
# Contextual fear conditioning in humans: The return of contextual anxiety and the influence of genetic polymorphisms

*Kontextuelle Furchtkonditionierung beim Menschen: die Wiederkehr von Kontextangst und der Einfluss von genetischen Polymorphismen*

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# Abbreviations

| | |
|---|---|
| **5-HT** | 5-Hydroxytryptamin or serotonin |
| **5-HTT** | serotonin transporter |
| **5-HTTLPR** | 5-HTT-linked polymorphic region |
| **ACC** | anterior cingulate cortex |
| **ANOVA** | analysis of variance |
| **ANS** | autonomic nervous system |
| **ASI** | Anxiety Sensitivity Index |
| **BAS** | Behavioral Approach System |
| **BIS** | Behavioral Inhibition System |
| **BLA** | basolateral amygdala |
| **BNST** | bed nucleus of the stria terminalis |
| **CeA** | central amygdala |
| **$CeA_L$** | lateral central amygdala |
| **$CeA_M$** | medial central amygdala |
| **CG** | central gray |
| **COMT** | catechol-O-methyltransferase |
| **CR** | conditioned response |
| **CRF** | corticotropin-releasing factor |
| **CS** | conditioned stimulus |
| **CS+** | fear cue |
| **CS-** | safety cue |
| **CXT+** | anxiety context |
| **CXT-** | safety context |
| **DMN** | dorsal motor nucleus of the vagus |
| **EC** | evaluative conditioning |
| **EDA** | electrodermal activity |
| **EMG** | electromyography |
| **fMRI** | functional magnetic resonance imaging |
| **GABA** | gamma aminobutyric acid |
| **GAD** | generalized anxiety disorder |
| **HMD** | head mounted display |
| **HPA** | hypothalamic-pituitary-adrenal (axis) |
| **IF** | infralimbic |
| **IPQ** | Igroup Presence Questionnaire |

| | |
|---|---|
| **ITC** | intercalated (neurons) |
| **ITI** | intertrial interval |
| **KO** | knockout |
| **LH** | lateral hypothalamus |
| **MeA** | medial amygdala |
| **MEQ** | Morningness-Eveningness-Questionnaire |
| **NPS** | neuropeptide S |
| **NPSR** | neuropeptide S receptor |
| **NS** | neutral stimulus |
| **PAG** | periaqueductal gray |
| **PANAS** | Positive And Negative Affect Schedule |
| **PFC** | prefrontal cortex |
| **PnC** | caudal pontine reticular nucleus |
| **PSQI** | Pittsburgh Sleep Quality Index |
| **PTSD** | posttraumatic stress disorder |
| **PVN** | paraventricular nucleus of the hypothalamus |
| **SCL** | skin conductance level |
| **SCR** | skin conductance response |
| **SNP** | single nucleotid polymorphism |
| **SSRI** | selective serotonin re-uptake inhibitor |
| **STAI** | State-Trait-Anxiety-Inventory |
| **TPH** | tryptophan-hydroxylase |
| **UR** | unconditioned response |
| **US** | unconditioned stimulus |
| **vmPFC** | ventromedial prefrontal cortex |
| **VR** | virtual reality |

## Abstract

Sustained anxiety is considered as a chronic and future-oriented state of apprehension that does not belong to a specific object. It is discussed as an important characteristic of anxiety disorders including panic disorder, generalized anxiety disorder (GAD) and posttraumatic stress disorder (PTSD). Experimentally, sustained anxiety can be induced by contextual fear conditioning in which aversive events are unpredictably presented and therefore the whole context becomes associated with the threat. This thesis aimed at investigating important mechanisms in the development and maintenance of sustained anxiety: (1) facilitated acquisition and resistant extinction of contextual anxiety due to genetic risk factors (Study 1), and (2) the return of contextual anxiety after successful extinction using a new reinstatement paradigm (Study 2).

To this end, two contextual fear conditioning studies were conducted in virtual reality (VR). During acquisition one virtual office was paired with unpredictable mildly painful electric stimuli (unconditioned stimulus, US), thus becoming the anxiety context (CXT+). Another virtual office was never paired with any US, thus becoming the safety context (CXT-). Extinction was conducted 24 h later, i.e. no US was presented, and extinction recall was tested another 24 h later on Day 3. In both studies context-evoked anxiety was measured on three different response levels: behavioral (anxiety-potentiated startle reflex), physiological (skin conductance level), and verbal (explicit ratings).

In Study 1, participants were stratified for 5-HTTLPR (S+ risk allele vs. LL no risk allele) and NPSR1 rs324981 (T+ risk allele vs. AA no risk allele) polymorphisms, resulting in four combined genotype groups with 20 participants each: S+/T+, S+/LL, LL/T+, and LL/AA. Results showed that acquisition of anxiety-potentiated startle was influenced by a gene × gene interaction: only carriers of both risk alleles (S+ carriers of the 5-HTTLPR *and* T+ carriers of the NPSR1 polymorphism) exhibited significantly higher startle magnitudes in CXT+ compared to CXT-. However, extinction recall as measured with anxiety-potentiated startle was not affected by any genotype. Interestingly, the explicit anxiety level, i.e. valence and anxiety ratings, was only influenced by the NPSR1 genotype, in a way that no risk allele carriers (AA) reported higher anxiety and more negative valence in response to CXT+ compared to CXT-, whereas risk allele carriers (T+) did not.

Study 2 adopted nearly the same paradigm with the modification that one group (reinstatement group) received one unsignaled US at the beginning of the experimental session on Day 3 before seeing CXT+ and CXT-. The second group served as a control group and received no US, but was immediately exposed to CXT+ and CXT-. Results showed a return of anxiety on the implicit and explicit level (higher startle responses and

anxiety ratings in response to CXT+ compared to CXT-) in the reinstatement group only. Most important, the return of contextual anxiety in the reinstatement group was associated with a change of state anxiety and mood from extinction to test, that is the more anxiety and negative mood participants experienced before the reinstatement procedure, the higher their return of anxiety was.

In sum, results of Study 1 showed that facilitated contextual fear conditioning on an implicit behavioral level (startle response) could be regarded as an endophenotype for anxiety disorders, which can contribute to our understanding of the etiology of anxiety disorders. Results of Study 2 imply that anxiety and negative mood after extinction could be an important facilitator for to the return of anxiety. Furthermore, the present VR-based contextual fear conditioning paradigm seems to be an ideal tool to experimentally study mechanisms underlying the acquisition and the return of anxiety. Future studies could investigate clinical samples and extend the VR paradigm to evolutionary-relevant contexts (e.g., heights, darkness, open spaces).

## Zusammenfassung

Als *Angst* bezeichnet man einen nicht auf spezifische Objekte gerichteten länger anhaltenden zukunfts-orientierten Zustand der Besorgnis. Diese ist kennzeichnend für Angststörungen wie Panikstörung, generalisierte Angststörung und Posttraumatische Belastungsstörung (PTBS). Experimentell kann Angst durch kontextuelle Furchtkonditionierung ausgelöst werden. Bei dieser Art der Konditionierung werden aversive Ereignisse als unvorhersehbar erlebt, wodurch der gesamte Kontext mit der Gefahr assoziiert wird. Diese Arbeit hat zum Ziel, Mechanismen der Entstehung und Aufrechterhaltung von Kontextangst zu untersuchen. Dies sind zum einem erleichterte Akquisition von Kontextkonditionierungen und deren fehlerhafte Extinktion. Hier ist vor allem die Fragestellung relevant, wie dies durch genetische Varianten moduliert wird (Studie 1). Zum anderen soll die Wiederkehr der Angst nach der Extinktion mit einem neuen Reinstatement-Paradigma untersucht werden (Studie 2).

Zur Untersuchung dieser Forschungsfragen wurden zwei kontextuelle Furchtkonditionierungsstudien in virtueller Realität (VR) durchgeführt. Während der Akquisition wurden leicht schmerzhafte elektrische Reize (unkonditionierter Stimulus, US) unvorhersehbar präsentiert, während die Probanden in einem virtuellen Büroraum waren. Dadurch wurde dieser Raum zum Angstkontext (CXT+). Ein zweiter Büroraum wurde nie mit dem US gepaart, deshalb wurde dieser Raum zum Sicherheitskontext (CXT-). Die Extinktion, in der die Kontexte ohne US präsentiert wurden, fand 24 h später statt, und ein Test zum Abruf der Extinktion bzw. zur Wiederkehr der Angst nochmals 24 h später. In beiden Studien wurde die Angst auf drei verschiedenen Ebenen gemessen: Verhalten (angstpotenzierter Schreckreflex), Physiologie (tonische Hautleitfähigkeit), und verbale Ebene (explizite Ratings).

Die Probanden für Studie 1 wurden anhand der 5-HTTLPR (S+ Risikoallel vs. LL nicht-Risikoallel) und NPSR1 rs324981 (T+ Risikoallel vs. AA nicht-Risikoallel) Polymorphismen stratifiziert, sodass vier kombinierte Genotyp Gruppen (S+/T+, S+/LL, LL/T+ und LL/AA) mit je 20 Probanden vorlagen. Es zeigte sich, dass der angstpotenzierte Schreckreflex durch die Interaktion zwischen beiden genetischen Polymorphismen moduliert wurde. Nur Träger beider Risikoallele (S+ Träger des 5-HTTLPR *und* T+ Träger des NPSR1 Polymorphismus) zeigten einen höheren Schreckreflex im CXT+ als im CXT- während der Akquisition. Der Abruf der Extinktion an Tag 3, gemessen anhand des Schreckreflexes, wurde allerdings nicht durch die Genotypen moduliert. Interessanterweise zeigte sich auf dem expliziten Angstlevel (Valenz- und Angstratings) nur ein Einfluss des NPSR1 Polymorphismus, und zwar bewerteten die

nicht-Risikoallel Träger (AA) den CXT+ mit negativerer Valenz und höherer Angst im Vergleich zum CXT-; die Risikoallel Träger (T+) taten dies nicht.

In der zweiten Studie wurde fast das gleiche Paradigma benutzt wie in der ersten Studie mit der Ausnahme, dass eine Versuchsgruppe (Reinstatementgruppe) den US noch einmal am Anfang des dritten Untersuchungstages vor der Präsentation von CXT+ und CXT- appliziert bekam. Die zweite Versuchsgruppe (Kontrollgruppe) erhielt keinen US, sondern wurde direkt durch CXT+ und CXT- geführt. Es zeigte sich, dass nur in der Reinstatementgruppe die Angst auf impliziter und expliziter Ebene wiederkehrte, d.h. die Probanden zeigten einen höheren Schreckreflex und höhere Angstratings auf den CXT+ im Vergleich zum CXT-. Wichtig war vor allem, dass die Wiederkehr der Angst in der Reinstatementgruppe mit der Veränderung der Zustandsangst und der Stimmung (von der Extinktion zum Test) korrelierte. D.h. je größer die Angst und je negativer die Stimmung wurden, desto höher war die Wiederkehr der Angst.

Zusammengefasst belegt Studie 1, dass erleichterte kontextuelle Furchtkonditionierung auf impliziter Ebene (Schreckreflex) ein Endophänotyp für Angststörungen sein könnte, was zu unserem Verständnis der Ätiologie von Angststörungen beitragen könnte. Die Ergebnisse der zweiten Studie legen nahe, dass eine ängstliche und negative Stimmung nach der Extinktion die Rückkehr von Angst begünstigen könnte. Darüber hinaus scheint das VR-basierte kontextuelle Furchtkonditionierungsparadigma ein geeignetes Mittel zu sein, um Mechanismen der Angstentstehung und Angstwiederkehr experimentell zu erforschen. Weiterführende Studien könnten nun auch Angstpatienten untersuchen und das Paradigma auf evolutionär-relevante Kontexte (z.B. Höhe, Dunkelheit, weite Plätze) ausweiten.

# 1. Introduction

Anxiety disorders have a high prevalence of 8.4% within 12 month or 14.5% for the lifetime within the EU. The highest 12-month prevalence has been observed for specific phobia (5.4%), followed by social phobia (1.6%), posttraumatic stress disorder (1.1%), generalized anxiety disorder (0.9%), panic disorder (0.7%) and agoraphobia without panic disorder (0.3%) (data are from the European Study of the Epidemiology of Mental Disorders, ESEMeD; Alonso & Lepine, 2007). It is estimated that there are approximately 41 million patients with anxiety disorders (panic disorder, phobias, obsessive-compulsive disorder and generalized anxiety disorder) in Europe which results in a total cost of more than 41 billon € dedicated to healthcare costs, direct non-medical costs and indirect costs (Andlin-Sobocki, Jonsson, Wittchen, & Olesen, 2005). These data definitely warrant further research on the origin and maintenance of anxiety disorders.

Especially, associative learning processes are discussed as crucial for the development and maintenance of anxiety disorders (Mineka & Zinbarg, 2006). Fear conditioning to specific cues has been suggested as a model for phasic fear learning and phobic fear (Grillon, 2002), because fear responses are initiated by a specific threat and are diminished, if the specific threat is not present anymore (e.g., spider phobics show fast fear responses towards a present spider, but their fear responses will decline, if the spider is not present anymore). In contrast, sustained anxiety and chronic worry can be observed firstly, in response to specific contexts where a specific phobic cue was formerly present (defined as background contextual fear conditioning; e.g., a cellar where a spider was present), and secondly, even without the cue the context itself elicits anxiety responses (as a model for foreground contextual fear conditioning). Experimentally, anxiety can be modeled by the contextual fear conditioning paradigm, where explicit cues signaling threat are lacking and therefore the whole context is associated with the unpredictable threat (Grillon, 2002). It is suggested that anxiety disorder patients, like panic disorder and posttraumatic stress disorder (PTSD), are more sensitive to unpredictable threat and acquire context-US associations more easily compared to cue conditioned associations (Grillon et al., 2008; Grillon, Pine, et al., 2009). Moreover, it has been shown that fear responses can return after extinction, if a traumatic event occurred, which has been proposed as a model for relapse after successful exposure therapy (Bouton, 2002). However, cued fear conditioning as a model for specific phobia has been studied intensively, but mechanisms for the acquisition of contextual fear conditioning and the return of contextual anxiety has not been investigating widely, despite its relevance for sustained anxiety states characterizing many anxiety disorders.

The main aim of this thesis is to extend the limited research on contextual fear conditioning in humans and to investigate potential inter-individual variables that facilitate contextual fear conditioning as a model for sustained anxiety. Firstly, genetic variants will be examined to further elucidate the role of genetic predispositions on sustained anxiety. Secondly, reinstatement of contextual anxiety after extinction will be investigated as a possible model for relapse after exposure therapy. In the following chapters, the theoretical background of contextual fear conditioning and extinction processes as well as involved neuronal structures will be clarified (Chapter 2). Afterwards, two experimental studies regarding the influences of genetic polymorphisms of the serotonin and neuropeptide S system (Study 1; Chapter 3) and the reinstatement of extinguished contextual anxiety (Study 2; Chapter 4) will be described. Finally, the results of both studies will be discussed together and implications and limitations will be derived (Chapter 5).

# 2. Theoretical background

## 2.1. Fear vs. anxiety

It is suggested that there is a clear distinction between two defensive mechanisms: *fear* and *anxiety*. Fear is linked to a specific threat and activated by specific cues, whereas anxiety is a more sustained state, future oriented, not related to a specific object or stimulus and initiated by threatening contexts (Grillon, 2002). Fear and anxiety responses can be described on several output systems – the physiological level (e.g., heart rate, skin conductance response), the emotional-cognitive level (e.g., verbal reports for valence and arousal), and the behavioral level (e.g. avoidance) (Bradley & Lang, 2000), which initializes evolutionary adaptive defensive behavior. However, these responses may vary with its distance towards the threatening object. Michael S. Fanselow has proposed a *predatory imminence continuum* (see Fanselow, 1994; Fanselow & Lester, 1988). If the predator has not yet been detected but to some extend is likely to be present, the animal becomes more vigilant and cautious (*pre-encounter behavior*). In a next step, if the predator becomes present, the prey starts freezing (*post-encounter behavior*), meaning it rests motionless. Freezing is the most adaptive animal functional behavior, because the animal is unlikely to be detected by the predator. Pre- or post-encounter threat can both evoke anxiety observed in humans (Davis, Walker, Miles, & Grillon, 2010). If the threat comes too close, the next adaptive response is escape, and if the animal is caught, the only possibility to survive is fighting. All these reactions close to contact with the predator are referred to as *circa-strike defensive behaviors*. It is assumed that these fight and flight reactions (circa-strike) in the presence of a clear threatening object resemble fear reactions (Davis et al., 2010). Fear and anxiety responses can be initiated firstly, via an innate fear system which is automatically activated by phylogenetic relevant stimuli (e.g., snakes and spider for specific cues vs. open fields for specific contexts) (Mineka & Öhman, 2002). Secondly, fear reactions can be learned quickly via Pavlovian conditioning, which is a very adaptive associative learning mechanism because it allows fast reactions to an environment with changing sources of threat (Fanselow & Lester, 1988).

## 2.2. Cued fear conditioning

Cued fear conditioning is a simple form of classical conditioning, where a previously neutral stimulus (NS) is predictably paired with a naturally aversive event (unconditioned stimulus, US). After several repetitions and due to the temporal contiguity between the two stimuli, the neutral stimulus becomes a conditioned stimulus (CS). The fear response which is naturally evoked by the US (unconditioned response, UR) is

transferred to the CS, which now elicits a conditioned response (CR) – a fear response which is similar to the UR (Pavlov, 1927). Normally, cues that serve as CS are discrete and presented only for a few seconds. In a discriminative fear conditioning paradigm, two neutral stimuli are used. Only one stimulus is followed by the aversive US and becomes a CS+, while another stimulus (CS-) is never paired with this US. The CS+ is contingently delivered with or shortly after the US and therefore becomes a valid time-bound predictor of threat (Grillon, 2002). Notably, CS+ works as a signal for threat eliciting strong fear responses, while CS- works as a safety signal (Seligman & Binik, 1977) and is capable to reduce fear responses. Logically, the CS+ evokes a stronger or higher CR than the CS-. However, the context where the fear conditioning took place can also entail information about the US. The principle of contextual fear conditioning as well a definition of context is provided in the next chapter.

## 2.3. Contextual fear conditioning

Firstly, a definition of context will be provided before different paradigms for studying contextual fear conditioning are described.

### 2.3.1. Definition of context

Bouton (2002) suggested dividing contexts into *exteroceptive* and *interoceptive* ones. Exteroceptive contexts are specific features and background stimuli, while interoceptive contexts are defined by the status of the individual, e.g. mood, drug state, hormonal state, deprivation state, recent events, expectation of events or passage of time. According to Rudy (2009) features defining a context have two properties. On the one hand, they have to be *stable* meaning that the features and their relationships to each other should remain the same and constant over time. Importantly, both exteroceptive and interoceptive contexts have to be stable throughout the learning experience to be considered as a context. Notably, features that are presented only shortly at certain time points within the context, for example a tone or a light, are therefore not considered as contextual stable features. On the other hand, features should also be *variable* (*component variation*), meaning a component and its relationship to other components can be rearranged. To this end, a new context can be created, despite it is not clear how many rearrangements are necessary to define a new context.

### 2.3.2. Paradigms to study contextual fear conditioning

Originally, contextual fear conditioning was observed in rodents which also showed freezing to the context, where a cue conditioning protocol took place, even if the CS was not presented (Blanchard & Blanchard, 1972; Phillips & LeDoux, 1992). This is

referred to as *background contextual fear conditioning*, because during the learning phase the CS is associated with the US becoming the best predictor of the US. However, the context in the background is only secondarily associated with the US. In humans, a background contextual fear conditioning study was realized with two virtual contexts which each contained two light cues (Baas, Nugent, Lissek, Pine, & Grillon, 2004). Only in one context (anxiety context) one light (CS+) was followed by the US, the second light was not (CS-). The other context (safety context) also contained the same lights (CS+, CS-) but they were never associated with any US. Contextual fear conditioning was measured during a test phase where no US was delivered following the acquisition phase. Results showed that anxiety responses to the context (between cues) were higher in the anxiety context compared to the safety context. Background contextual fear conditioning can be clinically relevant for specific phobia, because the context where a phobic stimulus has occurred might induce anxiety responses and consequently is avoided. For example, a room (e.g., bathroom or cellar) where a spider has been previously detected afterwards elicits physiological arousal and anxious expectations of the occurrence of the spider in phobics and this room will most likely be avoided.

