka (2001) is automaticity of fear activation. In order to provoke a defensive reaction in response to a fear-relevant stimulus, higher order cognitive processing is not necessary. This means, a very short and cursory perception can be enough for the stimulus to enter the fear system and elicit a defensive reaction. This has, amongst others, been tested by confronting human beings with subliminally presented fear-relevant stimuli like pictures of snakes or spiders. In this case, the picture is presented for approximately 30 ms, which is too short for higher order processing to take place. However, a physiological fear reaction has still been found (Öhmann & Soares, 1993).

Besides its automatic activation and selectivity with regard to input, the fear system described by Öhmann and Mineka (2001) can also be characterized as an encapsulated system. Once elicited, it is relatively resistant to conscious cognitive control (Mineka & Öhman, 2002). This can often been observed in people with phobic fears: Usually these

individuals are consciously aware that their fear is irrational and disproportional, but nonetheless they cannot gain rational control over their reaction to the phobic stimulus.

Furthermore, the fear system is mediated by a specific neuronal circuitry. Since the development of the fear system began early in evolution, the neuronal circuitry contolling an automatic fear response is, basically, similar across species. It is located in the older parts of the brain, the limbic system. Evidence for the central role of the amygdala in fear learning the production of a fear response derives mainly from rodent studies. Up to now, these findings from the animal model have been vastly extended and also confirmed in human studies (see for example LeDoux, 2000).

3.2. Classical conditioning

3.2.1. Pavlov´s conditioning paradigm

In a changing environment, it is essential for an organism to be capable of learning associations between environmental stimuli and corresponding appetitive or aversive events. In 1927, Ivan Pavlov began to study this kind of associative learning in the laboratory. He described the famous phenomenon called Pavlovian Conditioning: An initially neutral stimulus as a tone or a light is paired with primary reinforcer which can be appetitive (e.g. food) or aversive (e.g. an electric shock) in nature. After several of these pairings, the initially neutral conditioned stimulus (CS) is associated with the appetitive or aversive unconditioned stimulus (US) and evokes an appropriate conditioned response (CR) when presented on its own. Learning that a certain environmental stimulus predicts an aversive event is called fear conditioning. This is the mechanism, which allows us to learn to fear certain objects, animals, places or people (Maren, 2001). Without that ability, we would hardly be able to cope with survival threats by escaping or avoiding them.

In the past 25 years, fear conditioning has been studied intensely (e.g. Davis, 1992; Fendt & Fanselow, 1999; LeDoux, 2000; Maren, 1996; Fanselow, 1994). It is one of the most important animal models and has greatly improved the knowledge about mechanisms of fear and anxiety as well as fear mediating neuronal structures (Davis, 1997) in rodents as well as in humans (LeDoux, 2000). According to Joseph LeDoux (2000), it has also opened the way for a new wave of research on emotion in neuroscience, inter alia since fear conditioning is a paradigm which allows for examining emotional mechanisms within a cognitive learning paradigm.

Of course, the mechanisms of fear are also extremely important from a clinical point of view. An adaptive fear reaction, which requires functioning fear learning, enables an individual to cope with survival threats by escaping or avoiding them. However, fear reactions can become maladaptive when they are no longer appropriate to the actual situation. The ability to readjust behavior is especially important in a rapidly changing environment. In anxiety disorders, this ability usually is impaired (e.g. Rauch, Shin & Phelps, 2006). A person suffering from claustrophobia will react with a disproportional and irrational fear reaction when exposed to, for example, a narrow and crowded elevator. The high prevalence of anxiety disorders - in Germany approximately 15,3% within 12 months according to the DEGS1-MH (Jacobi et al., 2014) - has led to extensive research in the field of fear learning and unlearning as well as the neural systems involved in fear learning. In a classical fear conditioning paradigm, an initially neutral CS, mainly a tone ore a light, is paired with an initially aversive US, such as an electric shock. After several pairings, the CS is associated with the US and evokes a conditioned fear response (CR) when presented on its own. These are the basics of classical conditioning - however, there is a lot more to it than that.

3.2.2. Concepts and underlying processes of classical conditioning

3.2.2.1. Contiguity and contingency in classical conditioning

According to Robert A. Rescorla (Rescorla, 1988), *"Pavlovian Conditioning is not a stupid process by which an organism willy-nilly forms associations between any two stimuli that happen to co-occur. Rather, the organism is better seen as an information seeker using logical and perceptual relations among events, along with its own*

Figure 1
*Schematic of Two Conditioned Stimulus/
Unconditioned Stimulus (CS/US) Relations That
Share the Same Contiguity but Differ in the
Information the CS Gives About the US*
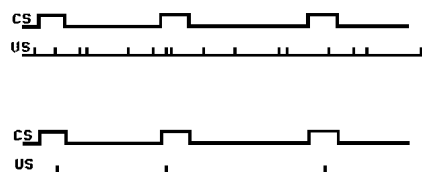
March 1988 • American Psychologist

*Figure 1: Reprinted from Rescorla, 1988;
Copyright 1988 by the American Psychological
Association*

preconceptions, to form a sophisticated representation of the world." Conditioning only occurs when an organism can see the coherence in the pairing of two stimuli and when it benefits from learning the association (e.g. because it can avoid a threat). The mere temporal proximity of two stimuli which is referred to as contiguity has long been thought of as responsible for conditioning to take place.

However, already in 1968, Rescorla (1968) demonstrated that contiguity is not sufficient for associative learning. A rat was exposed to tone and an electric shock. In one experimental condition (see figure 1), the tone and the shock occurred in the same two minute time window, but the tone did not actually provide information about the shock. This is the case when the shock can occur both shortly after the tone but also in time intervals without any tone, making the shock equally likely whether or not a tone is presented. In the second condition (see figure 1), tone and shock always coincided. That is, the tone provided all the information about the appearance of the shock. In this case, the contingency, i.e. the probability of the shock in the presence of the tone is 100%. The higher the contingency

between CS and US, the faster leaning will take place. When the CS signals an increase in the probability that the US will occur, one speaks of positive contingency. For example in fear conditioning, the amount of fear in reaction to the CS is positively correlated with the number of tone - shock (CS-US) pairings (see for example Miller, 2006). On the contrary, a subsequent negative contingency between the same two stimuli signals a decrease of probability of the shock when the tone occurs. This will lead to inhibitory conditioning and a conditioned inhibitory response: The fear reaction to the tone will decrease with the number of tone - no shock pairings. Since the 1960s when Rescorla presented his contingency theory, the concept has often been confirmed (e.g. Murphy & Baker, 2004) and often been criticized (for a review see for example Papini & Bitterman, 1990). However, it was an early explanation for a decrease of the fear reaction when – after excitatory conditioning – the CS is presented without the US for several times.

A theory derived from the contingency concept is the safety signal theory by Seligman and Blinik (1977). If an aversive event is signaled by a CS, the event becomes predictable. However, if there is no information available about the occurrence of the event, an organism lives in constant anxiety, since it cannot know when it will be exposed to the aversive stimulus again. According to the safety signal theory, it is necessary for an organism to learn the association between a predictive signal and the following aversive event in order to be able to identify periods of safety. A CS which is associated with an aversive outcome not only signals danger and elicits fear when it is presented. It also signals absence of danger when it is not present and therefore it serves as a safety signal. A similar process can be observed in a differential conditioning paradigm, in which one conditioned stimulus (CS+) is followed by the US and a second one (CS-) is not. In this case, both excitatory and inhibitory conditioning takes place: The positive contingency

between the CS+ and the US leads to a fear reaction in response to the CS+, whereas the CS- is associated with the absence of the US. Therefore the CS- serves as a safety signal or an inhibitor of the conditioned fear response. The safety-signal theory attracted a lot of attention, especially in the research in anxiety disorders like PD or GAD. Grillon and Ameli (2001) reported that high anxious individuals show an enhanced fear response to the CS- compared to low anxious ones. This has also been found in clinical studies comparing anxiety patients and healthy controls (see for example Lissek et al., 2005). Davis and colleagues (Davis, Falls and Gerwitz, 2000) suggested that the insufficient inhibition of a fear reaction in response to safety signals might be responsible for the development of anxiety disorders.

In more recent studies, PTSD patients have been found to show deficits in discriminative learning of the danger and the safety stimulus. They show higher physiological fear reactions and report higher expectancies of dangerous outcomes in response to the safety stimulus compared to healthy controls (Grillon, Pine, Lissek, Rabin, Bonne, & Vythilingam, 2009; Lissek et al., 2009).

3.2.2.2. Classical conditioning and predictability

Besides contiguity and contingency, a third factor has been shown to play an important role in classical conditioning. An experiment indicating that contiguity and contingency do not necessarily have to be sufficient for learning to take place was already conducted in 1968 (Kamin, 1968): Two groups of animals were exposed to a compound CS which was followed by a US. A compound stimulus could for example be realized by a tone and a light presented together. One of the two groups had already been trained before being exposed to the CS: The animals had learned that the light alone signals the US. The other group of animals had no prior learning experiences related to either of the

stimuli. Both groups were tested for conditioning of the tone, but only the group with no pretraining showed an adequate conditioned response to the CS (Rescorla, 1988). This result is known as the Kamin Blocking Effect. The two groups share the same contiguity and contingency information between the compound CS and the US. Then why does the pretraining group not learn the association between the tone and the US? This question was answered by Rescorla and Wagner, who suggested that the lacking component for the pretraining group is surprise. The animals already knew that the light signals the US. Thus, they were not surprised that the light in combination with the tone also signals the US – the US was already predictable for them. For the other group however, this information was completely new. In their famous Rescorla-Wagner Model, the two authors stated that learning (or change of associative strength) is only possible, if there is a discrepancy between expectancy and actual outcome (Resorla & Wagner 1972). The change of associative strength in one learning trial depends on the maximum possible associative strength and the current associative strength between the stimuli involved. Accordingly, in the first few learning trials, in which the discrepancy between expectancy and actual outcome is still large, the change of associative strength, i.e. learning, is greater than in later trials. This discrepancy is also referred to as prediction error. In fear conditioning one speaks of an aversive prediction error. If the discrepancy between actual outcome of a conditioning trial (λ) and expected outcome (ΣV, consisting of the sum of the associative strengths of all present stimuli) is positive, excitatory conditioning occurs. However, if it is negative and the actual outcome undermatches the expected one, conditioning is inhibitory (McNally & Westbrook, 2006).

3.2.3. What is learned?

Next to the factors that are necessary for the formation of associations, researchers came across another very fundamental question regarding classical conditioning: "What exactly is learned?". There are two basic theories about the formation of associations in conditioning. Back in 1927, Pavlov already suggested that an organism forms an association between the US and the CS (stimulus-stimulus model). In his opinion, the CS serves as a substitute for the US. This is referred to as Stimulus-Substitution Theory. In other words, when dogs are conditioned on a light which is paired with food, the light will become a substitute for the food and therefore elicit salivation. In an experiment conducted in 1941, Pavlov showed that the dogs even started to lick at the lamp after the light had been associated with the food. Today, this phenomenon is referred to as sign tracking. However, there is also evidence in favor of another theory called stimulus-response model, which has been suggested by Watson and Hull. According to them, the organism does not form an association between the CS and the US, but between the CS and the unconditioned response. In contrast to the just mentioned example, the light would be associated with salivation and not with the food. One finding in favor of the S-R (stimulus-response) model by Watson and Hull is, that the conditioned response is not always identical to the unconditioned response, sometimes not even similar (see for example Hull, 1943; Watson, 1913). For example in a fear conditioning experiment, in which a rat is conditioned on a tone that is a paired with a foot shock, the rat will not show the same reaction in response to the tone (CS) as to the foot shock (CS). The expected reaction to the foot shock would be jumping, however, in response to the tone the rat will most certainly show freezing behavior. This can be explained by the preparatory-response theory, which states that the CR prepares the organism for the US: Freezing behavior

prepares the rat for the foot shock just as salivation prepares the dog for food (compare (Powell, Honey & Symbaluk, 2012). In general, evidence for the S-S model is stronger than evidence for the S-R model. For example, in a US devaluation study conducted with rats, a tone (CS) was paired with food (US). The US was later devaluated by inducing nausea. When the CS is presented again after devaluation, the rats show a reduced CR (Holland & Straub, 1979). These findings argue for an association between the CS and the US. But, as stated above, there is also evidence in favor of the CS being a signal for the US and not a substitute. The two level hypotheses is an approach to solve this problem by including both types of learning. It suggests that two systems can be involved in the conditioning process: the associative and the cognitive system. The associative system is an automatic response system allowing for the formation of associations between stimuli without cognitive contributions. When expectations are built in a way that the CS serves as a signal for the US, the cognitive system in involved. There has been great interest in a two-level account in human fear conditioning (LeDoux, 2000; Hamm & Weike, 2005), which will be discussed in more detail in the next chapter.

3.3. Basic Phenomena of fear conditioning

3.3.1. Acquisition

As described above, in fear conditioning an initially neutral stimulus is repeatedly paired with an aversive US. After several pairings, the initially neutral CS is associated with the US and evokes an appropriate CR like freezing or jumping. This learning process is referred to as acquisition. In the first few conditioning trials (i.e. CS-US pairings) acquisition takes place quite rapidly, resulting in a steep learning curve which flattens after several trials and levels out when the asymptote of conditioning is reached (compare

Powell et al. 2012). The speed and sustainability of acquisition depends amongst other things on the nature and the intensity of the US. A stronger US leads to more rapid and sustainable conditioning. The same is the case for a US which has a strong relevance from an evolutionary perspective (like food or pain). Another important factor is the contingency during the acquisition. If the CS is always followed by the US (100% contingency), acquisition takes place much faster than in a paradigm in which for example only every second CS is paired with the US. However, it is also easier to extinguish the learned CR after a 100% contingency learning period.

3.3.1.1. Delay and trace conditioning

The timing of the CS - US pairing during acquisition also plays an important role in the learning process. Two common paradigms regarding event timing in fear conditioning are delay and trace conditioning. In delay conditioning, the US coterminates with or follows directly on the CS. In trace conditioning, a temporal gap lies between the offset of the CS and the onset of the US. This temporal gap is referred to as "trace interval". In this case, a "memory trace" is necessary in order to form an association between the CS and the US (Pavlov, 1927), because the CS is no longer present when the US occurs. For trace conditioning to occur, the temporal gap between the CS and the US should not be longer than a few seconds in most cases. Already back in 1954, Moeller tested whether aversive conditioning takes place after trace intervals of different duration. He found that the greatest learning effect can be achieved with a trace interval of only 0.5 seconds (Moeller, 1954). In more recent human fear conditioning studies, trace conditioning is possible after much longer trace intervals of 4 to 5 seconds (Weike, Schupp, & Hamm, 2007; Knight, Waters & Bandettini, 2006). Under special circumstances, for example when evolutionary relevant stimuli are involved, associations between a CS and a US can be formed after

much longer temporal gaps. Taste aversions for example also develop when the food (CS) has been consumed many hours before nausea is induced (Welzl, D'Adamo, & Lipp, 2001)

3.3.1.2. Contingency awareness

There is an ongoing debate about whether contingency awareness, which can be defined as the explicit knowledge of the association between CSs and UCS, is required for fear acquisition (Klucken, Tabbert, Schweckendiek, Merz, Kagerer, Vaitl & Stark, 2009). As suggested by (Lovibond & Shanks, 2002; Mitchell, De Houwer, & Lovibond, 2009), it is likely that awareness of contingencies between the CS and the UCS is necessary for establishing a conditioned response. There are findings which indicate that even an implicit CR cannot be found without explicit awareness of CS/UCS contingencies (Dawson, Rissling, Schell, & Wilcox, 2007; Klucken, Kagerer, Schweckendiek, Tabbert, Vaitl, & Stark, 2009a). However, there is also opposing evidence, demonstrating that implicit conditioned responses may occur without contingency awareness (Hamm, Weike, Schupp, Treig, Dressel & Kessler, 2003; Knight, Nguyen, & Bandettini, 2006; Weike et al., 2007). Weike and colleagues (2007) for example showed that in delay fear conditioning, acquisition was independent of contingency awareness, whereas in trace conditioning, a conditioned response was only found for participants who were able to explicitly name the association between the CS and the US. Knight and colleagues (2006) came to the same conclusion in their study. The concept of awareness will be discussed in more detail in chapter two.

3.3.1.3. The two level account of fear conditioning

As mentioned before, the amygdala plays a central role in the acquisition of fear. The amygdala is a small structure located in the medial temporal lobe which belongs to the older parts of the brain. From animal research derives the knowledge that the amygdala is crucial for the formation of a conditioned fear response to a CS that has been paired with a threatening stimulus (see for example Davis, 1992; LeDoux, 1995). These findings have been transferred to humans. For example Bechara et al. 1995 demonstrated that a patient with bilateral damage of the amygdala shows deficits in fear conditioning (Bechara, Tranel, Damasio, Adolphs, Rockland & Damasio, 1995). LaBar and colleagues (1995) found similar results in a group of patients with unilateral removal of the amygdala due to epileptic seizures. These participants also did not develop a conditioned fear response (LaBar, LeDoux, Spencer, & Phelps, 1995). Interestingly, patients' declarative learning in both studies was intact, meaning that they were able to correctly report the contingency between CS and US after acquisition. This indicates that the amygdala is not necessary for the formation of explicit contingency knowledge.

In neuro-imaging studies, increased functional activity in the amygdala can be observed during fear conditioning (eg. Büchel, Morris, Dolan, & Friston, 1998; Knight, Smith, Cheng, Stein, & Helmstetter, 2004). In more detail, in the first trials of acquisition, when the learning curve is still rising steeply, amygdala activation is higher during presentations of the CS+ compared to the CS-. Knight et al. (2004) reported that the amygdala was activated when contingencies between the stimuli changed and therfore they concluded that it is crucial for the formation of new associations.

Today we know that during fear conditioning information about the CS and the US is transmitted from sensory cortices via the thalamus to the amygdala, which controls the expression of the fear reaction via projections to the brainstem (LeDoux, 2000).

According to Joseph LeDoux, there are two different pathways over which threatening stimuli can be processed. The subcortical way or „low road" is a rather fast but also inaccurate way. This road is obviously very convenient when an organism finds itself in a situation of imminent danger, in which a fast reaction is necessary for survival. But there is also a „high road" involving higher order cognitive processing of the threatening stimulus. The amygdala is a central structure in both of these pathways. Following the low road, a signal is encoded and then transferred to to the amygdala via the thalamus. The amygdala directly triggers the fear reaction via the brainstem. Following the high road, the amygdala does not directly trigger a fear reaction, but the signal is transmitted from the amygdala to higher order sensory cortices like the primary visual or auditory cortex. Here, a cognitive representation and evaluation of the stimulus is generated.

In figure 2 (LeDoux, 2000) describing the pathway of an auditory CS, the low road is indicated by the arrow connecting the thalamus [medial division of the medial geniculate body (MGm/PIN)] and the lateral amygdala (LA).
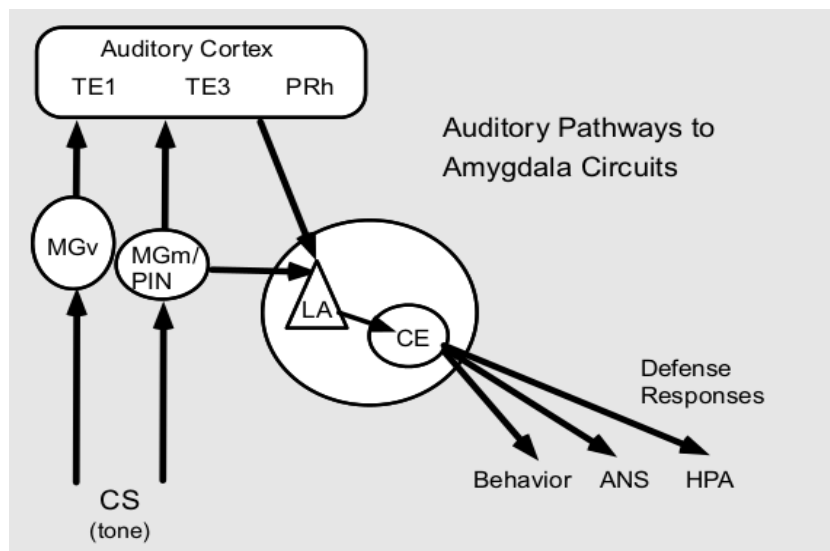
Figure 2: LeDoux, 2000; Copyright 2000 by Annual Reviews

In the lateral amygdala, information about the CS and the US get together. From there, the signal is transmitted to

the central amygdala (CE) and to the brainstem, eliciting a defensive response by influencing behavior, the autonomic nervous system (ANS) and the hypothalamic-pituitary axis (HPA) (LeDoux, 2000).

Correspondant to the idea of an automatic low road and a cognitive high road, Hamm and Weike (2005) proposed a two level account for fear conditioning (Hamm & Weike, 2005). One the one hand, the CS is capable of eliciting the subcortical fear network described above and thus automatically resulting in a fear response. But during human fear conditioning, usually also the declarative knowledge of the contingency between the CS and the US is formed, requiring higher order processing as described in the "high road". Hence, an expectancy is build that the CS will be followed by the US. Studies showing that delay fear conditioning is possible without contingency awareness indicate declarative knowledge is not always formed during fear conditioning.

An important index for measuring whether fear conditioning worked on a non conscious level is the fear potentiated startle (FPS) reflex. The startle reflex is a cross-species response to sudden, intense and unexpected stimulation, e.g. a sharp loud noise. In humans, presentation of such a startle tone leads to an eye-blink, i.e. to a rapid contraction of the M. Orbicularis oculi, which can be measured using electromyographic recordings (Blumenthal, Cuthbert, Filion, Hackley, Lipp & Van Boxtel, 2005; Lang, Bradley, & Cuthbert, 1990). Since the startle reflex is an automatic defensive response which does not depend on cortical input, it is often used to test learning processes on an unconscious and implicit level. Importantly, the magnitude of the reflex varies with the affective state of the organism: Fear-inducing stimuli for example increase the startle response, whereas positive stimuli reduce it. This effect is dependent on subcortical brain structures influencing the brainstem acoustic startle pathway (Koch, 1999; LeDoux, 2000).

Another reliable index for conditioned fear is the electro-dermal activity (EDA). Changes in skin conductance have been shown to be an indicator for fear conditioning on an explicit level. According to Hamm and Weike (2005), declarative knowledge about contingencies is associated with an increased skin conductance response (SCR). In numerous studies, a dissociation between FPS and SCR could be found. For example in the study conducted by Weike and collegues (2007) mentioned earlier, startle potentiation was independent of contingency awareness, a change in SCR however was only present in aware participant (see also Lovibond and Shanks, 2002). Tabbert and colleagues also found a dissociation between neural correlates of fear conditioning and SCR – they did find increased activation of the amygdala in both aware and unaware participants, however, a change in SCR was again only present in aware participants (Tabbert, Stark, Kirsch, & Vaitl, 2006).

As mentioned before, fear conditioning serves as a model to investigate the maladaptive processes resulting in anxiety disorders. In humans, FPS and SCR as well as imaging studies have greatly extended our knowledge of fear learning. However, for understanding the mechanisms of fear and the development and maintenance of anxiety disorders, studying the acquisition of fear is not sufficient. An anxiety disorder can result from either a disproportionately rapid or strong acquisition of fear or from the resistance to extinction of the fear reaction if it is no longer appropriate (Baas, van Ooijen, Goudriaan & Kenemans, 2008).

3.3.2. Extinction

3.3.2.1. Unlearning and relapse

After an organism has learned the association between a CS and a US and responds to the CS with the appropriate CR, the repeated presentation of the CS without

the US usually leads to a weakening of the CR. This process is called extinction. For example, when a rat is trained to associate a light with a foot shock, it will – after a few pairings - show freezing behavior in response to the light alone. If the light is now presented repeatedly without the aversive foot shock, the rat will eventually return to normal behavior as the freezing response will slowly decrease until it disappears completely.

This does not mean that the association between the light and the foot shock is extinguished completely. Today we know that, although the CR gradually dies out, the association between the CS and the US is not at all deleted completely during extinction. An organism does not forget or unlearn, but rather forms new memory inhibiting the acquired fear memory (Bouton, 2002; Bouton, 2004; Myers & Davis 2002, Quirk 2002; Milad & Quirk, 2002). According to Bouton (2002), there are several phenomena indicating that extinction does not result in unlearning (for a review see Bouton, 2002). One of them is called spontaneous recovery (SR), meaning that, some time after extinction training, a presentation of the CS will elicit the same (or a weakened) CR without having been paired with the US again (e.g. Brooks & Bouton, 1993). In a recent fear conditioning study including 43 participants, Norrholm and colleagues paired visual and acoustic CS with an aversive airblast serving as US. Twenty-four hours after extinction training, a presentation of the CS+ alone led to a significant return of the FPS reflex (Norrholm et al., 2011). Besides SR, reinstatement and renewal are phenomena which are regarded as evidence for CS-US associations not being deleted during extinction. In reinstatement, a presentation of the US after extinction leads to recovery of the fear reaction. This phenomenon is context-dependent, which means that the reaction will be stronger if the US is presented in the same context, in which the CS had been associated with the US

before. When acquisition takes place in a first context and extinction training in a second context, fear will return when the subject is confronted once more with the CS in the acquisition context. In more general terms, an extinguished fear response recovers when the subject is exposed to the CS in a context that is different from the extinction context. This is referred to as renewal. In a rodent study, Myers, Ressler and Davis (2006) showed that extinction, when conducted immediately after acquisition, might actually lead to the complete erasure of the fear memory. Rats which underwent extinction training only ten minutes after extinction showed neither SR, nor reinstatement or renewal in a FPS paradigm. By contrast, extinction conducted one hour after acquisition lead to partial recovery of the fear reaction, and extinction conducted several days after acquisition lead to clear recovery of the rear reaction. Their conclusion was that extinction training shortly after acquisition interrupts consolidation of the CS-US association. Once fear learning is consolidated, extinction only leads to the formation of a new memory trace which has an inhibitory effect on the original fear memory. Schiller et al. (2009) replicated these findings in humans. Before extinction training, they presented a reminder (a single presentation of the CS+) to activate the fear memory and open the reconsolidation window, in which the memory trace is labile after retrieval. Extinction training conducted within this window (10 minutes after presenting the reminder) lead complete extinction: The fear did not return, even up to one year after extinction training (Schiller, Monfils, Raio, Johnson, LeDoux & Phelps, 2009). These findings have not been replicated until recently (Oyarzún et al., 2012; Schiller, Raio, & Phelps, 2011).

3.3.2.2. Context dependency of extinction

Once an association between a CS and a US has been formed, this memory is very stable. Usually it is transferred to other contexts, and also to stimuli that are similar to the

original CS. For example, when one is bitten by the neighbor's dog in the neighbor's garden, one will most likely not only fear this particular dog in this particular garden, but also other dogs in other situations. In contrast, extinction – when not conducted during the reconsolidation window - is highly context dependent, meaning that extinction learning is not generalized to different contexts. In the described example this means that it is very difficult to completely extinguish the acquired fear of dogs by conducting an extinction training with one dog in a certain therapeutic setting. It is likely that extinction training leaves the original fear memory intact, unless it has been activated and therefore rendered labile before the training.

3.3.2.3. Neuronal correlates of extinction

As mentioned before, the CR becomes weaker and eventually disappears during extinction training. So how does this new information about the CS, namely that it is no longer followed by the US, inhibit the learned fear reaction? The knowledge about neuronal structures involved in the acquisition of fear exceeds that of structures involved in extinction learning. However, the amygdala is known to be crucial for both acquisition and extinction learning. In addition, after a few trials of extinction, activation of the prefrontal cortex has been found to be increased, which is assumed to inhibit the expression of conditioned fear as new learning takes place (Quirk, Garcia & González-Lima 2006). Animal models provide evidence that a new memory trace between the ventromedial prefrontal cortex (vmPFC) and amygdala is established during extinction learning (Sotres-Bayon, Bush & LeDoux, 2004; Sotres-Bayon, Bush & LeDoux, 2007), which has an inhibitory effect on the formerly conditioned fear memory and thus modulates the fear reaction. In human delay conditioning studies, the involvement of the vmPFC and the amygdala in extinction learning has been confirmed (Gottfried & Dolan, 2004; Milad,

Wright, Orr, Pitman, Quirk & Rauch, 2007; Phelps, Delgado, Nearing, & LeDoux, 2004). The neural circuit for extinction learning and recall is assumed to include, besides amygdala and vmPFC, also the hippocampus. Extinction learning takes place in the amygdala, and the vmPFC is responsible for the inhibition of fear during extinction recall. Presumably, the hippocampus contributes contextual information which determines the setting in which extinction memory can be recalled (Corcoran & Quirk, 2007). Kalisch et al (2006) showed that a network containing the vmPFC and the hippocampus provides for context dependent recall of extinction memory. More precisely, they found that during extinction a strong activation of the hippocampus correlates with a strong activation in the VMPFC, but only in the extinction context. According to Kalisch and colleagues, their findings add evidence to the notion that contextual information stored in the hippocampus facilitates recall of the extinction memory which is mediated by the vmPFC (Kalisch, Korenfeld, Stephan, Weiskopf, Seymour & Dolan, 2006).

A well functioning interaction between the vmPFC, the amygdala and the hippocampus is crucial for adaptation to a fast changing environment. Without this extinction mechanism, fear reactions are no longer flexible and can become inadequate and maladaptive, leading to emotional perseveration.

3.4. Fear and anxiety in classical conditioning

Fear is associated with a real and distinct source of danger, whereas the emotion one feels in a more sustained and unclear threatening situation is referred to as anxiety. An adequate defensive reaction in response to an imminent danger is of course crucial for survival. When an animal is threatened by a predator, it will naturally react with a fight-or-flight response which is triggered by the sympathetic nervous system. However, in periods

of diffuse but not imminent danger, a fight-or-flight response would be a waste of energy. More appropriate in this situation is a state of increased vigilance and tension, which enables the animal to adapt its behaviour rapidly if necessary. In humans, this state can be compared to a state of anxiety. Anxiety is more diffuse than fear, involving worry or anxious apprehension about possible and unpredictable future threats or dangers (Mineka & Oehlberg, 2008). From a clinical point of view, fear can lead to panic symptoms and is associated with for example specific phobias. Anxiety is more likely to lead to chronic worry, tension and enhanced arousal. Therefore, unlike fear, it is related to generalized anxiety disorder (GAD) and also to depression (see for example Hamm & Weike, 2005).

3.4.1. Context conditioning and anxiety

As already mentioned in the last chapter, the associations which are formed during fear conditioning are not exclusively associations between specific cues and an aversive stimulus. When contextual cues are present during acquisition, they form the background milieu of the learning process and play an important role in both the formation of associations and the extinction of conditioned fear (Baas, Nugent, Lissek, Pine, & Grillon, 2004). However, the context does not necessarily serve merely as background information during cue conditioning. It can also be the cue itself. During context conditioning, an experimental context like a cage or a room is paired with an aversive stimulus. Cue and context conditioning differ with regard to temporal information about the threatening stimulus. Whereas a distinct cue allows for an exact prediction of the onset of the associated US, a contextual cue offers no exact temporal information (see for example Grillon, 2008). This sort of conditioning leads to a response that is closer to a state of sustained anxiety than to distinct fear, because the organism finds itself in a setting of uncued and therefore less predictable danger. According to the safety-signal theory

described earlier, a distinct cue associated with a threatening stimulus does not only signal danger. It also serves as a safety signal, because it guarantees absence of danger when it is not present. Without distinct cues serving as safety signals, the organism experiences chronic anxiety. According to Baas and colleagues, successful cue conditioning both signals threat and provides information about safety periods, which in turn allows for a reduction of contextual anxiety (Baas et al., 2008). Consequently, a deficit in learning the association between a present cue and an aversive US might lead to enhanced contextual anxiety instead. Grillon postulates a causal relationship between the failure to learn the CS–US contingency and increased contextual anxiety (Grillon, 2002a). In consideration of this concept, not only hyper-conditionability can lead to maladaptive fear reactions or anxiety disorders like specific phobias. Also hypo-conditionability or the failure to associate a cue with a threat can result in anxiety disorders, however not to specific phobias but rather to GAD or phobic avoidance in panic disorders which are associated with sustained anxiety.