However, there are two additional paradigms which can be described as *foreground contextual fear conditioning.* Firstly, the US is presented in between the CS, therefore CS and US are unpaired. Secondly, the US is presented alone without any CS. During foreground contextual fear conditioning the US will be associated with the whole context because in these paradigms the context is the best predictor of the US (Luyten, Vansteenwegen, van Kuyck, Deckers, & Nuttin, 2011; Phillips & LeDoux, 1994). Another explanation comes from the safety-signal hypothesis of Seligman. If specific cues signaling threat occurrence are lacking, and the threat is unpredictable, the individual experiences a chronic status of anxiety because it cannot identify periods of safety (Seligman, 1968; Seligman & Binik, 1977). Therefore, foreground contextual fear conditioning may serve as a model for sustained and chronic anxiety because the US is not time-bound to a specific cue and therefore is experienced as unpredictable (Grillon, 2008). In humans, the CS-US unpaired contextual fear conditioning paradigm is extensively used by Grillon and colleges. Normally, they present three different experimental conditions. During the predictable condition (P) specific cues are paired with the US. In contrast, during the unpredictable condition (U) the US is presented between specific cues. During the neutral condition (N) only the cues are presented but no US is delivered. All three conditions are part of an instructed fear conditioning protocol, meaning that during the experiment participants are always instructed about the conditions. Using this NPU-paradigm Grillon, Baas, Lissek, Smith, and Milstein (2004) could show that contextual anxiety was higher in the unpredictable compared to the predictable condition and was only evident if an

aversive US was used (an electric shock in contrast to an airblast). Additionally, it was demonstrated that chronic expectations about the US were higher and avoidance behavior was more frequent in the unpredictable compared to the predictable condition (Grillon, Baas, Cornwell, & Johnson, 2006; Vansteenwegen, Iberico, Vervliet, Marescau, & Hermans, 2008). In the same view, adding a predictive cue which signals US occurrence to a context where the US was formerly presented unpredictably, reduced contextual anxiety as indicated by reduction of startle responses and expectancy ratings (Fonteyne, Vervliet, Hermans, Baeyens, & Vansteenwegen, 2009, 2010). Therefore, all these data support the role of unpredictable threat in the development of contextual anxiety.

Nevertheless, contextual fear conditioning can also established using only unpredictable US without presenting any CS in between. An animal study has demonstrated that both paradigms (CS-US unpaired vs. US only) are equally successful and did not differ in the strength of contextual anxiety as measured with fear-potentiated startle and freezing (Luyten, Vansteenwegen, van Kuyck, Deckers, et al., 2011). We were also able to demonstrate that a virtual spatial context paired with unpredictable USs (without any CS) evoked larger anxiety responses on different response levels (physiology, ratings, and avoidance behavior) compared to a safe context, in which no US was delivered (Glotzbach, Ewald, Andreatta, Pauli, & Mühlberger, 2012; Tröger, Ewald, Glotzbach, Pauli, & Mühlberger, 2012). Another human paradigm applying the US-only paradigm used background colors as contexts instead of virtual spatial contexts and provided successful context conditioning as indicated on physiological, neuronal and explicit-verbal levels (Lang et al., 2009). Confirming the sufficiency of this latter human paradigm, an animal study showed that long duration CS also elicit sustained anxiety (Waddell, Morris, & Bouton, 2006). Taken together all foreground contextual fear conditioning paradigms emphasize the importance of unpredictable threat for the establishment of contextual anxiety.

### 2.3.3. Contextual fear conditioning and anxiety disorders

From a clinical perspective, it has been suggested that increased contextual anxiety elicited by unpredictable aversive events may be an important pathogenic marker for panic disorder (Grillon et al., 2008), PTSD (Grillon, Pine, et al., 2009) and generalized anxiety disorder (GAD) (Luyten, Vansteenwegen, van Kuyck, Gabriels, & Nuttin, 2011). In the following, I will briefly describe the diagnostic criteria for these anxiety disorders and explain how the contextual fear conditioning paradigm might contribute to the development or maintenance of these disorders.

Firstly, panic disorder with agoraphobia is characterized by recurrent, unexpected panic attacks, persistent concerns about future panic attacks, and a strong avoidance of

places where panic attacks might occur and where patients feel helpless (American Psychiatric Association, 2000). As I have already stated elsewhere (Glotzbach et al., 2012), the panic attacks may be perceived as unpredictable, because the patients might have difficulties to identify specific environmental cues which predict their panic attacks (which may function as a US). As a result, the whole context (CXT+) might become associated with the panic attack. As a consequence the context (CXT+) might induce sustained and chronic anxiety states and avoidance behavior. By contrast, a context not associated with a panic attack might be perceived as safe (safety context; CXT-) and preferentially approached (Gorman, Kent, Sullivan, & Coplan, 2000).

Secondly, PTSD can develop after the exposure to a traumatic event. It is characterized by a persistent re-experience of the traumatic event, intense distress to internal or external cues that symbolize aspects of the traumatic event, avoidance of these stimuli and places associated with the trauma, and increased arousal levels (American Psychiatric Association, 2000). In relation to panic attacks in panic disorder, the traumatic event, which may function as a US, can also be experienced as unpredictable and uncontrollable (Grillon, Pine, et al., 2009). Therefore, not only specific stimuli but also the internal or external context can be conditioned with the traumatic event. In turn, confrontation with these contextual stimuli can evoke intense distress, physiological arousal and recollection of the trauma.

Thirdly, symptoms of GAD include chronic anxiety and worry, and difficulties to control the worry. Patients with GAD do not fear a specific situation or object (like in specific phobia), but show general apprehensive expectations according to many conditions (American Psychiatric Association, 2000). Contextual fear conditioning induces chronic expectations (Vansteenwegen et al., 2008), thus GAD might be better characterized by sustained anxiety than phasic fear (Luyten, Vansteenwegen, van Kuyck, Gabriels, et al., 2011).

## 2.4. Neural basis of fear and anxiety

Two neuronal pathways are discussed in the processing of threatening stimuli (an innate fearful stimulus or a learned CS). The *low road* or the *thalamo-amygdala pathway* directly conveys visual input of a potential threatening object via the thalamus to the amygdala. As a result, fear responses and defensive behavior are initialized very quickly but imprecise, also described as “quick and dirty” processing. The low road has the advantage that defensive behavior, like freezing or avoidance, is elicited very quickly which is evolutionary very adaptive. Furthermore, the quick activation of the amygdala

might prime it for the evaluation of additional information from cortical areas. And the subcortical route has even the function to interrupt attention and to shift it to threatening stimuli via amygdala-cortical projections. A more elaborate and precise processing of a threatening stimulus is done via the *high road* or the *thalamo-cortico-amygdala pathway*. Before the stimulus is processed in the amygdala it is first analyzed in sensory cortical areas (i.e., the occipital cortex for visual stimuli, and the auditory cortex for auditory stimuli) where a precise processing takes place resulting in a more correct and appropriate fear response, but it also takes longer to initialize it. Cortical areas seem not to be necessary for the conditioning to simple cues but may be crucial for the conditioning to complex ones. Both routes, the subcortical low road and the cortical high road, process the sensory stimulus automatically and unconsciously. However, the sensory cortex involved in the high road is not sufficient enough to evoke a conscious perception. Moreover, the prefrontal cortex (PFC) is needed to consciously process and to cognitively evaluate sensory threatening stimuli (LeDoux & Phelps, 2008; Monfils, Bush, & LeDoux, 2010).

Figure 1 depicts the neuronal pathways for the processing of fearful cues (CS) and electric painful US, their association in the amygdala, and output systems for the fear responses. The amygdala can mainly be divided into three nuclei: the basolateral nucleus or complex (BLA), the medial amygdala (MeA) and the central amygdala (CeA). The CS is processed in parallel via both, the thalamo-amygdala and thalamo-cortico-amygdala pathways, which both end up in the BLA. The US is also projected to the BLA via the somatosensory thalamus and somatosensory cortical areas. It is assumed that in the BLA the association between CS and US takes place. Additionally, the BLA does not only receive sensory information of the CS and US but also contextual information during the learning procedure from the hippocampus. This information is integrated and then transmitted to the CeA, which is the output part of the amygdala and capable to initialize various autonomic and behavioral fear responses (Kim & Jung, 2006; Maren, 2001). The CeA has projections to the hypothalamus and brainstem. The periaqueductal gray (PAG) produces freezing, the lateral hypothalamus (LH) and the dorsal motor nucleus of the vagus (DMN) lead to increased heart rate and blood pressure. The release of stress hormones, such as glucocorticoids, is done via the paraventricular nucleus of the hypothalamus (PVN) (Maren, 2001). Supportively, a review on human neuroimaging studies also revealed that amygdala activation is mostly found during fear acquisition and expression. Furthermore, the insula, which is thought to transmit a cortical representation of fear to the amygdala (Phelps et al., 2001), and the anterior cingulate cortex (ACC), which seems to play a role in approach and avoidance learning, are also important neuronal structures involved in human cued fear conditioning studies (Sehlmeyer et al., 2009).

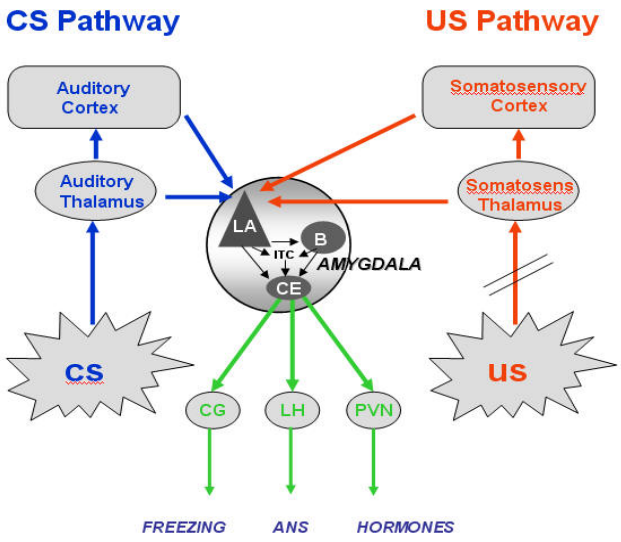


*Figure 1. Neuronal pathways involved in fear conditioning.*
From LeDoux (2007): http://www.scholarpedia.org/article/File:Emotional_Memory_fig3.jpg
ANS = autonomic nervous system; B = basal amygdala; CE = central amygdala; CG = central gray; CS = conditioned stimulus; LA = lateral amygdala; ITC = intercalated neurons; LH = lateral hypothalamus; PVN = paraventricular nucleus of the hypothalamus; US = unconditioned stimulus.

### 2.4.1. Hippocampus and contextual fear conditioning

The hippocampus is mainly involved in learning and memory, more precisely in the formation and retrieval of episodic memories - the memory for experiences as well as the time and place where they occurred (Rudy, 2008; Smith & Mizumori, 2006). Furthermore, the hippocampus is necessary to generate cognitive maps of the spatial environment, i.e. a mental representation of the environment (O'Keefe & Dostrovsky, 1971; O'Keefe & Nadel, 1978), and is strongly involved in spatial learning and recall (Kessels, de Haan, Kappelle, & Postma, 2001; Nadel & Hardt, 2004). Especially, the hippocampus is important for the formation and storage of temporal-spatial contextual information, and therefore plays a key role in episodic memory formation as well as in spatial mapping (Rudy, 2008; Smith & Mizumori, 2006). Additionally, the hippocampus is also involved in the conscious recollection of an episode and declarative knowledge, as a fear conditioning study in a patient with bilateral hippocampus lesions showed. This patient had no explicit contingency knowledge about the relationship between CS and US (Bechara et al., 1995).

Given the important role of the hippocampus in the formation and storage of contextual information, it is regarded as an crucial neuroanatomical structure in contextual fear conditioning in animals (Kim & Jung, 2006; Maren, 2001) and humans (Alvarez, Biggs, Chen, Pine, & Grillon, 2008; Alvarez, Chen, Bodurka, Kaplan, & Grillon, 2011; Lang et al., 2009; Marschner, Kalisch, Vervliet, Vansteenwegen, & Büchel, 2008). The hippocampus transmits the contextual information to the BLA, where it is associated with

the US, suggesting that the BLA is the site where contextual fear memories are formed and encoded (Barot, Chung, Kim, & Bernstein, 2009).

Rudy, Huff, and Matus-Amat (2004) proposed a two-process theory of contextual fear conditioning and described how the hippocampus is involved in contextual processing. They suggested two representation types of a context. Firstly, the context is represented as *independent features* and each of them can be associated with the US during conditioning. Secondly, the context is represented as the *conjunction* of the single features and the conjunctive presentation is associated with the US. Therefore, fear conditioning can be established by either associating independent features of a context or the conjunctive feature of the context with the US. Furthermore, distinct neurobiological substrates are assumed to be responsible for the two representation types. According to Rudy et al. (2004) the neocortical system stores independent features, whereas the conjunctive representation of a context is built through an interaction of the cortex with the hippocampus. Furthermore, the feature and conjunctive contextual representations compete for an association with the US during learning, but the conjunctive representation will be stronger, because the hippocampus inhibits the association between the feature representations of the context and the US. Therefore, if the hippocampus is intact during learning, the conjunctive representation of the context will be associated with the US. Conclusively, hippocampal damage *after* learning (called the *retrograde effect*) will lead to a decreased contextual fear response at test (Kim & Fanselow, 1992; Matus-Amat, Higgins, Barrientos, & Rudy, 2004), because the conjunctive representation cannot be retrieved and the feature representation cannot induce the fear responses, because it has not been established strongly enough during learning. On the other hand, Rudy et al. (2004) propose that contextual fear conditioning will occur, if the hippocampus is damaged *prior* to conditioning (*anterograde effect*) (Wiltgen, Sanders, Anagnostaras, Sage, & Fanselow, 2006). At first glance these results are quite surprising, but, as described above, a contextual representation also consists of independent single features stored in the cortex, and their association with the US is normally inhibited by the hippocampus. Hippocampal damage before the conditioning allows for associating the independent feature representation with the US. Hence, contextual fear conditioning can be established, because it is mediated via associations between amygdala and the cortex, where single contextual features are processed. However, other studies could not confirm the anterograde effect, but found that hippocampal lesions before conditioning impaired contextual fear conditioning (Phillips & LeDoux, 1992; Young, Bohenek, & Fanselow, 1994). These controversial findings may result from a different amount of learning trials, because it is assumed that the association between the independent feature

representation and the US would develop slower and would need more learning trials (Rudy et al., 2004; Wiltgen et al., 2006).

### 2.4.2. BNST and sustained anxiety

Another important structure involved in sustained anxiety is the bed nucleus of the stria terminalis (BNST). The BNST is part of the "extended amygdala" because it is connected to the CeA and MeA (Alheid & Heimer, 1988). Importantly, different subnuclei of the CeA seem to play different roles in phasic fear and sustained anxiety. A vary detailed description can be found in Davis et al. (2010). In short, both the medial CeA ($CeA_M$) and the lateral CeA ($CeA_L$) project to the BNST, but they differ in their projections to other brain regions. The $CeA_M$ is connected to the hypothalamus and the brain stem which are responsible for somatic and autonomic *fear* responses (e.g., fear-potentiated startle reflex), whereas the $CeA_L$ has projections to the substantia innominate, but mostly to the BNST. Furthermore, the corticotropin-releasing factor (CRF), which is specifically involved in the peripheral stress response, is highly associated with $CeA_L$ and BNST but to a much lesser extend with $CeA_M$. CRF is highly concentrated in the PVN, which projects to the $CeA_L$, possibly directly regulating stress responses via CRF-neurons. The $CeA_L$ and BNST also receive input from the insula, which is often activated in human fear conditioning studies during the anticipation of an aversive stimulus, and is suggested to play an important role in the cognitive representation of fear in humans (e.g., Andreatta et al., 2012; Phelps et al., 2001). Davis et al. (2010) assume that the pathway from the $CeA_M$ to the brain stem and the hypothalamus would rapidly initiate phasic fear reactions, even to a long duration CS or a context, whereas shortly thereafter a second pathway would be activated. The $CeA_L$ releases CRF into the BNST which initiates sustained anxiety responses, also mediated by the brain stem. Additionally, the BNST or $CeA_L$ would inhibit the $CeA_M$, thus disrupting the phasic fear response. In conclusion, phasic fear and sustained anxiety seem to some extend be mediate by different brain systems.

Evidence for an involvement of BNST in sustained but not phasic fear comes from animal as well as from human studies. For instance, in rodents lesions of the BNST decreased freezing and startle potentiation to a conditioned context, but not to a discrete cue (Luyten, van Kuyck, Vansteenwegen, & Nuttin, 2011; Sullivan et al., 2004). In humans, functional magnetic resonance imaging (fMRI) studies revealed BNST activation to several sustained anxiety-inducing situations: to a context, where unpredictable US were presented but not during predictable conditions (Alvarez et al., 2011), and during hypervigilant threat monitoring in anxious participants (Somerville, Whalen, & Kelley, 2010).

## 2.5. Measuring fear and anxiety in humans

### 2.5.1. Two-level account of fear conditioning

Hamm and Weike (2005) suggested a two-level account of fear conditioning: an implicit and explicit level. The implicit non-cognitive level does not require declarative and explicit knowledge about contingencies between CS and US and is regarded to mostly rely on the amygdala, as it is capable to automatically activate the defensive subcortical system. In contrast, the explicit cognitive level requires declarative knowledge about CS-US contingencies and might occur even without activation of the subcortical defensive system, but requires the hippocampus. The explicit cognitive fear level can be established by means of evaluative conditioning (EC), which describes the change in the subjective evaluation of the CS or CXT due to conditioning. EC transfers the negative affect of the US to the CS+ (De Houwer, Thomas, & Baeyens, 2001). The explicit cognitive level can be measured by means of verbal reports for valence, arousal and fear regarding conditioned cues or conditioned context (CS+ vs. CS-, or CXT+ vs. CXT-) (Andreatta et al., 2010; Glotzbach et al., 2012; Lipp, 2006; Tröger et al., 2012). Additionally, explicit learning is reflected in differential skin conductance response (SCR) and subjective contingency awareness (Hamm et al., 2003; Hamm & Vaitl, 1996), whereas implicit fear conditioning without cognitive awareness can be measured with the fear-potentiated startle reflex.

### 2.5.2. Electrodermal activity

Electrodermal activity (EDA) is influenced by the autonomic nervous system (ANS), especially the sympathetic nervous system, and can be measured by means of electrical conductivity of the skin at the fingers or the thenar of the non-dominat hand (Dawson, Schell, & Filion, 2000). On the one hand, many studies use SCR, the peak response after stimulus onset, in fear conditioning paradigms as an index of cued fear learning, with higher SCR for CS+ compared to CS- (Olsson & Phelps, 2004; Schiller et al., 2010; Tabbert et al., 2011). On the other hand, skin conductance level (SCL), the mean tonic conductivity during a stimulus presentation, has been established in contextual fear conditioning paradigms as an index for sustained anxiety with higher SCL in CXT+ compared to CXT- (Glotzbach-Schoon et al., 2013; Tröger et al., 2012). Possibly, EDA is influenced by, 1) the hypothalamus and the limbic system, controlling thermoregulation and emotional arousal, 2) the reticular formation in the brain stem controlling muscle tone, and 3) cortical areas like premotor cortex, responsible for fine motor control, and PFC controlling orienting and attention (Dawson et al., 2000). Supporting the role of the amygdala in EDA, Cheng, Knight, Smith, and Helmstetter (2006) could show that increased amygdala activity to CS+ correlated SCR. In the same view, patients with temporal lobectomy failed to exhibit conditioned discrimination between CS+ and CS- in SCR (LaBar,

LeDoux, Spencer, & Phelps, 1995), but this seems to be most prominent in unaware patients (Weike et al., 2005). Therefore, SCR might be regarded as a valid measure for amygdala output via ANS in fear conditioning. But SCR is not valence specific for negative affect, but only a measure for emotional arousal and stimulus significance. Various studies showed that SCR increased to both, negative and positive stimuli, thus, SCR can reflect an activation of the aversive as well as the appetitive system (for review see Bradley & Lang, 2007). Instead, SCR is considered as a mere measure of emotional arousal, because it is highest for high-arousing emotional stimuli. It has also been shown that SCR is dependent on contingency learning, meaning that a higher SCR to CS+ compared to CS- could only be observed in participants who explicitly learned and were able to report the association between CS+ and US, suggesting that conditioning of SCR might reflect an explicit, cognitive level of contingency learning (Hamm & Weike, 2005; Hamm & Vaitl, 1996; Weike, Schupp & Hamm, 2007).

### 2.5.3. The startle reflex

A valid behavioral measure for the translational research of fear and anxiety, which can be used across species, is the fear-potentiated startle response. The startle response is an ancestral reflex which is elicited by a sudden and intense acoustic, tactile or visual stimulus. The mostly used acoustic startle reflex is processed via a circuit of auditory cortices, the caudal pontine reticular nucleus (PnC) (a part of the brain stem) and motor neurons resulting in fast muscle contractions. Via electromyography (EMG) the muscle contractions can be measured, which are mostly pronounced around the face, neck and shoulders in rats, whereas in humans it can be measured at the M. *orbicularis oculi*, which is the muscle located around the eye controlling the eye-blink reflex (Blumenthal et al., 2005; Fendt & Fanselow, 1999). The startle response is regarded as a defensive reflex, as its possible functions are protection from injury from a predator and the reduction of the latency of flight behavior (Fendt & Fanselow, 1999; Koch, 1999). Importantly, the startle reflex is modulated by influences of the central amygdala (CeA) on the PnC (for reviews see Davis, 2006; Koch, 1999). As a consequence, negative, threatening and fear inducing stimuli like negative pictures or fear conditioned stimuli (CS+) activate the amygdala leading to startle potentiation. On the other hand, positive stimuli, like positive pictures or relief-associated stimuli lead to a reduction of the startle reflex (Andreatta, Mühlberger, Yarali, Gerber, & Pauli, 2010; Hamm, Greenwald, Bradley, & Lang, 1993; Lang, Bradley, & Cuthbert, 1990) because of the afferent projections from the nucleus accumbens (mainly involved in the processing of rewards) to the PnC (Koch, 1999). It is assumed that the $CeA_M$ directly innervates the brain stem (PnC), leading to a *fear*-potentiated startle reflex, whereas long lasting CS or contextual stimuli are processed via the $CeA_L$ which activates the brain stem (PnC) via the BNST, resulting in an *anxiety*-

potentiated startle reflex (Davis, 2006; Davis et al., 2010; Nagy & Paré, 2008; Walker, Miles, & Davis, 2009). According to the predator-imminence model (Fanselow, 1994), Davis et al. (2010) assumd that the fear-potentiated startle reflex would resemble circa-strike behavior, whereas the anxiety-potentiated startle reflex would depict post-encounter defensive behavior. Animal studies demonstrated that lesions of the amygdala, especially the BLA, lead to reduced or even abolished fear-potentiated and anxiety-potentiated startle (for reviews see Davis et al., 2010; Walker et al., 2009), whereas lesions of the BNST, as noted above, disrupt anxiety-potentiated startle, but not fear-potentiated startle (Sullivan et al., 2004). Supporting the involvement of the amygdala in fear-potentiated startle in humans, Weike et al. (2005) demonstrated that patients with temporal lobectomy failed to exhibit fear-potentiated startle to a CS+. Furthermore, amygdala activation correlated positively with startle-potentiation to the CS+ in an simultaneous fMRI and EMG study in healthy participants (van Well, Visser, Scholte, & Kindt, 2012). The fear-potentiated startle reflex was even observed in a cortically blind patient suggesting that this reflex may even occure without cortical processing (subcortical low road) (Hamm et al., 2003). Therefore, it is thought to be an implicit measure of fear learning greatly independent of cognitive processes and explicit contingency knowledge (Hamm & Weike, 2005). For example, Hamm and Vaitl (1996) showed that in humans differential conditioning can lead to fear-potentiated startle to the CS+ regardless of contingency awareness. An shematic overview of involved neuronal structures in fear- and anxiety-potentiated startle is provided in Figure 2.

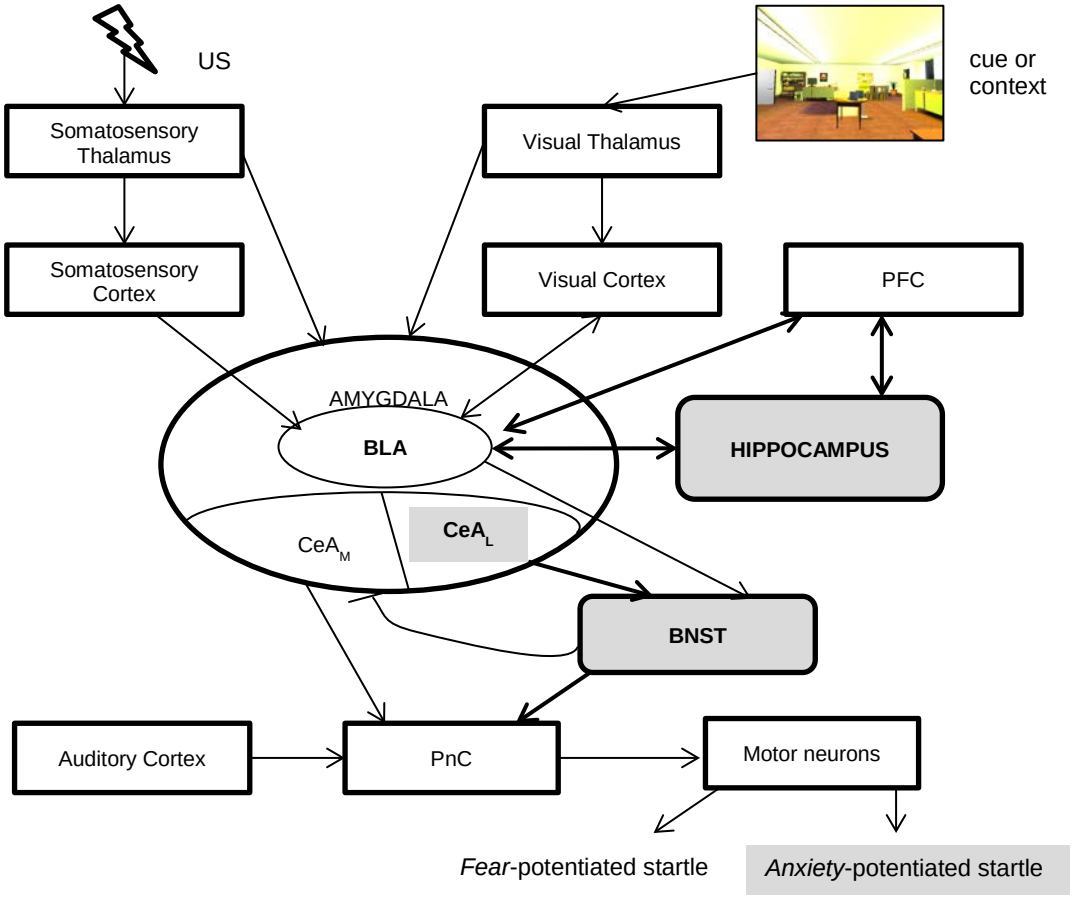


*Figure 2. A hypothetical neuroanatomical model of fear- and anxiety-potentiated startle reflex.* Important structures for the anxiety-potentiated startle reflex are shaded in gray. Adopted and modified from Davis et al. (2010), LeDoux (2007), Rudy et al. (2004), and Walker et al. (2009). BLA = basolateral amygdala; BNST = bed nucleus of the stria terminalis; $CeA_L$ = lateral central amygdala; $CeA_M$ = medial central amygdala; CS = conditioned stimulus; PFC = prefrontal cortex; PnC = caudal pontine reticular nucleus; US = unconditioned stimulus.

## 2.6. Extinction processes

Extinction is defined as the decrease of the CR when the CS is presented without any US. In terms of fear conditioning, if a feared object or context (CS or CXT) is repeatedly experienced without aversive consequences (US), fear reactions (CR) will decrease. Originally, Rescorla and Wagner suggested extinction as unlearning, meaning that the fear memory established during conditioning would be deleted (as cited in Bouton, 2002). Notably, a better comprehension of the mechanisms underlying extinction training is of crucial importance, because extinction training is an important mechanism for exposure-based therapies – a standard treatment of anxiety disorders (Boschen, Neumann, & Waters, 2009). Although, extinction training is highly effective in anxiety disorder patients, several patients suffer from relapse after exposure therapy (Boschen et al., 2009). Because of this clinical observation as well as experimental studies conducted in the laboratory which observed a return of fear after extinction sessions, it is assumed that a new safety

memory (CS-noUS) is formed during extinction that does not erase but instead inhibits the original fear memory (Bouton, 2002).

Furthermore, in line with the idea that extinction is not unlearning but new learning, it has been observed that extinction training is not equally successful for different levels of a fear response. Whereas physiological fear responses (SCR, startle potentiation) are relatively quickly extinguished, negative evaluations of a CS+ seem to be resistant to extinction. The CS+ can still be rated as more negative than the CS- even after several extinction trials, because it is assumed that the CS+ is still cognitively associated with the US, but the physiological arousal is decreased during extinction so that it is not sufficient enough to evoke a differential conditioned response (CS+ > CS-) in SCR or startle reflex (Vansteenwegen, Crombez, Baeyens, & Eelen, 1998).

### 2.6.1. Neural basis of extinction

On the neural level, Milad and Quirk (2002) showed that infralimbic (IL) neurons of the ventral medial PFC (vmPFC) fired when rats are tested for *extinction recall* one day after the initial extinction training. They concluded that the vmPFC would play a crucial role, not in extinction learning but in the long-term consolidation of the extinction memory. It has been shown that extinction involves a network of IL cortex, BLA, intercalated (ITC) neurons, hippocampus and CeA (Milad & Quirk, 2012). In detail, it is assumed that during extinction the IL cortex enhances the efficacy of the BLA which in turn activates GABAergic (GABA = gamma aminobutyric acid) ITC neurons via glutamate pathways. The ITC activation results in an inhibition of the $CeA_M$ (Amano, Unal, & Paré, 2010). Importantly, the inhibitory extinction memory is highly context dependent, so that a CS presented outside the extinction-context is still able to elicit a CR (Bouton, 2002). Notably, the context-dependency of fear extinction is mediated by the hippocampus. As noted above (2.4.1), the hippocampus is necessary to build a conjunctive representation of the context (Rudy et al., 2004). The hippocampus has projections to the IL cortex and to the BLA and therefore might influence fear extinction (Milad & Quirk, 2012). Ji and Maren (2007) provided two suggestions about how the hippocampus is involved in fear extinction. On the one hand, the hippocampus is directly connected to the amygdala and therefore targets fear expression or inhibition. On the other hand, the hippocampus might have an executive control over IL cortex, thus regulating context-dependent fear inhibition or fear expression indirectly via the amygdala. The hippocampus might have an excitatory influence on the IL cortex when the CS is tested in the extinction context, but on the contrary might have an inhibitory influence on the IL cortex when the CS is tested outside the extinction context. Neuroimaging studies confirmed the involvement of the vmPFC and the hippocampus in extinction. Activations of vmPFC and hippocampus have been found

during extinction recall (Kalisch et al., 2006; Milad et al., 2007) and the thickness of the vmPFC correlated positively with extinction performance (Milad, Quinn, et al., 2005).

### 2.6.2. Extinction of contextual fear memories

There are sufficient studies in humans focusing on cued fear learning and its extinction, but there is an apparently lack of research on contextual fear conditioning and especially on extinction learning and extinction consolidation of contextual fear memories. The neural circuits involved in extinction learning summarized above focused on cued fear extinction and the role of the background context involved in it, but there are only few studies investigating extinction of contextual conditioned anxiety. One study using the NPU-paradigm found greater startle potentiation during the unpredictable compared to the predictable and neutral conditions at the beginning of extinction learning, and this difference was extinguished at the end of the extinction session (Grillon et al., 2006). However, startle potentiation to a specific cue in the predictable condition remained significantly higher compared to the intertrial interval (ITI) and to the cue in the neutral condition, indicating that fear to a threat cue did not extinguish during the extinction session (Grillon et al., 2006). Similar results were obtained by an animal study which found that rats showed faster extinction of freezing behavior to the background context than to the conditioned cue (Phillips & LeDoux, 1992). Unfortunately, the authors did not discuss why extinction of sustained anxiety to the context was faster than the extinction of phasic fear to a specific cue. Maybe, fear conditioning to a context where cues are presented was weaker than fear responses to a cue. Supportively, fear-potentiated startle magnitudes were higher during the cues compared to the background context (Baas et al., 2004), suggesting that extinction of the contextual anxiety was facilitated. However, in the animal study by Phillips and LeDoux (1992) freezing to the cue was equally high compared to the background context during acquisition. On the other hand, other inhibitory mechanisms maybe involved during extinction of phasic fear vs. sustained anxiety applying different neural correlates. But on a neural level, extinction of contextual anxiety also seems to involve the medial PFC as indicated by an fMRI study (Lang et al., 2009). These authors reported amygdala activation during the beginning of the extinction learning (reflecting the acquired conditioned response), but medial PFC activation during the later extinction session. Moreover, a functional coupling between medial PFC, amygdala, and hippocampus during extinction learning was found (Lang et al., 2009), resembling a neural network involved in extinction of contextual anxiety which was also proposed by Milad and Quirk (2012), and Ji and Maren (2007) (see above).

In sum, only few studies exist which investigated extinction of contextual anxiety in humans. These studies suggested that extinction of contextual anxiety is faster

compared to cued fear, but involves similar neuronal circuits. To my knowledge there are no studies investigating the consolidation of a contextual fear memory tested during extinction recall, e.g., one day after extinction training in analogy to cued fear conditioning studies (e.g., Milad & Quirk, 2002). Given the pivotal role of impaired extinction learning in anxiety disorder patients (see below, 2.7.) and the importance of extinction mechanisms in behavioral therapy, the lack of extinction research in contextual fear conditioning is surprising and there is a need for further research on this topic.

### 2.6.3. Mechanisms of the return of fear after extinction

It is assumed that during extinction, the original fear memory is not erased, but a new safety memory is formed which inhibits the original fear memory (Bouton, 2002). Importantly, the inhibitory extinction memory is highly context dependent, so that a CS presented outside the extinction-context induces a return of fear after extinction, providing crucial evidence for the assumption that the original fear memory is still intact and not erased (Bouton, 2002). Several animal (for reviews see Bouton, 2002, 2004) and human cued fear conditioning studies demonstrated four mechanisms of return of fear after extinction which strongly dependent on the context. Fear reactions according to a CS can return after extinction training due to: 1) a passage of time (*spontaneous recovery*; Norrholm et al., 2008); 2) a change of the context (*renewal*; Alvarez, Johnson, & Grillon, 2007; Milad, Orr, Pitman, & Rauch, 2005; Vansteenwegen et al., 2005); 3) an unsignaled US presentation (*reinstatement*; Dirikx, Hermans, Vansteenwegen, Baeyens, & Eelen, 2004; Hermans et al., 2005); and 4) re-pairing of CS and US (*rapid reacquisition*; Kindt & Soeter, 2013). Based on the ideas that the extinction memory is highly context dependent and that background stimuli as well as internal states like mood, drugs or time can constitute a context, renewal can also be observed, if the physical or internal extinction-context is changed (Bouton, 2002). In the same vein, rapid reacquisition will be most pronounced, if the background stimuli, which were presented during extinction, are changed (Bouton, 2002). Spontaneous recovery is considered as a special kind of renewal because a change in time can also be considered as change of the temporal context (Bouton, 2002). Reinstatement is discussed to be dependent on the context, where the unsignaled US is given. Here, the idea is that this procedure leads to contextual fear conditioning, and therefore also leads to increased fear responses to CS presented in this context (Bouton, 2002). Notably, all these four mechanisms have been studied in cued fear conditioning paradigms, but to my knowledge there are no studies which investigated the return of contextual anxiety in humans.

## 2.7. Fear conditioning, extinction and anxiety disorders

The central "fear system" related to phasic fear and sustained anxiety in the animal and human brain seems to be the amygdala. In the amygdala the association between either distinct cues or contextual information and the US takes place and fear or anxiety responses are initiated. Mineka and Öhman (2002) have argued that the fear system can be even regarded as an encapsulated module. Namely, associations between evolutionary relevant stimuli like snakes, spiders or open places (contexts), and aversive events can be learned quickly (preparedness theory). As a consequence, the individual reacts with automatic, reflex-like fear reactions even without conscious processing (i.e., even if the CS was presented subliminally; subcortical route), which are mediated by the amygdala, and can result in a fear- or anxiety-potentiated startle reflex. According to Grillon (2002) anxiety disorders are characterized by an overactivation or an inappropriate activation of the fear system. This can result in faster fear conditioning and higher fear responses to a CS+ vs. CS- (higher conditionability) in anxiety disorder patients compared to healthy controls (Mineka & Oehlberg, 2008), which has been supported by a study in PTSD patients (Orr et al., 2000). Additionally, an overactivation of the fear system in pathologic anxiety has also been evidenced by a meta-analysis of neuroimaging studies in PTSD, social and specific phobia patients, showing greater amygdala and insula activation to negative stimuli compared to healthy controls (Etkin & Wager, 2007). Furthermore, the BNST, a part of the extended amygdala, seems to be especially relevant for sustained anxiety states. A heightened BNST activation has been reported during a task of high uncertainty in GAD patients (Yassa, Hazlett, Stark, & Hoehn-Saric, 2012). In sum, a hyperactivity of the amygdala and BNST to threatening stimuli and uncertain situation, as well as faster fear conditioning might contribute to exaggerated fear and anxiety responses seen in anxiety disorder patients.

Logically, it is also proposed that a dysregulation of the prefrontal-amygdala network plays an important role in anxiety disorders. Patients who suffer from anxiety disorders, show exaggerated fear responses which are not appropriate anymore (American Psychiatric Association, 2000). The exaggerated fear and anxiety levels might result from an inhibitory deficit to reduce and extinguish fear responses due to a hyperactive amygdala and an impaired vmPFC and hippocampus functioning (Milad & Quirk, 2012; Rauch, Shin, & Phelps, 2006). There are several studies showing extinction deficits, i.e. heightened CS+ responding during extinction, in PTSD and panic disorder patients indexed by SCR and evaluative conditioning (Blechert, Michael, Vriends, Margraf, & Wilhelm, 2007; Michael, Blechert, Vriends, Margraf, & Wilhelm, 2007; Wessa & Flor, 2007). In sum, not only enhanced conditionability but also reduced extinction capabilities are important pathologic learning mechanisms which might contribute to the

development and maintenance of anxiety disorders (Mineka & Oehlberg, 2008; Orr et al., 2000). Furthermore, it is also important to investigate the persistence of extinction and the consolidation of an extinction memory, for example during extinction recall one day after extinction learning (Milad & Quirk, 2012). Supportively, one study did not find any difference between PTSD patients and healthy controls during fear acquisition or extinction learning but during extinction recall in SCR data. Furthermore, the reduced extinction recall in SCR correlated with reduced activity in vmPFC and hippocampus (Milad et al., 2009).

In conclusion, identifying inter-individual risk factors which contribute to faster conditioning and/or reduced extinction learning and impaired extinction recall of contextual anxiety seems highly relevant. On the one hand, the personality variable *trait anxiety* has been found to modulate the acquisition of contextual anxiety. High-anxious participants showed faster contextual fear conditioning compared to low-anxious ones (Glotzbach-Schoon, Tadda, et al., 2013). On the other hand, certain genetic polymorphisms have been found to influence cued fear conditioning and extinction (see below, 2.8.), and to be associated with personality variables of anxiety (Schinka, Busch, & Robichaux-Keene, 2004). Genetic contributions to the etiology of anxiety disorders and fear conditioning will be described in the following chapter.