3.4.2. Predictability and anxiety

In humans, contextual conditioning has only recently become an area of interest. Due to its relevance for the development of anxiety disorders, it is very important to further investigate the way context conditioning is modulated and the way it elicits emotional responses in humans. Predictability of the aversive event is one major factor influencing contextual conditioning that has been studied in humans recently. Already in the 1970s, it has been shown in animal studies that unpredictable cues lead to more anxiety and more avoidance behaviour than predictable ones (Mineka and Kihlstrom 1978, Odling-Smee 1975). In humans, recent studies have come to similar results. Grillon (2002a) for example found higher levels of anxiety and greater avoidance among participants who were

unaware about the association between the CS and the US and thus experienced a higher level of unpredictability. In a study conducted in virtual reality contexts, Baas et al. (2008) found similar results: Participants who learned the contingency between the distinct cue and the US showed less contextual fear compared to participants unaware of the CS-US association. On average, the unaware subjects had higher levels of trait anxiety according to Spielberger's self report scale, indicating that there might be a connection between general trait anxiety and conditionability (Baas et al., 2008). Recently, Baas reported that higher trait anxiety is associated with a maladaptive modulation of contextual anxiety (Baas, 2013). In a clinical study, Grillon and colleagues compared sensitivity of patients with PTSD or GAD and healthy controls. They found that, compared to healthy controls, anxious reactivity to unpredictable aversive events was heightened in PTSD patients, but not in GAD patients (Grillon et al., 2009).

3.4.3. Virtual reality as a tool for studying anxiety in humans

Despite the important contributions context conditioning has to offer for the understanding of anxiety disorders, there is relatively few evidence from human studies. In animal research, context conditioning is widely used as a model to measure sustained anxiety. Usually, different cages are used during conditioning and/or extinction (e.g. Myers et al., 2006). One reason for the comparatively small number of human studies probably lies in difficulties to realize a change of context in the laboratory. In the last decade, a new tool has been used to realize contexts both in experimental settings and in therapy. Virtual reality (VR) has been shown to be quite effective in fear conditioned studies (see for example Baas et al., 2004; Grillon, Baas, Cornwell & Johnson, 2006; Mühlberger, Wieser & Pauli, 2008a; Alvarez, Biggs, Chen, Pine, & Grillon, 2008; Huff, Hernandez, Fecteau, Zielinski, Brady & LaBar, 2011; Glotzbach, Ewald, Andreatta, Pauli & Mühlberger, 2012)

and also in treatment of anxiety disorders such as aviophobia, public speaking anxiety or PTSD (Mühlberger, Weik, Pauli & Wiedemann, 2006; McLay et al., 2012). VR enables the observer to immerse into and to interact with the scene he or she is currently experiencing. Hoffmann and colleagues could show that immersive VR works as a powerful distractor and therefore as an effective pain reduction technique for burn patients during wound care (Hoffmann, Patterson, Seibel, Soltani, Jewett-Leahy & Sharar 2008). For a fear conditioning experiment, the learning environment can be designed a lot more complex and realistic in VR compared to a laboratory setting involving a 2D computer screen. In everyday life, people are confronted with complex learning environments involving ambiguous associations between cues and consequences. These situations can be reproduced in VR environments. Also, a conditioning or extinction context can be created and manipulated without the need to physically change for example the room in analogy to the cage in animal studies. VR allows the experimenter to control every detail of contexts as well as cues and aversive events presented during conditioning. Moreover, physiological fear responses like FPS or SCR and verbal reactions such as explicit fear ratings can be measures in a controlled way.

3.5. Aim of Dissertation

The aim of this dissertation is to study fear conditioning in humans in a setting which is close to a real life learning situation. So far, little effort has been put into studying fear condititing in the presence of many potential distractors which we usually encounter in everyday life. In such a setting, associations between different CS and US become less evident and therefore are more difficult to learn. One can expect that participants do not necessarily become aware of the contingencies between the CS and the US and that

learning is more likely to take place on an implicit level. In this dissertation I examine cue and contextual fear conditioning with regard to the effects of contingency awareness. For this purpose a VR paradigm with high ecological validity was created. In spite of its complexity, this learning environment allowed for exact manipulation of cues and contextual stimuli as well as the timing of the events.

In a first pilot study, which was conducted to test the paradigm for further studies, I examine whether conditioning is successful in the newly created virtual environment using a classic differential cue conditioning paradigm. Of special interest is whether participants would actually become aware of the associations between CS-, CS+ and US within the complex learning situation. Fear learning and extinction of fear are examined both on an implicit and on an explicit level.

For the second experiment, the complexity of the learning situation has been enhanced to investigate cue conditioning in combination with contextual conditioning. The contexts are realized by three different virtual rooms. I expect higher contextual fear in participants who fail to explicitly learn the association between CS and US. Besides information about the existing threat, this knowledge also provides information about safety periods. For unaware participants the threat is much more unpredictable, which has been shown to lead to higher contextual fear.

As third experiment I conduct an imaging study, in which I use the same virtual environment. Since there is relatively few evidence on neuronal structures involved in fear extinction, I apply the paradigm tested during the pilot study to take a closer look on neuronal structures involved in fear extinction of both implicit and explicit fear memory. Of particular interest in this study are the differences in neuronal activity between the extinction of fear memory acquired during delay and trace conditioning. There is evidence

that trace conditioning requires more cognitive resources than delay conditioning and does not occur on an implicit level. I am interested in whether I find neural activation correlating to extinction of fear memory in unaware participants after trace conditioning. Of special interest are also differences between extinction of trace and delay conditioning and differences of extinction in aware and unaware participants.

Taken together, this work investigates fear conditioning and extinction in complex and ecologically valid virtual environments with regard to awareness and its consequences on neurological processes and contextual anxiety.

4. Fear conditioning in virtual reality: Effects of awareness in a complex learning environment

4.1. Summary

Human studies of classical fear conditioning are often realized in a rather abstract way which might lead to a lack of ecological validity. The use of Virtual Reality has been shown to be a promising tool to create more complex environments, which are closer to real life situations. In a differential fear conditioning paradigm participants were guided through a virtual office, in which two different light colors served as CS and an electric stimulus as US. FPS responses and evaluative conditioning in form of subjective ratings were measured. My findings give evidence that eye blink conditioning is possible in a complex environment containing many distractors. Additionally, the FPS response did not depend on contingency awareness. However, conditioning was not reflected in valence, arousal and anxiety ratings of participants who did not explicitly learn the association between CS and US. On the contrary the aware group rated the CS+ as more arousing and more anxiety eliciting than the CS-. These results indicate that explicit memory of contingencies is necessary for attitude formation in fear conditioning.

4.2. Introduction

When confronted with an external threat an organism experiences fear, which leads to an appropriate defensive reaction. This reaction is crucial for the survival of the organism, since it results in adjustment to the imminent danger by triggering self protection mechanisms like reflexive escape responses. By evolution, some stimuli are innate

sources of fear, e.g. snakes, spiders, or heights. Additionally, the ability to learn associations between an initially neutral stimulus and a possible threat is essential for survival of an animal or a human being (Mineka & Öhman, 2002). The fear conditioning paradigm is used to study this mechanism in the laboratory. It is one of the most important animal models and has greatly improved our knowledge about mechanisms of fear and anxiety as well as fear mediation neuronal structures (Davis, 1997).

In a differential fear conditioning paradigm one initially neutral stimulus (CS+) is paired with an aversive event (US), for example an electric stimulus, whereas another stimulus (CS-) is never followed by this event. After a few pairings, the CS+ is associated with the US elicits a CR. This response usually involves behavioral and autonomic changes as well as increased activity in the neural fear network.

Once a stimulus has acquired fear eliciting properties, it activates the fear network in a rather automatic way. According to LeDoux (2000) there is a low road of fear processing, which follows the thalamo-amygdala pathway and does not involve cortical structures. This means that conscious processing of the stimulus is not necessary for eliciting a fear reaction. Yet, findings concerning the actual learning processes in fear conditioning are ambiguous regarding the role of awareness. There is an ongoing debate about whether contingency awareness which can be defined as the explicit knowledge of the association between CS and US is required for fear acquisition (Klucken et al., 2009b). As suggested by (Lovibond & Shanks, 2002; Mitchell, De Houwer, & Lovibond, 2009), it is likely that awareness of contingencies between the CS and the US is necessary for establishing a conditioned response. There are findings which indicate that even an implicit CR cannot be found without explicit awareness of CS/US contingencies (Dawson, Rissling, Schell, & Wilcox, 2007; Klucken et al., 2009a). However, there is also evidence that implicit

conditioned responses may occur without contingency awareness (Hamm et al., 2003; Knight, Nguyen, & Bandettini, 2006; Weike, Schupp, & Hamm, 2007).

The FPS response is often used to test whether cued fear conditioning had been successful on a non conscious level. The startle reflex is a cross-species response to sudden, intense stimulation, e.g. a sharp loud noise. In humans, presentation of a startle tone leads to an eye-blink, i.e. to a rapid contraction of the M. Orbicularis oculi, which can be measured using electromyographic recordings (Blumenthal et al., 2005; Lang, Bradley, & Cuthbert, 1990). Importantly, the magnitude of the reflex varies with the affective state of the organism: Fear-inducing stimuli for example increase the startle response, whereas positive stimuli reduce it. This effect is dependent on subcortical brain structures influencing the brainstem acoustic startle pathway (Koch, 1999; LeDoux, 2000). Since the startle reflex does not depend on cortical input, it may reflect learning processes on an unconscious and implicit level.

To test conditioning effects on an explicit level, subjective ratings of stimulus valence as well as levels of arousal and anxiety in response to the stimulus are often used. There is strong evidence that a change of valence is not possible without contingency awareness (Dawson, et al., 2007; Pleyers, Corneille, Luminet, & Yzerbyt, 2007; Stahl, Unkelbach, & Corneille, 2009).

One problem of most conditioning studies is their lack of ecological validity. They mostly realized context free conditioning paradigms with picture stimuli as CSs, presented on a blank screen (Klucken et al., 2009; Phelps, Delgado, Nearing, & LeDoux, 2004; Weike et al., 2007). In real life however, a person is usually situated in a complex and changing environment, when confronted with a new association between a specific stimulus and an aversive event. Thus, it might be quite difficult to become aware of possible contingencies,

because many and changing distracters are present simultaneously. Hence, it is very interesting to investigate fear conditioning in a complex environment in more detail.

Virtual Reality (VR) has proven to be a very useful tool to study fear conditioning in complex environments (Alvarez, Biggs, Chen, Pine, & Grillon, 2008; Baas, Nugent, Lissek, Pine, & Grillon, 2004; Grillon et al., 2006). Important advantages of VR paradigms are the high ecological validity and the simultaneous possibility to experimentally control all aspects of these stimuli (Baas et al., 2004; Mühlberger, Bülthoff, Wiedemann, & Pauli, 2007a; Mühlberger, Wieser, Kenntner-Mabiala, Pauli, & Wiederhold, 2007b; Mühlberger, Wieser, & Pauli, 2008a; Glotzbach, Ewald, Andreatta, Pauli & Mühlberger, 2012; Tröger, Ewald, Glotzbach, Pauli & Mühlberger, 2012). Participants view the environment via a Head Mounted Display. They immerse in this environment and can act like in a real world.

I created a differential cue conditioning paradigm in VR to study fear conditioning in a complex environment. The VR consisted of an office which participants could explore and were guided through. CSs were realized by turning on a lamp which illuminated the room in a specifically colored light. The offset of the CS+ was followed by a mildly painful electric stimulus serving as US. Based on studies mentioned above which found conditioned responses in spite of the absence of awareness, I expected startle responses to the CS+ compared to the CS- to be enhanced after the conditioning process, regardless of whether participants were able to explicitly report the association between CS+ and UCS. However, I expected a difference between aware and unaware participants for ratings of valence and arousal of the CSs, as well as for ratings of anxiety.

4.3. Method and materials

4.3.1. Participants

In total, 30 volunteers (16 female; age 20-31) participated in this study. Excluding criteria were past or present psychiatric disorders, use of antipsychotic drugs, present alcohol or drug abuse, hearing impairment and uncorrected amblyopia. Five participants had to be excluded due to low startle reactivity, regular drug consumption, or technical problems. The final sample consisted of 25 participants (16 female, mean age = 24.2 years, SD = 2.9 years). All participants gave their written informed consent. Participants gained 12€ for their participation. The investigation was approved by the Ethics Committee of the University of Wuerzburg.

4.3.2. Stimuli and apparatus

VR environment. I used the Valve Source engine (Valve Corporation, Bellevue, Washington, USA), which is also used in the computer game Half-Life 2, to create the virtual environment consisting of a corridor and an office. Two different lights, blue and yellow, served as CS+ and CS-, respectively (*see figure 3*). One light (CS+) was always followed by a mildly painful electric stimulus (US), while the other one (CS-) did not have any consequences. Colors of CS+ and CS- were counterbalanced across participants. The origin of the lights was a standard-lamp situated in the middle of the office. It was always switched on for 8 seconds and illuminated the whole room. Participants were guided through the VR environment on a prerecorded path but were able to change their field of view by moving their head. To manipulate the VR environment during the experiment I used the software CyberSession which has been written in-house. Rendering was completed by the Cortona VRML Renderer (ParallelGraphics, Dublin, Ireland). The virtual

environment was displayed by a Z800 3D Visor head-mounted display (HMD; eMagin, USA). In order to adapt the field of view to head movements and to assess head orientation, the head position was monitored with the Patriot electromagnetic tracking device (Polhemus Corporation, Colchester, Vermont, USA).

Figure 3: Office in neutral illumination and with yellow and blue light (serving as CS+ and CS-, counterbalanced across participants) switched on.

Electric stimuli. The US was a mildly painful electric stimulus generated by a current stimulator (Digitimer DG2A, Digitimer Ltd, Hertfordshire, England) and delivered through an electrode at the dominant inner forearm. Electric shocks were triggered automatically by CyberSession for 200 ms with a frequency of 50 Hz. They were sent by the simulator with a voltage of 400 V and duration of 2 ms. The intensity of the current could vary between 0 and 9.9 mA and was individually adjusted for each participant at their pain threshold. In four alternately ascending and descending series of electric stimuli the current was increased and decreased in steps of 0.5 mA, respectively, and the intensity of pain was rated by the participants on a scale from 0 ("no sensation at all") to 10 ("very strong pain"), whereas 4 meant "a pain just noticeable". The first ascending series started from 0 mA and was stopped when the electric stimulus was rated 4 or more. The descending series started 0.5 mA higher than the stopping point of the preceding ascending series and stopped when the electric shock was rated below 4. The next ascending series started 0.5 mA below this stopping point. After this procedure, the lowest current of each series that was rated at least 4 was taken to calculate a mean intensity of current. This mean was multiplied by 1.3 and the resulting current was used as individual US. In case that this final US was rated below 4, intensity was increased to the next half or full mA until rated above 4. In this sample, the realized electric stimuli had a mean current of 1.8 mA (SD = 0.8) and participants rated its intensity with a mean of 5.7 (SD = 1.4) at the beginning and 5.1 (SD = 1.5) at the end of the experiment.

Recording of Physiological Data. The startle reflex was measured by recording electromyographic activity (EMG) from the M. orbicularis oculi with two 13/7 mm miniature Ag-AgCl electrodes filled with electrolyte placed centrally beneath the left eye and about 1 cm closer to the outer corner of the left eye. Impedance level was kept below 10 kΩ. The

acoustic startle stimuli was a 103 dB burst of white noise presented for 50ms binaurally via headphones.

All physiological data were assessed using electrodes connected to a digital amplifier (V-Amp 16, Brain Products Inc., Munich, Germany) and recorded on a computer using Vision Recorder (Brain Products Inc., Munich, Germany), which also was used to check the impedances of the Ag/AgCl electrodes. 13/7 mm 10 miniature electrodes were fixed to the left and right mastoid each as reference and ground electrode, respectively. Impedances were kept below 10 kΩ.

4.3.3. Psychometric *measures*

Ratings. At several times during the experiment ratings of valence (very negative – very positive), arousal (not arousing at all – very arousing), anxiety (no anxiety – extreme anxiety) and contingency (not likely at all – very likely) were collected, each on scales from 0 to 100.

Awareness. Explicit knowledge of contingencies between CSs and US was assessed on the basis of the questions "Were the electric shocks predictable?" (possible answers: "Yes", "No", "Don´t know") and "During which light presentation did you receive electric shocks?". Participants who confirmed predictability and were able to state the correct light colour after the second acquisition run were labelled "aware", the others were labelled "unaware". Nineteen participants met the above mentioned criteria for awareness; the remaining six were labelled „unaware".

4.4. Procedure

After participants had given written informed consent, EMG electrodes and electric stimuli were adjusted as described above. In order to get accustomed to the volume of the

startle tone, participants were exposed to three startle probes before the start of the experiment. They were told that they will be able to predict the electric stimuli when they pay close attention to the experiment, and that the tones are not associated with the US.

This preparation phase was followed by a habituation block, in which participants were guided through the office once. Both lights were switched on and five startle tones were presented for startle habituation and as a baseline measurement. After habituation, participants rated the blue and yellow lights regarding valence and arousal.

The actual experiment consisted of 3 phases, two acquisition phases and one extinction phase. Each phase contained 3 trials, in which the office was visited once for 2 minutes each (*see Figure 4*). While moving through the office, CS+ and CS- appeared twice for 8 seconds. Hence, participants visited the office three times in one phase and were exposed to six CS+ and six CS-. During both acquisition phases the US was administered at the end of each CS+, resulting in a total of 12 electric stimuli during acquisition. During extinction no US was applied.

Three startle probes were delivered in the office during each acquisition trial: one during the inter stimulus interval (ISI), one during CS+ and one during CS- presentation (5-6 sec after stimulus onset), i.e., there were three startle probes per stimulus category (CS+, CS-, ISI) during each acquisition phase. This number was increased to 5 startle probes for each stimulus category in the extinction phase. I did not include more startle trials because I expected strong habituation effects. Moreover, too many startle trials during acquisition could lead to confusion regarding the contingency learning. If startle probes were more salient than the electric stimuli, participants might form an association between CSs and startle probes instead of the US. In the extinction phase I increased the number of startle probes because the learning process should already be completed.

Importantly, CS+ and CS- were presented when participants were at different locations in the office, thus preventing associations between cues like distinct pieces of furniture with startle probe or US administration. The startle probes were delivered every 15-30 sec during a trial. Additionally, there was an interval of at least 10 seconds between US and startle probe to avoid an influence of the shock on the startle reaction (Davis, 1998). Order of stimuli and duration of the ISI were pseudorandomized across participants. In total, there were four different event sequences, two of them with the blue light and two with the yellow light serving as CS+.
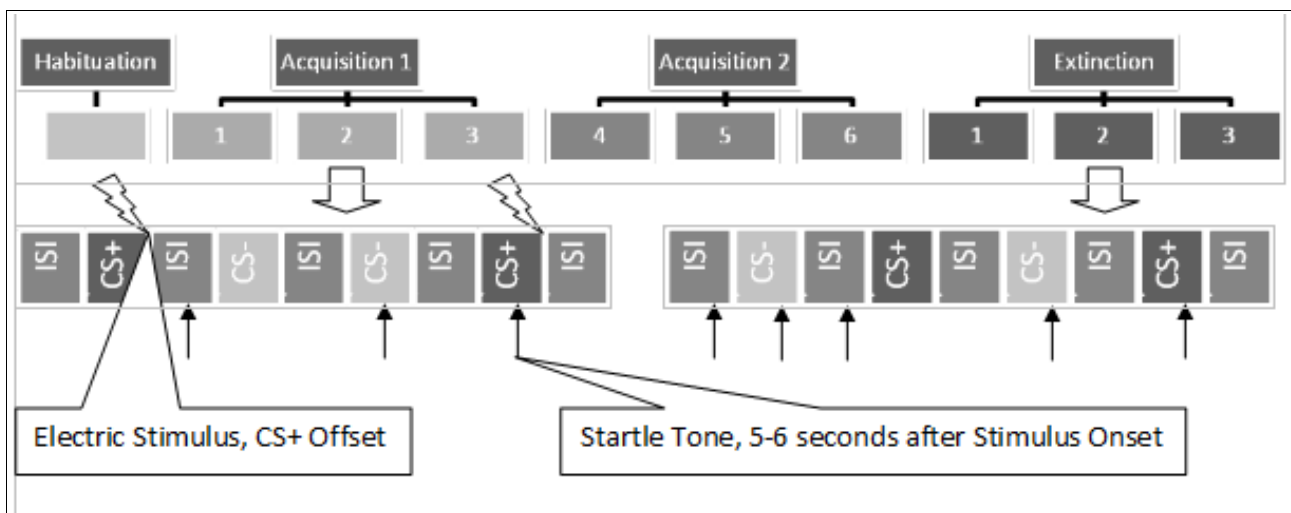
Figure 4: *Schematic drawing of the course of the experiment. The main experiment consisted of three phases (two acquisition blocks, one extinction block). Before conditioning, there was a short habituation trial. The three main blocks consisted of three trials each. During one trial participants were guided through the office for two minutes, CS+ and CS- were switched on twice for eight seconds. In the acquisition phases, startle probes were presented during 3 out of 6 CS presentations, in the extinction phase during 5 out of 6 CS presentations*

After each acquisition phase awareness was measured as described above. Participants rated valence and arousal of the two different lights after both acquisition phases and the extinction phase. Ratings of anxiety and contingency were conducted after acquisition 2

and extinction. For all ratings, situations in the experiment were described orally and questions were presented via headphones. Participants were told to relate their answers to the way they felt during the last phase of the experiment. Answers were given orally and recorded by the investigator. At the end of the experiment, they received 12 Euros for participation.

4.5. Data analysis

Alpha was set at .05 for all statistical tests, effect sizes are reported as partial η_p^2 scores. *t*-tests were conducted one-tailed because of directed hypotheses.

4.5.1. Startle reflex

Raw EMG signals were further processed with the Vision Analyzer (Brain Products Inc., Munich, Germany). The raw values recorded with the outer electrode were subtracted from the raw values recorded with the inner electrode. Then data epochs were extracted from 100 ms before to 1000 ms after the startle tone. A 500 Hz high cutoff filter, a 30 Hz low cutoff filter and a 50Hz notch filter was administered, the data were rectified (positive values remain the same and negative values are converted into positive values of the same magnitude) and moving averages of 50 ms were calculated. The epochs were baseline corrected using the 50 ms before probe onset. After that, a macro was used to search for startle peaks during 21-200 ms after probe onset. The placement of the peak markers was manually controlled and corrected if necessary. Invalid epochs containing artifacts were marked and removed. Artifacts were defined as follows: amplitude higher than 5 µV or lower than -5 µV during baseline. Peak data were exported to SPSS 16 where peak amplitudes below 5 µV were scored as zero (non-response). T-values for the startle amplitudes were calculated and the mean T-values for each Cue (CS+/CS-/ISI)

during each phase (acquisition1, aquisition2, extinction) were used for analysis. One participant was excluded from analysis because of less than two artifact-free responses per stimulus category.

Startle responses were analyzed separately for each phase with MANOVAs with repeated measures, including the factors Stimulus (CS+, CS-, ISI) and Contingency Awareness (aware, unaware).

4.5.2. Ratings

Contingency ratings were used as a manipulation check for the breakup of participants into aware and unaware. A multivariate analysis of variance (MANOVA) with repeated measures and the factors Awareness (aware, unaware), Phase (acquisition2, extinction) x Stimulus (CS+, CS-) was conducted to test whether participants labeled "aware" compared to those labeled unaware rated the CS+/US contingency as higher than the CS-/US contingency after conditioning. Unaware participants were expected to not differ in the contingency ratings of CS+ and CS-. For the ratings of valence and arousal MANOVAs with repeated measures and factors Phase (aquisition1, acquisition2, extinction) x Stimulus (CS+, CS-) x Awareness (aware, unaware) were conducted. The same analysis was applied to anxiety ratings, but the factor Phase contained only two steps (acquisition2, extinction). t-tests were conducted for valence and arousal ratings assessed after the habituation phase to check for initial differences between the two different lights.

4.6. Results

4.6.1. Manipulation check for awareness

Nineteen participants met the above mentioned criteria for awareness; the remaining six were labelled „unaware". The 2 (Awareness [aware, unaware]) x 2 (Phase [acquisition 2, extinction]) x 2 (Stimulus [CS+, CS-]) MANOVA of contingency ratings revealed significant main effects of Phase ($F(1, 23) = 83.34$, $p < .001$, $\eta_p^2 = .78$) and Stimulus ($F(1, 23) = 59.75$, $p < .001$, $\eta_p^2 = .72$) as well as significant interactions of Phase x Stimulus ($F(1, 23) = 49.43$, $p < .001$, $\eta_p^2 = .68$), Stimulus x Awareness ($F(1, 23) = 63.84$, $p < .001$, $\eta_p^2 = .74$) and Phase x Stimulus x Awareness ($F(1, 23) = 23.49$, $p < .001$, $\eta_p^2 = .59$). After acquisition 2, aware participants rated the US /CS+ contingency as almost 100% and as significantly higher ($t(18) = 31.14$, $p < .001$) than the CS-/US contingency. On the contrary, unaware participants did not show any significant differences between the contingency ratings of CS+ and CS- after the acquisition phase ($p > .77$). They rated the CS+/UCS contingency as significantly lower than aware participants ($t(6.106) = 4.38$, $p =.001$), the CS-/US contingency as significantly higher ($t(6.654) = -5.41$, $p =.005$). After the extinction phase, only in the aware group the difference between CS+/US and CS-/US contingency was marginally significant ($t(18) = 2.00$, $p =.061$). There were no significant group differences after extinction (all $ps > .47$) (*see figure 5*).
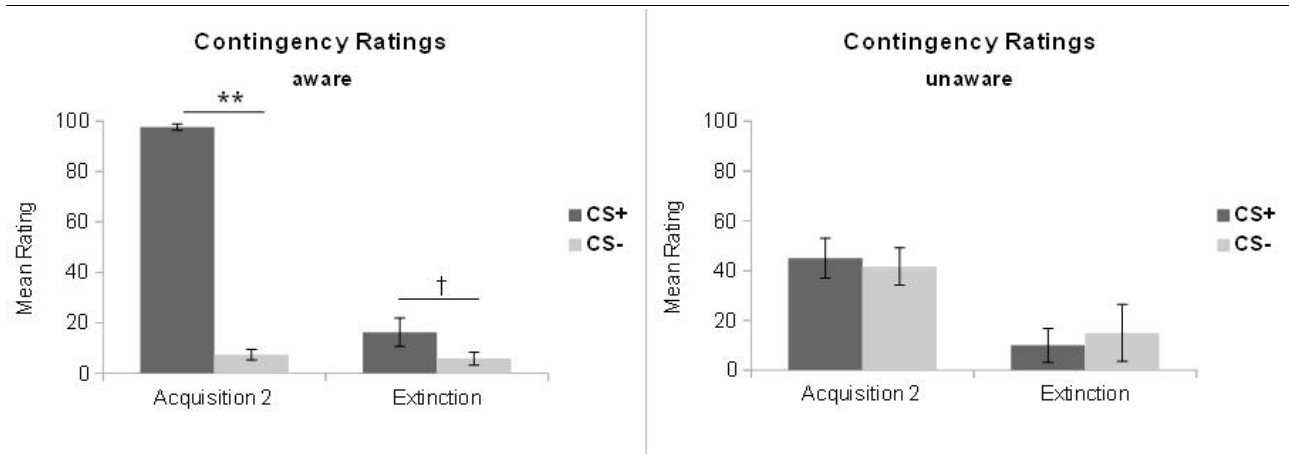
Figure 5: *Contingency ratings on a scale from 0% to 100% in the aware and unaware group.*

*** p<=.001; † p>=.01*

4.6.2. *Startle reflex*

As a first important finding, the factor awareness had no influence on the startle data in all three phases (acquisition 1, 2, and extinction). The MANOVAs for both acquisition blocks did not reveal any significant effects. In the extinction phase, which was the most important test phase for conditioning effects and FPS, I observed a significant main effect of Stimulus ($F(1, 22) = 6.07$, $p = .008$, $\eta_p^2 = .36$). Follow up *t*-tests revealed that difference between startle reactions to CS+ compared to CS- did not reach significance ($t(24) = 1.13$, $p = .13$), but were significantly increased compared to the ISI ($t(24) = 3.32$, $p = .002$). The difference between CS- and ISI also reached significance ($t(23) = 2.53$, $p = .010$) (*see figure 6*).

Startle Amplitude
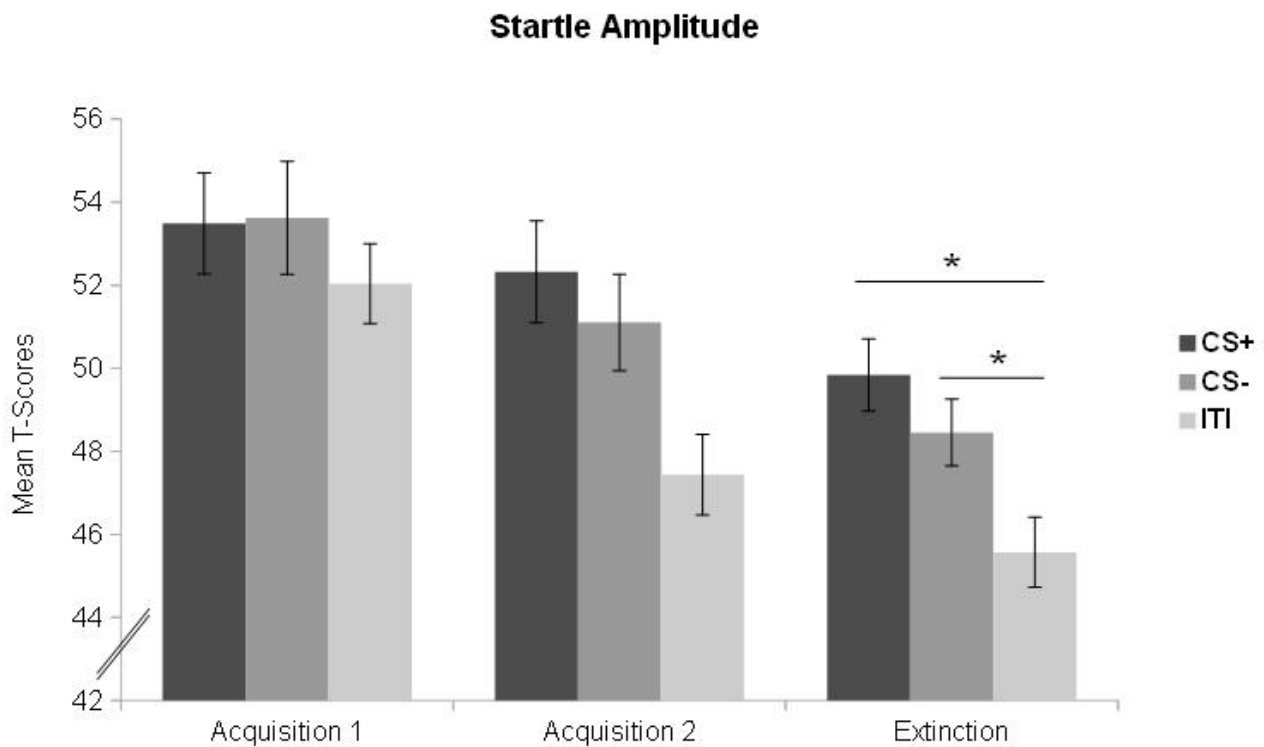
*Figure 6: Mean startle reactions (T-Scores) of the complete sample in acquisition 1, 2, and extinction. * p<=.005*

In a following explorative analysis I looked at reactions of aware and unaware participants separately, in order to confirm that both groups differed in the responses to the stimuli independent of awareness. FollowUp t-tests revealed differences between the stimuli according to the results in the whole group. In the aware group, I did not find a significant difference between CS+ and CS-. The reaction to CS+ and CS- both were significantly higher than the reactions to the ITI (CS+/ISI: $t(18) = 2.11$, p = .025, CS-/ISI: $t(18) = 1.81$, p = .044). Interestingly, next to significantly increased reactions of CS+ to ISI ($t(5) = 6.09$, p = .001) and CS- to ISI ($t(5) = 2.47$, p = .029), in the unaware group the startle reactions to CS+ were also marginally higher than the reactions to CS- ($t(5) = 2.11$, p = .077) (*see figure 7*).
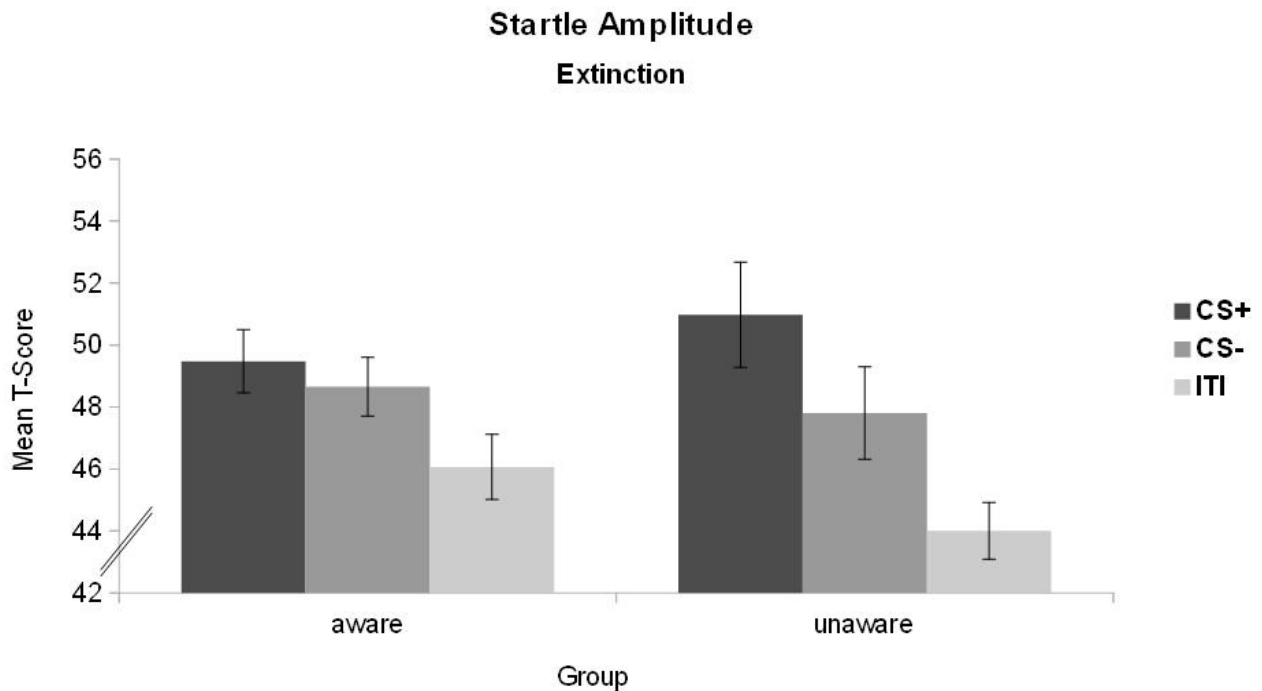
Figure 7: Mean startle reactions (T-Scores) of the aware and unaware participants during extinction.