## 2.8. Genetic variables

A genetic contribution to the etiology of anxiety disorders is likely, because a heritability of 43% for panic disorder and 32% for GAD has been estimated (Hettema, Neale, & Kendler, 2001). Interestingly, panic disorder, agoraphobia and GAD share similar genetic diathesis, which is different from the genetic diathesis of specific phobias (Hettema, Prescott, Myers, Neale, & Kendler, 2005), suggesting distinct genetic contributions to fear and anxiety. The most important and widely investigated genotype associated with anxiety disorders (PTSD and GAD) is a single nucleotid polymorphism (SNP) of the promoter region of the serotonin transporter gene (5-HTTLPR; S vs. L allelic variant) (for review see Norrholm & Ressler, 2009). Interestingly, a novel candidate gene for panic disorder, namely the neuropeptide S receptor gene (NPSR1; T vs. A allelic variant), has recently been identified (Domschke et al., 2011). However, a direct association between specific genes (genotype) and a complex psychiatric disorder, like an anxiety disorder (phenotype), is often hard to find, because genes solely encode proteins which constitute cells and neural circuits, but not behavior. Therefore, it is more promising to study *endophenotypes* (Leonardo & Hen, 2006). An endophenotype (or intermediate phenotype) lies in between the genotype and the disease (phenotype), and is

a measurable, mostly biological component (Gottesman & Gould, 2003). It is supposed that an association between a genotype and a biological endophenotype is much more likely to be detected than an association between a genotype and the phenotype, because the biological endophenotype lies closer to the genotype (Meyer-Lindenberg & Weinberger, 2006). Possible endophenotypes associated with anxiety disorders might be enhanced baseline startle reactivity (Anokhin, Golosheykin, & Heath, 2007), reduced SCR habituation and enhanced fear conditioning (Hettema, Annas, Neale, Kendler, & Fredrikson, 2003), resistance to extinction, an overactivation of the amygdala (Norrholm & Ressler, 2009), and anxiety sensitivity, which is the tendency to respond fearfully to one's own bodily reactions (Klauke et al., 2012). Some of these candidate endophenotypes have been associated with the 5-HTTLPR and NSPR1 polymorphisms (for a detailed discussion see below, 3.1. and 3.2.). Most important, 5-HTTLPR and NPSR1 both have been reported to influence fear conditioning. For instance, carriers of the S allele of the 5-HTTLPR polymorphism showed higher fear-potentiated startle to a CS+ compared to LL allele carriers (Klumpers, Heitland, Oosting, Kenemans, & Baas, 2012; Lonsdorf et al., 2009) and enhanced fear ratings to both, a CS+ and a CS-, have been found in T allele carriers of the NPSR1 polymorphism compared to AA allele carriers (Raczka et al., 2010). These two genetic polymorphisms have only been investigated separately for an association with cued fear conditioning and extinction (Hermann et al., 2012; Klucken et al., 2013; Lonsdorf et al., 2009; Raczka et al., 2010). However, a proof for an involvement of both polymorphisms in contextual fear conditioning as a model for sustained anxiety, its extinction learning and extinction recall, is still lacking.

## 2.9. Summary and goals of the thesis

In sum, abnormalities in fear conditioning and extinction could be important risk factors for the development and maintenance of anxiety disorders. In this thesis, contextual fear conditioning as a model for sustained anxiety and its extinction learning as well as extinction recall will be investigated using anxiety-potentiated startle, SCL and ratings as indices for conditioned responses.

1) In Study 1, it will be investigated whether contextual fear learning and its extinction processes are modulated by 5-HTTLPR and/or NPSR1 polymorphisms.
2) In Study 2, it will be investigated whether the reinstatement of a contextual fear memory after extinction can be established in analogy to cue conditioning studies and which variables influence the return of anxiety.

# 3. Study 1: Genetic modulation of contextual fear conditioning, and extinction

This study has been partly published in *Frontiers in Behavioral Neuroscience* (Glotzbach-Schoon, Andreatta, et al., 2013).

## 3.1. The serotonin system and the 5-HTTLPR polymorphism

Serotonin (5-Hydroxytryptamin, 5-HT) is a monoamine transmitter. Serotonergic neurons can be found in the raphe nuclei in the brain stem where they project to the amygdala, hippocampus, striatum, cortex, and spinal cord, regulating learning and memory, affect, cognition, circadian rhythmic, emotional and social behavior, thermoregulation, eating and sexual behavior, and fear and anxiety (Kriegebaum, Gutknecht, Schmitt, Lesch, & Reif, 2010a). The serotonin transporter (5-HTT) is responsible for the reuptake of transmitted serotonin back into the pre-synapse and therefore regulates serotonergic transmission and concentration in the synaptic cleft. Furthermore, 5-HTT is the target for antidepressant medication (selective serotonin re-uptake inhibitors; SSRI) (Kriegebaum et al., 2010a). On a neuronal level 5-HTT knockout (KO) mice have enhanced 5-HT concentrations in the synaptic cleft, a lowered concentration of 5-HT in serotonergic neurons, less serotonergic cells in the raphe nuclei, and altered 5-HT receptor densities in hypothalamus and hippocampus compared to wild type mice. On a behavioral level 5-HTT KO mice show anxious, depressive and aggressives behavior, and heightened sensibility to stress (Kriegebaum, Gutknecht, Schmitt, Lesch, & Reif, 2010b). Interestingly, 5-HTT KO mice showed normal cued fear acquisition and extinction, but seemed to have deficits in extinction recall compared to wild type mice (Narayanan et al., 2011; Wellman et al., 2007). In contrast, 5-HTT KO mice demonstrated enhanced contextual fear conditioning and delayed extinction of contextual fear memories (Dai et al., 2008). Possibly, the influence of 5-HT on contextual fear conditioning is mediated by the BNST. In fact, Hammack et al. (2009) suggested that normally 5-HT dampens BNST activity and leads to reduced anxiety, whereas altered 5-HT functioning in the BNST could increase anxiety and lead to pathologic anxiety.

A functional human polymorphism within the promoter region of the serotonin transporter (5-HTT) gene (SLC6A4) located on chromosome 17q11.1-q12 is discussed to play an important role in trait anxiety and anxiety disorders (Amstadter, Nugent, & Koenen, 2009; Lesch et al., 1996; Skelton, Ressler, Norrholm, Jovanovic, & Bradley-Davino, 2012). In detail, the short (S) allele encoded by the 5-HTT-linked polymorphic region (5-

HTTLPR) comprises less 5-HTT mRNA which leads to reduced serotonin reuptake compared to two copies of the long (LL) allelic variant (Hariri & Holmes, 2006). The S allele is associated with high trait anxiety and heightened amygdala reactivity towards emotional stimuli, and regarded as a risk allele for the development of depressive and anxiety disorders (Canli & Lesch, 2007; Dannlowski et al., 2010; Hariri et al., 2002; Lonsdorf et al., 2011). Human cued fear conditioning studies using fMRI revealed greater amygdala, thalamus, occipital cortex, and insula activation in S carriers compared to L carriers during acquisition (Hermann et al., 2012; Klucken et al., 2013). Additionally, cued fear conditioning studies measuring fear-potentiated startle revealed stronger fear conditioning in S allele compared to LL allele carriers (Klumpers et al., 2012; Lonsdorf et al., 2009). These findings suggest that S allele carriers are characterized by faster fear learning and/or stronger fear reactivity than LL allele carriers.

In line with studies in 5-HTT KO mice, the human 5-HTTLPR polymorphism also seems to affect cued fear extinction, but only in addition with another genetic polymorphism. S allele carriers, who additionally carried two met alleles (met/met) of the COMTval158met polymorphism of the catechol-O-methyltransferase (COMT) gene, exhibited enhanced startle responses to CS+ during extinction (Lonsdorf et al., 2009). Furthermore, an fMRI study found greater vmPFC activation during extinction in S allele carriers who additionally carried at least one T alleles of the TPH2 polymorphism of the tryptophan-hydroxylase-2 (TPH2) gene (Hermann et al., 2012). These two studies provided evidence for a gene × gene interaction on extinction of phasic fear in humans.

In sum, the S allele seems to be associated with an overactivated amygdala, enhanced cued fear conditioning and extinction deficits, suggesting an important role in phasic fear reactions and its regulation, and thus in the development of anxiety disorders characterized by phasic fear. However, animal studies also suggested an involvement of 5-HT and its transporter in sustained anxiety, contextual fear conditioning and extinction (Dai et al., 2009; Hammack et al., 2009), but so far no human studies investigated the influence of 5-HTTLPR on this topic.

## 3.2. The NPS system and the NPSR1 polymorphism

The recently discovered neuropeptide S (NPS) and its receptor (NPSR) also seem to impact arousal, fear and anxiety responses, as well as learning and memory. In the rat brain NPSR mRNA is highly expressed in olfactory processing areas, in the amygdala, hippocampus, parahippocampus, and PVN affecting fear, anxiety and memory, and in the thalamus and hypothalamus possibly regulating sleep and energy balance (Xu, Gall,

Jackson, Civelli, & Reinscheid, 2007). Especially, NPS binding to its receptor leads to increased glutamatergic transmission to intercalated GABAergic neurons in the amygdala (Jüngling et al., 2008), and as described above (2.6.1.) this results in an inhibition of the CeA. Hence, in rodents NPS injection has anxiolytic behavioral effects (Pape, Jüngling, Seidenbecher, Lesting, & Reinscheid, 2010). For example, cued fear conditioning studies in rodents showed reduced fear-potentiated startle to the CS (Fendt, Imobersteg, Bürki, McAllister, & Sailer, 2010), and enhanced extinction of conditioned freezing to the CS (Jüngling et al., 2008), when NPS was injected into the amygdala. In contrast, NPS injection into the endopiriform nucleus reduced freezing to the background context (Meis et al., 2008). Therefore, NPS might play a crucial role in cued fear and contextual anxiety.

In humans, there is a SNP (rs324981) in the human NPS receptor gene (NPSR1) located on chromosome 7p14.3. This functional A/T polymorphism leads an amino-acid exchange from Asn to Ile at position 107 resulting in a potentiated efficacy of NPS at NPSR in the T allele (Ile107) compared to the A allele (Asn107) carriers about tenfold (Reinscheid et al., 2005). The T allele has been suggested as a risk allele for panic disorder resulting from evidence that A allele carriers were found to be underrepresented in male panic disorder patients (Okamura et al., 2007), while T allele carries were overrepresented in a female panic disorder population (Domschke et al., 2011). Furthermore, in the latter study panic patients carrying the T allele exhibited increased anxiety sensitivity, higher heart rate and attenuated activity in PFC and ACC during processing of fearful faces. Moreover, two studies in healthy humans point into the same direction. First, T allele carriers showed increased basolateral amygdala activation to fearful faces compared to AA allele carriers (Dannlowski et al., 2011). Second, although T allele and AA allele carriers reported higher fear ratings for the fear signal (CS+) than the safety signal (CS-) during a cued fear conditioning paradigm, fear ratings for CS+ and CS- were higher in T allele carriers compared to AA allele carriers (Raczka et al., 2010). In conclusion, both animal and human studies indicate a fundamental role of the NPS in cued fear learning. However, animal studies suggest that NPS also affects contextual anxiety and extinction learning, but to date there are no studies in humans considering these aspects.

## 3.3. Environmental stress

Environmental stress has been reported to increase the risk for anxiety disorders (Melchior et al., 2007; Watanabe et al., 2005). However, environmental stress alone is often not sufficient enough to cause an anxiety disorder, but in addition with a genetic

diathesis the risk for the development of an anxiety disorder is increased (Nugent, Tyrka, Carpenter, & Price, 2011).

Regarding the 5-HTTLPR polymorphism, there is evidence for a gene-environment interaction (G × E) on the development of PTSD. Participants who experienced childhood adversity were more likely to develop PTSD in response to a traumatic event in adulthood, and this relationship was more pronounced in S allele compared to L allele carriers (Xie, Kranzler, Farrer, & Gelernter, 2012). An association between an anxiety disorder and the 5-HTTLPR polymorphism together with life stress is possibly mediated by the hypothalamic-pituitary-adrenal (HPA) axis, regulating the bodily stress response. S allele carriers who experienced a high number of stressful life events showed increased HPA axis response (measured by cortisol levels) to social stress, and increased amygdala-hypothalamic connectivity to fearful stimuli (Alexander et al., 2009, 2012). Possibly, a genetic predisposition and increased stressful experiences lead to a heightened HPA axis reactivity, resulting in an increased stress response and amygdala activity to upcoming stress. In line, also neural correlates of cued fear conditioning in healthy participants were affected by an interaction of the 5-HTTLPR polymorphism and traumatic life events. SS allele carriers with a history of traumatic life events showed enhanced activation of the occipital cortex and the insula in response to the CS+ during acquisition (; Klucken et al., 2013), whereas the second study demonstrated reduced amygdala activity in S+ carriers during extinction (Hermann et al., 2012).

Notably, an interaction between stressful experiences and the NPS system has recently been reported as well. In an animal study, stress-related CRF release was found to activate NPS neurons in the brain stem which resulted in NPS release into the amygdala, possibly regulating stress responsiveness (Jüngling et al., 2012). Importantly, an association between the NPSR1 polymorphism and stressful life events (childhood maltreatment) on anxiety sensitivity, an endophenotype for anxiety disorders, has been demonstrated. Namely, TT carriers who experienced childhood maltreatment showed increased anxiety sensitivity (Klauke et al., 2012). In the same vein, after acute stress T allele carriers showed enhanced cortisol levels compared to AA carriers (Kumsta, Chen, Pape, & Heinrichs, 2013). In sum, both an interaction between the NPSR1 polymorphism or between the 5-HTTLPR polymorphism and life stress has been reported, especially in regard to conditioned fear and anxiety responses.

## 3.4. Goals and hypotheses of Study 1

The goal of this study was to examine genetic influences, especially a gene × gene interaction of 5-HTTLPR and NPSR1 polymorphisms, on implicit and explicit indices of contextual fear conditioning, its extinction and extinction recall, to identify possible endophenotypes for sustained anxiety states. To this end, a foreground contextual fear conditioning paradigm using virtual reality contexts was employed. Healthy participants stratified for 5-HTTLPR (S+ vs. LL) and NPSR1 (T+ vs. AA) polymorphisms were divided into four combined genotype groups: high risk allele carriers S+/T+, intermediate risk allele carriers S+/AA and LL/T+, and no risk allele carriers LL/AA. Participants in each combined genotype were tested for contextual fear conditioning on Day 1, extinction learning on Day 2, and extinction recall on Day 3. Additionally, a modulation of possible genetic effects by environmental stress was exploratively tested by assessing each participant´s number of experienced life events.

I hypothesized that:

1) Contextual fear conditioning is influenced by a gene × gene interaction. During learning, risk allele carriers of both genetic polymorphisms, namely the S allele carriers of the 5-HTTLPR polymorphism and the T allele carriers of the NPSR1 polymorphism, show enhanced contextual fear conditioning. In detail, S and T allele carriers show higher anxiety-potentiated startle, SCL, more negative valence, and enhanced arousal, anxiety and US expectancy ratings in a context associated with an unpredictable US (CXT+, anxiety context) compared to a context in which no US occurred (CXT-, safety context).

2) S allele carriers of the 5-HTTLPR polymorphism show deficits during extinction learning and extinction recall. In detail, S allele carriers show enhanced anxiety responses (anxiety-potentiated startle, SCL, ratings) in the anxiety context compared to the safety context on Day 2 and 3 compared to LL allele carriers.

3) Genetic effects on contextual fear conditioning and extinction will additionally be associated with environmental stress:

    a. Contextual fear conditioning correlates positively with the number of stressful life events in risk allele carriers: the higher the number of stressful life events is, the more pronounced contextual fear conditioning is.

    b. Extinction of contextual fear conditioning correlates negatively with the number of stressful life events in risk allele carriers: the higher the number of stressful life events is, the lesser extinction of contextual anxiety is.

## 3.5. Materials and Methods

### 3.5.1. Participants

Ninety-three (60 female; mean age 23.96 years, *SD* = 3.14) healthy participants were recruited from a large sample of *N* = 497 (337 female; mean age 23.28 years, *SD* = 3.88, European descent) of the subproject Z02 of the Collaborative Research Center (SFB TRR 58). The project Z02 conducted general sample recruitment, screening for exclusion criteria, psychometric measurements and genotyping for several polymorphisms at the University Hospital of Psychiatry, Psychosomatics and Psychotherapy in Würzburg. For genotyping a blood sample (18 ml EDTA blood) was collected from each participant. Within the subproject Z02 participants were first screened for Caucasian ancestry, fluency in German speech and right-handedness. Additional exclusion criteria were a current and previous mental axis-I-disorder, severe somatic disorder and the use of illegal drugs, alcohol consumption of more than 140 g per week, daily smoking of more than 20 cigarettes a day, daily drinking of more than four cups of coffee, use of any central active medication and pregnancy. Secondly, possible participants were invited and the exclusion of current or prior diagnosis of DSM-IV axis-I, except specific phobia, was additionally confirmed by training psychologist using the Mini-International Neuropsychiatric Interview (MINI; Lecrubier et al., 1997). Illegal drug consumption was assessed by a urine drug screening for amphetamine, barbiturates, benzodiazepines, cocaine, ecstasy, methamphetamine, methadone, opiates, tricyclic antidepressants and tetrahydrocannabinol.

All participants recruited by the project Z02 completed sociodemographic and several fear and anxiety-related questionnaires. Additionally, the individual life stress history was assessed. For this contextual fear conditioning study, participants with specific phobia were included, if their STAI trait anxiety score (State-Trait-Anxiety-Inventory Spielberger, Gorsuch, & Edward, 1970; German version: Laux, Glanzmann, Schaffner, & Spielberger, 1981) was within a normal range (*M* ± 1 *SD*, these are *T*-scores between 40 and 60; participants´ gender and age were considered). Additionally, psychology students were excluded because of their familiarity to conditioning protocols. All participants gave their written informed consent. Participants gained 50 € for their participation. The study of was approved by the Ethics Committee of the Medical Faculty of the University of Würzburg.

### 3.5.2. Genotyping

Participants were genotyped for 5-HTTLPR and NPSR SNPR rs324981 A/T (Asn107Ile) polymorphisms as documented by Klauke et al. (2011) and Domschke et al. (2011). In a double blind design, participants heterozygous (SL) and homozygous (SS) for

the S allele of the 5-HTTLPR polymorphism were grouped together (S+), as well as participants heterozygous (TA) and homozygous (TT) for T allele of the NPSR1 polymorphism (T+), as done in previous studies (Domschke et al., 2011; Hariri et al., 2002; Lonsdorf et al., 2009; Raczka et al., 2010), resulting in four combined genotype groups: S+/T+, S+/AA, LL/T+ and LL/AA.

### 3.5.3. Questionnaires

Within the subproject Z02 participants completed the Trait version of the STAI, which measures relative firm inter-individual differences in the tendency to rate situations as threatening on a 20-item scale (Spielberger et al., 1970), the Anxiety-Sensitivity-Index (ASI; Reiss, Peterson, Gursky, & McNally, 1986; German version: Alpers & Pauli, 2001), which measures the tendency to respond fearfully to one's own bodily sensations on a 16-item scale, and the Behavioral Inhibition System and Behavioral Approach System (BIS-BAS; Carver & White, 1994; German version: Strobel, Beauducel, Debener, & Brocke, 2001), measuring the sensitivity to punishment (BIS) on a 7-item scale and sensitivity to reward (BAS) on a 13-item scale. BIS depicts an aversive motivational system which correlates with avoidance behavior and negative feelings such as fear and anxiety, whereas BAS is regarded as an appetitive motivational system which drives goal-directed behavior and positive affect (Gray, 1990). Life stress history was assessed with a 27-item self-report questionnaire (see Canli et al., 2006; Herrmann et al., 2009) based on the life history calendar (Caspi et al., 1996). Items were related to work, marriage plan, relocation and house renovation, financial and legal problems, own serious illness or of a friend or family member, physical or sexual abuse, and others. Participants had to indicate how many of these stressful life events they had experienced and a sum score was calculated.