4.6.3. Ratings

t-tests for valence and arousal ratings before conditioning revealed no differences between CS+ and CS- (all *ps* >.40).

For valence ratings after conditioning, the MANOVA did not show any significant effects or interactions. Explorative *t*-tests suggested that the CS+ was rated as more negative than the CS- after acquisition 2 only in the aware group ($t(18) = -2.40$, $p = .014$). Ratings of the unaware group did not differ significantly in any of the phases, (all $p_s > .30$).

For arousal ratings, the main effect stimulus ($F(1, 23) = 3.18$, $p = .088$, $\eta_p^2 = 0.12$) as well as the interaction Phase x Stimulus ($F(1, 22) = 2.74$, $p = .087$, $\eta_p^2 = 0.20$) were marginally significant, the interaction Stimulus x Awareness reached significance ($F(1, 23) = 4.92$, $p = .037$, $\eta_p^2 = 0.18$). One-tailed paired *t*-tests showed differences between arousal ratings after the acquisition phases only for the aware participants (acquisition 1: $t(18) =$

2.13, p = .02; acquisition 2: $t(18)$ = 3.95, p < .001). After extinction, this difference was only marginally significant ($t(18)$ = 1.51, p = .07). Again, ratings of the unaware group did not differ significantly in any of the phases, (all p_s > .14).

Anxiety was only measured after acquisition 2 and extinction. I found a marginally significant main effect Phase ($F(1, 23)$ = 3.25, p = .084, η_p^2 = 0.12) and a significant interaction of Stimulus x Awareness ($F(1, 23)$ = 4.50, p = .045, η_p^2 = 0.16). Once more only in the aware group anxiety ratings for the CS+ were significantly higher compared to the CS- after the second acquisition phase ($t(18)$ = 2.89, p = .005, d' = 0.89). The difference in ratings after the extinction phase was no longer significant ($t(18)$ = 1.45, p = .08). As well as for valence and arousal ratings there were no significant differences between the stimuli in the unaware group (all p_s > .07). Interestingly, unaware participants rated the CS+ as marginally less anxiety inducing than CS- after the second acquisition phase ($t(5)$ = -1.78, p = .07).

In sum, ratings indicate that evaluative conditioning was successful only in the aware group (*see figure 8*). After acquisition 2, the CS+ was rated as more arousing and more anxiety inducing compared to the CS-. After the extinction phase only the arousal rating for the CS+ was still marginally enhanced, the other differences had disappeared as expected. Results in the unaware group have to be interpreted with care due to very small sample sizes.
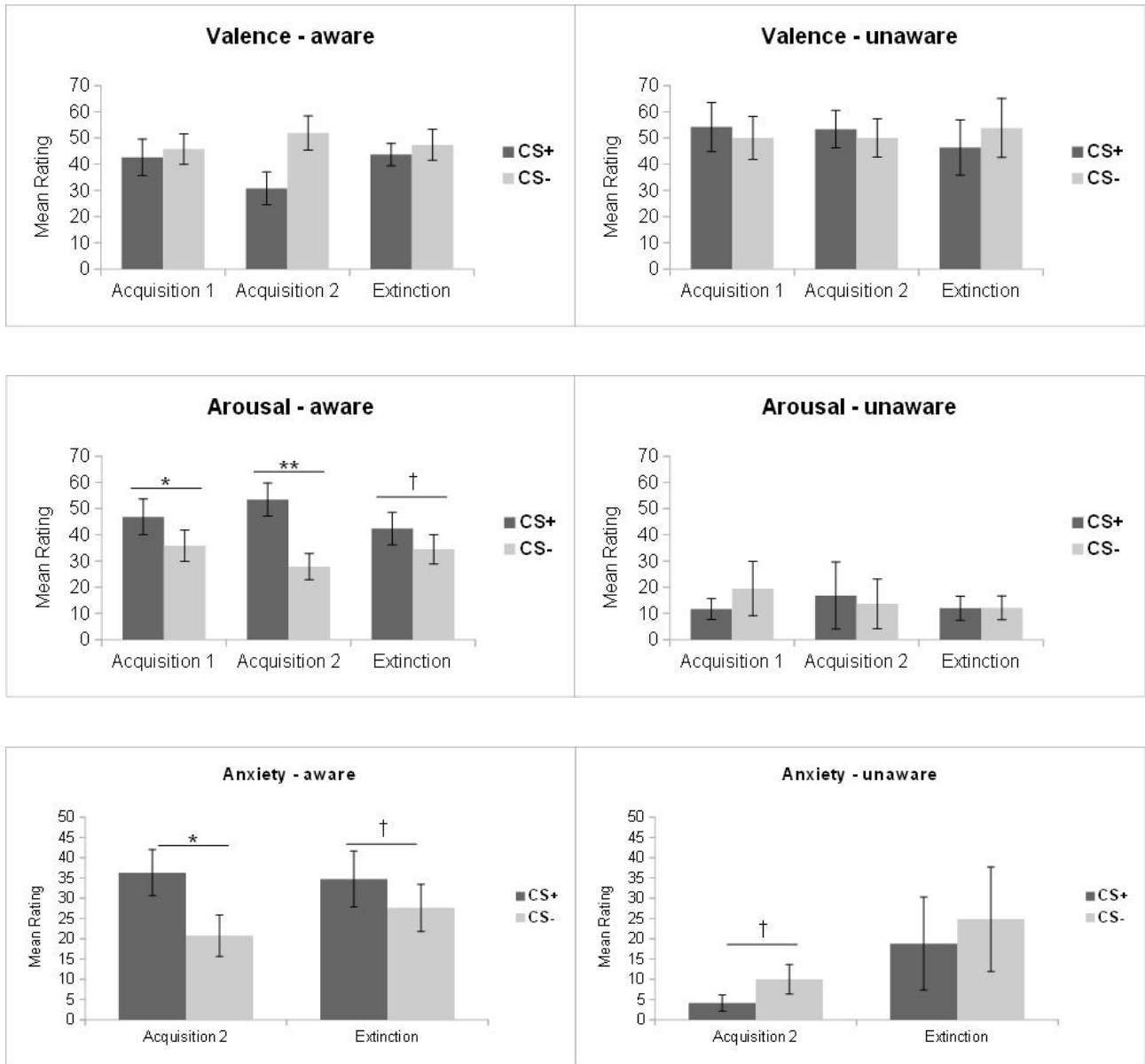
Figure 8: Ratings in the aware (left) and unaware (right) groups: (a) valence (from 0 = "very negative" to 100 = "very positive"), (b) arousal (from 0 = "not arousing at all" to 100 = "very arousing", (c) anxiety (from 0 = "no anxiety" to 100 = "extreme anxiety"). ** p<=.001; * $p<=.005$; † $p>=.01$

4.7. Discussion

In the present study I examined cued fear conditioning in a complex virtual environment. Results from startle data and subjective ratings showed that differential

conditioning was mostly successful. During the learning phase, I did not find any significant differences between startle reactions. During extinction, the test phase after the learning phases, reactions to the CS+ were significantly stronger than reactions during ITI. Compared to CS-, I found a trend for stronger reactions to CS+. This difference did not reach significance. As expected, the effect of awareness did not have an influence in any of the MANOVAs conducted on the startle data. These results are consistent with the view that awareness is not necessary for fear conditioning reflected in an implicit measurement such as the startle response. On the contrary, as revealed by separate exploratory analyses of aware and unaware participants, fear potentiation in response to the CS+ was even stronger in the unaware group compared to the aware group. Due to small sample sizes in both groups I cannot state clear evidence for a conditioning effect.

However, contingency awareness did play a significant role in subjective ratings. Here conditioning effects were only present in participants who explicitly learned the contingency between the US and the CS+. Aware participants rated the CS+ as more arousing and more anxiety inducing compared to the CS- after the second acquisition phase. Arousal ratings already differed after the first acquisition phase. After the extinction these differences had disappeared except for marginally enhanced arousal and anxiety ratings of the CS+. There were no significant differences between ratings of CS+ and CS- in the unaware group.

An important point regarding the separation of the sample into aware and unaware participants is the validity of contingency awareness measures. There is an ongoing debate about how to measure awareness to not misclassify aware participants as unaware or vice versa. In their review, Lovibond and Shanks (2002) suggest that assessment of awareness should occur under optimal retrieval conditions, or at least testing conditions

should be as similar to learning conditions as possible. Additionally, long time intervals can lead to forgetting. I asked participants after the first and second acquisition block verbally, whether electric stimuli were predictable and whether they could explicitly tell in which situation they received an electric shock. Since recognition tasks are more sensitive than free recall, I also included contingency ratings with visual analog scales for every situation. I did not ask for contingencies after every trial in between the acquisition bocks. This might lead to a higher number of aware participants because it directs attention towards the association between the stimuli (Dawson & Reardon, 1973). I checked whether the results of the contingency ratings were congruent with conclusions based on the open questions to control the quality of the assessment of awareness. A significant difference between ratings of the CS+ and the CS- after the conditioning process was present only for participants classified as aware. After the acquisition this group rated the contingency of CS+ and US as 80 to 100%, the contingency of CS- and US as 0 to 30%.

Taken together, startle data results are consistent with prior findings suggesting that explicit knowledge of contingency is not necessary for the production of a conditioned response in delay conditioning (Manns, Clark, & Squire, 2002; Smith, Clark, Manns, & Squire, 2005; Weike, et al., 2007). But due to the above mentioned small sample size, further research is required to answer this question more reliably. The here applied method of not manipulating awareness directly but exposing participants to a complex environment containing many distractors is a promising approach to investigate the influence of explicit knowledge of contingencies in close analogy to real life situations.

Contrary to the fact that awareness did not have an influence in the MANOVAS on the startle data, it did definitely have an impact on evaluative conditioning. The finding that unaware participants did not differentiate between CS+ and CS- in subjective ratings is in

line with a growing number of studies (Dawson, et al., 2007; Pleyers, et al., 2007; Pleyers, Corneille, Yzerbyt, & Luminet, 2009).

Findings from startle data and ratings taken together, my results provide further evidence for a dual process model of classical fear conditioning, which assumes that two different learning processes take place during conditioning. The first one leads to conscious awareness, whereas the second one leads to the production of a CR by forming an excitatory link between the CS and US nodes (Lovibond & Shanks, 2002) Results from imaging studies indicate that separate memory systems are involved in explicit memory of fear on the one hand, and the implicit and unconscious production of a fear response in classical fear conditioning on the other hand (Knight, Waters, & Bandettini, 2009; Tabbert, Stark, Kirsch, & Vaitl, 2006). For example Knight et al. (2009) showed that the medial temporal lobe, especially the hippocampus and parahippocampal gyrus are activated when explicit, aware learning occurs. However, these regions are not necessary for the production of a CR, which can be mediated by the amygdala complex.

In the third study I plan to take a closer look on neuronal structures involved in extinction of both explicit an implicit fear memory. Before that, I extended the paradigm tested in the present study to investigate both cue and context conditioning in a between-subjects-design under consideration of contingency awareness and individual differences regarding trait anxiety.

5. Effects of awareness revisited: Combined cue- and contextual conditioning in a virtual reality environment[1]

5.1. Summary

To examine acquisition and extinction of both fear associated cues and contexts, a a novel cue-context generalization paradigm was designed. Participants were guided through two distinguishable virtual rooms (again designed as offices), in which the two different lights already mentioned in the first study were presented. Additionally, one room served as fear context, the other one as safety context. During acquisition, one light (CS+) was always followed by an electric shock (US) in the fear context, the other light (CS-) had no consequences. In the safety context however, none of the lights was followed by the US. During extinction, a third novel context was introduced in addition to fear and safety context. Participants were guided through all three rooms, no US was delivered in any of the three contexts.

Participants showed enhanced startle responses on the CS+ compared to the CS- in the fear context. Thus, discriminative learning took place regarding both cues and contexts during acquisition. This was confirmed by subjective ratings, although only for participants who developed explicit contingency awareness. Unaware participants did not differentiate fear and safety cues and contexts. Generalization of the fear response to the novel context after conditioning could be found for all participants, though only on trend level. Participants with high trait anxiety developed fear of the CXT+ compared to the CXT- as indicated by startle responses to the contexts alone during the ISI.

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1 Data reported in this chapter are part of the study published by Mühlberger et al. (2013)

5.2. Introduction

Classical fear conditioning has been serving as a model for the development and maintenance of anxiety disorders for many years. Pathological states of phasic fear, such as specific phobias, have been studies by applying cued fear conditioning. Here, a distinct cue is paired with an aversive event, creating a rather predictable situation with an explicit threat. But the associations which are formed during fear conditioning are not exclusively associations between specific cues and an aversive stimulus. The two stimuli seldom exist in isolation, but are usually embedded in a certain context. When contextual cues are present during acquisition, they form the background milieu of the learning process and play an important role in both the formation of associations and the extinction of conditioned fear (Baas, Nugent, Lissek, Pine & Grillon, 2004). The context functions as a predictor of the relationship between the cue and the aversive event. This concept is referred to as occasion setting (Delamater 2012). However, the context does not necessarily serve merely as background information during cue conditioning. It can also be the cue itself. During context conditioning, an experimental context like a cage or a room is paired with an aversive stimulus. Cue and context conditioning differ with regard to temporal information about the threatening stimulus. Whereas a distinct cue allows for an exact prediction of the onset of the associated US, a contextual cue offers no temporal information (see for example Grillon, 2008). This sort of conditioning leads to a response that is closer to a state of sustained anxiety than to distinct fear, because the organism finds itself in a setting of uncued and therefore less predictable danger. According to the safety-signal theory by Seligman and Binik (1977) already described in chapter two, a distinct cue associated with a threatening stimulus does not only signal danger. It also serves as a safety signal, because it guarantees absence of danger when it is not present.

Without distinct cues serving as safety signals, the organism experiences chronic anxiety. According to Baas and colleagues, successful cue conditioning both signals threat and provides information about safety periods, which in turn allows for a reduction of contextual anxiety (Baas, van Ooijen, Goudriaan & Kenemans, 2008). Consequently, a deficit in learning the association between a present cue and an aversive US might lead to enhanced contextual anxiety instead. Grillon postulates a causal relationship between the failure to learn the CS–US contingency and increased contextual anxiety (Grillon, 2002a). In consideration of this concept, not only hyper-conditionability can lead to maladaptive strong fear reactions or anxiety disorders like specific phobias. Also hypo-conditionability or the failure to associate a cue with a threat can result in anxiety disorders, however not to specific phobias but rather to GAD or phobic avoidance in panic disorders which are associated with sustained anxiety. Grillon and colleagues showed that, compared to healthy controls, patients with post traumatic stress disorder as well as panic disorder react with increased anxiety in response to unpredictable aversive stimuli, but not to predictable (cued) ones (Grillon et al. 2009; Grillon, Lissek, Rabin, McDowell, Dvir, & Pine, 2008). Additionally, different neural systems have been found to be involved in phasic fear vs. sustained anxiety: The amygdala has been related to responses to cued threat (see for example Alvarez, Biggs, Chen, Pine & Grillon, 2008). The bed nucleus of the stria terminalis (Alvarez, Chen, Bodurka, Kaplan & Grillon, 2011) and the hippocampus (Alvarez et al., 2008; Marschner, Kalisch, Vervliet, Vansteenwegen & Büchel, 2008) have been associated with responses to unpredictable threat .

Besides hyper- and hypo-conditionability during acquisition of fear, also deficits during extinction can lead to the development or maintenance of pathological states of fear or anxiety. For example, conditioned fear often returns with the course of time after extinction

training in exposure therapy. This is a widely known problem in the treatment of anxiety disorders. Acquisition of fear is rather context independent and is usually generalized over different contexts and situations, whereas extinction of conditioned fear has been found to be highly context dependent (Bouton, 2004). There is strong evidence that extinction of conditioned fear does not imply forgetting or unlearning of an existing association between a cue and an aversive stimulus. Instead, it is generally assumed that new learning takes place. A new association is formed between the cue and the aversive event (or, in the case of extinction, the absence of the aversive stimulus), which then competes with the existing association. The context of extinction sets the occasion for which association is recalled in a specific situation . For example, if a person who suffers from fear of dogs is treated in a specific environment and probably also with the help of one particular dog, it is very likely that the fear will return in a different context and with a different dog.

Evidence for extinction being context specific comes from renewal studies. In such experiments, fear acquisition takes place in a first context (A), and extinction in a second context (B). After extinction, a test can be carried out either in context A or B, or in a third context C. The fear reaction returns in contexts A and C, and is only suppressed in context B, that is when extinction and test take place in the same context. Context specificity of extinction has been demonstrated in animal studies (Bouton, 2004), and more recently also in human studies (LaBar and Phelps 2005; Milad, Orr, Pitman & Rauch, 2005a; Vansteenwegen et al., 2005; Neumann, 2006).

According to animal models, the acquisition of contextual information during fear conditioning also involves different brain regions than forming a simple association between a specific cue and an aversive US. The standard view of fear conditioning is that information about the CS is encoded by the amygdala complex, whereas the hippocampus

is assumed to be the crucial structure for encoding contextual information (Yoon, Graham & Kim, 2011). There is also evidence that the orbitofrontal cortex is involved in context conditioning. In the amygdale, information about CS and context converge (Maren, 2001). The neural circuit for extinction learning and recall is assumed to include the amygdala, vmPFC, and hippocampus. Extinction learning takes place in the amygdala, and the vmPFC is responsible for the inhibition of fear during extinction recall. Presumably, the vmPFC receives contextual information from the hippocampus to determine the circumstances under which extinction or fear will be recalled (Corcoran & Quirk, 2007).

An individual factor which might play a role in the development and maintenance of anxiety disorders is trait anxiety (Mineka and Oehlberg, 2008). In contrast to state anxiety, trait anxiety is a stable tendency to interpret ambiguous situations as threatening and to react with state anxiety (Spielberger et al., 1970). In an fMRI study, Indovina and colleagues (Indovina, Robbins, Núñez-Elizalde, Dunn & Bishop, 2011) provided evidence for high trait anxiety being a risk factor for both the development of phobic fear and the maintenance of fear and GAD: High trait-anxious individuals reacted with increased amydala activation in response to distinct cues predicting an aversive event. Additionally, activation of the vmPFC was reduced in response to both cued and contexts after the US had been omitted, providing evidence for deficient inhibition of phasic fear and sustained anxiety. Startle studies illuminate another aspect of the influence of high trait anxiety in fear conditioning. Intuitively, one would expect that individuals scoring high on trait anxiety learn the association between a cue and an aversive event very quickly, because the pay close attention to the threat. Interestingly, there is evidence that high-anxious individuals do indeed show enhanced fear reactions, but have deficits in discriminating fear and safety signals. Grillon et al. (2002a) as well as Baas et al. (2008) found that individuals who did

not learn the association between the CS+ and the US in a differential fear conditioning paradigm scored higher on trait anxiety. In a complex fear learning procedure containing blocking and protection from overshadowing, Arnoudova et al. (2013) also found deficits in discriminatory fear learning in high-anxious individuals. Thus, high trait anxiety might lead to diminished discrimination between fear and safety cues and might therefore result in increased contextual anxiety (Glotzbach et al., 2013). This has recently been confirmed by Baas (2013), who found an inverse association between trait anxiety and adaptive modulation of contextual anxiety. In a virtual reality study by Glotzbach et al. (2013), examining isolated contextual conditioning, high trait-anxious participants showed faster contextual fear learning compared to low trait-anxious participants. Taken together, findings provide evidence that high trait anxiety is a vulnerability factor for deficient interpretations of safety-signals, increased contextual anxiety and resistance to extinction due to reduced inhibition. In this study, I plan to investigate cue and contextual conditioning under consideration of trait anxiety and contingency awareness of associations between cues and aversive event. For examining interactions of cue and contextual conditioning during acquisition and extinction learning a novel virtual reality paradigm was developed. During acquisition, differential cued fear conditioning was conducted in one context (fear context), whereas the cues were not followed by an aversive event (US) in a second context (safety context). In the extinction phase, a third (novel) context was introduced. The cues were presented in all three contexts, no US was delivered. As measures for fear reactions I recorded startle responses and collected subjective ratings. Trait anxiety was measures with the STAI questionnaire, contingency awareness by means of subjective reports. In general, I expected participants to react with increased fear in response to the CS+ compared to the CS-. This fear reaction should be

weaker in the safety context, but extended to the novel context during early extinction. Considering individual differences, I expected participants with high trait anxiety to show deficits in discriminating fear and safety cues. Therefore they should be more likely to be unaware of the association between CS+ and US on both an implicit and an explicit level, resulting in higher contextual fear. The effects of contingency awareness are interesting in and on itself. Not developing contingency awareness is not the same as not learning the association between the CS+ and the US. Differential cue conditioning can be successful on an implicit level, which can be shown fear potentiated startle (FPS) responses to the CS+, but at the same time unsuccessful on an explicit level, represented by subjective ratings (see for example Weike et al. 2007). I plan to investigate whether participants who are not explicitly aware of the CS+/US association but show FPS reactions all the same, develop higher contextual fear than aware participants.

5.3. Method and Materials

5.3.1. Participants

Sixty-one volunteers were recruited from the panel of participants collected for the Z2 project within the Collaborative Research Center SFB TRR 58. During the registration process for the Z2 panel participants had already completed various questionnaires and a structured clinical interview for DSM disorders (SCID). They had given written informed consent to being contacted for following studies. Excluding criteria were past or present psychiatric disorders, use of antipsychotic drugs, present alcohol or drug abuse, hearing impairment, uncorrected amblyopia and allochromasia (for blue and yellow). Moreover, students of psychology were not included in the study due to possible bias. The final sample consisted of 37 participants (28 female, mean age = 25.3 years, SD = 4.1 years). A

total of 24 participants had to be excluded due to low startle reactivity (overall mean amplitude below 5), regular drug consumption, technical problems with the course of events in the VR paradigm, or nausea (motion sickness). All participants gave their written informed consent and received 25 Euros for participation. The investigation was approved by the Ethics Committee of the medical faculty of the University of Würzburg.

5.3.2. Stimuli and apparatus

VR environment. As in the pilot study, the Valve Source engine (Valve Corporation, Bellevue, Washington, USA) was used to create the virtual environment for the experiment. It consisted of three offices (fear, safety and novel context) and a connecting quadratic corridor. The contexts differed in furniture and window views. Two colored lights, blue and yellow, served as CS+ and CS-, respectively (*see figure 9*). One light (CS+) was always followed by a mildly painful electric stimulus (US) in the fear context, while the other one (CS-) never had any consequences. Colors of CS+ and CS- were counterbalanced across participants. The origin of the lights was the same standard lamp situated in the middle of each office. CS+ and CS- were always presented for 8 seconds and illuminated the whole room. In a first habituation phase participants were required to navigate freely through the virtual rooms using a joystick. Later on they were guided through the VR environment on a prerecorded path but were able to change their field of view by moving their head. To manipulate the VR environment during the experiment, the in-house written software CyberSession was applied. Rendering was completed by the Cortona VRML Renderer (ParallelGraphics, Dublin, Ireland). The virtual environment was displayed by a Z800 3D Visor head-mounted display (HMD; eMagin, USA). Head positions were monitored with the Patriot electromagnetic tracking device (Polhemus Corporation,

Colchester, Vermont, USA), allowing the adaption of the field of view to head movements and the assessment of head orientation.
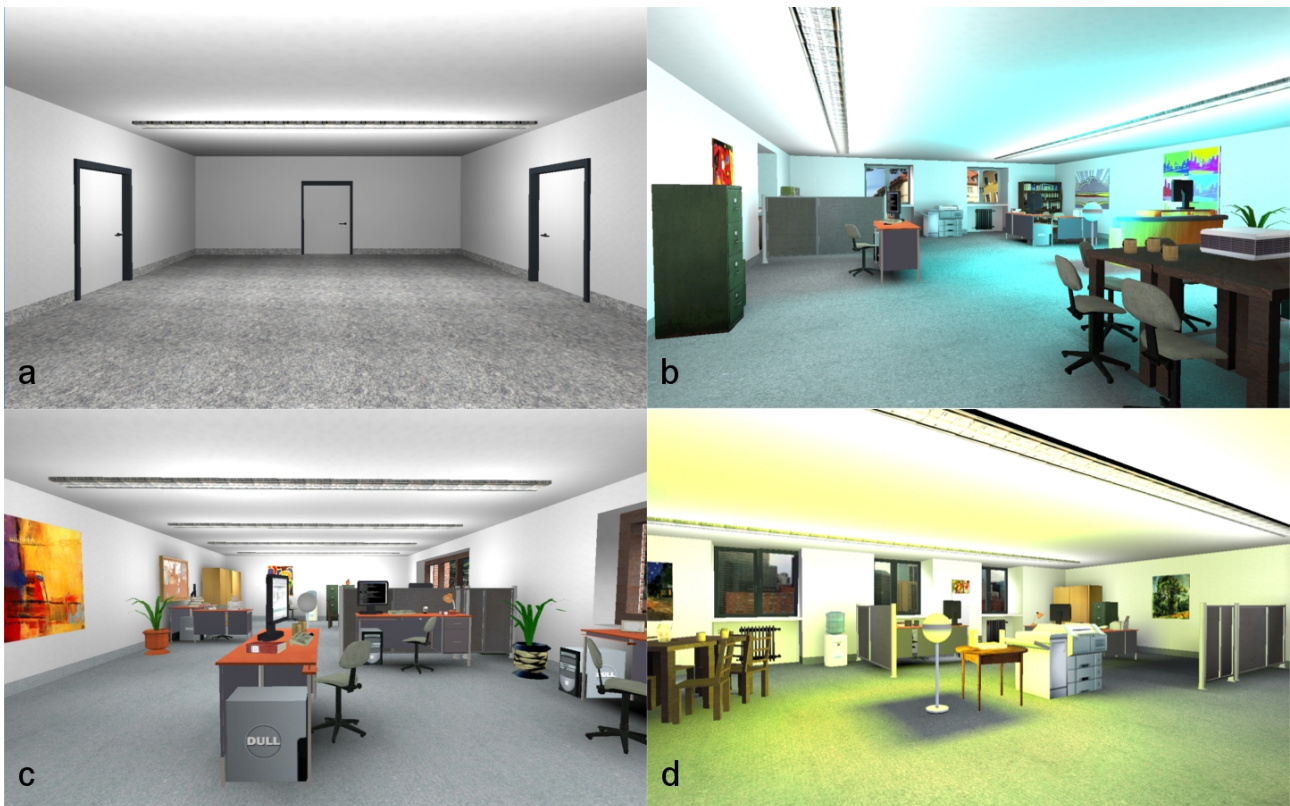
Figure 9: A corridor (a) served as starting position for each trial. Three different rooms serving as CXT+, CXT- and novel context were arranged around the corridor. In the contexts, two colored lights (b and d) served as CS+ and CS-. Contexts and lights were counterbalanced across participants.

Electric stimuli. The US was the same mildly painful electric stimulus as in the pilot study, also generated by a current stimulator (Digitimer DG2A, Digitimer Ltd, Hertfordshire, England) and delivered via two gold-plated stainless steel disk surface electrodes (9 mm diameter, 30 mm spacing) at the dominant inner forearm. Current of electric stimuli was adjusted for each participant according to their individual pain threshold. For details of application and adjustment of electric stimuli please see chapter 4.3.2. (page 55). In this sample, the realized electric stimuli had a mean current of 1.8 mA (*SD* = 0.9) and

participants rated its intensity with a mean of 5.4 (*SD* = 0.9) at the beginning and 4.9 (*SD* = 1.3) at the end of the experiment.

Recording of Physiological Data. As tested in the pilot study, the startle reflex was measured by recording electromyographic activity (EMG) from the M. orbicularis oculi with two 13/7 mm miniature Ag-AgCl electrodes. For a detailed description please see chapter 4.3.2. (page 55f). The acoustic startle stimuli was a 95 dB burst of white noise. It was presented binaurally via headphones for 50ms. For assessing and recording of physiological data, the same digital amplifier and software was used as described in the pilot study.

5.3.3. Psychometric measures

Questionnaires and Ratings. Participants were required to complete several questionnaires including personal information and excluding criteria. Additionally, I assessed general state anxiety with the STAI (State-Trait Anxiety Inventory, Spielberger, Gorsuch & Edward, 1970, German version from Laux, Glanzmann, Schaffner & Spielberger, 1981).

At several times during the experiment ratings of anxiety (no anxiety – extreme anxiety) and contingency (not likely at all – very likely) were collected, each on scales from 0 to 100.

Awareness. Explicit knowledge of contingencies between CS and UCS was assessed on the basis of the questions "Were the electric shocks predictable?" (possible answers: "Yes", "No", "Don´t know") and "During which light presentation and in which room did you receive electric shocks?". I differentiated between participants who did learn the contingency between the CS and the US and participants who learned both the contingency between lights and US and contexts and US. In the result section I report

analyses with group factor awareness regarding CS and UCS: Participants who confirmed predictability and were able to state the correct light colour after the second acquisition run were labelled "aware", the others were labelled "unaware". 23 participants met these criteria of awareness; the remaining 14 were labelled „unaware".

5.4. Procedure

After giving written informed consent, participants completed a questionnaire on personal information and excluding criteria. Then they filled in the STAI questionnaire. After having read written instructions, EMG electrodes and electric stimuli were adjusted as described above. In order to get accustomed to the volume of the startle tone, participants were exposed to three startle probes before the start of the experiment. They were told that they will be able to predict the electric stimuli if they pay close attention to the experiment, and that the tones are not associated with the electric stimulus.

These preparations were followed by a habituation phase (pre-acquisition), in which participants navigated freely through each room using a joystick, starting from the end of the corridor. They were instructed to stay in each room for two minutes and familiarize themselves with the environment. After exploring one room, they were re-placed to the starting point. Both lights were switched on and two startle tones were presented in each room for startle habituation and also as a baseline measurement. After habituation, participants rated each room with neutral illumination as well as with the blue and yellow light switched on regarding anxiety.

The main experiment consisted of two phases, an acquisition and an extinction phase. During acquisition, two of the three offices (fear and safety context) were visited for four times each in a pseudo randomized order. Participants were now guided through the

rooms on a prerecorded path, they were able to change their field of view by moving their head. The same room was never visited more than twice in a row. The whole acquisition phase consisted of eight trials, which lasted 2,5 minutes each, resulting in a total duration of 25 minutes. While moving through one office, CS+ and CS- appeared three times each for 8 seconds at a time. The US was administered at the end of the CS+ with a contingency of 100%, but only in one of the two rooms (fear context, CXT+), while in the other room (safety context, CXT-) the CS+ did not have any consequences. Hence, participants were exposed to 24 CS+ and 24 CS- during acquisition and received 12 electric shocks. The inter-stimulus interval (ISI), i.e. the time interval between one lights offset and the next lights onset lasted between 10 to 21 seconds (mean 15,5 s). The inter-trial interval (ITI) defined as the end of one trial and the entry of the next room varied from 20 to 30 seconds. In both contexts, six startle stimuli were presented during CS+, CS- and ISI, respectively. Overall, 36 startle probes were delivered during acquisition.

The extinction phase consisted of six trials. Each of the three rooms was visited twice, again for 2,5 minutes at a time. Frequency of the CS+ and the CS- presentations per trial were the same as in the acquisition phase, resulting in a total of 18 CS+ and 18 CS- during extinction, 6 presentations of each in every room. No UCS was applied. Classification of rooms as CXT+, CXT- and unconditioned (novel) context was pseudo-randomized across participants.

The number of startle probes was increased to five startle probes per context per stimulus category, resulting in 45 startle probes during extinction. I did not include more startle trials because I expected strong habituation effects. Moreover, too many startle trials during acquisition could lead to confusion regarding the contingency learning. If startle probes were more salient than the electric stimuli, participants might form an association between

CS and startle probes instead of the CS and the US. In the extinction phase I increased the number of startle probes because of fewer trials per context and because the learning process should already be completed.

Importantly, CS+ and CS- were presented when participants were at different locations in the office, thus preventing associations between cues like distinct pieces of furniture with startle probe or US administration. The startle probes were delivered every 15-30 sec during a trial. Additionally, there was an interval of at least 10 seconds between US and startle probe to avoid an influence of the shock on the startle reaction (Davis, 1998). Order of stimuli and duration of the ISI were pseudo-randomized across participants. In total, there were six different event sequences, three of them with the blue light and three with the yellow light serving as CS+.

After acquisition, awareness was measured by posing a free recall question as described above. Participants rated the two different rooms with neutral illumination and both lights switched on regarding valence, arousal, anxiety and contingency after acquisition and extinction. For all ratings (after pre-acquisition, acquisition and extinction), the HMD was removed and screen shots of situations in the experiment were presented on a screen in front of the participants. Questions were presented via headphones. Participants were told to relate their answers to the way they felt during the last phase of the experiment. Answers were given verbally and recorded by the investigator. At the end of the experiment, participants were once more exposed to the UCS and the startle tone and rated these on the appropriate scales. Then headphones, HMD and electrodes were removed and participants filled in the IPQ questionnaire and received 25 Euros for participation.

5.5. Data analysis

5.5.1. Startle reflex.

The Brain Vision Analyzer Software (Version 1.05; Brain Products Inc., Munich, Germany) was used to process the raw EMG signals. Raw values recorded with the outer electrode were subtracted from raw values recorded with the inner electrode. After that, data epochs were segmented and extracted from 100 ms before to 1000 ms after the startle tone. A 500 Hz high cutoff filter and a 28 Hz low cutoff filter were administered. For details on preparation of raw data (including rectifying, calculation of moving averages and baseline correction) please see chapter 4.5.1. (page 59). Identification of peaks and correction of artifacts was also executed according to the pilot study. Peak data were exported to SPSS 18 where peak amplitudes below 5 µV were scored as zero (non-response). Participant with less than two artifact-free and above zero responses per stimulus category were excluded from analysis. T-values for the startle amplitudes were calculated and the mean T-values for each Cue (CS+/CS-/ITI) during each phase (acquisition and extinction) were used for analysis.

Startle responses were analyzed separately for each phase as well as separately for cue in context and context conditioning. I looked at the complete acquisition phase, but since I expected a fast learning process and rapid habituation of startle responses I additionally divided the acquisition phase in two parts (acquisition 1 and acquisition 2) and analyzed those parts separately. For cue in context conditioning, repeated measures MANOVAs were calculated, including within factors Stimulus (CS+, CS-) and Context (CXT+, CXT-) for acquisition and Stimulus (CS+, CS-) and Context (CXT+, CXT-, Novel) for extinction. Between factor was Contingency Awareness (aware, unaware) for all phases. For context conditioning, data were analyzed with separated MANOVAs with Contingency Awareness

as between-subjects factor (aware, unaware) and the within-subjects factor Context (CXT+, CXT- for acquisition; CXT+, CXT-, Novel for extinction).

In addition to these analysis including the between factor Contingency Awareness, I calculated the same repeated measures MANOVAs with the between factor trait-anxiety. Results of analysis including contingeny awareness and those including trait-anxiety are reportet separately.

5.5.2. Ratings.

Contingency ratings were used as a manipulation check for the breakup of participants into aware and unaware. A multivariate analysis of variance (MANOVA) with repeated measures and the between factor Awareness (aware, unaware) and within factors Stimulus (CS+, CS-) and Context (CXT+, CXT-) was conducted to test whether participants labeled "aware" compared to those labeled unaware rated the CS+/UCS contingency as higher than the CS-/UCS contingency. For anxiety ratings, MANOVAs with repeated measures and between factor Awareness (aware, unaware) and within factors Stimulus (CS+, CS-) x Context (CXT+, CXT- for acquisition; CXT+, CXT-, Novel for pre-acquisition and extinction) were conducted. As for startle responses, I conducted separate MANOVAs with Contingency Awareness as between-subjects factor (aware, unaware) and the within-subjects factor Context (CXT+, CXT- for acquisition; CXT+, CXT-, Novel for pre-acquisition and extinction).

5.5.3. Questionnaires

For analyzing the effect of trait anxiety on cue and contextual conditioning, participants were divided into two groups (high-anxious and low-anxious) by means of a median split with respect to STAI Trait sum scores. The two groups were compared

regarding startle responses and subjective ratings. For this purpose, they were included into MANOVAs with repeated measures with between factor Trait Anxiety (high, low) in the same manner as in the analysis with between factor Contingency Awareness.

Alpha was set at .05 for all statistical tests, effect sizes are reported as partial η_p^2 scores. Multivariate procedures were used due to violation of sphericity. Follow up t-tests were conducted one-tailed because of directed hypotheses. Data was analyzed using SPSS for windows (Version 18.0.2, SPSS Inc.).

5.6. Results

5.6.1. Analysis including contingency awareness

5.6.1.1. Manipulation check

In the sample, 24 participants were classified as "aware" regarding the two different lights, meaning that they were able to state the correct light colour after the second acquisition run. The remaining 13 were classified as unaware, resulting in a total of 37 participants in the analysis including the factor awareness. The MANOVA on contingency ratings after acquisition revealed two significant main effects (Stimulus $F(1,34) = 50.23$, $p < 0.001$, $\eta_p^2 = 0.596$, Context $F(1,34) = 19.37$, $p < 0.001$, $\eta_p^2 = 0.363$), significant two way interactions (Stimulus x Awareness $F(1,34) = 43.96$, $p < 0.001$, $\eta_p^2 = 0.564$, Context x Awareness $F(1,34) = 26.33$, $p < 0.001$, $\eta_p^2 = 0.436$, Stimulus x Context $F(1,34) = 16.33$, $p < 0.001$, $\eta_p^2 = 0.324$) and also a significant three way interaction Stimulus x Context x Awareness ($F(1,34) = 22.92$, $p < 0.001$, $\eta_p^2 = 0.403$). In the aware group, the CS+/US contingency was rated significantly higher than CS-/US contingency in both the fear context $(t(23) = 13.69$, $p < 0.001)$ and the also safety context $(t(23) = 2.98$, $p = 0.004)$. The

CS+/US contingency in the fear context was rated higher than in the safety context $(t(23) =$ 6.29 $p < 0.001)$. In the unaware group, no significant differences were found (all ps > .117).

Compared to unaware participants, aware participants rated the CS+/US contingency as higher in the fear context $(t(35) = -7.68, p < 0.001)$ and the CS-/US contingency as lower in both the fear $(t(35) = 4.55, p < 0.001)$ and the safety context $(t(34) = 5.13, p < 0.001)$.

5.6.1.2. Conditioned responses to cues in context

Pre-Acquisition

Ratings: Analysis of anxiety ratings revealed a marginally significant interaction of Context x Awareness $(F(2,34) = 3.00, p = 0.063, \eta_p^2 = 0.150)$. In post hoc t-tests, no significant differences could be found.

Acquisition

Startle Response: Analysis of the acquisition phase revealed a significant interaction of Stimulus x Awareness $(F(1,35) = 4.12, p = 0.050, \eta_p^2 = 0.105)$ and additionally the interaction of Stimulus x Context $(F(1,35) = 3.68, p = 0.063, \eta_p^2 = 0.095)$ just failed to reach significance. A more detailed look on the first half of acquisition1 (A1) revealed a significant interaction of Stimulus x Context $(F(1,35) = 6.37, p = 0.016, \eta_p^2 = 0.154)$. No main effects or interactions involving the factor Awareness reached significance. The CS+ elicited higher startle responses than the CS- in the fear context $(t(36) = 2.25, p = 0.015)$. In the safety context however, I found the opposite pattern: Startle responses on CS- were higher than those on CS+ $(t(36) = -1.72, p = 0.048)$ *(see figure 10)*. In the second acquisition phase (A2), I found a significant interaction of stimulus x awareness $(F(1,35) = 9.44, p = 0.004, \eta_p^2 = 0.212)$. Again, no main effects reached significance, and also no interactions involving the factor context. Following t-tests showed that, regardless of context, startle responses to CS+ were generally higher compared to

CS- in the aware group ($t(23) = 4.20$, $p < 0.001$), but not in the unaware group ($t(12) = -0.67$, $p < 0.258$) *(see figure 10)*.

Anxiety Ratings: Analysis of anxiety ratings also revealed two significant main effects (Stimulus $F(1,35) = 4.27$, $p = 0.046$, $\eta_p^2 = 0.109$, Context $F(1,35) = 6.76$, $p = 0.014$, $\eta_p^2 = 0.162$), significant two way interactions (Stimulus x Awareness $F(1,35) = 10.57$, $p = 0.003$, $\eta_p^2 = 0.232$, Context x Awareness $F(1,35) = 9.43$, $p = 0.004$, $\eta_p^2 = 0.212$, Stimulus x Context $F(1,35) = 9.13$, $p = 0.005$, $\eta_p^2 = 0.207$) and a significant three way interaction Stimulus x Context x Awareness ($F(1,35) = 6.37$, $p = 0.016$, $\eta_p^2 = 0.154$). Since the three way interaction reached significance, I calculated separate MANOVAS for both groups. In the unaware group, no main effects or interactions remained significant. However, in the aware group, I again found significant main effects of Stimulus ($F(1,23) = 16.73$, $p < .001$, $\eta_p^2 = 0.421$) and Context ($F(1,23) = 15.40$, $p = 0.001$, $\eta_p^2 = 0.401$) as well as a significant interaction Stimulus x Context ($F(1,23) = 14.63$, $p = 0.001$, $\eta_p^2 = 0.389$) *(see figure 2)*: The CS+ was perceived as more anxiety inducing than the CS- in both the fear context *(t(23) = 4.17, p < 0.001)* and the safety context *(t(23) = 2.77, p = 0.006)*, Additionally, both CS+ *(t(23) = 4.24, p < 0.001)* and CS- *(t(23) = 2.54, p = 0.009)* elicited more anxiety in the fear context than in the safety context. Unaware participants did not differentiate between stimuli (all ps > .123). Compared to unaware participants, aware participants rated the CS+ as more anxiety inducing in the fear context *(t(35)=, -2.58, p=.007)*.
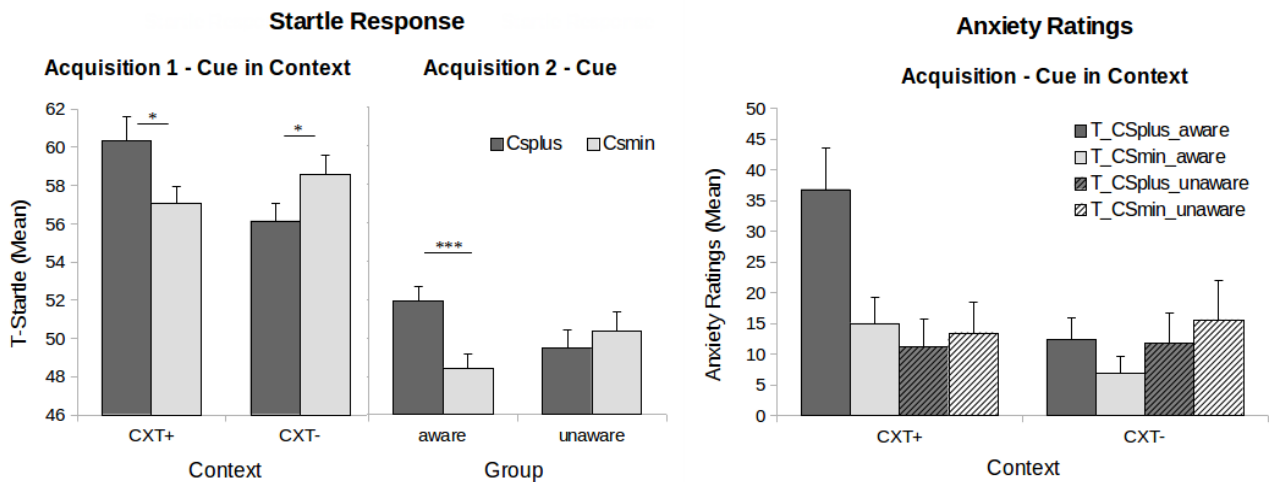
Figure 10. Startle response and anxiety ratings during acquisition: In A1 the CS+ elicited higher startle responses than the CS- in the CXT+, in the CXT- this pattern was reversed. In A2, startle responses to CS+ were generally higher than to CS- in the aware group. In the aware group, the CS+ was perceived as more anxiety inducing than the CS- in both contexts. Both stimuli elicited more anxiety in CXT+ than in CXT-. Unaware participants did not differentiate between stimuli. * $p<=.05$; *** $p<=.001$

Extinction

Startle Response: Analysis revealed a marginally significant interaction of Stimulus x Context ($F(2,34) = 2.73$, $p = 0.079$, $\eta_p^2 = 0.139$). Exploratory post hoc *t*-tests indicated that the CS+ elicited higher startle responses than the CS- only in the fear context ($t(36) = 2.44$, $p = 0.010$). Startle responses to the CS+ were significantly higher in the fear context than in the safety context ($t(36) = 2.58$, $p = 0.008$) and marginally higher than in the novel context ($t(36) = 1.53$, $p = 0.068$) *(see figure 11)*.

Anxiety Ratings: The interaction of Context x Awareness still reached significance ($F(2,34) = 5.79$, $p = 0.007$, $\eta_p^2 = 0.254$) after extinction learning, the main effect of Stimulus ($F(1,35) = 3.56$, $p = 0.067$, $\eta_p^2 = 0.92$) and the interaction Stimulus x Context ($F(2,34) = 2.71$, $p = 0.081$, $\eta_p^2 = 0.138$) reached trend level. In the aware group, stimuli presented in the fear context were rated as significantly more anxiety inducing than in the safety context

(t(23) = 2.59, *p* = 0.008). Interestingly, in the unaware group stimuli in the fear context were rated as less anxiety inducing compared to those in the safety context *(t*(12) = -2.13, *p* = 0.028), and marginally less than those in the novel context *(t*(12) = -1.48, *p* = 0.083). Explorative post hoc *t*-tests for further examination of the trend level results revealed that the CS+ was still rated as more anxiety inducing than the CS- in general *(t*(36) = 2.44, *p* = 0.010). This difference only reached significance in the fear context *(t*(36) = 3.33, *p* = 0.001), not in the safety *(p* = 0.067) or the novel context *(p* = 0.146).
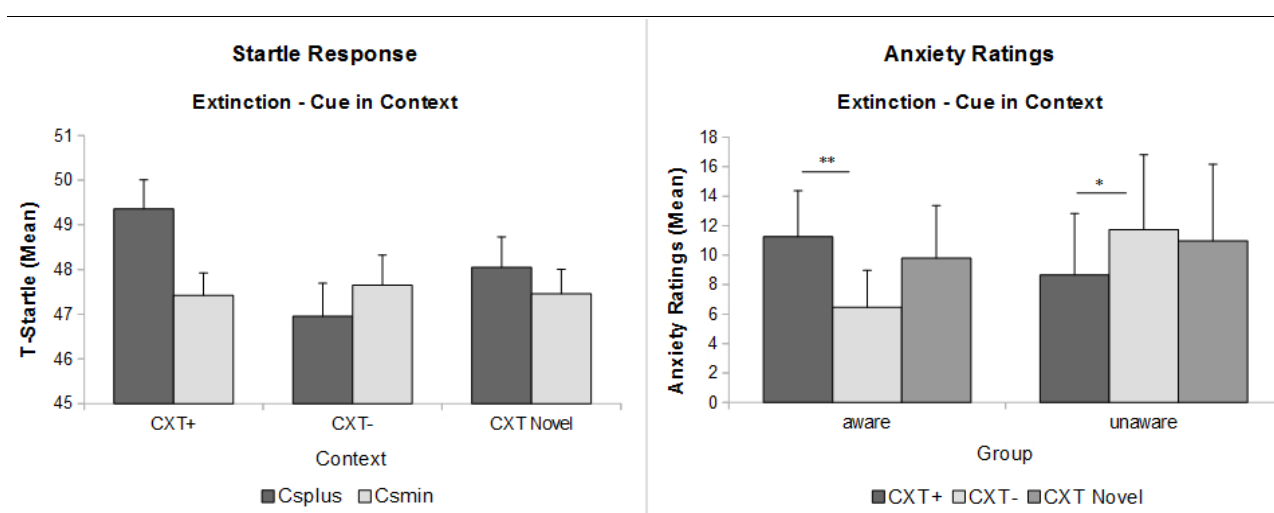
Figure 11. Startle response and anxiety ratings during extinction: Exploratory analysis showed that startle responses to the CS+ were significantly higher in CXT+ than in CXT- and marginally higher than in CXTnovel. In the aware group, stimuli presented in CXT+ were rated as more anxiety inducing than in CXT-. In the unaware group stimuli in CXT+ were rated as less anxiety inducing compared to those in CXT-.

* p<=.05; ** p<=.01

5.6.1.3. Conditioned response to contexts

Pre-Acquisition

Before conditioning, the three contexts did not differ in valence, arousal or anxiety ratings. No main effects or interactions reached significance (all ps > .159).

Acquisition

Startle response: The main effects of context and group did not reach significance, nor did the interaction Stimulus x Group. In explorative post hoc *t*-Tests, unaware participants displayed startle reactions to the CXT+ compared to aware participants ($t(35) = 2.08$, $p = 0.023$) and also marginally higher startle responses to the CXT+ compared to the CXT- ($t(12) = 1.57$, $p = 0.071$).

Ratings: After conditioning, analysis of anxiety ratings revealed only a marginally significant interaction of Context x Awareness ($F(1,35) = 3.03$, $p = 0. 090$, $\eta_p^2 = 0.080$), indicating that, in the aware group, CXT+ was experienced as more anxiety inducing than CXT- *(t(23) = 1.86, p = 0.038),* whereas in the unaware group no difference was found.

Extinction

Startle Response: As in the acquisition phase, neither main effects nor interaction reached the significance level in the extinction phase.

Ratings: Analysis of anxiety ratings also did not reveal any significant effects.

5.6.2. Analysis including trait anxiety

5.6.2.1. STAI trait analysis

A median split with respect to STAI Trait sum scores was calculated. The scores of three participants were identical with the median of the sample. These three participants were excluded from further analyzes including trait anxiety, resulting in two groups with 17 participants each and a total of 34 participants. Unaware participants tended to score higher on trait anxiety than participants who did learn the association between the CS and the US *(t(32) = -1.48, p = 0.074).*

5.6.2.2. Conditioned responses to cues in context

Pre-Acquisition

Ratings: I did not find any significant main effects or interactions the analysis of anxiety ratings before conditioning.

Acquisition

Startle Response: In the acquisition, I found a significant main effect of Stimulus ($F(1,32) = 4.88$, $p = 0.034$, $\eta_p^2 = 0.132$) as well as a marginally significant interaction of Stimulus x Context ($F(1,32) = 3.65$, $p = 0.065$, $\eta_p^2 = 0.102$). After splitting up acquisition in two parts, the Stimulus x Context interaction turned out to be significant in A1 ($F(1,32) = 4.95$, $p = 0.033$, $\eta_p^2 = 0.134$) , wheres the main effect of stimulus could still be found in A2 ($F(1,32) = 4.88$, $p = 0.034$, $\eta_p^2 = 0.132$) . No other effects, especially no interactions including the factor trait anxiety, reached significance level in any of the acquisition phases.

Post hoc tests revealed that, after the first part of acquisition, the CS+ elicited higher startle responses than the CS- in the fear context (t (33) = 2.38, $p = 0.017$), but not in the safety context ($p > 0.124$). In the second part of acquisition, the CS+ in general elicited higher startle responses than the CS- (t (33) = 2.479, $p = 0.009$) *(see figure 12)*.

Anxiety Ratings: The same pattern of significance was found for anxiety ratings (stimulus $F(1,32) = 7.34$, $p = 0.011$, $\eta_p^2 = 0.187$, context $F(1,32) = 9.44$, $p = 0.004$, $\eta_p^2 = 0.288$, stimulus x context $F(1,32) = 13.89$, $p = 0.001$, $\eta_p^2 = 0.303$). The CS+ was more anxiety inducing than the CS- in the CXT+ (t (33) = 3.26, $p = 0.002$), but not in the CXT- (p >.147). The CS also was more anxiety inducing in the CXT+ than the CXT- (t (33) = 3.52, $p = 0.001$) *(see figure 12)*.
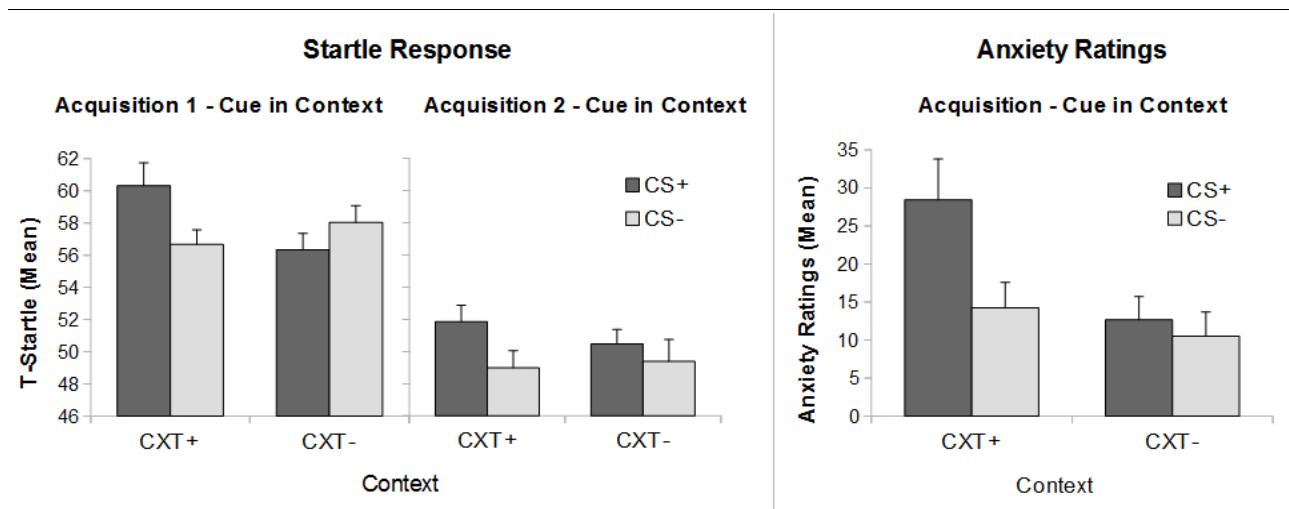
Figure 12. Startle response and anxiety ratings during acquisition: Trait anxiety did not have an effect on cued in context conditioning. After A1 the CS+ elicited higher startle responses than the CS- in CXT-, but not in the safety context (p > 0.124). In the second part of acquisition, the CS+ in general elicited higher startle responses than the CS-.

Extinction

Startle Response: The main effects of context and stimulus did not reach significance, nor did any interaction.

Anxiety Ratings: The main effect of Stimulus reached significance $F(1,32) = 4.72$, $p = 0.037$, $\eta_p^2 = 0.128$, as well as two way interaction Stimulus x Context $F(2,31) = 4.10$, $p = 0.026$, $\eta_p^2 = 0.209$. In the fear context, the CS+ elicited more anxiety than the CS- (t (33) = 3.13, $p = 0.002$). This was neither the case in the safety context nor in the novel context (ps > .124). Also, the CS+ induced more anxiety in the fear context compared to the safety context (t (33) = 1.87, $p = 0.036$) and also compared to the novel context (t (33) = 1.89, $p = 0.034$).

5.6.2.3. Conditioned response to contexts

Pre-Acquisition

Regarding subjective ratings, no effects reached significance before conditioning.

Acquisition

Startle response: In the acquisition phase, analysis revealed a significant interaction of Context x Trait anxiety ($F(1,32) = 4.94$, $p = 0.033$, $\eta_p^2 = 0.134$). Post hoc t-Tests showed that in the low anxiety group, CXT+ elicited marginally lower startle responses than CXT- ($t(16) = -1.49$, $p = 0.079$). In the high anxiety group, the opposite was the case: CXT+ elicited higher startle responses than CXT- ($t(16) = 1.67$, $p = 0.057$) *(see figure 13)*.

In A1 and A2 the main effects of context and group did not reach significance, nor did the interaction stimulus x group.
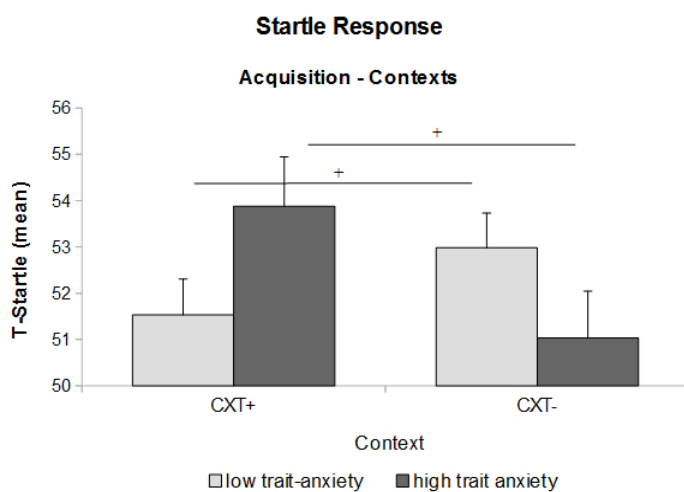
Figure 13. *Startle Responses during acquisition in the high and low anxiety group: In the low anxiety group, the CXT+ elicited marginally lower startle responses than CXT- during acquisition. In the high anxiety group, the opposite was the case: CXT+ elicited higher startle responses than CXT-. + p<=.01*

Anxiety Ratings: As after pre-acquisition, for subjective ratings no significant effects could be found.

Extinction

Startle Response: In the analysis of FPS, no effects reached significance during extinction training. An explorative post hoc analysis revealed that high-anxious participants

still reacted with slightly enhanced startle amplitudes in the CXT+ compared to the CXT- ($t(16)=1.39$, $p=.092$) and the novel context ($t(16)=1.66$, $p=.058$), though this was only observable on trend level (*see figure 14*). This was not the case for low-anxious participants (all ps > .269).
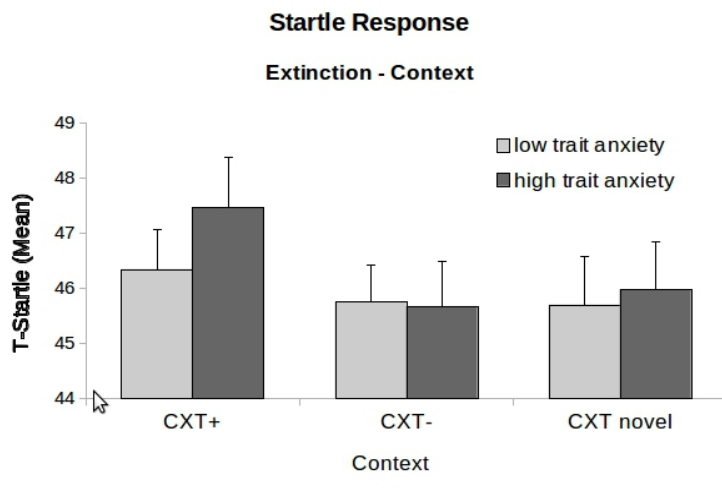
Figure 14. Startle Responses during extinction in the high and low anxiety group: Only high-anxious participants reacted with enhanced startle amplitudes in the CXT+ compared to the CXT- and the novel context on trend level.

Anxiety Ratings: For anxiety ratings, no main effects or interactions reached significance.

5.7. Discussion

Summary. Fur further understanding the mechanisms which facilitate the development and maintenance of anxiety disorders it is crucial to investigate the influence of context on the acquisition and expression of fear (Huff et al., 2011). Up to now, there are relatively few studies on human fear conditioning which include rich contextual cues due to practical difficulties. For studying contextual modulation of differential cue conditioning, a virtual reality paradigm was established including three different virtual rooms: One fear context, one safety context and one generalization context. I was interested in the differential responses to CS+ and CS-, its modulation by the contexts and contextual

anxiety measured in between presentation of cues. In general, participants reacted with increased fear to the CS+ compared to the CS-. Fear of cues was acquired preferential to contextual anxiety, but conditioned responses to cues were modulated by contexts: They were most prominent in the fear context and significantly weaker in the safety context. Regarding the novel context introduced in extinction, I found only trend level generalization of acquired contextual fear to the unknown and therefore ambiguous environment. Cued fear was not generalized to the novel context. Furthermore, I investigated fear conditioning against the background of individual differences in contingency learning and trait-anxiety. Participants who did not become aware of the CS-UC contingency during the experiment tended to score higher on trait-anxiety than participants who could explicitly report the association between CS and US. I found a dissociation between implicit and explicit fear reactions to the CS regarding contingency awareness, providing further evidence for a dual process model of fear conditioning. Individual difference in trait-anxiety primarily influenced contextual anxiety: Only high-anxious individuals displayed increased anxiety in the fear context compared to the safety context. However, against my expectation, high-anxious participants did not show deficits in discriminating fear and safety cues.

Contingency Awareness - Acquisition. Results of both startle responses and anxiety ratings clearly indicate successful cue conditioning. Participants showed FPS responses to the CS+ specifically in the fear context in the first acquisition phase, independent of contingency awareness. Hence, discriminative learning took place regarding both cues and contexts. In the safety context, startle reactions to the CS- were stronger than those to CS+, though not as high as reactions to CS+ in the fear context. Possibly, the safety context did not serve as a safety signal from the very beginning. Participants realized that CS-US contingency was different in this second context, possibly

resulting in ambiguity regarding the CS- during the first trials. In A2, this effect was no longer present, the safety context had become inhibitory. Surprisingly, contingency awareness had an influence on startle responses in the second acquisition phase: Only aware participants showed FPS responses to the CS+ in the fear context, unaware participants did no longer discriminate between the stimuli or the contexts. As expected, contingency awareness had a strong effect on anxiety ratings: In both contexts, aware participants rated the CS+ as more anxiety inducing than the CS-. Additionally, both the CS+ and the CS- were more alarming when presented in the fear context. Not only the CS- but also the safety context seemed to have become a safety-signal for aware participants. Unaware participants again did not differentiate between cues or contexts regarding anxiety ratings.

Effects of contingency awareness on isolated contextual conditioning, tested on the basis of startle reactions to and anxiety ratings of the different contexts in between stimulus presentation, did not reach significance. On an explicit level tested with subjective anxiety ratings, I found a marginal difference indicating that only aware participants rated the fear context as more anxiety inducing than the safety context. This finding is quite surprising, since I expected participants who did not explicitly become aware of the CS-UC association to develop higher contextual fear than aware participants. This assumption is based on prior studies (e.g. Grillon, 2002a; Baas et al., 2008) showing that deficient cue conditioning leads to higher contextual fear because the absence of a cue cannot signal safety for unaware participants. Hence, in an exploratory analysis, I took a closer look on startle responses to the contexts during acquisition. Unaware participants displayed stronger fear reactions to the CXT+ compared to aware participants and also marginally higher startle responses to the CXT+ compared to the CXT-. These findings point to a

dissociation between implicit and explicit measures regarding contextual fear. But, since the interaction Context x Awareness did not reach significance in the first place, these results have to be interpreted with care.