Before the experimental sessions of each day started, participants were required to complete the State version of the STAI (Laux et al., 1981), and the Positive And Negative Affect Schedule (PANAS; Watson, Clark, & Tellegen, 1988; German version: Krohne, Egloff, Kohlmann, & Tausch, 1996) to control for between-group differences in state anxiety and mood before each session. Because sleep is regarded to have an impact on memory consolidation (Diekelmann & Born, 2010) and may selectively enhance contextual memories (Cai, Shuman, Gorman, Sage, & Anagnostaras, 2009), whereas sleep deprivation before contextual fear conditioning may result in reduced contextual anxiety (Ruskin & Lahoste, 2008), participants filled out the PSQI (Pittsburgh Sleep Quality Index; Buysse, Reynolds III, Monk, Berman, & Kupfer, 1989, German version: Riemann & Backhaus, 1996) which determines the sleep quality of the last four weeks. Additionally, participants evaluated their sleep quality of the last night on a 4-point Likert scale (0 = *very good*, 1 =

*good*, 2 = *bad*, 3 = *very bad*). Additionally, the Morningness-Eveningness-Questionnaire (MEQ; Horne & Ostberg, 1976; German version: Griefahn, Künemund, Bröde, & Mehnert, 2001) was required, which measures the individual chronotype on a 19-item scale. At the end of each day, participants completed the Igroup Presence Questionnaire (IPQ; original version in German: Schubert, Friedmann, & Regenbrecht, 2001), which measures the experience of feeling presence in a virtual reality environment retrospectively on a 14-item scale.

### 3.5.4. Ratings

To obtain explicit ratings for valence, arousal, anxiety and US-expectancy, participants were shown a screenshot of each context and were instructed via headphones. The rating scale was presented below the screenshots. Participants had to answer loudly and the experimenter noted the rated value. Rating scales ranged from 0 (*very negative/ very calm/ no anxiety at all/ no expectancy at all*) to 100 (*very positive/ very excited/ very high anxiety/ definitely expected*).

### 3.5.5. Stimuli and Apparatus

*3.5.5.1. Contextual stimuli*

The virtual reality environment was created with the Source Engine from the Valve Corporation (Bellevue, USA), which is also used for the computer game Half-Life 2. The VR environment consisted of two offices that were arranged opposite each other and separated by a corridor. Participants were situated in the middle of the corridor in such a way that they could see only one room. This was the starting point for all passages through the rooms. The two offices served as the conditioned contexts. They differed in layout, window style, carpet color, view (big city vs. small village), and arrangement of furniture (Figure 3). The VR environment, instructions, and ratings were presented with a Z800 3D Visor head mounted display (HMD; eMagin, Hopewell Junction, USA) with a resolution of 600 × 800 Pixels. The head position was monitored by an electromagnetic tracking device (Patriot, Polhemus Corp., Colchester, USA) in order to adapt the field of view to head movements and to assess head orientation. The experimental procedure was controlled by the software Cyber Session (version 5.3.38), developed in the Department of Psychology I, University of Würzburg.

*Figure 3. Screenshots of the virtual reality environment.*
Pictures show the two offices (left and right) and in the middle the connecting corridor (intertrial interval, ITI). During acquisition one office room was paired with mildly painful electrical stimuli (anxiety context, CXT+), whereas the other office room was never paired with electrical stimuli (safety context, CXT-).

*3.5.5.2. Unconditioned stimulus*

The US was an electric stimulus generated by a current stimulator (Digitimer DS7A, Digitimer Ltd, Welwyn Garden City, UK; duration of 2000 µs, maximum of 9.99 mA, 400 V) and was triggered by a frequency of 50 Hz and a duration of 200 ms by the software Cyber Session. The electric stimulus was applied by a surface electrode placed on the dominant forearm. The intensity of the current was individually adjusted to each participant's pain threshold. Two rows of ascending and descending current intensities were used. The participants had to rate the intensity of the electric stimulus on a visual scale with anchors at 0 = *no feeling at all*, 4 = *just noticeable pain*, and 10 = *very strong pain*. Starting at 0 mA, in steps of 0.5 mA the current was increased until the participant rated the intensity with a least 4 (ascending row). Next, the descending row started with 0.5 mA above the last intensity of the ascending row. The current was decreased until the participant rated the intensity below 4 in steps of 0.5 mA. The next ascending row started with 0.5 mA below the last intensity and the current was increased in steps of 0.5 mA until the intensity was again rated with at least 4. The last descending row was conducted as described above. The current intensity depicting the pain threshold was calculated as the mean current of all four rows rated with at least 4. This mean was rounded to full or half mA intensities and increased by 30% to avoid habituation. This stimulus intensity was again administered and participants were required to rate its intensity. The investigator asked again, if this intensity was just painful and acceptable. If participants disagreed, the intensity of the US was re-adjusted by either increasing or decreasing the intensity in steps of 0.5 mA. If participants agreed, the final current intensity served as US throughout the experiment. Afterwards, participants rated the final US for valence and arousal (pre-conditioning) which was repeated at the end of the experimental session on Day 1 (post-conditioning).

Neither current intensity, nor pain ratings, nor valence ratings of the US were influenced by any genotype (all *p*s > .08; see Table 1). But there was a significant main effect of NPSR1 genotype for US arousal, $F(1, 75) = 5.15$, $p = .026$, $\eta_p^2 = .06$. That is, AA carriers rated the US as more arousing than T+ carriers, see Table 1. [1]

*Table 1. Study 1: US properties and ratings of the genotype groups.*

| | Genotype groups | | | |
|---|---|---|---|---|
| | **S+/T+** | **S+/AA** | **LL/T+** | **LL/AA** |
| Current intensity in mA | 2.85 (1.26) | 2.72 (1.30) | 2.96 (1.99) | 2.28 (1.04) |
| Pain rating | 5.10 (1.07) | 4.90 (0.85) | 5.00 (0.92) | 5.20 (1.61) |
| US valence pre | 42.75 (15.43) | 32.63 (13.16) | 41.00 (15.78) | 38.00 (19.49) |
| US valence post | 37.00 (20.29) | 36.53 (16.96) | 38.50 (16.55) | 31.00 (16.91) |
| US arousal pre | 41.25 (24.38) | 49.79 (24.47) | 44.00 (22.86) | 58.25 (19.55) |
| US arousal post | 47.75 (24.31) | 50.00 (30.00) | 45.25 (16.34) | 60.75 (18.87) |

Note: Displayed are means (SD). Pain ratings were from 0 (*no feeling at all*) to 10 (*very strong pain*) with 4 = *just noticeable pain*. Valence and arousal ratings ranged from 0 (*very negative/ very calm*) to 100 (*very positive/ very excited*).

*3.5.5.3. Recording of physiological data*

Startle probes of 50 ms, 103 dB (A) white noise were presented for physiological measures through Sennheiser HD 215 headphones. Startle reflex was measured by EMG from the M. *orbicularis oculi* with two 13/7 mm Ag-AgCl electrodes filled with electrolytes, which were placed centrally under and next to the lateral canthus of the left eye. Ground and reference electrodes were placed at the left and right mastoids, respectively. To keep impedances below 10 kΩ, the skin was first cleaned with alcohol, than peeled with a skin preparation gel, and again cleaned with alcohol. The EMG signal was filtered online with a 50 Hz notch filter and sampled at 1000 Hz. SCL was measured on the thenar of the left hand by two 13/7 mm Ag-AgCl electrodes filled with 0.5% NaCl paste. Physiological data were assessed using a digital amplifier (V-Amp 16, Brain Products Inc., Munich, Germany) and recorded by Vision Recorder software (version 1.03.004, Brain Products Inc., Munich, Germany). SCL was recorded during each context presentation (CXT+, CXT-), i.e., between entrance and exit.

[1] Current intensity and pain ratings of the US were analyzed separately with univariate ANOVAs 5-HTTLPR genotype (S+ vs. LL) × NPSR1 genotype (T+ vs. AA). US valence and arousal ratings were analyzed with separate ANOVAs with factors Time (pre-conditioning vs. post-conditioning) × 5-HTTLPR genotype (S+ vs. LL) × NPSR1 genotype (T+ vs. AA). Post conditioning ratings of one participant (S+/AA) were missing.

### 3.5.6. Procedure and Design

The experiment was run on three consecutive days separated by 24 h. Two acquisition phases (Acquisition 1, Acquisition 2) were performed on Day 1, with US administered in one office room (anxiety context, CXT+) but never in the second office room (safety context, CXT-). On Day 2, two extinction phases (Extinction 1, Extinction 2) were conducted without any US administration, and on Day 3 two additional extinction phases were performed to test extinction recall (Re-Extinction 1, Re-Extinction 2).

The experiment on Day 1 started with a familiarization phase on joystick handling. Participants were placed on a virtual street scene and had to move themselves through this scene by using the joystick. They were instructed to move forward, backward, left and right and to turn their head. After 60 s a black screen appeared and the training session was finished. Next, participants were placed in the virtual corridor and in front of them they saw a closed door (see Figure 3). Here, the shock workup procedure took place as described above. Next, the pre-acquisition phase started. Participants were instructed to freely explore both office rooms via the joystick for two minutes each. They should stay in the office rooms until they were requested to leave the rooms via headphones. Participants were told that no US would be delivered. Afterwards startle habituation was conducted. Four startle tones were presented at intervals of 15 to 17 s to reduce the initial startle reactivity.

Before the first acquisition phase on Day 1 participants were instructed that they could not use the joystick anymore, but that they were passively guided through the virtual rooms and were able to freely move their head to look around. They were also told to figure out the relationship between contexts and US (Schiller et al., 2010). Subsequently, two acquisition phases started. Each phase consisted of three runs each lasting about 210 s. During one run participants entered each context once. Thus, participants started in the corridor and went through one office room (ca. 85 s), then through the corridor (ITI; ca. 35 s) into the other office room (ca. 85 s) and back into the corridor (one run). After each run the display turned into black before a new run was started. Participants were passively moved through the VR environment, i.e. they could not influence the way through the office rooms and corridor. The paths leading through the corridor and office rooms were prerecorded and played back. However, participants were always able to adapt their line of sight in the VR by head movements. Participants received one to three mildly painful electric stimuli in CXT+ per run, but never in CXT- or in the corridor. The corridor served as a control context and as an ITI between CXT+ and CXT- in one run. A total of twelve electric stimuli were presented during both acquisition phases at different locations in CXT+ preventing the participants from associating specific cues within this context with

shock administration. Per run, two to three startle probes were presented within each context (CXT+, CXT-) and one to two startle probes were presented within the corridor (ITI) at intervals of 10 to 34 s. The interstimulus interval between a US and a subsequent startle probe was at least 10 s. Each day there were 15 startle probes per context and nine startle probes during ITI. The office rooms were randomly assigned to the two conditions (CXT+ vs. CXT-) and counterbalanced across participants and groups. The sequence of context presentations was pseudo-random and also counterbalanced across participants and groups, with the restriction that the same context was not entered more than twice in a row (see Figure 4 and Annex F). At the end of Day 1, again ratings for valence and arousal of the US were obtained. The virtual reality session was finished and the HMD and all electrodes were detached. Finally, participants filled in the IPQ.

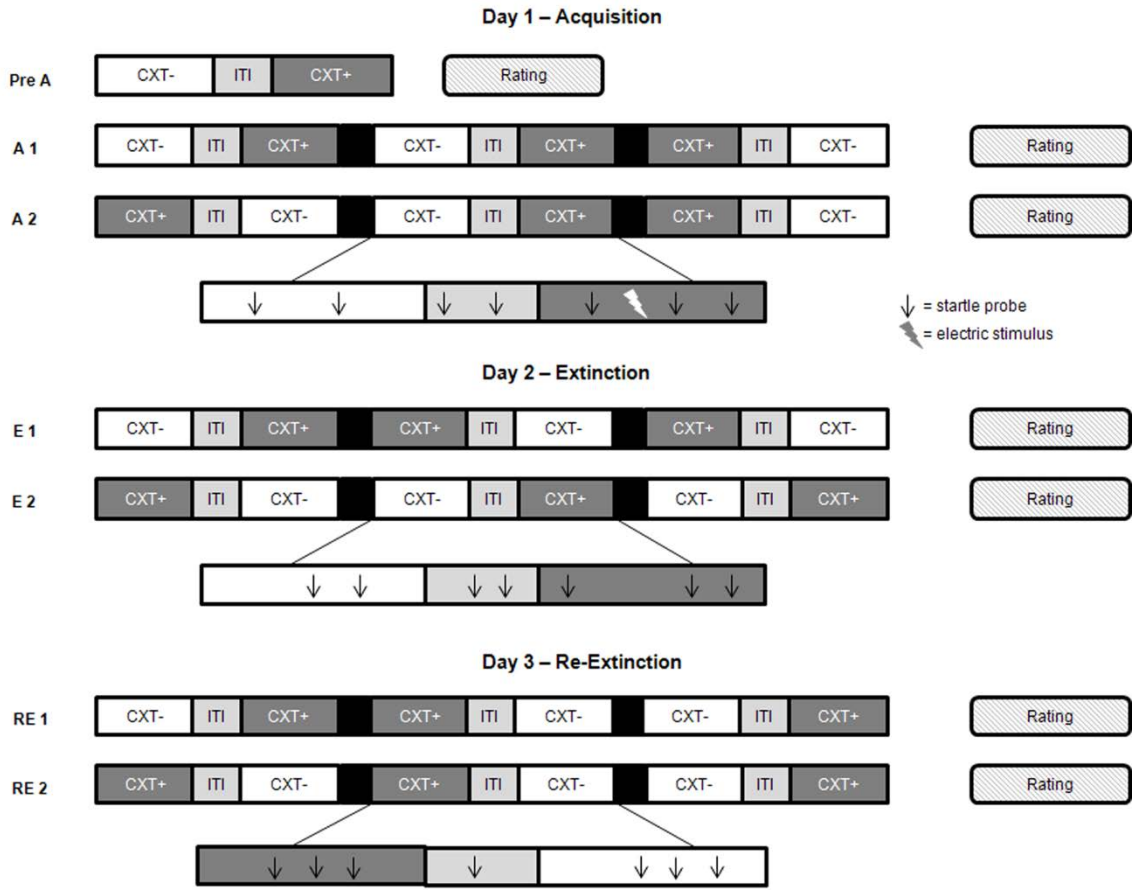


*Figure 4. Design of Study 1.*
Pre A = pre-acquisition, A 1 = Acquisition 1, A2 = Acquisition 2, E 1 = Extinction 1, E 2 = Extinction 2, RE 1 = Re-Extinction 1, RE 2 = Re-Extinction 2. During pre-acquisition participants entered each context (anxiety context = CXT+, safety context = CXT-, intertrial interval = ITI) once by using a joystick. The following phases consisted of three runs each. During one run, each context was entered once and afterwards the display turned black. Detailed examples of several runs are displayed under each day. Startle probes were presented during each context and ITI. Electric stimuli (US) were delivered in CXT+ only during acquisition on Day 1. Ratings for valence, arousal, anxiety and US-expectancy were collected after each phase.

The experimental sessions on Days 2 and 3 were nearly the same. All electrodes, including the one for US presentation were attached again. On both days, the experiment started with the startle habituation phase. Afterwards participants were instructed that they now were passively guided through the virtual offices but still were able to adapt their field of view to their head movements. Importantly, there was no comment on the US. During Day 2 two extinction phases (Extinction 1, Extinction 2) were conducted where no US was administered. On Day 3 two additional phases for extinction recall (Re-Extinction 1, Re-Extinction 2) followed, again without any US presentation. Like on Day 1, each phase consisted of three runs where participants were passively moved through the two contexts and the corridor once. An equal amount of startle tones was presented during CXT+, CXT- and ITI presentations like on Day 1. At the end of Days 2 and 3, participants again filled in the IPQ.

During each day, ratings for valence, arousal, anxiety and US-expectancy of the two conditioned contexts (CXT+, CXT-) were obtained after each phase of the experiment regarding the previously experienced phase (Day 1: Acquisition 1, Acquisition 2; Day 2: Extinction 1, Extinction 2; Day 3: Re-Extinction 1, Re-Extinction 2). After pre-acquisition on Day 1, only ratings for valence, arousal and anxiety were assessed. Awareness of the CXT+-US contingency was assessed with an open question ("In which room did you receive electrical stimuli?") after Acquisition 1 and 2 of Day 1 and participants had to describe the room. If participants described only the CXT+ as associated with the US, they were labeled as 'aware'. If they stated that the US would have been administered in both contexts (CXT+ and CXT-), they were labeled as 'uncertain'. Finally, if they stated the wrong context (CXT-), they were labeled as 'unaware'. In total, there were nine uncertain participants, who were equally distributed over 5-HTTLPR (S+: $n = 5$; LL: $n = 4$), $\chi^2(1, N = 80) = 0.13$, $p = .723$, and NPSR1 genotype groups (T+: $n = 5$; AA: $n = 4$), $\chi^2(1, N = 80) = 0.13$, $p = .723$. Nobody was unaware.

### 3.5.7. Data reduction

*3.5.7.1. Startle response*

Eyeblink EMG Data was offline processed with Vision Analyzer software (version 1.05.005, Brain Products Inc., Munich, Germany). The signal was filtered offline with a 500 Hz High Cut off and a 30 Hz Low Cut off Filter. The signal was rectified, smoothed (50 ms moving average) and baseline corrected (50 ms before startle probe onset). The peak magnitude was identified within a time window from 21 to 300 ms after the probe onset. Artifact rejection was made manually excluding responses with baseline shifts above or below 5 μV and pre-blinks 50 ms before probe onsets higher than 5 μV. Magnitudes smaller than 5 μV were coded as zero. Responders vs. non-responders were defined on the

basis of sufficient valid responses, meaning artifact free and higher than 5 μV. If there were less than two valid responses per stimulus category (CXT+, CXT-, ITI) in a given phase (Acquisition 1, Acquisition 2, Extinction 1, Extinction 2, Re-Extinction 1, Re-Extinction 2), the participant was excluded from further analysis. Magnitudes in the acquisition, extinction and re-extinction phases were standardized into *T*-scores for each participant.

*3.5.7.2. Skin conductance level*

SCL data was filtered with 1 Hz High Cut-off. The mean tonic SCL was determined over each context presentation (excluding epochs from US presentation to 10 s after US presentation to avoid an increased SCL due to US presentation). SCL data were log-transformed (log10[SCL +1]) to normalize the distribution.

*3.5.7.3. Statistical analysis*

Statistical analysis was performed with IBM SPSS Statistics 19 (IBM Corporation, Armonk, New York, U.S.A.). First, physiological data were averaged for each phase (Acquisition 1, Acquisition 2, Extinction 1, Extinction 2, Re-Extinction 1, Re-Extinction 2) across three runs. Anxiety-potentiated startle was determined as the difference score between the mean startle response during contexts and ITI (CXT+ - ITI, or CXT- - ITI). Startle, SCL and rating data were analyzed separately for each day. Pre-acquisition data were analyzed with a 2 (Context: CXT+, CXT-) × 2 (5-HTTLPR: S+, LL) × 2 (NPSR1: T+, AA) univariate Analysis of Variance (ANOVA). Acquisition, extinction and re-extinction data were analyzed separately with 2 (Context: CXT+, CXT-) × 2 (Phase: 1, 2) × 2 (NPSR1: T+, AA) × 2 (5-HTTLPR: S+, LL) ANOVAs. To clarify significant main effects or interactions *F* contrasts were calculated. To exploratively test for an association between the number of stressful life events and conditioning and extinction effects in certain genotype groups, Pearson correlations were conducted. Conditioning and extinction effects were defined as the difference score between CXT+ and CXT-.

Explorative analyses were conducted for questionnaire data and baseline measurements (initial startle reactivity during habituation). Trait questionnaire data (age, ASI, STAI-Trait, BIS, BAS, PSQI, MEQ, Life Events) were analyzed with 2 (5-HTTLPR: S+, LL) × 2 (NPSR1: T+, AA) univariate ANOVAs. Post hoc analyses were carried out with *F* contrasts. State questionnaire data (STAI-State, negative affect [NA], positive affect [PA], daily sleep quality, IPQ) and initial startle reactivity were assessed with 3 (Day: 1, 2, 3) × 2 (5-HTTLPR: S+, LL) × 2 (NPSR1: T+, AA) ANOVAs. Significant interactions were further analyzed with univariate ANOVAs and selected *F* contrasts. In all analyzes the alpha level was set at $p \leq .05$. If the assumption of sphericity for within-factors with three levels was

violated ($p$ < .20) Greenhouse-Geisser correction was applied and Greenhouse-Geisser Epsilon (GG-ε) was reported. Effect sizes were calculated using the partial eta ($\eta_p^2$).