Baas et al. (2008) investigated the influence of contingency awareness on cued and contextual fear. They found that both aware and unaware participants displayed clear contextual conditioning, and those participants who did not learn the CS-US contingency did not report reduced fear in the absence of the CS. They were in a state of anxiety in the fear context, because for them the absence of the CS did not imply a period of safety. In contrast, I only found a trend of contextual conditioning, in unaware participants on an implicit level and in aware participants on an explicit level. However, Baas et al. did not investigate differential cue conditioning within contexts. Only one cue was presented in form of a neutral light illuminating the virtual rooms. The contexts were more prominent than the cue, explaining their clear change in associative strength during acquisition. Compared to the neutral light applied by Baas et al. (2008), both cues in the present study were much more salient, which might have caused the cue contingency to be learned preferential to the context contingency. My findings regarding cue conditioning are similar to those of Baas et al. (2008). Only aware participants reported differential subjective fear to CS+ and CS- in the fear context. Fear potentiation of startle reactions was not influenced by contingency awareness at the beginning. Yet in the second part of acquisition, startle responses of unaware participants did not differ significantly between CS+ and CS-. This is surprising because the startle reflex depends on sub-cortical brain structures and thus does not require cortical input. In the debate about whether contingency awareness is necessary for establishing a conditioned response, my results are – at first glance - not explicit. But although unaware participants did not show a

conditioned response to the CS+ in A2, they did so in A1 – showing that contingency awareness is not a necessary precondition for the occurrence of a conditioned response. Taken together with results of the pilot study, this indicates a dissociation between implicit and explicit conditioned responses and provides further evidence for a dual process model of fear conditioning (for details see for example Lovibond and Shanks 2002), which postulates two independent learning processes, one propositional in nature and leading to conscious awareness, the second, lower level process non-propositional and activating the CR via a direct mechanism (Lovibond and Shanks 2002). Carter and colleagues (Carter, Hofstotter, Tsuchiya & Koch, 2003) showed that the higher the cognitive load during conditioning, the more contingency awareness is necessary for successful conditioning. Possibly, my results regarding the influence of contingency awareness on differential cue conditioning can be explained by a relatively high cognitive load due to a complex conditioning paradigm. Examining the correlation of working memory capacity, contingency awareness and development of a conditioned response in the complex virtual environment would be an interesting extension of my study and should be considered in further research. Consand et al. (2008) showed that high working memory capacity facilitates contingency learning in a cognitive masking paradigm, but to my knowledge this has not yet been investigated in a virtual reality fear conditioning study.

Contingency Awareness – Extinction. Startle responses of aware and unaware participants did no longer differ significantly in the extinction phase. Exploratory analysis of trend-level results regarding cues in context indicated that the CS+ elicited higher startle responses than the CS- only in the fear context. This result could be confirmed by anxiety ratings. Apparently, aversive cue conditioning did not generalize to a novel context. Context specificity of cued fear has also been shown in another human virtual reality study

by Huff et al. (2011). They tested whether cued fear acquired in one context would still be detectable during the test phase conducted 24 hours later in either the same or a different context. Fear was specific to the CS+ compared to the CS- only in participants tested in the same context as during acquisition during the test phase. According to the authors, contextually cued fear retention challenges "the assumption that fear conditioning to a cue is not initially context-specific relative to extinction memories" (Huff et al. 2011) and provides evidence for the transformation view of memory storage, stating that initial storage of episodic events is context dependend and involves activation of the hippocampus (Wiltgen & Silva, 2007). My results indicate that this might also be true for stimuli which are not biologically prepared like the stimuli applied by Huff et al. (2011). However, since these results did not reach significance, they have to be interpreted with care.

The aware group rated stimuli presented in the fear context as more anxiety inducing than in the safety context. Interestingly, in the unaware group stimuli in the fear context were rated as less anxiety inducing compared to those in the safety context. A reason for this might be, that the lack of a clear threat signal (like the CS followed by the US) also means a lack of safety-signals. In the safety context no shock was administered, and since unaware participants did not have a clear and explicit representation of the CS-US paining, the safety context might have been more unpredictably for them than the fear context. For unaware participants, the CXT- did not obtain the properties of a safety-signal. This leads to higher contextual fear, as has been shown for example by Grillon et al. (2006).

   Trait-Anxiety – Acquisition. In the study cited above, Baas et al. (2008) reported that trait-anxiety tended to be higher in unaware participants than aware participants. This has also been shown by Grillon (2002a) and can be explained by a deficient discrimination

of fear and safety cues in high-anxious individuals (e.g. Arnoudova et al., 2013). In my sample, unaware participants displayed marginally higher levels of trait-anxiety than aware participants. However, unaware participants did learn the CS-US association at least on an implicit level (see FPS in A1). Thus, they did not fail to discriminate fear and safety cues as has been shown by both Baas et al. (2008) and Grillon (2002a). In a more recent study, Baas (2013) investigated the effect of trait-anxiety on cue and contextual fear conditioning in more detail. In the first part of the experiment, deficient cue conditioning resulted in sustained contextual anxiety, indicating that learning the CS-US contingency is important for a successful reduction of contextual fear in the absence the threatening cue. Trait anxiety per se did not affect learning of CS-US contingencies. In the second part of the experiment was designed somewhat differently, In the first blocks only contexts were paired with the US, later a CS+ and a CS- were added to the conditioning paradigm. High-anxious participants displayed less adaptive responding as a function of the presence or absence of the CS in the shock context, and also higher levels of contextual anxiety (Baas 2013). Apparently, once high-anxious participants had learned to associate the fear context with the US, the cues could not obtain enough inhibitory associative strength to serve as safety-signals. Cosand et al. (2008) showed that lower levels of arousal are associated with better contingency learning. Possibly, contextual fear developed in the first trials was associated with higher levels of arousal, leading to deficient cue conditioning. In my study, participants were confronted with both cues and contexts from the beginning on. High-anxious individuals did not show deficits in cue conditioning. In all participants the CS+ elicited higher startle responses in the fear context in A1, in A2 this was the case also in the safety contexts. Additionally, high-anxious individuals showed only mild deficits in discriminating fear and safety cues in the later part of acquisition. On the other hand, my

findings affirm the assumption that high trait-anxiety is associated with increased contextual anxiety. Glotzbach et al. (2013) for example showed that high trait-anxious participants showed faster contextual fear learning compared to low trait-anxious participants. In the present sample, high-anxious participants showed higher startle responses in the fear context than in the safety context during acquisition, which was not the case for low-anxious participants. Interestingly, this effect could only be found on an implicit level – anxiety ratings did not differ significantly between groups or contexts.

Trait-Anxiety - Extinction. Regarding trait-anxiety, I did no longer find significant differences during extinction, though on a trend-level, high-anxious participants still reacted with enhanced startle amplitudes in the CXT+ compared to the CXT- and the novel context, which was not the case for low-anxious participants. To some extend, high-anxious individuals generalized contextual fear to the novel ambiguous context. This is in line with evidence coming from patients suffering from anxiety disorders associated with sustained anxiety like for example panic disorder (see Grillon et al. 2008). Wessa and Flor (2007) showed that not only acquisition was facilitated in PTSD patients exposed to trauma reminders during fear conditioning, but also extinction was impaired, resulting in more negative evaluations of the conditioned stimuli, enhanced peripheral and brain responses compared to healthy participants. There were no differences between trait-anxiety groups in subjective anxiety ratings. In general, the CS+ was rated as more anxiety-inducing in the fear context, but neither in the safety nor in the novel context.

Clinical implications. Interestingly, in my sample, contingency awareness had an effect on differential cue conditioning, wheres trait-anxiety did only influence contextual learning. Taking account of the evidence that lack of awareness might lead to higher trait-anxiety (e.g. Baas et al., 2008; Grillon, 2002a) as well as evidence that attentional

processes play a role in fear conditioning in a way that they facilitate acquisition of cued or contextual fear (Baas et al., 2008; Fani et al. 2012), it seems very important to further disentangle the associations between attentional processes, contingency awareness, and trait anxiety.

Trait anxiety is seen as a risk factor for developing anxiety disorders, but nevertheless evidence on the matter is ambiguous. High trait anxiety has been associated with impaired safety learning (e.g. Baas et al., 2008, Gazendam, Kamphuis & Kindt, 2013, Lissek et al. 2009), impaired extinction (Gazendam et al., 2013), overgeneralization of fear (Lissek et al., 2010; Wessa & Flor, 2007) and enhanced contextual fear (Glotzbach-Schoon et al. 2013, Baas 2013). There is also evidence that both acquisition and generalization of fear is not impaired in high-anxious individuals (Torrents-Rodas et al., 2013). In the present sample I found evidence for enhanced contextual fear learning as well as hints for impaired extinction and generalization of contextual anxiety to a novel context, although the latter results did not reach significance. Even so, I think that it would be of special interest to transfer the paradigm to a clinical setting to investigate the modulation of context-dependent cue conditioning in panic-disorder patients, as well as the generalization of cued fear and contextual anxiety to the novel context. As pointed out for example by Glotzbach et al. (2013), existing theories about safety behaviour and safety signals in panic-disorder patients are controversial: For example Lissek et al. (2009) state that panic-disorder patients display impaired discriminative fear conditioning, as indexed by enhanced startle potentiation to learned safety-cues and aberrant reactivity to danger cues. I could not replicate this finding in the high trait-anxiety group. Curiously, I did find a similar pattern of results – i.e. enhanced startle responses in reaction to the CS- - in the unaware group. But regarding trait anxiety as a non clinical model for panic-disorder, my

results point to Rachman`s safety signal perspective (Rachman, 1984), assuming that panic disorder patients avoid fearful situations and seek safety. Consequentially they should not only show enhanced fear reactions in the fear but also in the novel context compared to the safety context during extinction.

Limitations. I found hints for deficient cue conditioning in unaware participants, enhanced contextual conditioning in high-anxious participants and a mild correlation between contingency awareness and trait-anxiety. These findings combined do not allow for a clear distinction whether high-anxious individuals suffer from an over-activated fear network, or rather from an altered inhibitory system. It has to be mentioned that in my study, participants were divided into a high-anxious and a low-anxious group by means of a median split. Therefore, they do hardly represent extreme groups regarding trait anxiety. A larger sample allowing for the examination of more extreme groups would be beneficial, as well as the above mentioned transfer of the paradigm to a clinical setting. Including a larger sample might also solve this problem.

Conclusions. In sum, the novel virtual reality paradigm proved to be suitable for examining differential fear conditioning embedded in distinguishable contexts, as well as generalization of cued and contextual fear to a novel environment. My results provide further evidence for differential modulation of phasic fear by contingency awareness and contextual anxiety by trait anxiety. Interestingly, individual difference in trait-anxiety influenced contextual anxiety only, high-anxious participants did not show deficits in discriminating fear and safety cues. Additionally, only trend level generalization of contextual anxiety to a novel context was present, whereas cued fear was not generalized at all. As contextual conditioning is an appropriate paradigm for investigating the development and maintenance of anxiety disorders, the chances opened up by virtual

reality research for translating findings from animal research to humans should be used and extended. Next to gaining a deeper understanding of the role of trait-anxiety in the development of anxiety disorders, it would be of special interest to further study neural processes underlying contextual anxiety.

6. Delay and trace fear conditioning in a complex virtual learning environment - neural substrates of extinction[2]

6.1. Summary

During extinction, existing fear memory is not erased, but rather new memory is formed which inhibits an initially acquired fear response. The vmPFC plays an important role in this process. According to existing evidence, an inhibitory memory trace is formed between vmPFC and amygdala during extinction learning of delay fear conditioning. To my knowledge, the role of the prefrontal cortex (PFC) in the extinction of trace conditioning has not been examined so far. In an fMRI study conducted in VR, I compared neuronal activation during extinction of delay and trace fear conditioning in a between-subjects-design. A mildly painful electric stimulus served as US and two different lights represented conditioned stimuli (CS). The CS+ coterminated with the US in the delay condition, whereas in the trace condition the CS+ was followed by a 4s trace interval. Interestingly, the delay (DCG) and the trace conditioning group (TCG) showed differences in prefrontal activation during early extinction. As expected, the vmPFC was activated in the DCG. In the TCG however I observed activation of the dorsolateral prefrontal cortex (dlPFC). This dissociation indicates that extinction processes after trace fear conditioning differ from those after delay fear conditioning. Activation of the vmPFC probably reflects the inhibition of the fear response. In contrast, activation of the dlPFC could be associated with modulation of working memory processes which are involved in bridging the trace interval and hold information in short term memory.

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2   The following chapter ist based upon Ewald & Glotzbach-Schoon et al. (2014)

6.2. Introduction

Fear can be critical for survival when an individual faces a threatening situation. It serves as an important alert mechanism which prepares us for the adequate reaction, such as escaping or avoiding a predator. However, fear can become maladaptive when a fear reaction is unproportional and no longer appropriate in the actual situation. In anxiety disorders, the ability to readjust behavior to the actual situation is usually impaired (Rauch et al., 2006; Schiller et al., 2011). According to Jacobi et al. (2014), anxiety disorders including PD, agoraphobia, GAD, and social and specific phobias, are the most frequent mental disorders in Germany with a twelve month prevalence of 15.3%. Not surprisingly, this has led to extensive research in the field of fear and anxiety, also regarding the neural systems involved in fear learning and its extinction. Fear learning in animal and human research is mainly examined on the basis of Pavlovian fear conditioning (Pavlov, 1927). According to timing aspects of the CS–US pairing in classical fear conditioning, several forms can be distinguished. Two important forms are delay fear conditioning and trace fear conditioning. In delay conditioning, the US follows directly on the CS (or the CS and the US coterminate). In contrast, in a trace conditioning paradigm, a trace interval follows the CS, meaning that there is a temporal gap between CS offset and US onset. This trace interval needs to be bridged in the learning process, meaning that a "memory trace" between CS and US has to be formed in order to learn the CS/US association (Pavlov, 1927). This is not possible without contingency awareness (Weike et al., 2007) and requires higher order cognitive processing.

Trace and delay fear conditioning have been found to be associated with different neural structures. There is vast evidence that the amygdala is crucial for acquisition of fear. Information about the CS and the US is transmitted from sensory cortices via the thalamus

first to the lateral and then to the central amydala (for details see *figure 2* in chapter 3.3.1.3., page 35f). From there, a fear reaction is triggered via projections to the brainstem (e.g., LeDoux, 2000). Besides the amygdala, the ACC has been shown to be involved in fear acquisition in delay conditioning. For example, Knight et al. (2004) and Milad et al. (2007) provided evidence that the ACC is involved in the expression of the fear response. Moreover, activation of the ACC has been associated with the anticipation of pain. More precisely, it is assumed to integrate nociceptive input with memory in cooperation with the anterior insula (Büchel et al., 1998)., allowing for an appropriate response to subsequent stimuli (e.g. Coghill et al., 1994). Price (1988) suggested that the links between insula and limbic system might be essential for the integration of current pain with memory, which in turn is necessary for evaluation and interpretation of a stimulus under consideration of previous experience. In line with this assumption, Phelps, O'Connor, Gatenby, Gore, Grillon & Davis (2001) proposed that the insula is involved in transmitting a cortical representation of fear to the amygdala. Hartley, Fischl & Phelps (2011) examined brain structure correlates of individual differences in fear conditioning. They found that greater thickness in the posterior insula was associated with larger conditioned responses during acquisition. Hippocampal activation has been associated with fear conditioning as well, particularly with trace fear conditioning. Human delay fear conditioning has been reported to occur without explicit hippocampal activity (LaBar, Gatenby, Gore, LeDoux & Phelps, 1998; Phelps et al., 2004; Schiller et al., 2008). Büchel et al. (1999) reported activation of the hippocampus only in trace, but not in delay conditioning. They suggested that the hippocampus playes an important role for bridging the trace interval between CS and US. Moreover, Clark, Manns & Squire (2002) showed that activations of the cerebellum and the brainstem are sufficient for delay eyeblink conditioning, whereas trace eyeblink

conditioning additionally depends on the hippocampus and the neocortex. According to O'Keefe and Dostrovsky (1971), contextual information is represented in the hippocampus. A context can not only be of spacial nature. If there is a temporal gap between the CS and the US, conditioning requires the formation of a temporal context. Another possible explanation for the involvement of the hippocampus in trace conditioning is that hippocampal activation is necessary for establishing contingency awareness (e.g. Klucken et al., 2009; Tabbert et al., 2011). Taken together, I conclude that the main neural structures involved in delay fear conditioning are the amygdala, the insula, and the ACC (Sehlmeyer et al., 2009). During trace fear conditioning, declarative memory is formed, which requires additional activity of the hippocampus.

For gaining further understanding of the development and maintenance of anxiety disorders, it is not sufficient to study the acquisition of fear. Maladaptive responses to threatening stimuli can also result from a deficit in extinction of the fear (Baas et al., 2008). According to existing evidence, fear memory is not erased during extinction learning, but rather new memory is formed which inhibitis the acquired fear memory (Bouton, 2002, 2004; Milad & Quirk, 2002; Myers & Davis, 2002; Quirk, 2002). To date, the neural correlates of extinction are less understood than those of fear acquisition. In both acquisition and extinction, activation of the amygdala has been shown to play an important role. The prefrontal cortex is assumed to inhibit the expression of conditioned fear, after new memory has been formed during extinction (Quirk et al., 2006). In more detail, evidence from rodent models of extinction suggest that an inhibitory memory trace between vmPFC and amygdala is established during extinction (Sotres-Bayon et al., 2004, 2007), by means of which the expression of fear is inhibited. The vmPFC activates GABAergic intercalated cells in the amygdala which in turn inhibit the central nucleus of

the amygdala (Quirk et al., 2006; Sotres-Bayon et al., 2007). Burgos-Robles, Vidal-Gonzalez, Santini & Quirk (2007) reported that the infusion of an N-methyl-D-aspartate receptor (NMDAR) antagonist into the vmPFC prior to or directly after extinction training impaired 24 hour recall of extinction. They assumed that stabilization of extinction memory requires activation of NMDARs in the vmPFC. Evidence for the formation of an inhibitory memory trace between vmPFC and amygdala during extinction has also been provided by lesion studies (Morgan & LeDoux, 1993; Quirk, Russo, Barron & Lebron, 2000; Morgan, Schulkin & LeDoux 2003; Lebron, Milad & Quirk, 2004). Rats with lesions of the medial PFC were for example resistant to extinction learning in a delay fear conditioning paradigm (Morgan & LeDoux, 1993). Human studies on the extinction of delay conditioning have confirmed the role of the amygdala and the vmPFC (Phelps et al., 2004; Milad et al., 2007). Other brain structures associated with extinction learning in humans are the insula and the ACC (Gottfried & Dolan, 2004; Phelps et al., 2004). The hippocampus has been reported to be important in extinciton, since extinction recall is highly context dependent. According to Kalisch et al. (2006), during context dependent recall of extinction memory, a network containing the vmPFC and the hippocampus is activated. The striatum has been shown to be involved in affective learning too. More precisely, it is associated with processing of prediction errors which occur when the expected result does not coincide with the actual result (Delgado, Schiller & Phelps, 2008). Evidence for involvement of the striatum in the processing of prediction errors in classical fear conditioning comes for example from Jensen, McIntosh, Crawley, Mikulis, Remington & Kapur (2003). Delgado et al. (2008) reported activation in the striatum in response to prediciton errors in fear conditioning paradigms with both primary reinforcers (such as shocks) and secondary reinforcers (such as monetary loss). The absence of the US during extinction represents a

positive prediction error, because the expected aversive event does not occur. Consequently, the striatum should be considered as an important region in the extinction of conditioned fear. Imaging studies investigating neural correlates of extinction focused mainly on delay conditioning so far. Therefore little is known about extinction of trace conditioning in humans. Since differences in neuronal activation have been found during acquisition of delay and trace fear conditioning (see discussion above), it is likely that also in extinction different brain structures are involved. When fear is learned in real life situations, usually a temporal gap between lies between the predictive stimulus and the aversive event. Hence, those situations are closer to trace than to delay fear conditioning in the laboratory. Given the relevance of fear extinction for the treatment of anxiety disorders, it is important to detect possible differences in the neural networks involved in different types of fear conditioning.

A model of prefrontal organization postulates different funtions of the ventrolateral and the dorsal part of the PFC: The ventrolateral part of the PFC is assumed to be involved in the maintenance of information, such as retaining a sequence of letters in working memory. The dorsal part of the PFC is more important when it comes to manipulation of information, such as reordering the sequence into alphabetical order (D'Esposito, Postle, Ballard & Lease, 1999). Lesion studies also revealed that both nonhuman primates and humans with lesions of the dlPFC are less capable of adjusting their behavior appropriately in delayed response tasks (e.g., D'Esposito, Postle & Rypma 2000). In these tasks it is necessary to retain information in working memory over a short period of time before making choices and decisions based on this information. This demand is similar to forming and adjusting a memory trace necessary for bridging the trace interval during trace fear conditioning. Against this background one can assume that the dlPFC is involved in the extinction of

trace memory, either exclusively or in addition to the vmPFC. Evidence for the involvement of the dlPFC in trace eyeblink conditioning as already been found in the animal model. Weiss and Disterhoft (2011) propose a neural network in which the dlPFC orchestrates neural activity that bridges the trace interval in cooperation with prelimbic areas.

In line with existing evidence I expected that extinction of both delay and trace fear conditioning is associated with activation in the amygdala and the vmPFC. Additionally, I anticipated involvement of the insular and the anterior cingulate cortices as well as the striatum. Next to joint activation in both types of conditioning, I assumed differences between extinction of delay and trace conditioning regarding the involvement of the prefrontal cortex. Finally, since the hippocampus is assumed to be involved in the formation of declarative memory during fear conditioning, I expected it to play a greater role in the extinction of trace than delay memory.

In the present I implemented both delay and trace fear conditioning in a VR paradigm. VR is a powerful tool for investigating fear reactions in ecologically valid environments (Mühlberger et al., 2007a; 2007b; 2008a; Mühlberger, Neumann, Wieser & Pauli, 2008b). It has successfully been applied in conditioning studies (Baas et al., 2004, 2008; Alvarez et al., 2008; Glotzbach et al., 2012; Tröger et al., 2012; Glotzbach-Schoon et al., 2013) as well as in the actual treatment of specific phobias such as fear of flying (Mühlberger et al., 2006). In VR, full control of events is possible. Due to its complexity, the conditioning situation is closer to real life learning situations than in most laboratory designs. The virtual environment applied in the present study was identical with the paradigm tested in the pilot study. It consisted of a corridor and an office, through which subjects were passively guided while lying in the scanner. In both the DCG and the TCG, a blue and a yellow light presented in the office served as CS+ and CS−, respectively. The US consisted of mildly

painful electric stimulus. Differences in BOLD responses to CS+ minus CS− served as indices of brain responses related to conditioning. The main focus was on differences between delay and trace fear conditioning during extinction. Bold responses during acquisition were not analyzed because of an overlap of brain responses to the CS and US in the learning phase due to their temporal closeness.

6.3. Method and Materials

6.3.1. Participants

For the experiment, 43 right-handed volunteers (29 female; age 19–29) were recruited and randomly assigned to one of the two groups (DGC or TCG). Excluding criteria were assessed by self-report. Participants who reported past or present psychiatric disorders, use of antipsychotic drugs, regular alcohol or drug consumption, or allochromasia (for blue and yellow) were not included in the analysis. Twelve subjects had to be excluded due to technical problems, one subject due to regular drug consumption and one subject because of extensive head movements during scanning. Since only three subjects did not become aware of the CS/US contingency, these participants were also excluded. The small group size did not allow for an analysis of differences related to contingency awareness. The final sample consisted of 26 participants, 13 in the DCG (8 female, mean age = 23.1 years, SD = 3.0 years) and 13 in the TCG (9 female, mean age = 23.5 years, SD = 2.5 years). All participants gave their written informed consent and received 12 Euros per hour for participation. The study protocol was approved by the Ethics Committee of the Medical Faculty of the University of Würzburg.

6.3.2. Stimuli and apparatus

VR environment. In this experiment, the same virtual environment was applied as in the pilot study. In the office, the blue and the yellow light served as CS+ and CS-, respectively *(see figure 3,* chapter 4.3.2., page 54*).* The US, again a mild electric stimulus, was paired with the CS+ with 100% contingency, the CS− was never followed by the US. Colors of CS+ and CS− were counterbalanced across participants and conditioning groups. The delay (DCP) and trace conditioning protocol (TCP) differed in timing of the CS: In the delay protocol the lights were switched on for 8s and the CS+ coterminated with presentation of the US. In the trace protocol the lights were presented only for 4s, meaning that the CS+ was followed by a 4s trace interval. The US was presented 8s after onset of the CS+ in both protocols (*see figure 15*).
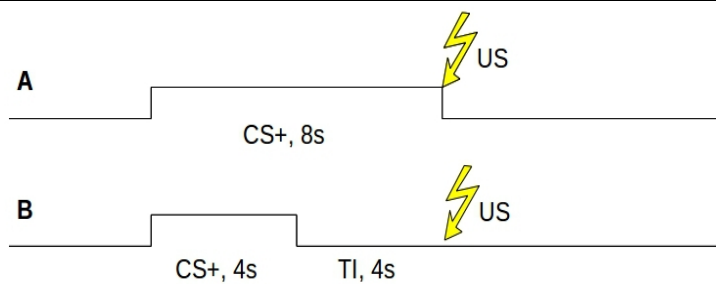
Figure 15

A, DCP: The CS+ is presented for 8s and coterminates with the onset of the US

B, TCP: The CS+ is presented for 4s, followed by a 4s trace interval (TI) before presentation of the US

Participants were guided through the virtual environment on a prerecorded path in order to enhance control over the course of events during the experiment, but also to prevent strong movement in the scanner. Events in the virtual environment were – as in the former experiments – manipulated by means of the in-house written VR simulation software CyberSession. VR rendering was done by an image generator running the in-house written Source SDK modification VRSessionMod 0.3. The virtual environment was displayed via MRI-compatible goggles (VisuaStim; Magnetic Resonance Technologies, Northridge, CA, USA).

Electric stimuli. The US was a mildly painful electric stimulus generated by a current stimulator (Digitimer DS7A, Digitimer Ltd, Hertfordshire, England). It was delivered at the left index finger through surface bar electrodes. Electrodes consisted of two durable gold-pasted stainless steel disk electrodes with 9mm diameter, 30mm spacing and with an impedance of max 5 kΩ. Electric stimuli were triggered automatically by CyberSession for a duration of 200 ms and with a frequency of 50 Hz. Before conditioning, current intensity was determined for each participant according to the individual pain threshold (for a detailed description see chapter 4.3.2., page 55). Both conditioning groups did not differ in current intensity (delay group: M = 2.25 mA, SD = 0.99; trace group:M = 2.18, SD = 0.90), t(23) = 0.19, p = 0.853, and pain rating (delay group: M = 5.00, SD = 0.84; trace group: M = 5.04, SD = 1.57), t(23) = −0.08, p = 0.934, of the US.

6.3.3. Psychometric measures

Ratings. Participants rated screenshots of the room with either the CS+ or the CS− switched on regarding valence (from "very negative" to "very positive"), arousal (from "not arousing at all" to "very arousing"), fear (from "no fear" to "extreme fear") and CS/US contingency (from "not likely at all" to "very likely"), each on scales from 0 to 100. In this study, all questions and screenshots were presented via the MRI-compatible goggles. Participants were supposed to relate their answers to the way they felt during the last phase of the experiment. The investigator recorded the answers, which were given orally via the speaker system of the scanner room.

Awareness. On the basis of the question "During which light presentation did you receive electric shocks?" I assessed explicit knowledge of CS/US contingencies. Twenty-six participants met the criteria of awareness and were labelled "aware", meaning that they were able to state the correct color of light after the second acquisition run. Three

participants in the DCG failed to learn the contingency and were labeled "unaware." There were no unaware participants in the TCG.

6.4. Procedure

As a first step participants were informed about the scanning procedure. After that they completed questionnaires on personal information and excluding criteria and received written instructions related to the experiment. They gave their written informed consent after having read these instructions. When participants were in the scanner room, they were brought into position for scanning and electrodes for electric stimulation were attached to the left index finger. Before the experiment startet, the individual pain threshold was determined.

After preparations were completed, a preacquisition block followed. Participants were guided through the virtual office to get used to the environment and the two different lights (each light was presented once). The first ratings of valence, arousal and fear were collected at this point. Before conditioning started, participants were informed by the investigator that they would be able to predict the electric stimuli if they paid close attention to the experiment. The following experiment consisted of three blocks, two acquisition blocks and one extinction block. Each of these blocks consisted of two passages through the virtual office. Participants were exposed to four CS+ and four CS− during one passage, which lasted 172s. This means that one block lastet approximately 6 min. During both acquisition blocks taken together, participants were exposed to 16 CS+ and 16 CS− and they received 16 US. The extinction block included two visits to the office with the same duration and CS frequency as the acquisition trials (i.e., 8 CS+ and 8 CS−). No US was applied during extinction. After each of the two acquisition blocks, awareness was

measured as described above and CS/US contingencies were measured. Additionally, ratings of valence, arousal and fear were collected after each acquisition block and after extinction.

Whether the blue or the yellow light served as CS+ or CS-, respectively, was pseudo-randomized across participants. The same was true for the order of stimuli. There were four different courses of events, two of them with the blue light and two with the yellow light serving as CS+. The length of the interstimulus interval (ISI) varied between 11 and 13 s in steps of 250 ms was also pseudo randomized.

Magnetic resonance imaging: For acquisition of structural and functional brain images, a 1.5-T whole-body magnetic resonance tomograph (MagnetomAvanto, SiemensHealthcare, Erlangen, Germany) with standard 12-channel head coil and integrated head holder was used. Recording of structural images was conducted at the end of the experiment. Structural imaging consisted of 160 T1-weighted sagittal magnetization-prepared rapid gradient-echo imaging (MPRAGE) 3D MRI sequence (MPRAGE, 1 mm slice thickness, TR = 2250 ms, TE = 3.93 ms, flip angle: 8°, FOV: 256 mm, matrix: 256 × 256, voxel size: 1 × 1 × 1 mm³).

Functional imaging was conducted in all of the four phases of the experiment (pre-acquisition, first and second acquisition phase and extinction). After every phase, subjective ratings were collected. Imaging was paused in the meantime. For functional imaging, a total of 161 volumes was registered using a T*2-weighted gradient echo-planar imaging sequence (EPI) with 25 axial slices (slice thickness 5-mm with 1-mm gap, interleaved (descending) order) covering the whole brain (TR: 2500 ms; TE: 40 ms; flip angle: 90°; FOV: 240 mm × 240 mm; matrix size: 64 × 64; voxel size: 3.1 × 3.1 × 3mm³).

Axial slices were orientated parallel to the AC-PC line. In each of the four phases, the first eight images were excluded from analysis to allow for T1 equilibration.

Image preprocessing and statistical analysis

Imaging. Analysis of fMRI data was performed with Statistical Parametric Mapping (SPM8, Wellcome Department of Cognitive Neurology, London) integraded in MatLab 7.0 (Mathworks Inc., Sherborn, MA). In image preprocessing, functional images were realigned after slice time correction. T1-scans were co-registered to each participant´s mean of the realigned images. Then the mean functional images were normalized to the Montreal Neurological Institute (MNI) single-subject template (Evans et al. 1992). Normalization parameters (attained from the previous segmentation procedure of co-registered T1 images) were applied and images were resampled (voxel size 2 × 2 × 2 mm³). For spatial smoothing of EPI images, an 8-mm full-width-half-maximum (FWHM) Gaussian kernel was applied and images were filtered with a 128 ms high pass filter.

I modeled the different experimental conditions using a boxcar reference vector convolved with a canonical hemodynamic response function (general linear model, Kiebel and Holmes, 2003). To regard variance caused by residual movement I included the six movement parameters of the rigid body transformation, applied by the realignment procedure. By means of a first-order autoregressive model, low-frequency signal drift was filtered. For the calculation of parameter estimates for each voxel, weighted least squares were used to provide maximum likelihood estimates based on the non-sphericity assumption (in order to get identical and independently distributed error terms).

In this experiment the extinction phase served as main test phase, since I was especially interested in neural correlates of the extinction of fear memory. Moreover, not only a steep learning curve, but also a rapid decrease of fear reactions is to be expected in a

conditioning paradigm with a CS-US contingency of 100% during acquisition. To account for this, the extinction phase was divided into two parts of equal duration and an equal number of stimuli. The first and second half of extinction were analyzed separately. Additionally, I compared activation during early extinction (first to fourth CS+) with activation during late extinction (fifth to eighth CS+).

In the second-level analysis, first level individual contrast images (CS+ > CS-) were used (one sample *t*-test). First the contrast CS+ > CS- was analyzed separately for the DCG and the TCG in both early and late extinction. Subsequently, I analyzed the contrast early extinction (CS+ > CS-) > late extinction (CS+ > CS-) for both groups. For the amygdala, the hippocampus, the insula, the ACC (Brodmann areas 24, 32 and 33) the striatum (caudate and putamen) and the ventromedial (medial orbital frontal gyrus) and dorsolateral prefrontal cortices (middle frontal gyrus) ROI analyses were carried out at an uncorrected threshold of p = .005 and with a minimum cluster size of 10 voxel. I also conducted an explorative whole brain analysis at an uncorrected threshold of p = .001 and with a minimum cluster size of 5 voxel. ROIs were based on masks of the WFU Pick Atlas (Maldjian et al 2003) and Brodmann Areas (BA).

Ratings. For the preacquisition and the extinction phase, ratings of valence, arousal, and fear were analyzed with ANOVAS with between factors stimulus (CS+, CS-) and group (delay, trace). Ratings collected after the first and second acquisition phase were analyzed with repeated measures ANOVAs with the between factors stimulus (CS+, CS-) and group (delay, trace) and the additional within factor phase (Acquisition 1, Acquisition 2). Contingency ratings were not collected after preacquisition and thus were only analyzed for acquisition and extinction (according to valence, arousal and fear ratings).

All rating data were analyzed using SPSS for Windows (Release 17.0). Alpha was set at .05 for all statistical tests, effect sizes are reported as η_p^2 scores.

6.5. Results

Ratings

Baseline.

CS+ and CS- did not differ in valence, arousal, or fear in either of the groups after the pre-acquisition phase (all ps > .23).

Acquisition.

Valence ratings. For valence ratings, I found a significant main effect of stimulus, ($F(1, 24) = 13.49$, $p = .001$, $\eta_p^2 = .360$) and a marginally significant interaction of Phase × Stimulus ($F(1, 24) = 3.92$, $p = .059$, $\eta_p^2 = .140$). The CS+ was rated more negative than the CS- in general (CS+: M = 36.83, SD = 20.49; CS-: M = 65.38, SD = 27.88).

Arousal rating. As for valence ratings the main effect of stimulus ($F(1, 24) = 33.05$, p < .001, $\eta_p^2 = .579$), reached significance. Additionally, the analysis revealed a significant three way interaction of Phase x Stimulus x Group ($F(1, 24) = 4.30$, p = .049, $\eta_p^2 = .152$). Post hoc *t*-Tests showed that the CS+ elicited more arousal than the CS- in both the delay group [$t(12) = 2.578$, p = .024 (CS+: M = 40.38, SD = 25.70; CS-: M = 14.62, SD = 19.84)], and the trace group, [$t(12) = 5.41$, p < .001 (CS+: M = 51.15, SD = 28.88; CS-: M = 8.46, SD = 9.87)] after the first acquisition phase. Results were similar after the second acquisition phase in the trace group [$t(12) = 4.38$, p = .001 (CS+: M = 40.77, SD = 26.91; CS-: M = 6.92, SD = 9.47)) and even more pronounced in the delay group [$t(12) = 3.534$, p = .004, (CS+: M = 43.46, SD = 27.03; CS-: M = 9.62, SD = 18.76)].

Fear ratings. For fear ratings I also found a significant main effect of stimulus ($F(1, 24) = 22.32$, $p < .000$, $\eta_p^2 = .482$), indicating that the CS+ elicited overall more fear than the CS- (CS+: $M = 36.25$, $SD = 30.40$; CS-: $M = 5.58$, $SD = 11.57$).

Contingency ratings. After the acquisition, participants rated the CS+ as considerably more likely to be followed by the US than the CS-, the main effect of stimulus was highly significant [$F(1, 24) = 201.45$, $p < .001$, $\eta_p^2 = .894$ (CS+: $M = 88.65$, $SD = 18.72$; CS-: $M = 9.62$, $SD = 14.69$)]. The interaction of Phase x Stimulus also reached significance ($F(1, 24) = 5.66$, $p = .026$, $\eta_p^2 = .191$). Post hoc *t*-Tests revealed that the CS+ was rated as more likely to be followed by the US than the CS- [$t(25) = 6.681$, $p < .001$ (CS+: $M = 81.73$, $SD = 29.29$; CS-: $M = 15.00$, $SD = 27.75$)] already after the first phase, but after the second phase this difference between CS+ and CS- had further increased [$t(25) = 26.239$, $p < .001$ (CS+: $M = 95.58$, $SD = 14.45$; CS-: $M = 4.23$, $SD = 11.38$)]. In general, the contingency between the CS+ and the US was rated higher after the second than after the first phase ($t(25) = -2.612$, $p = .015$).

Extinction

Valence ratings. Valence of CS+ and tCS- did no differ significantly any longer after extinction ($F(1, 24) = 3.48$, $p = .075$, $\eta_p^2 = .127$), although a marginal difference was still present.

Arousal ratings. I still found a significant main effect of stimulus ($F(1, 24) = 20.30$, $p < .000$, $\eta_p^2 = .458$) as well as a significant interaction of Stimulus x Group ($F(1, 24) = 5.26$, $p = .050$, $\eta_p^2 = .151$) after extinction. In the DCG, CS+ and CS- did not differ significantly any longer. In contrast, the TCG still rated the CS+ as more arousing than the CS- [$t(12) = 4.368$, $p = .001$ (CS+: $M = 21.76$, $SD = 6.03$; CS-: $M = 10.05$, $SD = 2.91$)].

Fear ratings. Also the main effect of stimulus still reached significance after the extinction phase ($F(1, 24) = 11.61$, $p = .002$, $\eta_p^2 = .326$). Additionally, I found a marginal interaction of Stimulus x Group ($F(1, 24) = 3.76$, $p = .064$, $\eta_p^2 = .136$), indicating similar results as for arousal ratings: The CS+ was still associated with more fear than the CS- after extinction [$t(12) = 3.726$, $p = .003$ (CS+: $M = 25.77$, $SD = 21.39$; CS-: $M = 6.15$, $SD = 10.44$)] only in the TCG. There was no such difference in the DCG ($t(12) = 1.054$, $p = .313$).

Contingency ratings. The main effect of stimulus persisted during the extinction phase also for contingency ratings ($F(1, 24) = 18.39$, $p < .001$, $\eta_p^2 = .434$). The CS+ was still rated as more likely to be followed by the US than the CS- (CS+: $M = 47.31$, $SD = 35.98$; CS-: $M = 12.31$, $SD = 20.06$).

Taken together, results of fear and arousal ratings suggest that extinction learning proceeded more slowly in the TCG than in the DCG. After extinction, fear and arousal ratings of the DCG did no longer differ between CS+ and CS-. In contrast, the TCG rated the CS+ still as more arousing and more frightening than the CS-.

Imaging data

<u>Early extinction</u>

ROI analysis: During early extinction, both the DCG and the TCG showed activation in the insula and the striatum in the contrast CS+ minus CS-. Importantly, the groups differed in their prefrontal activation *(see figure 16)*. Analyses revealed significant activation of the vmPFC (medial orbital frontal gyrus R) in the DCG, while in the TCG the dlPFC was significantly activated (middle frontal gyrus R). Additionally, the TCG showed activation of the dorsal part of the ACC (BA 33).

Whole brain analysis: Next to activation in areas defined as ROIs the whole brain analysis revealed significant activation in several other regions. The cuneus (L), the left motor cortex (precentral gyrus L), and the middle occipital gyrus (R) were activated in the DCG. In the TCG, analysis revealed activations in the somatosensory cortex (postcentral gyrus L), the calcarine (R), the rolandic operculum (R), and the ventral ACC (middle cingulate cortex L, BA 24).

For exact coordinates see *table 1*.

Table 1
Significant activations during early extinction in whole brain (WB) and regions of interest analysis (ROI) for contrast CS+ > CS-

Group	Brain structure	x	y	z	Z	Cluster size	p
Delay	Cuneus R (WB)	12	-76	24	3,7	29	**< 0.001**
	Precentral gyrus (WB)	-22	-14	62	3,67	26	**< 0.001**
	Caudate body (WB)	-18	20	8	3,67	7	**< 0.001**
	Medial orbital frontal gyrus R / BA 10 (WB)	12	58	-12	3,53	5	**< 0.001**
	Middle occipital gyrus (WB)	-34	-66	18	3,43	7	**< 0.001**
	Insula R (ROI)	44	2	0	3,01	10	**< 0.001**
	Caudate L (ROI)	-18	20	8	3,53	17	**< 0.001**
	Putamen R (ROI)	36	-12	-8	3,07	14	**< 0.001**
	Medial orbital frontal gyrus R (ROI)	12	58	-12	3,43	11	**< 0.001**
Trace	Postcentral gyrus L (WB)	-42	-32	54	4,70	6	**< 0.001**
	Rolandic Operculum R (WB)	42	-22	26	4,37	86	**< 0.001**
	Putamen L (WB)	-30	-14	2	4,11	8	**< 0.001**
	Calcarine R (WB)	12	-92	12	4,09	19	**< 0.001**
	Middle frontal gyrus R (WB)	40	6	40	3,55	7	**< 0.001**
	Ventral ACC L (WB)	-12	10	30	3,55	8	**< 0.001**
	Insula R (ROI)	36	-18	22	3,78	15	**< 0.001**
	Dorsal ACC R (ROI)	4	22	34	2,93	11	**0.002**
	Putamen L (ROI)	-30	-14	2	4,11	14	**< 0.001**
	Middle frontal gyrus R (ROI)	40	6	40	3,55	12	**< 0.001**

alpha < 0.001 uncorrected for WB analysis, alpha < 0.005 for uncorrected ROI analysis minimum cluster size k = 5 (WB) or k = 10 (ROI); L = left and R = right hemisphere

The cluster with the largest number of significant voxels within each region is reported. Coordinates x,y and z of peak voxels are given in Montreal Neurological Institute Space.

Late extinction

ROI analysis: In the second phase of extinction I observed significant activation in the ventral part of the ACC, though only in the DCG.

Whole brain analysis: The ventral ACC (R), the inferior frontal gyrus (R), and the supramarginal gyrus (R) were activated in the DCG during late extinction. In the TCG I found significant activation of the precuneus (both L and R).

For exact coordinates see _table 2_.

Group	Brain structure	x	y	z	Z	Cluster size	p
Delay	Ventral ACC R (WB)	6	10	30	4,84	13	**< 0.001**
	Triangular part of inferior frontal gyrus R (WB)50	50	18	14	3,67	35	**< 0.001**
	Supramarginal gyrus R (WB)	60	-34	28	3,66	25	**< 0.001**
	Ventral ACC R (ROI)	6	10	30	4,84	17	**< 0.001**
Trace	Precuneus R (WB)	14	-58	24	3,74	62	**< 0.001**
	Precuneus L (WB)	-10	-62	30	3,37	10	**< 0.001**
	ROI analysis: no significant voxel						

Table 2
Significant activations during late extinction in whole brain (WB) and regions of interest analysis (ROI) for contrast CS+ > CS-

alpha < 0.001 uncorrected for WB analysis, alpha < 0.005 for uncorrected ROI analysis minimum cluster size k = 5 (WB) or k = 10 (ROI); L = left and R = right hemisphere

The cluster with the largest number of significant voxels within each region is reported. Coordinates x,y and z of peak voxels are given in Montreal Neurological Institute Space.

Early extinction > late extinction

ROI analysis: In an additional analysis I examined in which areas activation was stronger in the early extinction compared to the late extinction (in the contrast CS+ > CS+). In the DCG, activation in the insula (L) was stronger in the early than in the late extinction,

whereas in the TCG the hippocampus (R) and the striatum (putamen L) showed greater activation.

Whole brain analysis: Whole brain analysis revealed additional activation of the ACC (ventral anterior cingulate area), the precentral gyrus (L), and the transverse temporal gyrus (Heschl L) in the DCG. In the TCG I also found activation of the ventral ACC (L), and additionally in the parahippocampal area.

For exact coordinates see table 3.

Table 3							
Significant activations in whole brain (WB) and regions of interest analysis (ROI) for contrast CS+ > CS- : Early extinction (CS+ > CS-) > late extinction (CS+ > CS-)							
Group	**Brain structure**	**x**	**y**	**z**	**Z**	**Cluster size**	**p**
Delay	Precentral gyrus L (WB)	-22	-14	62	4,10	59	**< 0.001**
	Ventral ACC L (WB)	-16	0	44	3,65	14	**< 0.001**
	Heschl L (WB)	-32	-28	16	3,29	5	**< 0.001**
	Insula L (ROI)	-38	-20	14	3,05	25	**< 0.001**
Trace	Putamen L (WB)	-30	-14	2	3,74	13	**< 0.001**
	Ventral ACC L (WB)	-10	14	30	3,61	10	**< 0.001**
	Parahippocampus R (WB)	32	-34	-12	3,51	11	**< 0.001**
	Hippocampus R (ROI)	30	-32	-8	3,87	11	**< 0.001**
	Putamen L (ROI)	-30	-14	2	3,74	23	**< 0.001**
alpha < 0.001 uncorrected for WB analysis, alpha < 0.005 for uncorrected ROI analysis minimum cluster size k = 5 (WB) or k = 10 (ROI); L = left and R = right hemisphere							
The cluster with the largest number of significant voxels within each region is reported. Coordinates x,y and z of peak voxels are given in Montreal Neurological Institute Space.							

6.6. Discussion

To my knowledge, this is the first study investigating neural correlates of extinction after acquisition of both delay and trace fear memory in humans. During early extinction, the two conditioning groups showed common activation in the insular cortex and the striatum, but more importantly they differed in prefrontal activation. In line with existing evidence on extinction of delay fear conditioning, the vmPFC was activated during extinction in the DCG. In contrast, I observed activation of the dlPFC in the TCG. In the late part of extinction I only found significant activation of the ventral ACC in the DCG. In the predefined ROIs no significant activation could be found in the TCG. Moreover, activation in the insula (delay group), the hippocampus, and the striatum (trace group) was greater during early extinction compared to late extinction.

Prefrontal cortex. The most important finding in the present study is the dissociation of prefrontal activation during early extinction in delay vs. trace conditioning. The vmPFC has been shown to play an important role in the extinction of fear memory. It is assumed that during extinction an inhibitory memory trace is established between the vmPFC and the amygdala, which allows for the modulation of the fear response. This has been shown the animal model (see for example Sotres-Bayon et al., 2004, 2007), but also been confirmed in human fear conditioning studies (e.g., Phelps et al., 2004). To my knowledge, evidence from human studies for this model comes mainly from delay fear conditioning. Activation of the vmPFC during early extinction of delay fear memory in the present sample provides further evidence for its involvement in inhibition of the conditioned fear response. Interestingly, I did not find activation of the vmPFC in the TCG, but instead activation of the dlPFC. This finding points to different processes during extinction in delay

and trace conditioning. According to a model of functional organization of the lateral PFC, the vmPFC is mainly involved in the mere maintenance of information, whereas the dorsal part is assumed to be involved in the manipulation of information, requiring more working memory capacities (D'Esposito et al., 1999). As mentioned in the introduction, lesion studies provide evidence that the dlPFC is crucial for adjusting behavior appropriately in delayed response tasks (e.g., D'Esposito et al., 2000). In a delayed response task, decisions have to be made on the basis of this information, which had to be kept in working memory for a short period of time. In contrast to delay conditioning, trace conditioning and its extinction afford higher working memory contributions to bridge the trace interval and hold information in short term memory. Results of subjective ratings in this study indicate that extinction learning preceded more slowly in the TCG compared to the DCG. Arousal and fear ratings of the CS+ and the CS− did no longer differ after extinction in the DCG. However, in the TCG, the CS+ was still rated as more arousing and more frightening than the CS−. The slower extinction process after trace conditioning might be an indicatior for a higher working memory contribution in the extinction of trace compared to delay conditioning. Besides possible parallels with evidence regarding the role of the dlPFC in working memory processes, there is also an interesting connection between my results and findings in trace eyeblink conditioning in rabbits. According to Weiss and Disterhoft (2011), the dlPFC plays an important role in the acquisition of trace conditioning. More precisely, they showed that activation of dlPFC and hippocampus potentiates the effect of the CS at pontine nuclei on the way to the cerebellum and by this means bridges the trace interval during acquisition. Structures mediating the conditioned response reorganize after consolidation of the association between CS und US. While the hippocampus becomes less important, the dlPFC becomes more important. For

investigating to what extend these findings from the rabbit model can be transferred to human fear conditioning, further research with regard to both acquisition and extinction of trace conditioning is necessary.
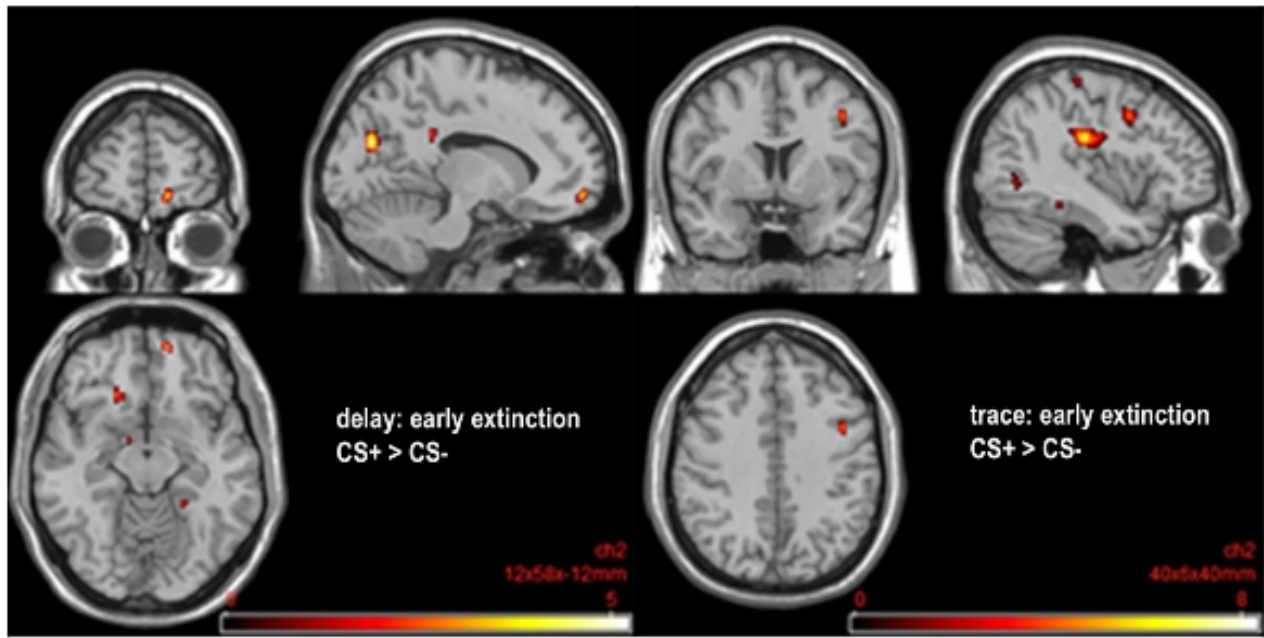
Figure 16: BOLD Signal(CS+ > CS−) during early extinction (ROI, α < 0.005, uncorrected). In both DCG and TCG, Insula and Putamen were activated during early extinction. In the DCG, significant activation of the vmPFC (medial orbitalfrontal gyrus R) was observed, while in the TCG the dlPFC (middle frontal gyrus R) was significantly activated.

Striatum. During acquisition, an initially neutral stimulus is paired with an aversive event such as an electric stimulus. Consequently, an individual forms the expectation that the aversive event follows the stimulus. This expectation is violated during extinction, because the aversive event does no longer occur. Such a discrepancy between the expected and the actual outcome is referred to as prediction error (Schultz, Dayan & Montague, 1997). In both appetitive and aversive classical and instrumental conditioning, the striatum has been shown to be involved in the coding of prediction errors. This is true for both primary reinforcers such as pain (Phelps et al., 2004; Seymour, O'Doherty,

Koltzenburg, Wiech, Frackowiak & Friston, 2005), and also for secondary reinforcers ones such as monetary gains (e.g., Delgado et al., 2007). In the present study, striatal activation was significant in both the DCG and the TCG. These results provide evidence for an important role of the striatum not only in the acquistion (Jensen et al., 2003; Delgado et al., 2008; Klucken et al., 2009; Tabbert et al., 2011), but also in the extinction of fear memory. Raczka et al. (2011) showed that a functional polymorphism of the dopamine transporter gene, which is mainly expressed in the striatum, has an influence extinction learning. The 9-repeat allele is associated with enhanced phasic dopamine release and higher learning rates in the extinction of conditioned fear. They reported that 9R carriers showed stronger activation of the ventral striatum in response to prediction errors during extinction. Because of these findings they proposed that extinction, rather than a learning process driven by an aversive prediction error, is an appetitive-like learning process mediated by the mesostriatal dopamine system.

Insula and ACC. During early extinction, I found significant activation of the insular cortex in both conditioning groups. Additionally, though only in the DCG, insular activation was greater in the early compared to the late extinction. Phelps et al. (2001) provided evidence for the involvement of the insula in classical fear conditioning. According to them, insular activation in the conditioning paradigm occurred not until the later trials of acquisition, when participants were consciously aware of the association between the CS+ and the US. In contrast, in an instructed fear paradigm, they found activation of the insula already in early trials. This is in line with evidence coming from pain research, in which the insula has been found to play an important role in the anticipation of pain (e.g., Ploghaus et al., 1999; Wiech, Brodersen, Bingel, Ploner & Tracey, 2010). Phelps et al. (2004) suggested that the anticipation of pain leads to cortical representation of fear, and that this

representation is transmitted to the amygdala via the insular cortex. During extinction, the CS+ is no longer paired with a painful stimulus. However, particularly in the first trials of extinction, it is still associated with the US and thus it still leads to the anticipation of pain. In addition to the insula, the dorsal ACC was activated during early extinction. Interestingly, this was only the case in the TCG, but not in the DCG. In the comparison of early and late extinction, I found that ACC activation was stronger during early extinction. This could be observed in both groups. Coghill et al. (1994) discussed that the combined activation of ACC and insula represents a pathway for the integration of nociceptive input in memory processes. Following this model, not only the insula, but also the ACC is involved in the adjustment of behavior in response to a stimulus predicting pain (Büchel et al., 1998). There is broad evidence that sustained attention is essential for trace fear conditioning, though not for delay conditioning. Without contingency awareness, the formation of a conditioned fear response is not possible in trace conditioning. For delay conditioning, participants do not necessarily have to establish declarative memory of the association between the CS and the US (e.g., Manns, Clark & Squire, 2001; Clark et al., 2002; Weike et al., 2007). In a conditioning study conducted by Yágüez et al. (2005), neural correlates of actual and anticipated visceral pain were investigated. They report that the ACC was activated both during the learning phase as well as during anticipation and extinction phases. They suggest that ACC activation might be dependent on sustained attention toward the stimulus followed by pain. Han et al. (2003) provided additional evidence for the association of ACC activation and sustained attention during trace fear conditioning. They reported that attention-distracting stimuli interfere only with trace, but not delay or contextual fear conditioning in mice. Moreover, in the ACC of mice that had undergone trace fear conditioning, a higher density of c-fos-positive cells was found than in mice that

had undergone delay conditioning. Additionally, they observed that lesions of the ACC selectively impaired trace conditioning. These results offer an explanation why we found combined activation of the insula and the ACC only during the early extinction of trace but not delay fear memory.

Hippocampus. The hippocampus is assumed to be involved in the representation of the temporal context in a conditioning process. Especially in trace conditioning paradigms this context plays an important role, due to the temporal gap between CS and US (e.g., Phillips & LeDoux, 1992). Accordingly, I found hippocampal activation only in the TCG. Knight et al. (2004) reported a rapid decrease of hippocampal activation during the early trials of extinction, matching the fact that in this sample, hippocampal activation was significantly greater in the early compared to the late extinction. Clark et al. (2002) stated that the hippocampus is crucial for explicit or declarative memory processes. There is vast evidence that trace conditioning is not possible without contingency awareness and requires declarative knowledge about the CS/US association (e.g. Clark et al., 2002; Weike et al., 2007). Unfortunately, I could not investigate the association between contingency awareness and hippocampal activity directly, since only three participants remained unaware.

Amydala. Against my expectation, I did not find significant activation of the amygdala in either one of the two groups. Echoplanar imaging is highly vulnerable to susceptibility artifacts, which occur near the interfaces of substance of different magnetic susceptibility and thus are likely in structures of the medial temporal lobe, like the amygdala (Bellgowan, Bandettini, van Gelderen, Martin & Bodurka, 2006; Stöcker et al., 2006). LaBar et al. (1998) address this problem. The amygdala is a small structure located near sinus cavities, which cause susceptibility artifacts. This might have been one reason

for the lack of significance. Additionally, I applied a conditioning paradigm with 100% contingency between CS+ and US during acquisition. This has been shown to lead to rapid habituation of amygdala activity during extinction learning, making it harder to detect this acticvity (e.g., LaBar et al., 1998).

    Conclusion. In summary, these results are in line with existing evidence of the involvement of the PFC, insula, ACC, striatum, and hippocampus in the extinction of conditioned fear memory. Additionally, my findings also confirm that the hippocampus and ACC are mainly involved in trace conditioning. Most importantly, different parts of the PFC were activated during extinction of delay vs. trace fear conditioning: the vmPFC in the DCG and the dlPFC in the TCG. These results point to different processes underlying the extinction of he two types of conditioning. However, results have to be interpreted with care because of limited power. Moreover, a relatively liberal level of significance was applied. Nevertheless, results provide valuable input for the discussion about the role of the PFC in the extinction of fear. Further evidence is needed to elucidate the role of the PFC in the extinction of trace conditioning in more detail and to translate results from the animal model to trace fear conditioning in humans.

7. General discussion

The intention of this work was to study fear conditioning in a virtual reality setting close to real life learning situations, in which explicit learning of associations between threatening stimuli and aversive events was manipulated by the complexity of the environment. By placing participants into a virtual environment, different contexts can be simulated and manipulated easily, allowing for a translation of animal research in contextual conditioning to humans. The virtual reality paradigm can also be applied in imaging studies, which led to new possibilities in this field of research. I planned to test the paradigm and its effects on contingency awareness in a first pilot study, followed by the extension of the paradigm for studying context-dependent differential fear conditioning and generalization of fear in a second study. Additionally, the paradigm was applied in an imaging study using fMRI, in which I focused on neural correlates of extinction learning with regard to timing of stimuli.

7.1. Contingency awareness

Several interesting findings result from the three experiments. First of all, they provide further evidence for the two level account of classical fear conditioning (Hamm & Weike, 2005), which assumes that fear learning can take place on two different levels: For the development of a conditioned response, an implicit, unconscious and automatic processing of the CS via the subcortical fear network consisting of the amygdala complex and the brainstem can be sufficient. However, humans usually also develop explicit knowledge of the association between CS and US, referred to as contingency awareness. This kind of learning requires higher order processing. Tabbert et al. (2011) investigated

135

the influence of contingency awareness on neural, electrodermal and evaluative fear responses. They found amygdala acitvation in both aware and unaware subjects, but differential responses in the dorsal anterior cingulate, insula and ventral striatum were only present in aware subjects. The hippocampus was identified as especially important for the development of contingency awareness. Only aware subjects showed conditioned subjective and electrodermal responses. Thus, fear conditioning takes place irrespective of contingency awareness, but aware and unaware participants display different neural and physiological responses (see also Cacciaglia, Pohlack, Flor & Nees, 2014).

Hamm and Vaitl (1996) showed that FPS responses were completely unrelated to contingency awareness, whereas enhanced skin conductance in response to the CS+ was only present in participants who had acquired a cognitive representation of the CS-UCS contingencies and were able to report them. In contrast to the startle reflex, skin conductance response is associated with higher order processing, for example with prefrontal activations (Hamm & Weike,

Figure 17: *reprinted from Hamm and Vaitl (1996); copyright 1996 by Cambridge University Press*

2005) *(see figure 17).* This dissociation between startle response and skin conductance response has frequently been replicated until today (e.g. Weike, Schupp & Hamm 2007, Singh, Dawson, Schell, Courtney & Payne, 2013; Sevenster, Beckers & Kindt, 2014). In both my first and second study I found evidence for a dissociation between implicit and

explicit measures in unaware participants, i. e. between startle response and subjective ratings: Unaware participants did not differentiate between the fear and the safety stimulus in subjective ratings, only participants who explicitly learned the CS-US association rated the CS+ as more anxiety-inducing than the CS-. Evidence for evaluative conditioning taking place as a function of contingency awareness comes also from many other studies (e.g. Dawson et al., 2007; Pleyers et al., 2007; Pleyers et al., 2009; Tabbert et al., 2011; Hütter & Sweldens, 2013). However, both aware and unaware participants reacted with an enhanced startle response to the fear stimulus compared to the safety stimulus. Since the FPS reflex is modulated by cubcortical structures via the brainstem (LeDoux, 2000) and does not depend on cortical input, it is often consulted as an implicit measure of fear, anxiety or enhanced arousal. The results point to the production of a conditioned response without contingency awareness. Although, in the second study, I found an effect of awareness on the startle response in the later part of acquisition: In the unaware group I did no longer find a significantly enhanced startle response to the CS+ compared to the CS-. These results regarding contingecny awareness being to some point conflicting is reflected in the ongoing debate about whether awareness is necessary for establishing a conditioned response or not. Findings concerning the actual learning processes in fear conditioning are ambiguous regarding the role of awareness. Next to the vast evidence for a two level account reflected by a dissociation of subjective ratings and FPS responses (e.g. Weike et al., 2007; Tabbert et al., 2011; Sevenster et al., 2014), there are also results indicating that even an implicit CR cannot be found without explicit knowledge of CS/UCS contingencies (Dawson et al., 2007; Klucken et al., 2009, Grillon, 2002a; Purkis & Lipp, 2001). However, recent evidence mainly supports a dual process theory of classical conditioning, assuming that conditioning on an implicit level can occur without explicit

contingency awareness. In the second study, a differential startle reaction and thus an implicit conditioned response had already been present at the beginning of acquisition in the unaware group. I suspect that my results regarding the influence of contingency awareness on differential cue conditioning in the later part of extinction are a consequence of the high cognitive load due to a complex conditioning paradigm. For example Carter et al. (2003) showed that the higher the cognitive load during conditioning, the more contingency awareness is necessary for successful conditioning and the production of a CR. To further contribute to the debate about the role of contingency awareness in the acquisition of fear, it would be enlightening to investigate the interplay of cognitive load, contingency awareness and development of a conditioned response.

7.2. Delay and trace fear conditioning

Apparently, contingency awareness is not necessary for fear conditioned startle potentiation. However, this is not true for all types of fear conditioning. Above mentioned evidence is built on studies using delay fear conditioning, where the aversive stimulus usually coterminates with the CS+. In trace conditioning, there is a short temporal gap between CS and US, meaning that individuals have to keep the representation of the CS in short-term memory to bridge this gap. Active processing of the stimulus is required for this, which is supported by findings indicating that trace conditioning is not possible without contingency awareness. Weike at al. (2007) compared delay and trace fear conditioning in detail. Next to replicating previous findings regarding startle and skin conductance responses in delay conditioning, they found that FPS was only present in participants who were aware of the CS–UCS contingency in trace conditioning.

This difference between delay and trace conditioning are reflected in neural structures underlying fear acquisition. As mentioned above, a subcortical fear network consisting of the sensory cortices, thalamus, amygdala and brainstem is sufficient for successful delay fear conditioning. During trace conditioning however, declarative memory is formed, which is associated with hippocampus activity. Since neural structures involved in the acquisition of fear in both delay and trace conditioning as well as the development of contingency awareness are relatively well understood, I focused on the extinction of fear after both types of learning in more detail. Although I planned to do so I unfortunately could not evaluate neural mechanisms of extinction as a function of contingency awareness, because only three participants did not explicitly learn the CS-US association.