## 3.6. Results

### 3.6.1. Sample characteristics

Thirteen participants had to be excluded because of technical problems ($n$ = 7), low startle reactivity ($n$ = 3; for startle response quantification see Materials and Methods), excessive artifacts in startle data ($n$ = 2), and simulator sickness ($n$ = 1). The final sample consisted of 80 participants with 20 participants per combined genotype group (S+/T+, S+/AA, LL/T+, LL/AA).[2] Demographic and psychometric characteristics of genotype groups are displayed in Table 2. There were less male than female participants in the final sample (31 male, 49 female), $\chi^2(1, N = 80) = 4.05$, $p = .044$, but male participants were not statistically overrepresented in any NPSR1, $\chi^2(1, N = 80) = 2.58$, $p = .108$, or 5-HTTLPR genotype group, $\chi^2(1, N = 80) = 0.47$, $p = .491$, (see Table 2). Additionally, genotype groups did not differ in age ASI, BIS, BAS, MEQ, PSQI and the number of stressful life events (all $p$s > .20). However, AA allele carriers of the NPSR1 polymorphism reported slightly higher trait anxiety than T+ allele carriers, $F(1, 76) = 4.10$, $p = .046$, $\eta_p^2 = .05$.

The analyses of state questionnaire data revealed no significant effects for state anxiety (all $p$s > .08). For negative affect as well as for positive affect there were significant main effects of day, negative affect: $F(2, 152) = 4.26$, $p = .018$, $\eta_p^2 = .05$, GG-ε = .95, positive affect: $F(2, 152) = 3.93$, $p = .022$, $\eta_p^2 = .05$, with higher positive affect on Day 1 ($M$ = 29.89, $SD$ = 6.11) compared to Day 2 ($M$ = 28.76, $SD$ = 6.93), $F(1, 76) = 5.31$, $p = .024$, $\eta_p^2 = .07$, and higher negative affect on Day 2 ($M$ = 12.45, $SD$ = 3.27) compared to Day 3 ($M$ = 11.37, $SD$ = 2.47), $F(1, 76) = 6.66$, $p = .012$, $\eta_p^2 = .08$. For positive affect there was also a significant interaction 5-HTTLPR × NPSR1 genotype, $F(1, 76) = 4.24$, $p = .043$, $\eta_p^2 = .05$, with slightly higher positive affect in LL/AA ($M$ = 30.72, $SD$ = 5.30) compared to S+/AA carriers ($M$ = 27.38, $SD$ = 5.99), $F(1, 40) = 3.47$, $p = .070$, $\eta_p^2 = .08$. The analysis of sleep quality revealed a significant main effect of NPSR1 genotype, $F(1, 76) = 12.03$, $p = .001$, $\eta_p^2 = .14$, and a significant interaction NPSR1 × 5-HTTLPR, $F(1, 76) = 5.00$, $p = .028$, $\eta_p^2 = .06$.

[2] There were less homozygous SS ($n$ = 14) or TT ($n$ = 15) carriers than heterozygous SL ($n$ = 26) or TA ($n$ = 25) carriers. But homozygous SS carriers were equally distributed over NPSR1 subgroups (SS/AA: $n$ = 6; SS/TA: $n$ = 5; SS/TT: $n$ = 3), and homozygous TT carriers were equally distributed over 5-HTTLPR subgroups (SS/TT: $n$ = 3; SL/TT: $n$ = 4; LL/TT: $n$ = 8), $\chi^2(4, N = 80) = 0.58$, $p = .97$.

T+ carriers reported better sleep quality than AA carriers, but only if they additionally carried an S allele (better sleep quality in S+/T+ ($M$ = 0.55, $SD$ = 0.41) vs. S+/AA carriers ($M$ = 1.17, $SD$ = 0.48), $F(1, 40) = 19.25$, $p < .001$, $\eta_p^2 = .34$. Finally, the analysis of IPQ data only revealed a significant main effect of day, $F(2, 152) = 12.85$, $p < .001$, $\eta_p^2 = .15$, GG-ε = .95, with higher presence on Day 1 ($M$ = -1.49, $SD$ = 13.39) compared to Day 2 ($M$ = -4.93, $SD$ = 15.16), $F(1, 76) = 9.84$, $p = .002$, $\eta_p^2 = .12$.

*Table 2. Study 1: Demographic and psychometric data of the genotype groups.*

| **NPSR1** | **5-HTTLPR** | | **Total** |
|---|---|---|---|
| | *S+* | *LL* | |
| *T+* | 10 female, 10 male | 11 female, 9 male | 21 female, 19 male |
| | Age = 24.05 years (2.46) | Age = 24.20 years (4.43) | Age = 24.13 years (3.54) |
| | STAI Trait = 34.20 (6.61) | STAI Trait = 32.50 (7.33) | **STAI Trait = 33.35 (6.95)** |
| | ASI = 15.05 (6.25) | ASI = 13.65 (7.71) | ASI = 14.35 (6.97) |
| | BIS = 19.25 (2.59) | BIS = 18.20 (4.65) | BIS = 18.73 (3.76) |
| | BAS = 43.60 (3.95) | BAS = 41.85 (5.42) | BAS = 42.73 (4.77) |
| | MEQ = 49.60 (10.23) | MEQ = 52.00 (10.09) | MEQ = 50.80 (10.10) |
| | PSQI = 4.50 (2.07) | PSQI = 4.80 (3.09) | PSQI = 4.65 (2.60) |
| | SLE = 10.55 (4.35) | SLE = 8.50 (4.64) | SLE = 9.53 (4.56) |
| | $n$ = 20 | $n$ = 20 | $n$ = 40 |
| *AA* | 13 female, 7 male | 15 female, 5 male | 28 female, 12 male |
| | Age = 23.50 years (2.65) | Age = 24.35 years (3.75) | Age = 23.92 years (3.23) |
| | STAI Trait = 36.70 (6.73) | STAI Trait = 36.35 (7.34) | **STAI Trait = 36.53 (6.95)** |
| | ASI = 15.30 (7.12) | ASI = 16.75 (7.68) | ASI = 16.03 (7.34) |
| | BIS = 19.30 (3.05) | BIS = 20.05 (2.98) | BIS = 19.68 (3.00) |
| | BAS = 41.90 (3.89) | BAS = 42.60 (3.22) | BAS = 42.25 (3.54) |
| | MEQ = 49.25 (9.98) | MEQ = 50.20 (8.92) | MEQ = 49.72 (9.36) |
| | PSQI = 4.85 (2.56) | PSQI = 4.70 (2.39) | PSQI = 4.78 (2.44) |
| | SLE = 10.15 (4.49) | SLE = 10.75 (4.96) | SLE = 10.45 (4.68) |
| | $n$ = 20 | $n$ = 20 | $n$ = 40 |
| **Total** | 23 female, 17 male | 26 female, 14 male | 49 female, 31 male |
| | Age = 23.78 years (2.54) | Age = 24.28 years (4.05) | Age = 24.03 (3.37) |
| | STAI Trait = 36.70 (6.73) | STAI Trait = 36.35 (7.34) | STAI Trait = 34.94 (7.09) |
| | ASI =15.18 (6.61) | ASI = 15.20 (7.76) | ASI = 15.19 (7.16) |
| | BIS = 19.28 (2.79) | BIS = 19.13 (3.97) | BIS = 19.20 (3.41) |
| | BAS = 42.75 (3.97) | BAS = 42.23 (4.42) | BAS = 42.49 (4.18) |
| | MEQ = 49.43 (9.98) | MEQ = 51.10 (9.44) | MEQ = 50.20 (9.69) |
| | PSQI = 4.68 (2.30) | PSQI = 4.75 (2.73) | PSQI = 4.71 (2.51) |
| | SLE = 10.35 (4.37) | SLE = 9.63 (4.88) | SLE = 9.99 (4.61) |
| | $n$ = 40 | $n$ = 40 | $N$ = 80 |

*Note:* Displayed are frequencies and means (SD). Significant group differences are displayed in bold. ASI = Anxiety Sensitivity Index; BAS = Behavior Avoidance Scale; BIS = Behavior Inhibition Scale; MEQ = Morningness-Eveningness-Questionnaire; PSQI = Pittsburgh Sleep Quality Index; SLE = number of stressful Life Events; STAI = State-Trait-Anxiety-Inventory.

### 3.6.2. Baseline measurements

*3.6.2.1. Initial startle reactivity*[3]

At the beginning of every day, four startle probes were presented to habituate the initial startle response. Raw magnitudes were considered for this analysis. The ANOVA revealed a significant main effect of day, $F(2, 146) = 18.70$, $p < .001$, $\eta_p^2 = .20$, and a significant interaction Day × 5-HTTLRP × NPSR1, $F(2, 146) = 4.77$, $p = .010$, $\eta_p^2 = .06$. Post hoc univariate ANOVAs separately for each day only revealed a significant 5-HTTLPR × NPSR1 interaction for Day 1, $F(1, 73) = 5.86$, $p = .018$, $\eta_p^2 = .07$, with higher baseline raw startle magnitudes only for S+/T+ carriers ($M = 100.06$, $SD = 39.83$) compared to S+/AA carriers ($M = 75.98$, $SD = 39.53$), $F(1, 73) = 4.54$, $p = .036$, $\eta_p^2 = .06$.

*3.6.2.2. Pre-acquisition*[4]

There was neither a significant difference between contexts nor any effect of genotype during pre-acquisition in SCL data (all *p*s > .20; see Figure 6), valence (CXT+: $M = 55.78$, $SD = 13.47$; CXT-: $M = 58.35$, $SD = 16.03$) and anxiety ratings (CXT+: $M = 5.89$, $SD = 10.40$; CXT-: $M = 5.95$, $SD = 11.63$), all *p*s > .25. But there was a significant main effect of context for arousal ratings, $F(1, 75) = 3.90$, $p = .052$, $\eta_p^2 = .05$, with slightly higher arousal ratings for CXT+ ($M = 19.67$, $SD = 19.18$) compared to CXT- ($M = 16.66$, $SD = 17.02$) before conditioning on Day 1.

### 3.6.3. Acquisition (Day 1)

*3.6.3.1. Anxiety-potentiated startle*

Most important, the ANOVA revealed a significant three-way interaction of Context × 5-HTTLPR × NPSR1, $F(1, 76) = 6.42$, $p = .013$, $\eta_p^2 = .08$. This interaction was driven by the fact that anxiety-potentiated startle in CXT+ compared to CXT- was only apparent in the carriers of both risk alleles, S+ and T+, $F(1, 19) = 3.98$, $p = .061$, $\eta_p^2 = .17$, whereas carriers of one risk allele (S+/AA and LL/T+, both *F*s < 1), or carriers of no risk allele (LL/AA), $F(1, 19) = 1.59$, $p = .223$, $\eta_p^2 = .08$, did not show differential contextual fear conditioning, see Figure 5. The only other relevant effect was a significant main effect of phase, $F(1, 76) = 3.87$, $p = .053$, $\eta_p^2 = .05$, indicating that startle responses habituation from Acquisition 1 ($M = 4.07$, $SD = 4.80$) to Acquisition 2 ($M = 2.72$, $SD = 4.27$). The only marginal conditioning effect in the S+/T+ group, was due to the fact that startle responses were averaged across both acquisition phases, because during Acquisition 1 the difference between CXT+ and CXT- was not significant, $F(1, 19) < 1$, but during Acquisition 2, $F(1, 19) = 6.79$, $p = .017$, $\eta_p^2 = .26$.

[3] Data of three participants were missing (S+/AA, LL/AA, LL/T+).

[4] Rating data after pre-acquisition and acquisition phases of one participant (LL/T+) were missing due to technical problems.

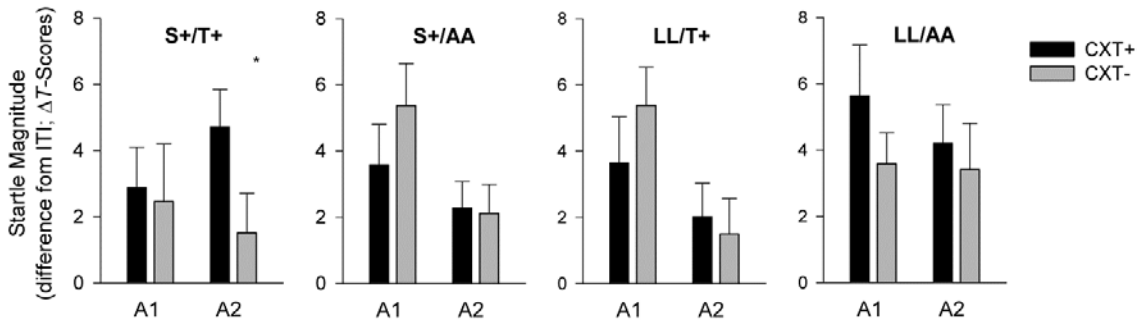


*Figure 5. Study 1: Anxiety-potentiated startle during acquisition depending on both genotypes.*
Results are shown separately for Acquisition 1 (A1) and Acquisition 2 (A2) and for each combined genotype group of 5-HTTLPR (S+ vs. LL) and NPSR1 (T+ vs. AA) polymorphisms: S+/T+, S+/AA, LL/T+, LL/AA. Error bars represent standard error of the mean (SEM). * $p < .05$.

### *3.6.3.2. Skin conductance*

Successful contextual fear conditioning was reflected in a significant main effects of context, $F(1, 76) = 48.24$, $p < .001$, $\eta_p^2 = .39$, with enhanced SCL in CXT+ ($M = 0.690$, $SD = 0.198$) compared to CXT- ($M = 0.679$, $SD = 0.198$), see Figure 6. In addition, SCL habituated from Acquisition 1 ($M = 0.691$, $SD = 0.195$) to Acquisition 2 ($M = 0.677$, $SD = 0.203$), as indicated by the main effect of phase, $F(1, 76) = 10.32$, $p = .002$, $\eta_p^2 = .12$. All main or interaction effects involving the factor genotype did not reach significance (all $ps > .11$).

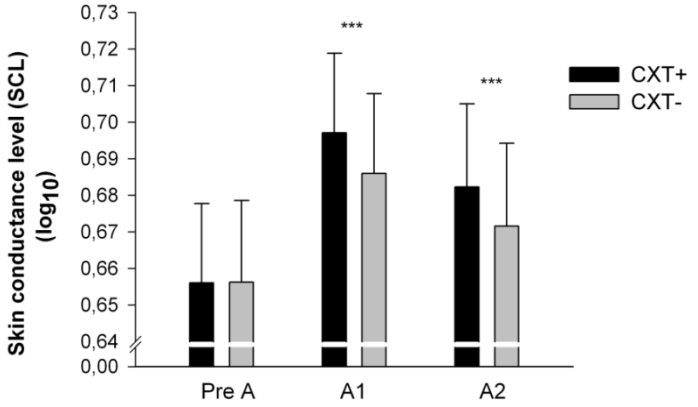


*Figure 6. Study 1: SCL during pre-acquisition and acquisition phases on Day 1.*
Results are shown separately pre-acquisition (Pre A), Acquisition 1 (A1), and Acquisition 2 (A2). Error bars represent standard error of the mean (SEM). *** $p < .001$.

### *3.6.3.3. Valence rating*

There was a significant main effect of context, $F(1, 75) = 19.10$, $p < .001$, $\eta_p^2 = .20$, and a significant interaction Context × NPSR1, $F(1, 75) = 7.59$, $p = .007$, $\eta_p^2 = .09$. The interactions Phase × 5-HTTLPR, $F(1, 75) = 2.97$, $p = .089$, $\eta_p^2 = .04$, and Phase × Context, $F(1, 75) = 3.13$, $p = .081$, $\eta_p^2 = .04$, just failed to reach significance. The main effect of context indicated that CXT+ was rated as more negative ($M = 40.13$, $SD = 17.25$) than CXT-

(*M* = 50.03, *SD* = 15.64). This evaluative conditioning effect was influenced by the NPSR1 genotype: only AA carriers reported more negative valence for CXT+ compared to CXT-, $F(1, 39) = 30.58$, $p < .001$, $\eta_p^2 = .44$, whereas T+ carriers did not, $F(1, 38) = 1.12$, $p = .298$, $\eta_p^2 = .03$, see Figure 7. Additionally, AA carriers rated CXT+ as more negative compared to T+ carriers, $F(1, 77) = 4.41$, $p = .039$, $\eta_p^2 = .05$, whereas both groups did not differ in valence ratings for CXT-, $F(1, 77) = 1.64$, $p = .204$, $\eta_p^2 = .02$.

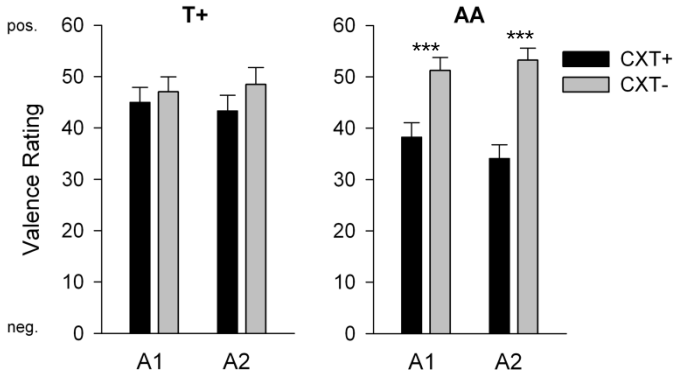


*Figure 7. Study 1: Valence ratings after acquisition phases depending on NPSR1 genotype.*
Valence ratings ranged from 0 (*very negative*) to 100 (*very positive*) and were collected after Acquisition 1 (A1) and Acquisition 2 (A2). Results are shown separately for NPSR1 genotype groups: T+ (left) vs. AA carriers (right). Error bars represent standard errors of the mean (SEM). *** $p \leq .001$.

*3.6.3.4. Arousal rating*

The analysis of arousal ratings revealed significant main effects of context, $F(1, 75) = 36.98$, $p < .001$, $\eta_p^2 = .33$, and phase, $F(1, 75) = 23.35$, $p < .001$, $\eta_p^2 = .24$, indicating successful conditioning (CXT+: *M* = 42.37, *SD* = 23.65; CXT-: *M* = 32.06, *SD* = 22.48) and habituation across both conditioning phases (Acquisition 1: *M* = 41.99, *SD* = 23.51; Acquisition 2: *M* = 32.44, *SD* = 23.45), see Figure 8. The main effect of 5-HTTLPR, $F(1, 75) = 3.38$, $p = .070$, $\eta_p^2 = .04$ (S+: *M* = 32.78, *SD* =22.59; LL: *M* = 41.76, *SD* = 20.25), and the interaction Context × NPSR1 just failed to reach significance, $F(1, 75) = 3.06$, $p = .084$, $\eta_p^2 = .04$.[5]

[5] Because there were differences in arousal ratings between contexts before conditioning (see pre-acquisition), a difference score (Acquisition – Pre-Acquisition) was calculated. So, it was possible to consider effects of the acquisition phase corrected for baseline differences. The ANOVA conducted with this difference scores also revealed main effects of phase, $F(1, 75) = 23.35$, $p < .001$, $\eta_p^2 = .24$, and context, $F(1, 75) = 9.07$, $p = .004$, $\eta_p^2 = .11$, suggesting similar conditioning effects as the standard analysis. Additionally, the main effect of NPSR1 genotype now turned significant, $F(1, 75) = 6.08$, $p = .016$, $\eta_p^2 = .08$, with higher overall arousal reported by AA compared to T+ carriers.

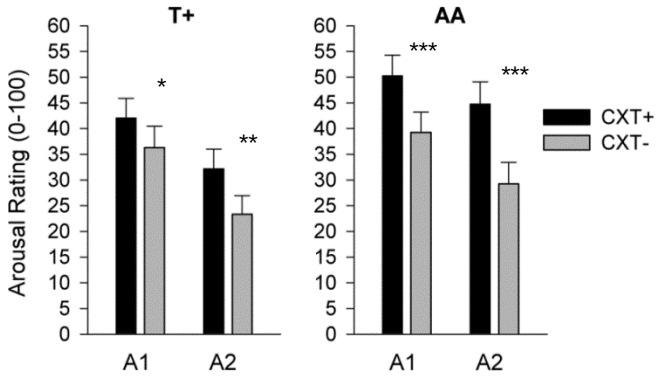


*Figure 8. Study 1: Arousal ratings after acquisition phases depending on NPSR1 genotype.*
Arousal ratings ranged from 0 (*very calm*) to 100 (*very excited*) and were collected after Acquisition 1 (A1) and Acquisition 2 (A2). Results are shown separately for NPSR1 genotype groups: T+ (left) vs. AA carriers (right). Error bars represent standard errors of the mean (SEM). * $p < .05$, ** $p < .01$, *** $p \leq .001$.

*3.6.3.5. Anxiety rating*

The ANOVA revealed significant main effects of context, $F(1, 75) = 14.21$, $p < .001$, $\eta_p^2 = .16$, and phase, $F(1, 75) = 14.74$, $p < .001$, $\eta_p^2 = .16$, as well as significant interactions of Context × NPSR1, $F(1, 75) = 5.67$, $p = .020$, $\eta_p^2 = .07$, and Phase × 5-HTTLPR, $F(1, 75) = 7.05$, $p = .010$, $\eta_p^2 = .09$. The main effect of context showed that CXT+ was rated as more anxiety eliciting ($M = 25.92$, $SD = 26.27$) than CTX- ($M = 20.23$, $SD = 23.37$), indicating successful contextual fear conditioning. But this evaluative conditioning effect was also influenced by the NPSR1 genotype, as the Context × NPSR1 interaction showed. In line with valence ratings, only AA carriers displayed differential conditioning; they reported higher anxiety in CXT+ compared to CXT-, $F(1, 39) = 15.65$, $p < .001$, $\eta_p^2 = .29$, whereas T+ carriers did not, $F(1, 38) = 1.19$, $p = .281$, $\eta_p^2 = .03$, as depicted in Figure 9. The main effect of phase reflected an overall habituation of explicit anxiety from Acquisition 1 ($M = 25.70$, $SD = 25.69$) to Acquisition 2 ($M = 20.45$, $SD = 23.68$). Interestingly, this habituation effect was additionally influenced by the 5-HTTLPR genotype, as the Phase × 5-HTTLPR interaction revealed. Only in LL carriers, anxiety ratings declined from Acquisition 1 ($M = 30.71$, $SD = 26.20$) to Acquisition 2 ($M = 21.74$, $SD = 23.50$), $F(1, 38) = 12.43$, $p = .001$, $\eta_p^2 = .25$, but not in S+ carriers, $F(1, 39) = 2.41$, $p = .128$, $\eta_p^2 = .06$, (Acquisition 1: $M = 20.81$, $SD = 24.52$; Acquisition 2: $M = 19.19$, $SD = 24.10$).

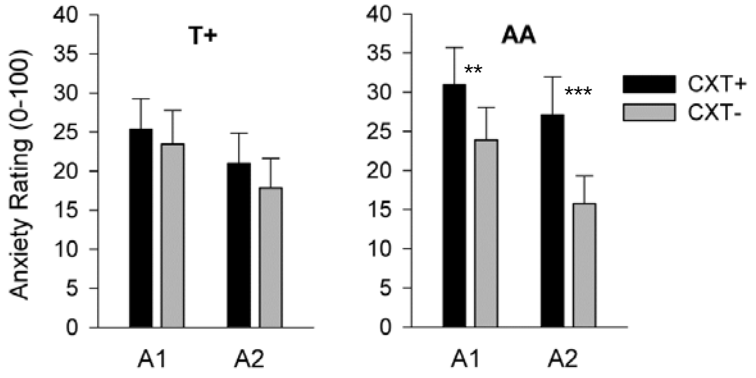


*Figure 9. Study 1: Anxiety ratings after acquisition phases depending on NPSR1 genotype.*
Anxiety ratings ranged from 0 (*no anxiety at all*) to 100 (*very high anxiety*) and were collected after Acquisition 1 (A1) and Acquisition 2 (A2). Results are shown separately for NPSR1 genotype groups: T+ (left) vs. AA carriers (right). Error bars represent standard errors of the mean (SEM). ** $p < .01$, *** $p \leq .001$.

*3.6.3.6. US-expectancy rating*

There was a significant main effect of context, $F(1, 75) = 246.48$, $p < .001$, $\eta_p^2 = .77$, and significant interactions of Phase × Context, $F(1, 75) = 56.64$, $p < .001$, $\eta_p^2 = .43$, and Context × NPSR1 × 5-HTTLPR, $F(1, 75) = 5.64$, $p = .020$, $\eta_p^2 = .07$. After Acquisition 1 and 2 participants rated the expectancy of receiving the US in CXT+ (Acquisition 1: $M = 74.81$, $SD = 25.22$; Acquisition 2: $M = 90.44$, $SD = 17.45$) as higher compared to CXT- (Acquisition 1: $M = 38.86$, $SD = 31.31$; Acquisition 2: $M = 19.87$, $SD = 26.89$), $F(1, 78) = 66.42$, $p < .001$, $\eta_p^2 = .46$, and $F(1, 78) = 316.37$, $p < .001$, $\eta_p^2 = .80$, respectively. However, this difference increased from Acquisition 1 to Acquisition 2, as indicated by the significant interaction Phase × Context, suggesting successful contextual fear conditioning, see Figure 10. The three-way interaction was due to the fact that S+/AA carriers displayed a greater difference in expectancy ratings between CXT+ and CXT- compared to all other combined genotype groups (all $ps \leq .05$), although all four combined genotype groups reported higher US-expectancy in CXT+ compared to CXT- across both acquisition phases (all $ps < .001$).

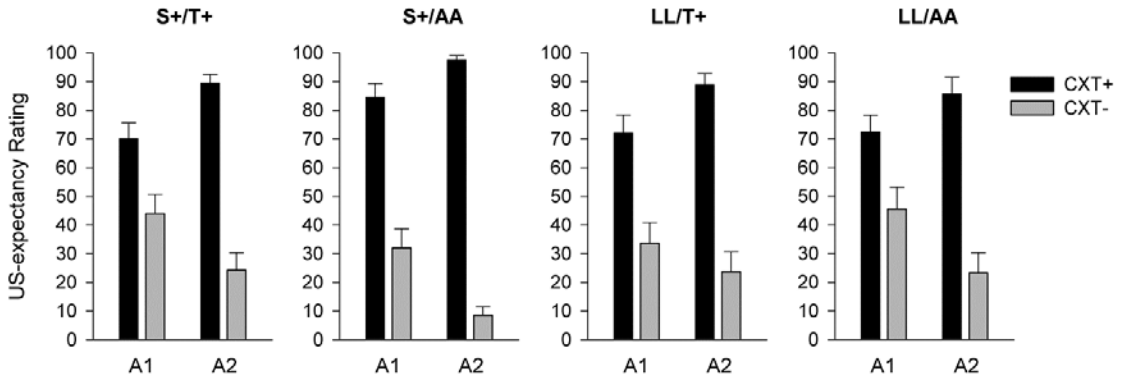


*Figure 10. Study 1: US-expectancy ratings after acquisition phases depending on both genotypes.* US-expectancy ratings ranged from 0 (*no expectancy at all*) to 100 (*definitely expected*) and were collected after Acquisition 1 (A1) and Acquisition 2 (A2). Results are shown separately for each combined genotype group of 5-HTTLPR (S+ vs. LL) and NPSR1 (T+ vs. AA) polymorphisms: S+/T+, S+/AA, LL/T+, LL/AA. Error bars represent standard error of the mean (SEM).

### 3.6.4. Correlations between conditioning effects and number of stressful life events

To elucidate the interaction between genotype effects and life stress on contextual fear conditioning effects and especially to shed light on the absent conditioning effects in valence and anxiety ratings in T+ allele carriers, correlations with the number of stressful life events were calculated. To this end, conditioning effects were assessed as the difference between anxiety responses triggered by CXT+ and CXT-. These difference scores for startle and ratings data were then correlated with the number of stressful life events reported by each participant. For startle data and US-expectancy ratings, four correlation analyses were carried out separately for each combined genotype group (S+/T+, S+/AA, LL/T+, LL/AA), as the interaction between both polymorphisms had influenced conditioning. For valence and anxiety ratings correlation analyses were only conducted for each NPSR1 genotype group, irrespective of 5-HTTLPR genotype group, as only the NPSR1 genotype had an influence on rating data. The results for startle data and US-expectancy ratings are displayed in Table 3, which shows that there were no significant correlations between conditioning effects neither in startle data nor in US-expectancy ratings and the number of stressful life events.

*Table 3. Study 1: Correlations between the number of stressful life events and conditioning effects depending on both genotypes.*

| **Genotype group** | Correlation between **APS** during acquisition and **SLE** | Correlation between **US-expectancy ratings** during acquisition and **SLE** |
|---|---|---|
| S+/T+ | $r$ = -.361, $p$ = .118 | $r$ = -.073, $p$ = .761 |
| S+/AA | $r$ = -.090, $p$ = .707 | $r$ = .125, $p$ = .601 |
| LL/T+ | $r$ = -.071, $p$ = .773 | $r$ = .053, $p$ = .824 |
| LL/AA | $r$ = -.227, $p$ = .336 | $r$ = -.304, $p$ = .192 |

Note: APS = anxiety-potentiated startle, SLE = stressful life events. APS and ratings depict the difference between CXT+ and CXT-.

Table 4 depicts the results for valence and anxiety ratings. For valence ratings there were no significant correlations but for anxiety ratings. In the T+ allele group, the difference between anxiety ratings for CXT+ and CXT- correlated negatively with the number of stressful life events, indicating that the lower the conditioning effect the higher the number of stressful life events. For AA allele carriers the same correlation was not significant (see Figure 11).

*Table 4. Study 1: Correlations between the number of stressful life events and conditioning effects depending on NPSR1 genotype.*

| **NPSR1 genotype group** | Correlation between **valence ratings** and **SLE** | Correlation between **anxiety ratings** and **SLE** |
|---|---|---|
| T+ | $r$ = 0.94, $p$ = .570 | ***r* = -.345, *p* = .032*** |
| AA | $r$ = .253, $p$ = .116 | $r$ = -.186, $p$ = .251 |

Note: SLE = stressful life events. Ratings depict the difference between CXT+ and CXT-. Significant correlations are displayed in bold. * $p$ < .05.

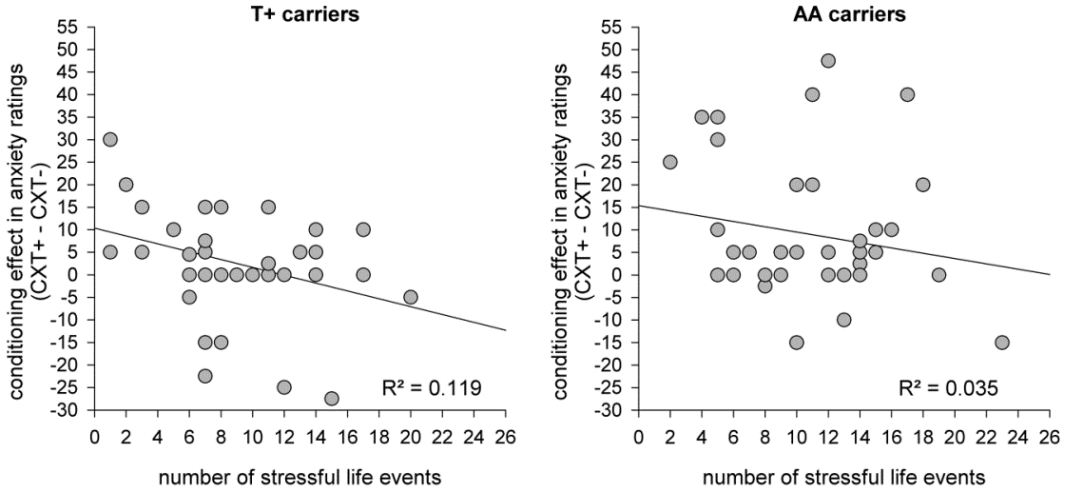


*Figure 11. Study 1: Scatterplots depicting the correlation between the conditioning effect in anxiety ratings and the number of stressful life events depending on NPSR1 genotype.*
Conditioning effect in anxiety ratings on Day 1 are displayed as the difference between CXT+ and CXT-. Results are shown separately for each NPSR1 genotype groups: risk allele carriers T+ (left) and no risk allele carriers AA (right).

### 3.6.5. Extinction (Day 2)

*3.6.5.1. Anxiety-potentiated startle*

The ANOVA revealed a significant main effect of context, $F(1, 76) = 6.12$, $p = .016$, $\eta_p^2 = .07$, and a significant interaction of Phase × Context, $F(1, 76) = 6.75$, $p = .011$, $\eta_p^2 = .08$, indicating successful extinction. While startle magnitudes were higher in CXT+ ($M = 3.95$, $SD = 7.30$) compared to CXT- ($M = 1.87$, $SD = 5.45$), $F(1, 76) = 10.69$, $p = .002$, $\eta_p^2 = .12$, during Extinction 1 as a consequence of the previous acquisition phase, this effect lost significance during Extinction 2, CXT+ ($M = 1.88$, $SD = 4.91$) and CXT- ($M = 1.55$, $SD = 5.19$), $F(1, 76) < 1$. The Phase × Context × NPSR1 interaction just failed to reach significance, $F(1, 76) = 3.49$, $p = .066$, $\eta_p^2 = .04$. There were no other significant interaction effects involving any genotype factor (all *p*s > .10). Nevertheless, as there was a modulation of both genotypes on the acquisition of anxiety-potentiated startle, I exploratively analyzed the time course of extinction of the four genotype groups separately (see Figure 12). During Extinction 1 carriers of one risk allele, S+/AA, $F(1, 19) = 6.38$, $p = .021$, $\eta_p^2 = .25$, and LL/T+, $F(1, 19) = 4.18$, $p = .055$, $\eta_p^2 = .18$ (marginal), showed significantly higher startle magnitudes in CXT+ compared to CXT-. Carriers of both risk alleles (S+/T+) and with no risk allele (LL/AA) did not significantly differentiate between both contexts during Extinction 1 (all *p*s > .27). All four genotype groups extinguished anxiety-potentiated startle during Extinction 2 (all *p*s > .22).

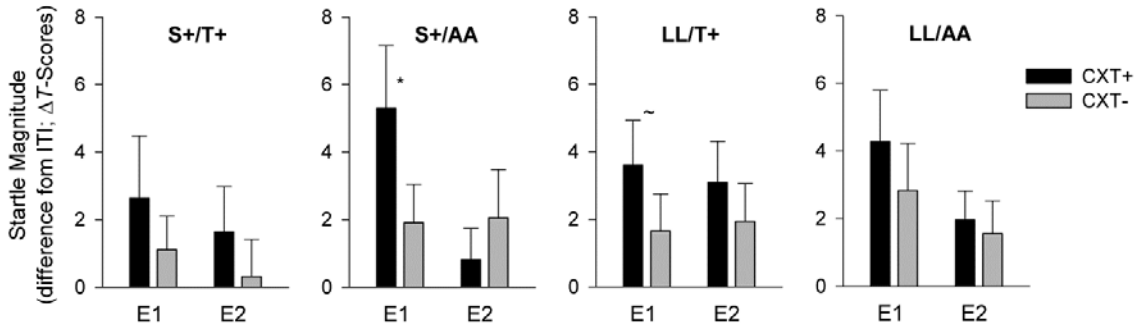


*Figure 12. Study 1: Anxiety-potentiated startle during extinction depending on both genotypes.*
Results are shown separately for Extinction 1 (E1) and Extinction 2 (E2) and for each combined genotype group of 5-HTTLPR (S+ vs. LL) and NPSR1 (T+ vs. AA) polymorphisms: S+/T+, S+/AA, LL/T+, LL/AA. Error bars represent standard error of the mean (SEM). ~ $p < .06$, * $p < .05$.

*3.6.5.2. Skin conductance*

SCL decreased from Extinction 1 ($M$ = 0.635, $SD$ = 0.235) to Extinction 2 ($M$ = 0.622, $SD$ = 0.228), $F(1, 76) = 5.78$, $p = .019$, $\eta_p^2 = .07$, (main effect of phase). Additionally, there was a marginally significant interaction of Context × Phase, $F(1, 76) = 3.50$, $p = .065$, $\eta_p^2 = .04$, indicating successful extinction. During Extinction 1, SCL was higher in CXT+ compared to CXT, $F(1, 79) = 6.15$, $p = .015$, $\eta_p^2 = .07$, but this difference disappeared during Extinction 2, $F(1, 79) < 1$, see Figure 13. There was also a significant main effect of 5-HTTLPR genotype, $F(1, 76) = 5.48$, $p = .022$, $\eta_p^2 = .07$, due to S+ carriers ($M$ = 0.569, $SD$ = 0.226) having reduced overall SCL during extinction compared to LL carriers ($M$ = 0.688, $SD$ = 0.222), not shown in Figure 13.

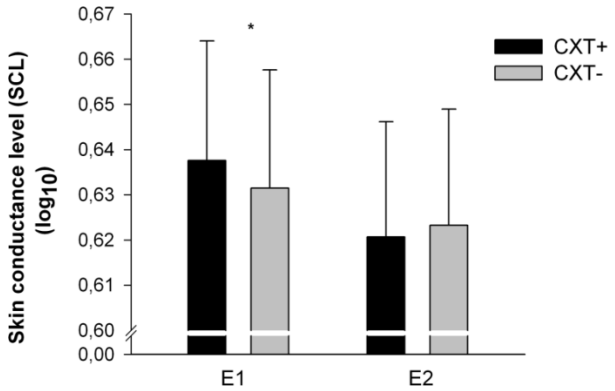


*Figure 13. Study 1: SCL during extinction phases on Day 2.*
Results are shown separately for Extinction 1 (E1) and Extinction 2 (E2). Error bars represent standard error of the mean (SEM). * $p < .05$.

*3.6.5.3. Valence rating*

After the extinction phases CXT+ (*M* = 48.78, *SD* = 14.34) was still rated as more negative than the CXT- (*M* = 55.78, *SD* = 14.08), as reflected in the main effect of context, $F(1, 76) = 18.09$, $p < .001$, $\eta_p^2 = .19$. All other main or interaction effects were not significant (all *p*s > .16). To keep constant with the presentation of valence ratings after the acquisition phases on Day 1, valence ratings after the extinction phases on Day 2 are also displayed separately for NPSR1 genotype groups in Figure 14.

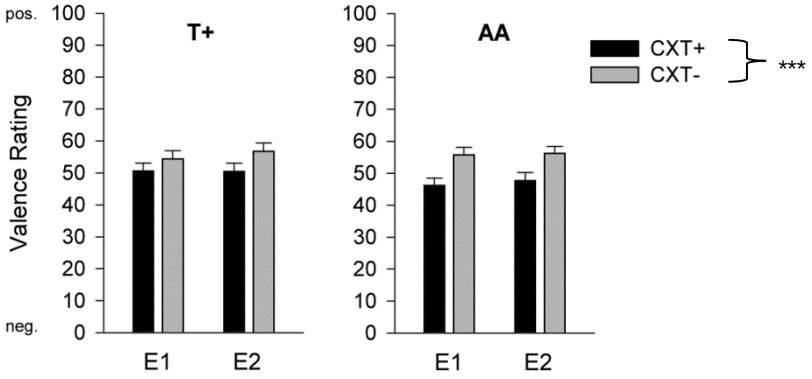


*Figure 14. Study 1: Valence ratings after extinction depending on NPSR1 genotype.*
Valence ratings ranged from 0 (*very negative*) to 100 (*very positive*) and were collected after Extinction 1 (E1) and Extinction 2 (E2). Results are shown separately for NPSR1 genotype groups: T+ (left) vs. AA carriers (right). Only the main effect of context reached significance (*** $p < .001$), meaning that across all groups and both phases valence ratings were more negative for CXT+ compared to CXT-. Error bars represent standard errors of the mean (SEM).