This is interesting on itself, since I applied the same paradigm I used in the pilot study, in which about one third of participants remained unaware. A common problem in human fear conditioning paradigms is that unconditioned stimuli are usually much less intense than in animal research, because participants get the chance to determine the averseness of for example an electric shock themselves. I tried to control this by increasing the intensity of current chosen by participants by one third after the electric stimulus was individually adjusted, but of course the US was nowhere as aversive as stimuli in animal fear conditioning. Moreover, participants choose freely to take part in an experiment: They know that nothing bad is going to happen to them and that they can stop the experiment at any time. An animal in a fear conditioning experiment on the other hand finds itself in extreme danger, leading to high arousal and probably much stronger fear reactions. If a participant is not sufficiently engaged in the experiment, it might as well happen that he does not learn CS-US contingencies. In an fMRI study however, the situation is more threatening for most participants, especially when they have never been exposed to an

fMRI scanner before. It is likely that this leads to increased arousal and a facilitated acquisition process, resulting in less unaware participants. Due to the lack of unaware participants I concentrated on neural activation during extinction of fear memory acquired in delay and trace fear conditioning. Taken together, results support previous findings of involvement of PFC, insula, ACC, striatum, and hippocampus in the extinction of conditioned fear memory. In both groups I found activation of the insula, which has been associated with the anticipation of pain leading to a cortical representation of fear (Phelps et al., 2004). In a fear conditioning paradigm using painful stimuli as US, one can expect that participants still anticipate pain during the first trials of extinction until they have learned that no US is administered any more in this phase of the experiment. Additionally, the striatum was activated in both the delay and the TCG during extinction. Striatal activation is also related to anticipation of the US, or more precisely to the discrepancy between an expected and the actual outcome which is experienced when the US as no longer present during extinction. This discrepancy is referred to as prediction error. The striatum has been shown to be involved in the coding of prediction errors in aversive classical conditioning (e.g. Seymour et al., 2005). Accordingly, it also is assumed to play a crucial role in the development of contingency awareness. Klucken et al. (2009) found striatal activation during acquistion only in learned aware participants, but not in unaware or instructed aware participants. Schiller et al. (2008) reported increased activation when a change in contingencies had to be learned. This is in line with the change of contingency during early extinction. Differences between the two groups were found in the involvement of ACC, hippocampus and especially in prefrontal activation. Both the ACC and the hippocampus have been found to be important for trace but not necessarily for delay conditioning, which my results confirm. The ACC has been linked to sustained attention,

which is necessary to bridge the trace interval between CS and US. The gap between CS and US forms a temporal context which is represented by the hippocampus (Phillips & LeDoux, 1992). Also, as mentioned above, it is involved in explicit memory processes (Clark et al., 2002) and important for the development of contingency awareness (Tabbert et al., 2011), which is a prerequisite for trace conditioning.

However, the most interesting finding is the dissociation in prefrontal activation I observed in the extinction of delay versus trace fear conditioning. During early extinction, I found activation of the vmPFC after delay conditioning, but activation of the dlPFC after trace conditioning, indicating distinct underlying processes between extinction of the two types of conditioning. There is considerable evidence that the vmPFC is involved in the extinction of fear memory in human (e.g. Phelps et al., 2004; Milad et al., 2007; Müller, Panitz, Hermann & Pizzagalli, 2014; Lonsdorf, Haaker & Kalisch, 2014) as well as animal research (e.g. Moustafa et al., 2013). It is assumed that a memory trace between the vmPFC and the amygdala is formed during extinction, which enables the inhibition of the conditioned response (Sotres-Bayon et al 2004, 2007; Motzkin, Philippi, Wolf, Baskaya & Koenigs, 2014). In the DCG I can add further evidence to this model, however, to my knowledge, there are no such findings for trace conditioning so far. In a study designed by Haritha and colleagues (Haritha, Wood, Ver Hoef & Knight, 2013), neural underpinnings of trace conditioning were examined in detail, especially with regard to the differences in activation related to the cue vs. to the trace interval. Interestingly, they found a similar pattern of activation during the trace interval as I did during early extinction of trace fear memory, namely in the dorsomedial PFC (dmPFC), posterior cingulate cortex (PCC), right dlPFC, right IPL, right superior/middle temporal gyrus, and bilateral insula. Activity was greater in right than in left dlPFC, IPL, and superior/middle temporal gyrus. They conclude

that a right-lateralized fronto-parietal circuit might be crucial for trace conditioning. Right dlPFC activation might be involved in bridging the temporal gap between the CS and the US during both acquisition of trace conditioning and during early extinction. In a very recent study, Vytal, Overstreet, Charney, Robinson & Grillon (2014) investigated the neural mechanisms of the maintenance of anxiety responses. They argued that the amygdala is only responsible for instantaneously eliciting a fear reaction, but not for maintaining a state of anxiety. Amygdala responding is assumed to be involved in the initiation of a conditioned response shortly following the onset of the CS (Cheng et al., 2008). Rodent studies have identified the medial prefrontal cortex, studies with nonhuman primates the dorsal ACC to be crucial for maintaining defensive states in response to uncertain threats (Vytal et al. 2014). In humans, there is no direct equivalent for these regions, but dorsal parts of the PFC have been related to these functions: Robinson et al. (2012) reported that anxiety significantly increased positive dmPFC – amygdala connectivity. According to Vythal et al. (2014), today`s evidence indicades that the dmPFC – amygdala network might be involved in both the modulation and preservation of anxiety states. In their study, they showed that anticipatory anxiety in response to an uncertain threat increases amygdala – dmPFC coupling, indicating that these regions work in concert to support anxious responding or defensive readiness. Also, high trait-anxiety was associated with increased amydala – dmPFC coupling, whereas Kim et al. (2011) found a positive correlation between this coupling and state anxiety. My results can of course not be compared to the findings about amydala – dmPFC coupling directly. First of all, in the TCG, the dorsal prefrontal activation I found is situated much more lateral. Also, I cannot compare amygdala activation since it did not reach significance in my study, which I – at least partially, attribute to susceptibility artifacts during echo-planar imaging. Nevertheless,

there are some interesting similarities which should be considered here: First if all, according to a model of the functional organization of the lateral PFC, the dlPFC is associated with the manipulation of information, which requires a lot of working memory capacity (D´Esposito et al., 1999). Evidence from lesion studies also indicates that the dlPFC activation is crucial for adjusting behavior appropriately in delayed response tasks (e.g. D'Esposito et al., 2000), in which information has to be kept in working memory before an individual can make choices and decisions based on it. As mentioned above, trace conditioning and its extinction afford higher working memory contributions than delay conditioning to bridge the trace interval and hold information in short term memory. It is also conceivable that trace conditioning is not only associated with higher working memory contributions but also with more sustained anxiety than delay conditioning. At least for the duration of the trace interval, participants cannot know whether the CS will be followed by a US, especially after a change in contingencies at the beginning of extinction. In this case, the initiation of a conditioned response shortly following the onset of the CS is not sufficient. The defensive state has to be maintained at least during the trace interval. Accordingly, one can assume that during early extinction, the TCG is in a state closer to sustained anxiety elicited by uncertain threat than the DCG. According to subjective ratings, extinction proceeded more slowly in the trace group compared to the delay group. The DCG no longer reported differences in arousal and fear ratings between CS+ and CS- after extinction. However, the CS+ was still rated more arousing and more frightening than the CS- in the TCG. A slower extinction process in the TCG can be seen as an indication for higher working memory contribution as well as a longer lasting threatening situation in the extinction of trace conditioning. Up to now, prefrontal contributions to extinction are not clear. It would be very interesting, also from a clinical point of view, to further elaborate

functions and contributions of the different parts of the prefrontal cortex and their connectivity. For example Vytal and colleagues (2014) point out that the PFC-amygdala coupling during anticipatory anxiety being correlated to trait anxiety might constitute a potential vulnerability marker for anxiety disorders. A problem in the treatment of anxiety disorders is that the responding behavior of patients to different psychotherapeutic of pharmacological interventions is highly variable and cannot be predicted very well. Shin, Davis, VanElzakker, Dahlgren & Dubois (2013) reviewed studies using neuroimaging measures to predict response to different types of anxiety disorders. Interestingly, higher pre-treatment activity or gray matter density in the mPFC predicted better response to behavioral or cognitive behavioral therapy in Obsessive Complusive Disorder (OCD) and PTSD as well as to venlafaxine medication in GAD. Also, lower amygdala activation before treatment is associated with better response to Cognitive Behavioral Therapy in PTSD and venlafaxine in GAD. In their own study, they found that increased sustained metabolic activity in the dmPFC is a risk factor for PTSD (Shin et al., 2009). Evidence in this area is still small, however, exact knowledge about the role of different parts of the prefrontal cortex for the extinction of fear and anxiety promises to be of great help in improving the treatment of anxiety disorders.

7.3. Fear and anxiety in classical conditioning

7.3.1. Predictability and anxiety

There is a long history of studying the development and maintenance of anxiety disorders by means of the classical fear conditioning paradigm. The difference between fear and anxiety has been modeled by predictable versus unpredictable threat: An individual confronted with predictable or cued threat experiences phasic and directed fear,

whereas an individual confronted with an unpredictable danger such as a threatening environment or context experiences a more sustained state of apprehension and anxiety (Davis et al., 2010). Not being able to predict danger has been shown to lead to enhanced anxiety: If an individual does not learn the contingency between CS and US, the environment becomes the best predictor of a danger, leading to higher contextual anxiety (Grillon, 2002a; Baas et al., 2008, Seligman & Blinik, 1977). In most anxiety disorders, both fear and anxiety are involved. The scope ranges from specific phobias, which are mainly associated with phasic fear, to GAD, mainly characterized by sustained fear. Disorders such as PTSD and PD involve both fear and anxiety (for details see Grillon et al., 2008; Grillon et al., 2009). Studying sustained fear by means of contextual conditioning is important for understanding the mechanisms behind anxiety disorders characterized by diffuse states of anxiety rather than fear (Andreatta & Glotzbach-Schoon et al., 2015; Glotzbach et al., 2013; Grillon, 2002). There is evidence that clinically high-anxious individuals are overly sensitive to unpredictable threat: Grillon et al. (2009) for example showed that PTSD patients react with FPS to the same extend as healthy controls when confronted with predictable threatening cues, but with raised contextual anxiety in an unpredictable condition. Similar findings about panic-disorder patients have also been reported by Grillon and colleagues (Grillon et al., 2008).

In sub-clinical studies, high trait-anxiety, according to the State-Trait-Anxiety Model the stable tendency to interpret ambiguous situations as threatening and to react with state anxiety (Spielberger et al., 1970), has been identified as a risk factor for the development and maintenance of anxiety disorders. For example, high-anxious individuals display deficient discriminative learning of fear and safety cues (Arnoudova et al., 2013), trait-anxiety tends to be higher in unaware participants than aware participants (Baas et al.,

2008; Grillon, 2002a), and individuals with higher trait-anxiety develop higher levels of contextual anxiety in unpredictable contexts (Baas 2013). To investigate context-dependent cue conditioning under consideration of individual differences in awareness and trait-anxiety, I increased the complexity of the virtual environment used in the pilot study. Participants were exposed to differential cue conditioning in a fear and a safety context during acquisition, and to a third novel context during extinction. Although unaware participants displayed deficits in cue conditioning on an explicit level (anxiety ratings) and only by trend also in startle responses, they did not react with enhanced contextual fear as one could expect on the basis of existing evidence. However, an important difference between my paradigm and for example the paradigm of Baas and colleagues (Baas et al., 2008, Baas 2013) is the relative salience of the cues compared to the contexts. Additionally, Baas et al. used one cue instead of two as in a differential cue conditioning paradigm. One can assume that in my study, cue conditioning was learned preferentially to context conditioning, and contexts only modulated this memory in an inhibitory way.

7.3.2. The role of the context in extinction

In contrast to evidence provided by Bass et al. (2004, 2008) for cues being generalized from the fear to the safety context even though participants were aware of the fact that cues in CXT- are not followed by shocks, I did not find generalization of fear responses to cues presented in the safety or the novel context during acquisition or extinction. Somehow surprisingly participants reacted with enhanced startle responses to the CS- in the safety context during acquisition. This contradicts the assumption that a preferentially learned excitatory association with the cue causes the generalization of cued fear to the CXT- because it is stronger than the inhibitory association with the safety-context. However, these findings should be interpreted with care, because increased

startle reactions to the CS- tended to invert in aware participants in the later part of acquisition and to disappear in unaware participants.

During extinction, the FPS responses to the CS+ in the fear context were also not generalized to the safety or even to the novel context. This was confirmed by anxiety ratings. In extinction research, there are different explanations for the problem that the associative strength between a CS and a US is rarely - if ever – extinguished completely (for an overview see Delamater 2012). Rescorla (2003) for example described the protection from extinction mechanism: A context acquires inhibitory associative strength during extinction, because the absence of the US in presence of the CS elicits a prediction error which can only be explained by the new contextual information. The inhibitory associative strength then sums up with the excitatory associative strength of CS and US, lowering the expectation of the US. The association between context and US will finally lead to the retention of a residual excitatory CS-US association, which is referred to as „protection from extinction". The fact that extinction does not completely erase fear memory can be demonstrated by eliciting the renewal effect (Vervliet, Craske & Hermanns, 2013). The already extinguished fear response recovers when an individual is exposed to the CS in a context that is different from the context of extinction. Besides the protection from extinction mechanism, other explanations for the renewal effect are discussed. The context in which acquisition takes place could for example gain own excitatory strength in addition to the CS, leading to a stronger conditioned response to the CS due to summation of associative strength of CS and context. Bouton (2004) explained the renewal effect on the basis of occasion setting. Assuming that higher order learning processes are involved in extinction, one stimulus can acquire both excitatory and inhibitory associations with the US (during acquisition and extinction, respectively), which then compete with each other.

In this case, the context sets the occasion, i.e. it determines which conditioned response is appropriate: while the acquisition context activates the excitatory association resulting in a fear response, the extinction context elicits the inhibitory association.

Although I did not investigate renewal, my results support the theory of occasion setting to some extend: I did not find isolated context conditioning, indicating that the contexts itself did not gain much associative strength. Additionally, cued fear was not generalized to the safety context. One explanation for this could be that association between the CS+ and the US was already ambiguous regarding associative strength and the safety context served as an occasion setter. However, it remains surprising that participants did not react with an enhanced fear response in the novel context, because without any clear contextual information, the excitatory association should have been retrieved preferentially. Unfortunately, small sample size and interindividual variance in startle responses in the study prevent further interpretations of the lack of generalization. This aspect should be given more attention in future studies, also in terms of renewal and the possible mechanisms involved in extinction learning.

7.4. Trait-anxiety and fear conditioning

As mentioned before, I also analyzed the effect of individual differences in terms of trait-anxiety. In line with findings reported by Baas et al. (2008), participants who did explicitly learn the CS-US contingency during acquisition scored higher on trait-anxiety than aware participants. However, trait-anxiety did not have an effect on differential cue conditioning. In fact, I did not find differences between high – and low-anxious participants. More importantly, it did have a clear effect on context conditioning. The high-anxious group reacted with increased anxiety in the fear context compared to the safety context, whereas

the low-anxious group did not display successful context conditioning at all. The fact that high-anxious individuals did not show deficient cue conditioning is somewhat surprising. According to rodent studies, high anxious rats have a poor ability to discriminate between cues but show high contextual freezing (see for example Duvarci, Bauer & Paré, 2009). Similar results have been shown in humans. Arnoudova et al. (2013) also reported deficient discriminative learning of fear and safety cues in high-anxious individuals. According to Grillon (2002a), this lack of discrimination between cues leads to a higher level of unpredictability, since the CS- is not perceived as a safety-signal. Consequently, deficient cue conditioning leads to higher contextual anxiety. With these findings I cannot completely support this explanation. High-anxious individuals exhibited only mild deficits in discriminating fear and safety cues in the second part of acquisition. Nevertheless, they reacted with higher contextual anxiety to the fear context. According to existing evidence one can assume that not fear learning per se is impaired, but that high-anxious individuals have difficulties to inhibit a fear reaction to a presented stimulus. There are several possible explanations why high-anxious participants in my study did not displayed deficient discriminative learning. First of all, both groups did not differ strongly in trait-anxiety, since participants were split into the two groups by means of a median split. Possibly, a sample with higher trait-anxiety might have reacted differently. Also, evidence on the association between high trait-anxiety and deficient cue conditioning is not completely unambiguous. Lissek and colleagues (2005) reviewed studies on fear conditioning comparing anxiety patients and healthy controls. Results indicate both greater activation of fear in response to threatening cues (CS+) among patients, but also impaired abilities to inhibit a conditioned response to safety cues. In the study conducted by Baas (2013), the hypotheses that unaware participants would score high on trait anxiety could not be

confirmed. Instead, aware subjects displayed higher levels of attentional control as measured with the Attentional Control Scale. Baas (2013) assumed that higher order cognition was involved due to the complexity of the conditioning paradigm with a less than 100% reinforcement schedule and many distractors. Instead of trait-anxiety, attentional control might also have played a crucial role in the complex setting.

Nevertheless, my findings confirm existing evidence that high trait-anxiety is associated with increased contextual anxiety. In a virtual reality study investigating the influence of trait-anxiety on contextual fear, Glotzbach-Schoon et al. (2013) also reported faster contextual conditioning in high-anxious compared to low-anxious participants. Indovina et al. (2011) conducted an imaging study using fMRI to investigate neuronal processes underlying both acquisition and extinction of conditioned fear in high-anxious individuals compared to low-anxious participants. They found two main differences between neurocognitive functions in the two groups. Firstly, high-anxious participants showed enhanced amygdala responsivity to fear cues. Secondly, they found variability in ventral prefrontal cortex (vPFC) mechanisms to downregulate cued and contextual fear: Results revealed increased vPFC activation in response to both fear cues and unpredictable contexts in individuals with low trait-anxiety, which was associated with a downregulation of fear or sustained anxiety, respectively. Interestingly, this activation was found before the US was no longer presented, which might be a protective mechanism against the development of anxiety disorders. However, high-anxious individuals showed reduced vPFC activation compared to low-anxious participants, indicating a higher risk for the development and maintenance of pathological fear.

7.5. Virtual Reality in human fear conditioning

Taken together, recent evidence mainly indicates that high-anxious individuals both react with enhanced acquisition of fear in the presence of a fear signal and with deficient downregulation of fear in the presence of a safety signal, or, according to Indovina and colleagues (2011), to both safety and fear signals. Due to the devastating effects fear and anxiety can have when they become and remain maladaptive, it is crucial to further understand individual risk factors for and also mechanisms to go against anxiety disorders. Virtual reality extends the possibilities we have to investigate these processes in humans. Individual risk factors can only partially be studied in the animal model, thus we need to find ways to transfer fear conditioning paradigms to human research in an ecologically valid way. By moving participants into a virtual environment we can simulate different contexts, which can easily be controlled and manipulated. The situation becomes more real and probably also more aversive, leading to fear conditioning closer to threatening situations in real life. An astounding example for the immersion virtual reality can induce are the studies by Hoffman and colleagues (Hoffman, Patterson & Carrougher, 2000; Hoffman, Doctor, Patterson & Carrougher, 2000a), who used virtual reality as a distractor to reduce pain experienced during wound care and physical therapy of patients with severe burns. They found that VR functioned as a strong nonpharmacologic pain reduction technique. Besides being a useful tool to study fear conditioning in complex environments (e.g. Baas 2013; Glotzbach-Schoon et al., 2013; Mühlberger et al. 2013), VR has proven to be very effective in exposure therapy. Mühlberger and colleagues (Mühlberger, Wiedemann & Pauli, 2003; Mühlberger et al., 2006) for example demonstrated that a one-session VR treatment effectively reduced fear of flying, and that this effect was still present one year after treatment. In a more recent study, Shiban, Pauli & Mühlberger (2013)

showed that exposure therapy in virtual reality successfully reduced fear of real spiders in spider phobics. Additionally, exposing spider phobics to virtual spiders in multiple virtual contexts reduced the likelihood of renewal. After reviewing thirty-eight studies using virtual reality, Diemer, Mühlberger, Pauli & Zwanzger (2014) concluded that VR exposure is capable of eliciting psychophysiological fear reactions in patients and healthy individuals, which is essential for successful exposure treatment. Hence, VR exposure treatment seems to be an up-and-coming addition to cognitive behavioral therapy.

7.6. Conclusions

Conclusively, the virtual reality paradigm has proven to be a promising tool for creating a more complex environment which is closer to a real life situation. For advancing our understanding of the development, maintenance and treatment of pathological anxiety, we need to include contextual information into studies since threatening stimuli never occur in isolation in real life situations. For this purpose, virtual reality is well suited.

With these studies I added further evidence to a dual process model of fear conditioning, indicating that two independent learning processes exist: one propositional in nature and leading to conscious awareness, the second, lower level process non-propositional and activating the CR via a direct mechanism. A complex conditioning paradigm containing many distractors can be used to passively manipulate the development of contingency awareness. Not all participants gained explicit knowledge about the CS-US associations, but they still developed an implicit conditioned response reflected by a FPS reaction. I found successful differential cue conditioning modulated by contextual information when participants were confronted with cues in a threatening and a safe environment. Increased fear reactions to the threatening stimulus were only present in the fear, not in the safety

context, indication that discriminative learning took place regarding both cues and contexts. Trait-anxiety proved to be a vulnerability factor for contextual conditioning: High-anxious individuals reacted with enhanced anxiety to the fear context, which was not the case for participants with low trait-anxiety. Generalization of contextual anxiety to a novel and therefore unpredictable context was only found on trend level, whereas cued fear was not generalized at all to the novel context. Especially gaining a deeper understanding of the role of the context in extinction of conditioned fear is essential for further research. Additionally, the mechanisms making trait-anxiety a vulnerability factor are not fully understood up to now: Safety learning is assumed to be impaired in high-anxious individuals, leading to higher sustained and contextual anxiety. My results in fact add evidence to enhanced contextual anxiety in high-anxious participants, however I could not confirm impaired discriminative learning between fear and safety cues.

I also examined neuronal structured involved in extinction of delay and trace fear conditioning. Contingency awareness has been found to be a prerequisite for trace conditioning, because higher working memory contributions are necessary to bridge the temporal gap between CS and US. My results confirm that the two times of learning differ significantly: Besides common activation in neuronal structures which have frequently been shown to be involved in extinction of fear memory such as the insula and the striatum, both groups differed primarily in prefrontal activation. The vmPFC was activated in the DCG, whereas the TCG showed activation of the dlPFC during extinction. These results point to different extinction processes in the two types of conditioning, presumably in form of increased involvement of working memory processes in trace conditioning compared to delay conditioning.

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9. Annex

A First Study

(1) Information for participants

(2) Written informed consent

(3) Demographic data and exclusion criteria

(4) Written instructions

(5) Determination of pain threshold

(6) List of subjective ratings

(7) Example of trial order and pseudo-randomization of stimuli

B Second Study

For "Determination of pain threshold" and "Written instructions" please see first study

(1) Preliminary interview (by phone)

(2) Information for participants

(3) Written informed consent

(4) Demographic data and exclusion criteria

(5) List of subjective ratings

C Third Study

For "Written informed consent", "Determination of pain threshold" and "Demographic data and exclusion criteria" please see first study

(1) Information for participants

(2) Written instructions

(3) List of subjective ratings

A (1)

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Würzburg, Januar 2009

Probandeninformation zur Studie

Teilprojekt B1 „Strukturelle/funktionelle Korrelate von kontextueller Furchtkonditionierung beim Menschen"
im Rahmen des SFB Transregio 58 Furcht, Angst, Angsterkrankungen

Sehr geehrte Versuchsteilnehmerin, sehr geehrter Versuchsteilnehmer,

Sie haben Gelegenheit, an einer von der Deutschen Forschungsgemeinschaft geförderten Studie teilzunehmen, mit der wir untersuchen wollen, unter welchen Bedingungen bestimmte Gegenstände oder Umwelten unangenehme Gefühle (z. B. Angst) auslösen. Sie werden aus der Teilnahme keinen unmittelbaren Nutzen für sich ziehen können. Wir hoffen jedoch, durch unsere Arbeit mehr darüber zu erfahren, wie Angststörungen entstehen und welche Bedingungen sie aufrecht erhalten, um so langfristig die Behandlung zu verbessern.

Vor der Untersuchung werden Sie einige Fragebögen ausfüllen, in denen wichtige Daten bezüglich Ihrer Person festgehalten werden. Dann wird der Versuchsleiter zur Messung Ihrer Herzrate, Ihrer Schweißdrüsenaktivität und Ihrer Muskelspannung mehrere Messelektroden in Ihrem Gesicht und auf Ihrer Brust anbringen. Dazu wird Ihre Haut mit Alkohol gereinigt, um den elektrischen Widerstand zwischen Haut und Messelektrode so gering wie möglich zu halten. Aufgrund dieser Hautreinigung kann es zu Hautrötungen oder leichten Hautirritationen kommen, die aber normalerweise innerhalb kurzer Zeit abklingen.

Im ersten Teil der Untersuchung werden wir Ihnen eine Virtuelle Welt, d. h. von einem Computer erzeugte Räume, zeigen. Sie sollen diese Räume und die darin enthaltenen Gegenstände aufmerksam betrachten. In seltenen Fällen kann die Virtuelle Realität Übelkeit oder Schwindel auslösen, ähnlich wie eine Karussellfahrt. Falls dies passiert, so teilen Sie uns das bitte sofort mit.

Manchmal werden Sie elektrische Reize am Unterarm verspüren. Diese elektrischen Reize sind etwas schmerzhaft, aber sehr kurz und nicht gefährlich. Die Stärke der elektrischen Reize wird individuell ermittelt und vor Versuchsbeginn festgelegt.

Im zweiten Teil der Untersuchung bekommen Sie die virtuelle Welt ohne Einschränkung nochmals präsentiert, wobei wir Ihre körperlichen Reaktionen in diesen Räumen aufzeichnen werden.

Während dieser Untersuchungen werden Sie manchmal über Kopfhörer ein kurzes, lautes Geräusch hören. Dieses Geräusch kann etwas unangenehm für Sie sein, es ist aber unschädlich. Bitte lassen Sie sich dadurch nicht stören.

Damit Sie sich den Untersuchungsablauf und die darin vorkommenden Virtuellen Welten, elektrischen Reize und Geräusche besser vorstellen können, werden wir Ihnen zu Beginn der Untersuchung jeweils Beispiele dafür präsentieren.

Alle Daten dienen ausschließlich Forschungszwecken, werden vertraulich behandelt und ohne Namensgebung unter einer Codenummer abgespeichert. Die Daten werden für unbestimmte Zeit gespeichert. Der Codierungsschlüssel wird ein Jahr nach Abschluss der Studie vernichtet. Bis dahin können Sie, auch noch nach der Untersuchung, die Löschung ihrer Daten verlangen.

Die Teilnahme an der Untersuchung ist völlig freiwillig. Sie können jederzeit - ohne Angabe von Gründen - die Teilnahme abbrechen. Dadurch entstehen Ihnen keinerlei persönliche Nachteile.

Falls Sie noch weitere Frage haben, stellen Sie diese bitte jetzt.

A (2)

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Würzburg, Januar 2009

Einverständniserklärung zur Datenerhebung im Rahmen der Studie

Teilprojekt B1 „Strukturelle/funktionelle Korrelate von kontextueller Furchtkonditionierung beim Menschen"
im Rahmen des SFB Transregio 58 Furcht, Angst, Angsterkrankungen

Durch meine Unterschrift bestätige ich:
Ich nehme freiwillig an der Untersuchung „Strukturelle/funktionelle Korrelate von kontextueller Furchtkonditionierung beim Menschen" teil und bin damit einverstanden, dass die erhobenen Daten in anonymisierter Form wissenschaftlich ausgewertet werden. Ich bin auch damit einverstanden, dass die Ergebnisse der Studie in Gruppen zusammengefasst wissenschaftlich veröffentlicht werden.

Über mögliche Risiken wurde ich aufgeklärt. Ich weiß auch, dass es nicht möglich ist, Informationen über individuelle Untersuchungsergebnisse zu erhalten.

Ich hatte ausreichend Zeit, mir zu überlegen, ob ich an der Datenerhebung teilnehmen will, sowie die Gelegenheit, Fragen zu stellen. Mit den erhaltenen Antworten bin ich zufrieden. Ich habe darüber hinaus eine Probandeninformation und eine Kopie dieser Einverständniserklärung (datiert und unterschrieben) erhalten. Ich wurde darauf hingewiesen, dass ich jederzeit von dieser Untersuchung zurücktreten kann, ohne dass mir dadurch ein Nachteil entsteht. Die Daten werden in diesem Falle vernichtet.

Name des Teilnehmers: .... (bitte Blockbuchstaben)

....
Ort, Datum

....
Unterschrift des Teilnehmers

....
Unterschrift des aufklärenden Mitarbeiters

A (3)

Untersuchung:

Datum: VP-Code:

Angaben zur Person:

Bitte kreuzen Sie die für Sie zutreffenden Antworten an!

Alter _____ Jahre

Geschlecht

weiblich   ☐

männlich   ☐

Höchster Schulabschluss

Volks-,Hauptschulabschluss   ☐

mittlere Reife   ☐

Fachhochschulreife   ☐

Hochschulreife   ☐

(Fach-)Hochschulabschluss   ☐

Derzeitige Tätigkeit

Student/in   ☐

Wenn ja: Studienfach ……………………………………………………………………

in Ausbildung   ☐

teilzeitbeschäftigt   ☐

voll berufstätig   ☐

Hausfrau, - mann   ☐

Rentner/in   ☐

arbeitslos   ☐

Händigkeit

rechts   ☐

links   ☐

Untersuchung:

Datum: **VP-Code:**

Ein-/Ausschlusskriterien

Bitte kreuzen Sie an:

1.	Sind Sie zurzeit in psychotherapeutischer/nervenärztlicher Behandlung?	Ja	Nein
2.	Hatten Sie in der Vergangenheit eine behandlungsbedürftige psychische oder neurologische Erkrankung? Wenn ja: Was? Wann?	Ja	Nein
3.	Nehmen Sie gegenwärtig Psychopharmaka ein? Wenn ja: Was? Dosierung?	Ja	Nein
4.	Wird Ihnen während Karussell-, Schiffs- oder Flugzeugfahrten schnell schwindlig oder übel?	Ja	Nein
5.	Konsumieren Sie regelmäßig Alkohol? Wenn ja: Durchschnittliche Menge pro Tag:	Ja	Nein
6.	Konsumieren Sie Drogen? Wenn ja: Was? Wie häufig (Menge pro Tag):	Ja	Nein
7.	Tragen Sie im Moment Kontaktlinsen?	Ja	Nein
8.	Sind Sie farbenblind? Wenn ja: Für welche Farben?	Ja	Nein
9.	Leiden Sie unter Hörproblemen?	Ja	Nein

A (4)

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Instruktion zur Studie

Sehr geehrte Versuchsteilnehmerin, sehr geehrter Versuchsteilnehmer,

Vielen Dank, dass Sie sich bereit erklärt haben, an unserem Experiment teilzunehmen.

Im Laufe des Experiments werden wir Sie über ein Head Mounted Display in einen virtuellen Flur versetzen, von dem eine Tür abgeht. Hinter dieser Tür befindet sich ein Büro, durch das Sie mehrmals geführt werden.
Die Führungen durch das Büro werden passiv erfolgen, d.h. Sie können nicht aktiv in den Verlauf eingreifen, aber durch Kopfbewegungen Ihr Blickfeld verändern. Auf diese Weise ist es Ihnen eingeschränkt möglich, den Raum frei zu erkunden. Sie können dies im Vorfeld ausprobieren.

Der eigentliche Versuch besteht aus mehren Phasen. In jeder Phase werden Sie mehrmals durch den virtuellen Büroraum geführt.

Nach jeder Phase werden Ihnen verschiedene Fragen gestellt, z.B.:

Wie groß war Ihre Angst in einer bestimmten Situation?
Nennen Sie bitte eine Zahl von **0 (keine Angst)** bis **100 (sehr starke Angst)** auf der unten angegebenen Skala.

....
0   50   **100**

Wie wahrscheinlich war es, einen elektrischen Reiz in dieser Situation zu erhalten?
Nennen Sie bitte eine Zahl von **0 (unmöglich)** bis **100 (sicher)** auf der unten angegebenen Skala.

....
0   50   **100**

Wie positiv oder negativ empfanden Sie diese Situation?
Nennen Sie bitte eine Zahl von **0 (sehr negativ)** bis **100 (sehr positiv)** auf der unten angegebenen Skala.

....
0   50   **100**

Wie aufgeregt waren Sie in dieser Situation?

Nennen Sie bitte eine Zahl von **0 (sehr ruhig)** bis **100 (sehr aufgeregt)** auf der unten angegebenen Skala.

....
0   50   **100**

Bitte prägen Sie sich diese Skalen gut ein. Wenn Sie später danach gefragt werden, antworten Sie bitte mündlich und möglichst spontan und zügig.

Bevor das Experiment startet, möchten wir zunächst Ihre persönliche Schmerzschwelle bestimmen. Zum einen können wir so die auf Sie passende Reizintensität auswählen, zum anderen können Sie die Reize, die Sie im Experiment erhalten werden, kennenlernen. Sie werden gleich das Head Mounted Display aufsetzen und in den virtuellen Flur versetzt werden. Über die Elektrode an Ihrem Unterarm werden Sie dann verschieden starke Reize erhalten. Manche Reize sind eventuell so schwach, dass Sie diese gar nicht spüren können. Ihre Aufgabe ist es, dem Versuchsleiter nach jedem Reiz mitzuteilen, wie stark dieser war.

Dazu wird Ihnen folgende Frage gestellt:

Wie hoch war dieser elektrische Reiz auf der Skala von 0 bis 10?

....
0   1   2   3   **4**   5   6   7   8   9   **10**
↑   ↑   ↑
nichts   **eben**   **sehr**
gespürt   **wahrnehmbarer**   **starker**
                                 Schmerz   **Schmerz**

Bitte prägen Sie sich diese Skala gut ein. Wenn Sie das Head Mounted Display und die Kopfhörer aufgesetzt haben, werden Sie nach jedem Reiz mündlich dazu aufgefordert, den Reiz zu beurteilen. Sie werden die Skala nicht mehr sehen. Nennen Sie dann bitte die entsprechende Zahl.

Falls Sie noch weitere Fragen haben, stellen Sie diese bitte jetzt.

A (5)

Untersuchung:

Datum: **VP-Code:**

Schmerzschwellenbestimmung – Intensität

	Serie1-Ansteigen	Serie1-Absteigen	Serie2-Ansteigen	Serie2-Absteigen
8 mA				
7,5 mA				
7 mA				
6,5 mA				
6 mA				
5,5 mA				
5 mA				
4,5 mA				
4,0 mA				
3,5 mA				
3 mA				
2,5 mA				
2 mA				
1,5 mA				
1 mA				
0,5 mA				
0 mA				

Mittelwert der Intensität (gerundet):   _____

+ 30% (x 1.3):   _____

Rating Schmerzschwelle: _____

A (6)

Untersuchung:

Datum: **VP-Code:**

Ratings

Nach Habituation

Valenz Licht gelb   _____

Valenz Licht blau   _____

Arousal Licht gelb   _____

Arousal Licht blau   _____

Nach Akquisition 1

Waren die elektrischen Reize vorhersagbar?   _____

Wann kam der elektrische Reiz?   _____

Valenz Licht gelb   _____

Valenz Licht blau   _____

Arousal Licht gelb   _____

Arousal Licht blau   _____

Nach Akquisition 2

Angst Licht gelb   _____

Angst Licht blau   _____

Waren die elektrischen Reize vorhersagbar?   _____

Wann kam der elektrische Reiz?   _____

Kontingenz Licht gelb   _____

Kontingenz Licht blau   _____

Valenz Licht gelb   _____

Valenz Licht blau   _____

Arousal Licht gelb   _____

Arousal Licht blau   _____

Nach Extinktion

Angst Licht gelb   _____

Angst Licht blau   _____

Kontingenz Licht gelb   _____

Kontingenz Licht blau   _____

Valenz Licht gelb   _____

Valenz Licht blau   _____

Arousal Licht gelb   _____

Arousal Licht blau   _____

Rating elektrischer Reiz   _____

Rating Startle Ton   _____

A (7)

Acquisition 1 (Trials 1 - 3)

ITI 27s	CS + 8s	ITI 20s		CS - 8s	ITI 10s	CS - 8s	ITI 16s		CS + 8s	ITI 15s
		11s	9s			6s			6s	

ITI 22s	CS + 8s	ITI 14s	CS - 8s	ITI 12s	CS + 8s	ITI 24s			CS - 8s	ITI 16s
			5s		6s	14s	10s			

ITI 17s	CS - 8s	ITI 17s	CS + 8s	ITI 11s	CS + 8s	ITI 13s	CS - 8s	ITI 30s	
					5s		5s	13s	17s

Acquisition 2 (Trials 4 – 6)

ITI 15s	CS - 8s	ITI 13s	CS - 8s	ITI 17s	CS + 8s	ITI 27s		CS + 8s	ITI 16s
			6s		5s	20s	7s		

ITI 24s	CS + 8s	ITI 12s	CS + 8s	ITI 10s	CS - 8s	ITI 22s		CS - 8s	ITI 20s
			6s			10s	12s	5s	

ITI 25s	CS - 8s	ITI 20s		CS + 8s	ITI 10s	CS + 8s	ITI 18s	CS - 8s	ITI 15s
		15s	5s			5s		6s	

Extinction (Trials 7 - 9)

ITI 18s	CS - 8s	ITI 11s	CS + 8s	ITI 18s		CS + 8s	ITI 15s	CS - 8s	ITI 26s	
	6s↑			6s↑	12s	5s↑		5s↑	14s	12s ↑

ITI 28s		CS + 8s	ITI 19s	CS - 8s	ITI 10s	CS - 8s	ITI 14s		CS + 8s	ITI 17s
16s	12s ↑	5s↑	15s	4s ↑		5s↑			6s↑	

ITI 21s	CS - 8s	ITI 13s	CS + 8s	ITI 12s	CS - 8s	ITI 26s			CS + 8s	ITI 16s
	6s↑		5s↑		5s↑	13s	13s ↑		5s↑	

B (1)

SFB Transregio 58 / TP B1 – Studie CCC1, Diss. H.Ewald „Furcht, Angst und Angsterkrankungen"

Teilnehmer-Code:_____   Datum:_____

Telefonische Vorbefragung(Ein- Ausschlusskriterien)

1. Wie viele Gläser Alkohol trinken Sie pro Woche? Menge:_____

   Weniger als 15 Gläser Alkohol pro Woche: □ **ja**   □ nein

2. Wie viele Zigaretten rauchen Sie täglich? Menge:_____

   Nicht mehr als 20 Zigaretten pro Tag: □ **ja**   □ nein

3. Konsumieren Sie illegale Drogen: □ ja   □ **nein**

4. Nehmen Sie regelmäßig verschreibungspflichtige Medikamente ein?: □ ja   □ **nein**

   Falls ja: Welche?_____

   Kontraindikation: Zentral wirksame Medikamente, z.B. Neuroleptika, Antidepressiva, Antiepileptika, Opiate, Benzodiazepine

5. Leiden Sie an einer psychischen Erkrankung (Angststörungen, Depression, Schizophrenie, Alkohol-, Drogen-, Medikamentenabhängigkeit? □ ja   □ **nein**

   Falls ja: Welche?_____

   isolierte Phobien (z.B. Spinnen, Spritzen) auch ausschließen!

6. Leiden Sie an einer neurologischen Erkrankung? □ ja   □ **nein**

   Falls ja: Welche?_____

   Kontraindikation: Erkrankungen mit Beteiligung des ZNS, z.B. Schlaganfall, Gehirnblutungen, Epilepsie, Parkinson, MS

7. Leiden Sie an einer sonstigen Erkrankung (Herz-Kreislauf, Blut, Lunge, Leber, Nieren, Schilddrüse, Augen, Haut, Magen-Darmtrakt, Stoffwechsel): □ ja   □ **nein**

   Falls ja: Welche?_____

   Kontraindikation: schwere Erkrankungen

8. <u>Bei Frauen:</u> Sind Sie schwanger? □ ja   □ **nein**

9. Wird Ihnen während Karussell-, Schiffs- oder Flugzeugfahrten schnell schwindelig oder übel? □ ja   □ **nein**

10. Sind Sie farbenblind? □ ja   □ **nein**

11. Leiden Sie unter Hörproblemen? □ ja   □ **nein**

Termin VR-Experiment: _____

B (2)

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Würzburg, Januar 2009

Probandeninformation zur Studie

Teilprojekt B1 „Strukturelle/funktionelle Korrelate von kontextueller Furchtkonditionierung beim Menschen"
im Rahmen des SFB Transregio 58 Furcht, Angst, Angsterkrankungen

Sehr geehrte Versuchsteilnehmerin, sehr geehrter Versuchsteilnehmer,

Sie haben Gelegenheit, an einer von der Deutschen Forschungsgemeinschaft geförderten Studie teilzunehmen, mit der wir untersuchen wollen, unter welchen Bedingungen bestimmte Gegenstände oder Umwelten unangenehme Gefühle (z. B. Angst) auslösen. Sie werden aus der Teilnahme keinen unmittelbaren Nutzen für sich ziehen können. Wir hoffen jedoch, durch unsere Arbeit mehr darüber zu erfahren, wie Angststörungen entstehen und welche Bedingungen sie aufrecht erhalten, um so langfristig die Behandlung zu verbessern.

Vor der Untersuchung werden Sie einige Fragebögen ausfüllen, in denen wichtige Daten bezüglich Ihrer Person festgehalten werden. Dann wird der Versuchsleiter zur Messung Ihrer Herzrate, Ihrer Schweißdrüsenaktivität und Ihrer Muskelspannung mehrere Messelektroden in Ihrem Gesicht und auf Ihrer Brust anbringen. Dazu wird Ihre Haut mit Alkohol gereinigt, um den elektrischen Widerstand zwischen Haut und Messelektrode so gering wie möglich zu halten. Aufgrund dieser Hautreinigung kann es zu Hautrötungen oder leichten Hautirritationen kommen, die aber normalerweise innerhalb kurzer Zeit abklingen.

In der Untersuchung werden wir Ihnen eine Virtuelle Welt, d. h. von einem Computer erzeugte Räume, zeigen. Sie sollen diese Räume und die darin enthaltenen Gegenstände aufmerksam betrachten. In seltenen Fällen kann die Virtuelle Realität Übelkeit oder Schwindel auslösen, ähnlich wie eine Karussellfahrt. Falls dies passiert, so teilen Sie uns das bitte sofort mit.

Manchmal werden Sie elektrische Reize am Unterarm verspüren. Diese elektrischen Reize sind etwas schmerzhaft, aber sehr kurz und nicht gefährlich. Die Stärke der elektrischen Reize wird individuell ermittelt und vor Versuchsbeginn festgelegt.

Während dieser Untersuchungen werden Sie manchmal über Kopfhörer ein kurzes, lautes Geräusch hören. Dieses Geräusch kann etwas unangenehm für Sie sein, es ist aber unschädlich. Bitte lassen Sie sich dadurch nicht stören.

Damit Sie sich den Untersuchungsablauf und die darin vorkommenden Virtuellen Welten, elektrischen Reize und Geräusche besser vorstellen können, werden wir Ihnen zu Beginn der Untersuchung jeweils Beispiele dafür präsentieren.

Angststörungen nehmen bisweilen einen sehr unterschiedlichen Verlauf und treten gelegentlich auch familiär gehäuft auf. Vermutlich gibt es genetische Faktoren, die einen Einfluss auf die Entstehung oder Aufrechterhaltung von Angsterkrankungen haben. Diese Untersuchung dient auch der Suche nach genetischen Einflussfaktoren, die sich auf Lernmechanismen im Zusammenhang mit der Entstehung von Angststörungen auswirken können. Im Rahmen der Studie „Furcht, Angst und Angsterkrankungen: funktionelle Genomik und Gen-Umwelt-Interaktionen in dimensionalen Endophänotypen für Furcht und Angst" (MEGA-Studie) wurde Ihnen eine Blutprobe entnommen, aus der Informationen über die Ausprägung bestimmter Gene gewonnen wurden.

Für die aktuelle Studie wurden Probanden unterschiedlicher Genausprägungen ausgewählt. Die Auswahl erfolgte durch eine unabhängige Schlüsselperson, die für de Dauer der Untersuchung Zugang zu Ihren Daten aus der MEGA-Studie hat. Ihre Daten wurden von dieser Person pseudonymisiert, d.h. Sie wurden durch einen Code verschlüsselt. Der „Schlüssel", der die Zuordnung dieses Codes zu Ihrer Genausprägung erlaubt, wird getrennt von Ihren hier erhobenen Daten von der unabhängigen Schlüsselperson aufbewahrt. Der Untersucher hat dazu keinen Zugang und somit keine Kenntnis über Ihre Genausprägung. Er erhält lediglich den Code zu Ihrem Namen, der aber ohne den Schlüssel keine Zuordnung Ihrer hier erhobenen Daten zu Ihrer Genausprägung möglich macht. Alle hier erhobenen Daten werden nicht unter Ihrem Namen, sondern unter dem Code abgespeichert.
Nach Abschluss der Studie wird Ihre Genausprägung von der unabhängigen Schlüsselperson den hier erhobenen Daten zugeordnet. Danach wird der Schlüssel zusammen mit dem Code gelöscht. Ab diesem Zeitpunkt sind die Daten vollständig anonymisiert, d.h. Eine zuordnung der Daten zu Ihrem Namen ist nicht mehr möglich. Sie können deshalb nur bis zum Abschluss der Studie und somit bis zur Vernichtung von Schlüssel und Code die Löschung Ihrer hier erhobenen Daten verlangen. Die anonymisierten Daten werden auf unbestimmte Zeit gespeichert.

Die erhobenen Daten dienen rein wissenschaftlichen Zwecken und werden ohne Bezug auf konkrete Personen ausgewertet und in wissenschaftlichen Fachzeitschriften veröffentlicht.

Die Teilnahme an der Untersuchung ist völlig freiwillig. Sie können jederzeit - ohne Angabe von Gründen - die Teilnahme abbrechen. Dadurch entstehen Ihnen keinerlei persönliche Nachteile.

Falls Sie noch weitere Frage haben, stellen Sie diese bitte jetzt.

B (3)

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Würzburg, Januar 2009

Einverständniserklärung zur Datenerhebung im Rahmen der Studie

Teilprojekt B1 „Strukturelle/funktionelle Korrelate von kontextueller Furchtkonditionierung beim Menschen"
im Rahmen des SFB Transregio 58 Furcht, Angst, Angsterkrankungen

Durch meine Unterschrift bestätige ich:
Ich nehme freiwillig an der Untersuchung „Strukturelle/funktionelle Korrelate von kontextueller Furchtkonditionierung beim Menschen" teil und bin damit einverstanden, dass die erhobenen Daten wissenschaftlich ausgewertet werden. Ich bin auch damit einverstanden, dass die Ergebnisse der Studie in Gruppen zusammengefasst wissenschaftlich veröffentlicht werden.

Über mögliche Risiken wurde ich aufgeklärt. Ich weiß auch, dass es nicht möglich ist, nformationen über individuelle Untersuchungsergebnisse (z. B. persönliche Risikokonstellationen) zu erhalten.

Ich hatte ausreichend Zeit, mir zu überlegen, ob ich an der Datenerhebung teilnehmen will, sowie Gelegenheit, Fragen zu stellen. Mit den erhaltenen Antworten bin ich zufrieden. Ich habe darüber hinaus eine Probandeninformation und eine Kopie dieser Einverständniserklärung (datiert und unterschrieben) erhalten. Ich wurde darauf hingewiesen, dass ich die Untersuchung jederzeit abbrechen kann, ohne dass mir dadurch ein Nachteil entsteht. Die im Rahmen dieser Studie erhobenen Daten werden in diesem Falle vernichtet.

Ich kann auch nach der Teilnahme noch bis zum Abschluss der Studie die Löschung der hier erhobenen Daten verlangen. Nach Abschluss der Studie wird der Codierungsschlüssel gelöscht und damit ist die Zuordnung meines Namens zu meinen hier erhobenen Daten (und damit auch die Löschung der Daten) nicht mehr möglich.

Name des Teilnehmers: .... (bitte Blockbuchstaben)

....
Ort, Datum   Unterschrift des Teilnehmers

                                                                ....
                                                                Unterschrift des aufklärenden Mitarbeiters

B (4)

Untersuchung:

Datum: **VP-Code:**

Angaben zur Person:

Bitte kreuzen Sie die für Sie zutreffenden Antworten an!

Alter   _____   Jahre

Geschlecht

weiblich ☐

männlich ☐

Höchster Schulabschluss

Volks-,Hauptschulabschluss ☐

mittlere Reife ☐

Fachhochschulreife ☐

Hochschulreife ☐

(Fach-)Hochschulabschluss ☐

Derzeitige Tätigkeit

Student/in ☐

            Wenn ja: Studienfach: _____

in Ausbildung ☐

teilzeitbeschäftigt ☐

voll berufstätig ☐

Hausfrau, - mann ☐

Rentner/in ☐

arbeitslos ☐

Händigkeit

rechts ☐

links ☐

Untersuchung:

Datum: **VP-Code:**

Ein-/Ausschlusskriterien

Bitte kreuzen Sie an:

1.	Sind Sie zurzeit in psychotherapeutischer/nervenärztlicher Behandlung?	Ja	Nein
2.	Hatten Sie in der Vergangenheit eine behandlungsbedürftige psychische oder neurologische Erkrankung? Wenn ja: Was? Wann?	Ja	Nein
3.	Nehmen Sie gegenwärtig Psychopharmaka ein? Wenn ja: Was? Dosierung?	Ja	Nein
4.	Wird Ihnen während Karussell-, Schiffs- oder Flugzeugfahrten schnell schwindlig oder übel?	Ja	Nein
5.	Konsumieren Sie regelmäßig Alkohol? Wenn ja: Durchschnittliche Menge pro Tag:	Ja	Nein
6.	Konsumieren Sie Drogen? Wenn ja: Was? Wie häufig (Menge pro Tag):	Ja	Nein
7.	Tragen Sie im Moment Kontaktlinsen?	Ja	Nein
8.	Sind Sie farbenblind? Wenn ja: Für welche Farben?	Ja	Nein
9.	Leiden Sie unter Hörproblemen?	Ja	Nein
10.	**Nur weibliche** Versuchsteilnehmer: Verwenden Sie hormonelle Verhütungsmittel? Wenn **ja**: Was? (Art und Name/Marke):	Ja	Nein
	Sind Sie gerade in der 7-Tage Pause?	Ja	Nein
	Wenn **nein**: Der wievielte Tag seit dem 1. Tag Ihrer letzten Periode ist heute? Wie viele Tage umfasst normalerweise ein Zyklus bei Ihnen?		

B (5)

Untersuchung: **VP-Code:**

Datum: **Ablauf:**

Rating Startle-Ton (Valenz)   _____

Prä-Akquisition Raumauswahl   1. Raum   _____

                        2. Raum   _____

                        3. Raum   _____

Nach Habituation/ Prä-Akquisition

Valenz Fear Room   _____

Valenz Fear: CS+   _____

Valenz Fear: CS-   _____

Valenz Safety Room   _____

Valenz Safety: CS+   _____

Valenz Safety: CS-   _____

Valenz Neutral Room   _____

Valenz Neutral: CS+   _____

Valenz Neutral: CS-   _____

Arousal Fear Room   _____

Arousal Fear: CS+   _____

Arousal Fear: CS-   _____

Arousal Safety Room   _____

Arousal Safety: CS+   _____

Arousal Safety: CS-   _____

Arousal Neutral Room   _____

Arousal Neutral: CS+   _____

Arousal Neutral: CS-   _____

Angst Fear Room   _____

Angst Fear: CS+   _____

Angst Fear: CS-   _____

Angst Safety Room   _____

Angst Safety: CS+ _____

Angst Safety: CS- _____

Angst Neutral Room _____

Angst Neutral: CS+ _____

Angst Neutral: CS- _____

Nach Akquisition

In welchem Raum und bei welchem Licht gab es elektrische Reize?

………………………………………………………………………………………………………

………………………………………………………………………………………………………

Valenz Fear Room _____

Valenz Fear: CS+ _____

Valenz Fear: CS- _____

Valenz Safety Room _____

Valenz Safety: CS+ _____

Valenz Safety: CS- _____

Arousal Fear Room _____

Arousal Fear: CS+ _____

Arousal Fear: CS- _____

Arousal Safety Room _____

Arousal Safety: CS+ _____

Arousal Safety: CS- _____

Angst Fear Room _____

Angst Fear: CS+ _____

Angst Fear: CS- _____

Angst Safety Room _____

Angst Safety: CS+ _____

Angst Safety: CS- _____

Kontingenz Fear Room _____

Kontingenz Fear: CS+ _____

Kontingenz Fear: CS-   _____

Kontingenz Safety Room   _____

Kontingenz Safety: CS+   _____

Kontingenz Safety: CS-   _____

Nach Extinktion

Valenz Fear Room   _____

Valenz Fear: CS+   _____

Valenz Fear: CS-   _____

Valenz Safety Room   _____

Valenz Safety: CS+   _____

Valenz Safety: CS-   _____

Valenz Neutral Room   _____

Valenz Neutral: CS+   _____

Valenz Neutral: CS-   _____

Arousal Fear Room   _____

Arousal Fear: CS+   _____

Arousal Fear: CS-   _____

Arousal Safety Room   _____

Arousal Safety: CS+   _____

Arousal Safety: CS-   _____

Arousal Neutral Room   _____

Arousal Neutral: CS+   _____

Arousal Neutral: CS-   _____

Angst Fear Room   _____

Angst Fear: CS+   _____

Angst Fear: CS-   _____

Angst Safety Room   _____

Angst Safety: CS+   _____

Angst Safety: CS-   _____

Angst Neutral Room   _____

Angst Neutral: CS+   _____

Angst Neutral: CS-   _____

Nach Extinktion

Kontingenz Fear Room   _____

Kontingenz Fear: CS+ _____

Kontingenz Fear: CS- _____

Kontingenz Safety Room _____

Kontingenz Safety: CS+ _____

Kontingenz Safety: CS- _____

Kontingenz Neutral Room _____

Kontingenz Neutral: CS+ _____

Kontingenz Neutral: CS- _____

Rating elektrischer Reiz_____

Rating Startle-Ton (Valenz)_____

Notizen:

…..

…..

…..

…..

C (1)

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Würzburg, Januar 2009

Probandeninformation zur Studie

Teilprojekt B1 „Strukturelle/funktionelle Korrelate von kontextueller Furchtkonditionierung beim Menschen"
im Rahmen des SFB Transregio 58 Furcht, Angst, Angsterkrankungen

Sehr geehrte Versuchsteilnehmerin, sehr geehrter Versuchsteilnehmer,

Sie haben Gelegenheit, an einer von der Deutschen Forschungsgemeinschaft geförderten Studie teilzunehmen, mit der wir untersuchen wollen, unter welchen Bedingungen bestimmte Gegenstände oder Umwelten unangenehme Gefühle (z. B. Angst) auslösen. Sie werden aus der Teilnahme keinen unmittelbaren Nutzen für sich ziehen können. Wir hoffen jedoch, durch unsere Arbeit mehr darüber zu erfahren, wie Angststörungen entstehen und welche Bedingungen sie aufrecht erhalten, um so langfristig die Behandlung zu verbessern.

Vor der Untersuchung werden Sie einige Fragebögen ausfüllen, in denen wichtige Daten bezüglich Ihrer Person festgehalten werden. Dann wird der Versuchsleiter zur Messung Ihrer Schweißdrüsenaktivität Messelektroden auf Ihrer Hand anbringen.

Während der Untersuchung werden wir Ihnen eine Virtuelle Welt, d. h. von einem Computer erzeugte Räume, zeigen. Sie sollen diese Räume und die darin enthaltenen Gegenstände aufmerksam betrachten.

Manchmal werden Sie elektrische Reize am Finger verspüren. Diese elektrischen Reize sind etwas schmerzhaft, aber sehr kurz und nicht gefährlich. Die Stärke der elektrischen Reize wird individuell ermittelt und vor Versuchsbeginn festgelegt.

Damit Sie sich den Untersuchungsablauf, die darin vorkommenden Virtuellen Welten und die elektrischen Reize besser vorstellen können, werden wir Ihnen zu Beginn der Untersuchung jeweils Beispiele dafür präsentieren.

Alle Daten dienen ausschließlich Forschungszwecken, werden vertraulich behandelt und ohne Namensgebung unter einer Codenummer abgespeichert. Die Daten werden für unbestimmte Zeit gespeichert. Der Codierungsschlüssel wird ein Jahr nach Abschluss der Studie vernichtet. Bis dahin können Sie, auch noch nach der Untersuchung, die Löschung Ihrer Daten verlangen.

Die Teilnahme an der Untersuchung ist völlig freiwillig. Sie können jederzeit - ohne Angabe von Gründen - die Teilnahme abbrechen. Dadurch entstehen Ihnen keinerlei persönliche Nachteile.

Falls Sie noch weitere Frage haben, stellen Sie diese bitte jetzt.

C (2)

Lehrstuhl für Psychologie I - Prof. Dr. Paul Pauli
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Instruktion zur Studie

Sehr geehrte Versuchsteilnehmerin, sehr geehrter Versuchsteilnehmer,

Vielen Dank, dass Sie sich bereit erklärt haben, an unserem Experiment teilzunehmen.

Im Laufe des Experiments werden wir Sie über ein Head Mounted Display in einen virtuellen Flur versetzen, von dem eine Tür abgeht. Hinter dieser Tür befindet sich ein Büro, durch das Sie mehrmals geführt werden.
Die Führungen durch das Büro werden passiv erfolgen, d.h. Sie können nicht aktiv in den Verlauf eingreifen, aber durch Kopfbewegungen Ihr Blickfeld verändern. Auf diese Weise ist es Ihnen eingeschränkt möglich, den Raum frei zu erkunden. Sie können dies im Vorfeld ausprobieren.

Der eigentliche Versuch besteht aus mehren Phasen. In jeder Phase werden Sie mehrmals durch den virtuellen Büroraum geführt.

Nach jeder Phase werden Ihnen verschiedene Fragen gestellt, z.B.:

Wie groß war Ihre Angst in einer bestimmten Situation?
Nennen Sie bitte eine Zahl von **0 (keine Angst)** bis **100 (sehr starke Angst)** auf der unten angegebenen Skala.

....
0   50   **100**

Wie wahrscheinlich war es, einen elektrischen Reiz in dieser Situation zu erhalten?
Nennen Sie bitte eine Zahl von **0 (unmöglich)** bis **100 (sicher)** auf der unten angegebenen Skala.

....
0   50   **100**

Wie positiv oder negativ empfanden Sie diese Situation?
Nennen Sie bitte eine Zahl von **0 (sehr negativ)** bis **100 (sehr positiv)** auf der unten angegebenen Skala.

....
0   50   **100**

Wie aufgeregt waren Sie in dieser Situation?
Nennen Sie bitte eine Zahl von **0 (sehr ruhig)** bis **100 (sehr aufgeregt)** auf der unten angegebenen Skala.

....
0   50   **100**

Bitte prägen Sie sich diese Skalen gut ein. Wenn Sie später danach gefragt werden, antworten Sie bitte mündlich und möglichst spontan und zügig.

Bevor das Experiment startet, möchten wir zunächst Ihre persönliche Schmerzschwelle bestimmen. Zum einen können wir so die auf Sie passende Reizintensität auswählen, zum anderen können Sie die Reize, die Sie im Experiment erhalten werden, kennenlernen. Sie werden gleich das Head Mounted Display aufsetzen und in den virtuellen Flur versetzt werden. Über die Elektrode an Ihrem Unterarm werden Sie dann verschieden starke Reize erhalten. Manche Reize sind eventuell so schwach, dass Sie diese gar nicht spüren können. Ihre Aufgabe ist es, dem Versuchsleiter nach jedem Reiz mitzuteilen, wie stark dieser war.
Dazu wird Ihnen folgende Frage gestellt:

Wie hoch war dieser elektrische Reiz auf der Skala von 0 bis 10?

....
0   1   2   3   **4**   5   6   7   8   9   **10**
↑   ↑   ↑
nichts   **eben**   **sehr**
gespürt   **wahrnehmbarer**   **starker**
                                    Schmerz   **Schmerz**

Bitte beachten Sie:
0 bedeutet **„nichts gespürt"**,
1-3 bedeuten **„etwas gespürt, aber nicht schmerzhaft"**,
4 bedeutet „eben wahrnehmbarer **Schmerz**".

Bitte prägen Sie sich diese Skala gut ein. Wenn Sie das Head Mounted Display und die Kopfhörer aufgesetzt haben, werden Sie nach jedem Reiz mündlich dazu aufgefordert, den Reiz zu beurteilen. Sie werden die Skala nicht mehr sehen. Nennen Sie dann bitte die entsprechende Zahl.

Falls Sie noch weitere Fragen haben, stellen Sie diese bitte jetzt.

C (3)

Untersuchung: **Datum:**   **VP-Code:**

Ratings

Nach Habituation

Valenz Licht blau   _____

Valenz Licht gelb   _____

Arousal Licht blau   _____

Arousal Licht gelb   _____

Angst Licht blau   _____

Angst Licht gelb   _____

Nach Akquisition 1

Valenz Licht blau   _____

Valenz Licht gelb   _____

Arousal Licht blau   _____

Arousal Licht gelb   _____

Angst Licht blau   _____

Angst Licht gelb   _____

Waren die elektrischen Reize vorhersagbar?   _____

Wann kam der elektrische Reiz?   _____

Kontingenz Licht blau   _____

Kontingenz Licht gelb   _____

Nach Akquisition 2

Valenz Licht blau   _____

Valenz Licht gelb   _____

Arousal Licht blau   _____

Arousal Licht gelb   _____

Angst Licht blau   _____

Angst Licht gelb   _____

Waren die elektrischen Reize vorhersagbar?   _____

Wann kam der elektrische Reiz?   _____

Kontingenz Licht blau   _____

Kontingenz Licht gelb   _____

Nach Extinktion

Valenz Licht blau   _____

Valenz Licht gelb   _____

Arousal Licht blau   _____

Arousal Licht gelb   _____

Angst Licht blau   _____

Angst Licht gelb   _____

Kontingenz Licht blau   _____

Kontingenz Licht gelb   _____

List of publications

Research articles in peer-reviewed journals:

- **Ewald H**, Glotzbach-Schoon E, Gerdes AB, Andreatta M, Müller M, Mühlberger A, Pauli P. Delay and trace fear conditioning in a complex virtual learning environment-neural substrates of extinction. Front Hum Neurosci. 2014 May 27; 8:323. doi: 10.3389/fnhum.2014.00323.

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- Glotzbach-Schoon E, Andreatta M, Reif A, **Ewald H**, Tröger C, Baumann C, Deckert J, Mühlberger A, Pauli P. Contextual fear conditioning in virtual reality is affected by 5HTTLPR and NPSR1 polymorphisms: effects on fear-potentiated startle. Front Behav Neurosci. 2013 Apr 23; 7:31. doi: 10.3389/fnbeh.2013.00031.

- Glotzbach-Schoon E, Tadda R, Andreatta M, Tröger C, **Ewald H**, Grillon C, Pauli P, Mühlberger A. Enhanced discrimination between threatening and safe contexts in high-anxious individuals. Biol Psychol. 2013 Apr; 93(1): 159-66. doi: 10.1016/j.biopsycho.2013.01.011.

- Glotzbach E, **Ewald H**, Andreatta M, Pauli P, Mühlberger A. Contextual fear conditioning predicts subsequent avoidance behaviour in a virtual reality environment. Cogn Emot. 2012; 26(7): 1256-72. doi: 10.1080/02699931.2012.656581.

- Tröger C, **Ewald H**, Glotzbach E, Pauli P, Mühlberger A. Does pre-exposure inhibit fear context conditioning? A Virtual Reality Study. J Neural Transm. 2012 Jun; 119(6): 709-19. doi: 10.1007/s00702-011-0757-8.

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- Glotzbach, E., Andreatta, M., Reif, A., **Ewald, H.,** Tröger, C., Baumann, C., Deckert, J., Mühlberger, A., & Pauli, P. (2012). The impact of 5-HTTLPR and NPSR1 polymorphisms on contextual fear conditioning. Psychologie und Gehirn, Jena, Germany.

- Andreatta, M., **Ewald, H.,** Glotzbach, E., Pauli, P., & Mühlberger, A. (2011). Der Einfluss des Kontexts in Cue Konditionierung. Psychologie und Gehirn, Heidelberg, Germany.

- Glotzbach, E., **Ewald, H.,** Tröger, C., Pauli, P., & Mühlberger, M. (2011). Extinction and spontaneous recovery of contextual fear memories. Cognitive Neuroscience Society (CNS), San Francisco, USA.

- Glotzbach, E., **Ewald, H.,** Pauli, P., & Mühlberger, A. (2010). Vermeidungsverhalten nach kontextueller Furchtkonditionierung ist abhängig von der subjektiven Bewertung. Psychologie und Gehirn, Greifswald, Germany.

- **Ewald, H.,** Glotzbach, E., Tröger, C., Pauli, P. & Mühlberger, A. (2009). Fear conditioning in immersive virtual reality. Psychophysiology, 46, S128.

- Mühlberger, A., Tröger, C., **Ewald, H.**, Glotzbach, E., & Pauli, P. (2009). Context conditioning in virtual reality and the influence of pre-exposure. Psychophysiology, 46, S26-S27.
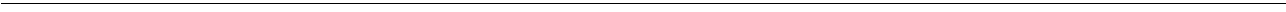
Affidavit

I hereby confirm that my thesis entitled

„Influence of context and contingency awareness on fear conditioning –

an investigation in virtual reality"

is the result of my own work.

I did not receive any help or support from commercial consultants. All sources and / or

materials applied are listed and specified in the thesis.

Furthermore, I confirm that this thesis has not yet been submitted as part of another

examination process neither in identical nor in similar form.

…...   …..

   Place, Date   Signature