*3.6.5.4. Arousal rating*

Arousal ratings for CXT+ (*M* = 33.04, *SD* = 22.69) were higher than for CXT- (*M* = 23.69, *SD* = 20.63) after the extinction phases (main effect of context, $F(1, 76) = 25.29$, $p < .001$, $\eta_p^2 = .25$), but declined from Extinction 1 (*M* = 31.53, *SD* = 22.45) to Extinction 2 (*M* = 25.20, *SD* = 10.12) (main effect of phase, $F(1, 76) = 15.40$, $p < .001$, $\eta_p^2 = .17$). Moreover, the main effect of NSPR1 genotype was significant, $F(1, 76) = 4.34$, $p = .041$, $\eta_p^2 = .05$. AA carriers (*M* = 32.94, *SD* = 19.42) reported higher arousal compared to T+ (*M* = 23.79, *SD* = 19.82) carriers after the extinction phases according to both CXT+ and CXT-, see Figure 15. The Phase × Context interaction just failed to reach significance, $F(1, 76) = 3.18$, $p = .079$, $\eta_p^2 = .04$. All other effects were not significant (all *p*s > .12).

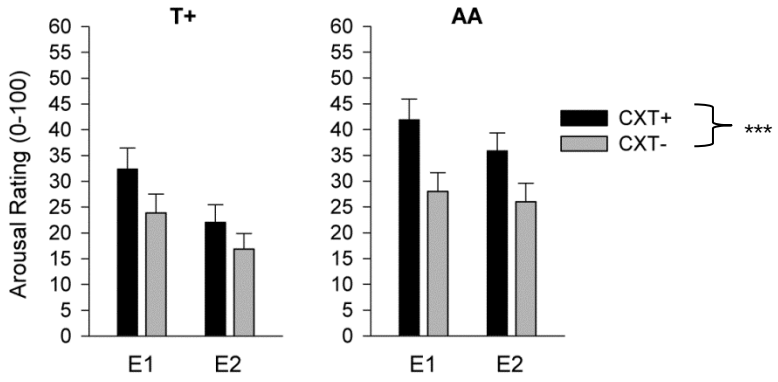


*Figure 15. Study 1: Arousal ratings after extinction phases depending on NPSR1 genotype.*
Arousal ratings ranged from 0 (*very calm*) to 100 (*very excited*) and were collected after Extinction 1 (E1) and Extinction 2 (E2). Results are shown separately for NPSR1 genotype groups: T+ (left) vs. AA carriers (right). The main effect of context reached significance (*** $p < .001$), meaning that across all groups and both phases arousal ratings were higher for CXT+ compared to CXT-. Error bars represent standard errors of the mean (SEM).

*3.6.5.5. Anxiety rating*

The ANOVA revealed significant main effects of phase, $F(1, 76) = 13.60$, $p < .001$, $\eta_p^2 = .15$, and context, $F(1, 76) = 21.60$, $p < .001$, $\eta_p^2 = .22$, and significant interactions of Context × NPSR1, $F(1, 76) = 4.71$, $p = .033$, $\eta_p^2 = .06$, and Phase × Context × NPSR1, $F(1, 76) = 3.93$, $p = .051$, $\eta_p^2 = .05$. Post-hoc contrast regarding the three-way interaction showed that AA carriers reported higher anxiety ratings for CXT+ compared to CXT- after both Extinction 1, $F(1, 39) = 18.88$, $p < .001$, $\eta_p^2 = .33$, and Extinction 2, $F(1, 39) = 16.39$, $p < .001$, $\eta_p^2 = .30$. In contrast, T+ carriers only reported higher anxiety for CXT+ compared to CXT- after Extinction 2, $F(1, 39) = 4.18$, $p = .048$, $\eta_p^2 = .10$. But after Extinction 2, anxiety ratings for CXT+ were higher in AA compared to T+ carriers, $F(1, 78) = 4.39$, $p = .039$, $\eta_p^2 = .05$, see Figure 16.

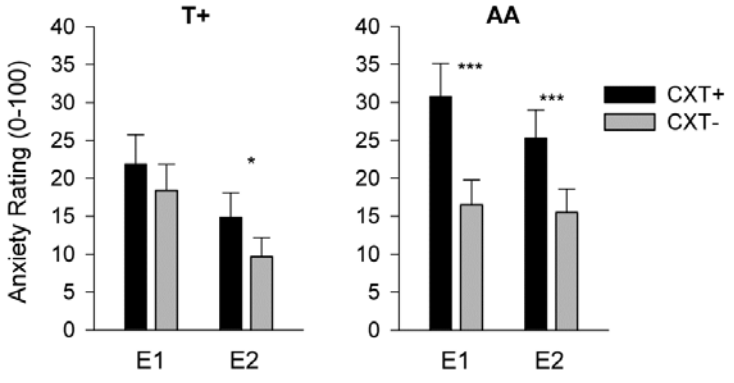


*Figure 16. Study 1: Anxiety ratings after extinction phases depending on NPSR1 genotype.*
Anxiety ratings ranged from 0 (*no anxiety at all*) to 100 (*very high anxiety*) and were collected after Extinction 1 (E1) and Extinction 2 (E2). Results are shown separately for NPSR1 genotype groups: T+ (left) vs. AA carriers (right). Error bars represent standard errors of the mean (SEM). * $p < .05$, *** $p \leq .001$.

*3.6.5.6. US-expectancy rating*

There were significant main effects of phase, $F(1, 76) = 16.14$, $p < .001$, $\eta_p^2 = .18$, and context, $F(1, 76) = 112.56$, $p < .001$, $\eta_p^2 = .60$, and significant interactions of Phase × Context, $F(1, 76) = 27.11$, $p < .001$, $\eta_p^2 = .26$, and Context × NPSR1, $F(1, 76) = 4.38$, $p = .040$, $\eta_p^2 = .06$. AA carriers reported higher US-expectancy for CXT+ compared to T+ carriers after the extinction phases, $F(1, 78) = 6.29$, $p = .014$, $\eta_p^2 = .08$, nevertheless AA, $F(1, 39) = 86.56$, $p < .001$, as well as T+ carriers, $F(1, 39) = 35.54$, $p < .001$, $\eta_p^2 = .48$, reported higher US-expectancy in CXT+ compared to CXT-, see Figure 17. Post hoc contrasts regarding the Phase × Context interaction revealed that US-expectancy for CXT+ was rated as higher as for CXT- after both extinction phases (all *p*s < .001), but the difference between ratings for CXT+ and CXT- decreased from Extinction 1 to Extinction 2, indicated by the significant interaction Phase × Context, thus suggesting extinction.

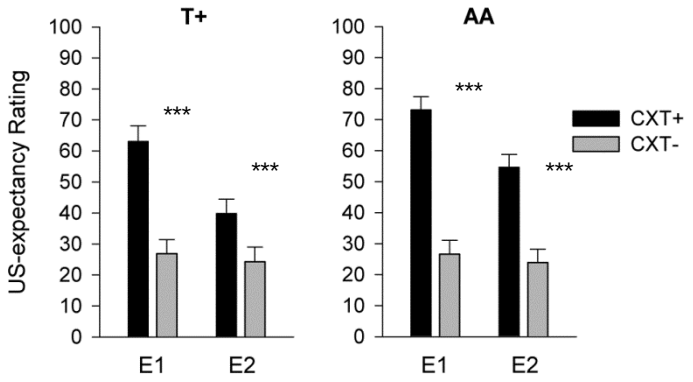


*Figure 17. Study 1: US-expectancy ratings after extinction phases depending on NPSR1 genotype.* US-expectancy ratings ranged from 0 (*no expectancy at all*) to 100 (*definitely expected*) and were collected after Extinction 1 (E1) and Extinction 2 (E2). NPSR1 genotype groups: T+ (left) vs. AA carriers (right). Error bars represent standard errors of the mean (SEM). *** $p < .001$.

### 3.6.6. Correlations between extinction effects and number of stressful life events

In analogy to conditioning data, extinction effects (difference scores between CXT+ and CXT-) in genotype subgroups were correlated with the number of stressful life events. For startle data, four correlation analyses were carried out separately for each combined genotype group for Extinction 1 (S+/T+, S+/AA, LL/T+, LL/AA). For anxiety and US-expectancy ratings correlation analyses were only conducted for each NPSR1 genotype group. For anxiety ratings separate correlations were conducted for Extinction 1 and Extinction 2, as the three-way interaction Phase × Context × NPSR1 turned significant. For US-expectancy ratings correlation analyses were conducted for the whole extinction data, as only the two-way interaction Context × NPSR1 was significant. The results for startle data are displayed in Table 5, which shows that there were no significant correlations between extinction effects in startle data and the number of stressful life events.

*Table 5. Study 1: Correlations between the number of stressful life events and extinction effects in anxiety-potentiated startle.*

| **Genotype group** | Correlation between **APS** during Extinction 1 and **SLE** |
|---|---|
| S+/T+ | $r$ = -.293, $p$ = .210 |
| S+/AA | $r$ = .018, $p$ = .939 |
| LL/T+ | $r$ = -.003, $p$ = .991 |
| LL/AA | $r$ = -.228, $p$ = .334 |

Note: APS = anxiety-potentiated startle, SLE = stressful life events. APS depicts the difference between CXT+ and CXT-.

Table 6 depicts the results for anxiety and US-expectancy ratings; there were no significant correlations.

*Table 6. Study 1: Correlations between the number of stressful life events and extinction effects in anxiety and US-expectancy ratings.*

| Correlation between **SLE** and | **Genotype group** | |
|---|---|---|
| | NPSR1: **T+** | NPSR1: **AA** |
| Anxiety rating E1 | $r$ = -.196, $p$ = .225 | $r$ = -.189, $p$ = .242 |
| Anxiety rating E2 | $r$ = -.265, $p$ = .098 | $r$ = -.124, $p$ = .445 |
| US-expectancy rating E | $r$ = -.063, $p$ = .701 | $r$ = -.302, $p$ = .058 |

Note: SLE = stressful life events; E1 = Extinction 1; E2 = Extinction 2; E = mean across both extinction phases. Ratings depict the difference between CXT+ and CXT-.

### 3.6.7. Extinction Recall (Day 3)

#### *3.6.7.1. Anxiety-potentiated startle*

There were no significant effects (all *p*s > .14) suggesting prolonged extinction within all genotype groups, see Figure 18.

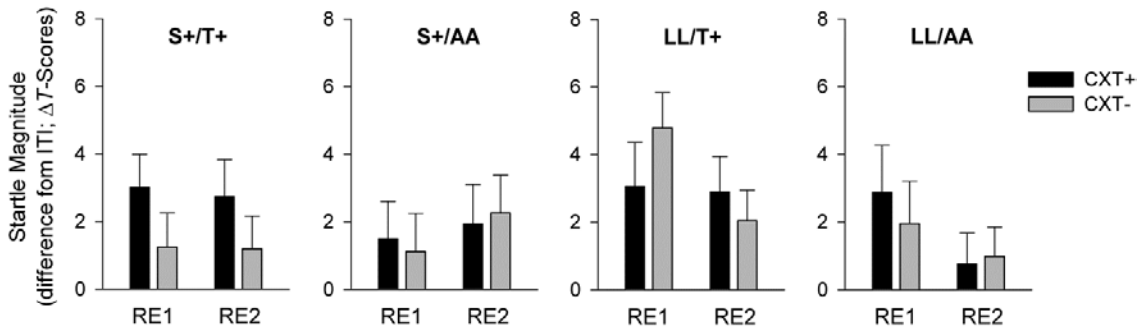


*Figure 18. Study 1: Anxiety-potentiated startle during re-extinction depending on both genotypes.*
Results are shown separately for Re-Extinction 1 (RE1) and Re-Extinction 2 (RE2) and for each combined genotype group of 5-HTTLPR (S+ vs. LL) and NPSR1 (T+ vs. AA) polymorphisms: S+/T+, S+/AA, LL/T+, LL/AA. Error bars represent standard error of the mean (SEM).

### *3.6.7.2. Skin conductance*

In line with the extinction data, S+ carriers had descriptively reduced overall SCL ($M$ = 0.566, $SD$ = 0.199) compared to LL carriers ($M$ = 0.646, $SD$ = 0.197) during re-extinction, but this main effect of 5-HTTLPR genotype just failed to reach significance, $F(1, 76) = 3.19$, $p = .078$, $\eta_p^2 = .04$. No other effects were significant, (all $ps > .20$) suggesting prolonged extinction effects within all genotype groups, see Figure 19.

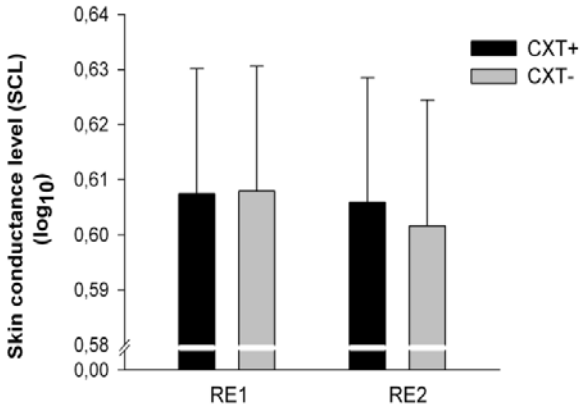


*Figure 19. Study 1: SCL during re-extinction phases on Day 3.*
Results are shown separately for Re-Extinction 1 (RE1) and Re-Extinction 2 (RE2). Error bars represent standard error of the mean (SEM).

### *3.6.7.3. Valence rating*

Even after the re-extinction phases on the third day CXT+ ($M$ = 51.22, $SD$ = 14.20) was still rated as more negative than CXT- ($M$ = 56.04, $SD$ = 13.59), as indicated by the main effect of context, $F(1, 76) = 14.40$, $p < .001$, $\eta_p^2 = .16$, therefore suggesting no extinction. The Context × NPSR1 interaction failed to reach significance, $F(1, 76) = 3.10$, $p = .082$, $\eta_p^2 = .4$, but indicated similar results like for acquisition data. Descriptively, AA carriers reported more negative valence for CXT+ compared to CXT-, whereas T+ carriers

did not, see Figure 20. No other main or interaction effects reached significance (all *p*s > .20).

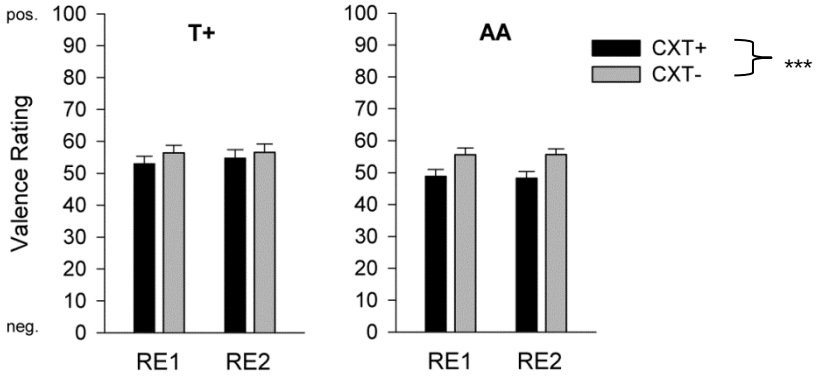


*Figure 20. Study 1: Valence ratings after re-extinction phases depending on NPSR1 genotype.*
Valence ratings ranged from 0 (*very negative*) to 100 (*very positive*) and were collected after Re-Extinction 1 (RE1) and Re-Extinction 2 (RE2). Results are shown separately for NPSR1 genotype groups: T+ (left) vs. AA carriers (right). Only the main effect of context reached significance (*** $p$ < .001), meaning that across all groups and both phases valence ratings were more negative for CXT+ compared to CXT-. Error bars represent standard errors of the mean (SEM).

*3.6.7.4. Arousal rating*

Arousal ratings for CXT+ ($M$ = 24.50, $SD$ = 20.59) were still higher than for CXT- ($M$ = 16.90, $SD$ = 17.49) after the re-extinction phases, main effect of context, $F(1, 76) = 29.99$, $p < .001$, $\eta_p^2 = .28$, but declined from Re-Extinction 1 ($M$ = 23.91, $SD$ = 20.28) to Re-Extinction 2 ($M$ = 17.49, $SD$ = 17.41), main effect of phase, $F(1, 76) = 27.14$, $p < .001$, $\eta_p^2 = .26$. Furthermore, the significant main effect of NSPR1 genotype, $F(1, 76) = 5.26$, $p = .025$, $\eta_p^2 = .07$, indicated that AA carriers ($M$ = 25.22, $SD$ = 19.18) reported higher arousal compared to T+ carriers ($M$ = 16.18, $SD$ = 15.93) for both CXT+ and CXT- after the re-extinction phases, see Figure 21.

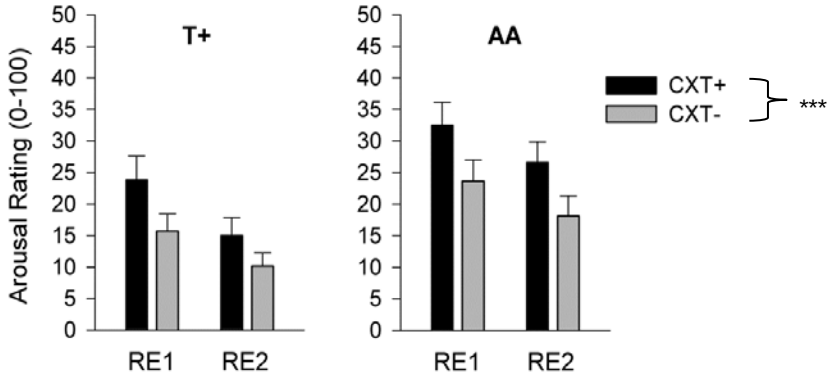


*Figure 21. Study 1: Arousal ratings after re-extinction phases depending on NPSR1 genotype.*
Arousal ratings ranged from 0 (*very calm*) to 100 (*very excited*) and were collected after Re-Extinction 1 (RE1) and Re-Extinction 2 (RE2). Results are shown separately for NPSR1 genotype groups: T+ (left) vs. AA carriers (right). The main effect of context reached significance (*** $p$ < .001), meaning that across all groups and both phases arousal ratings were higher for CXT+ compared to CXT-. Error bars represent standard errors of the mean (SEM).

*3.6.7.5. Anxiety rating*

The analysis of anxiety ratings also revealed similar effects: ratings for CXT+ ($M$ = 14.59, $SD$ = 19.04) were higher than for CXT- ($M$ = 9.72, $SD$ = 14.69) after the re-extinction phases, main effect of context, $F(1, 76) = 15.35$, $p < .001$, $\eta_p^2 = .17$, but declined from Re-Extinction 1 ($M$ = 14.22, $SD$ = 18.43) to Re-Extinction 2 ($M$ = 10.09, $SD$ = 14.70), main effect of phase, $F(1, 76) = 17.71$, $p < .001$, $\eta_p^2 = .19$. There were no effects involving any genotype (all $ps > .10$), see Figure 22.

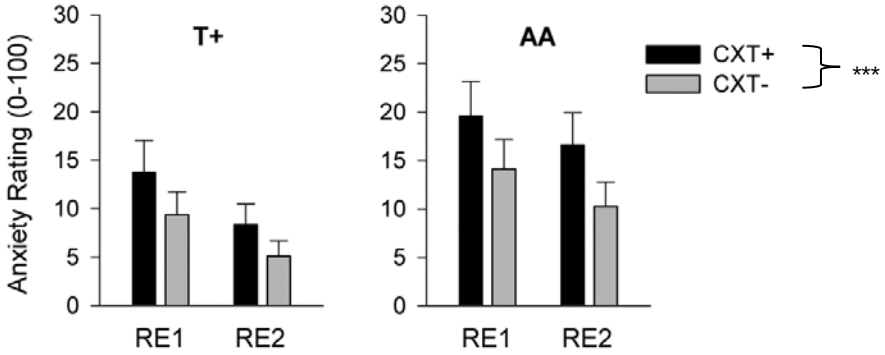


*Figure 22. Study 1: Anxiety ratings after re-extinction phases depending on NPSR1 genotype.*
Anxiety ratings ranged from 0 (*no anxiety at all*) to 100 (*very high anxiety*) and were collected after Re-Extinction 1 (RE1) and Re-Extinction 2 (RE2). Results are shown separately for NPSR1 genotype groups: T+ (left) vs. AA carriers (right). The main effect of context reached significance (*** $p < .001$), meaning that across all groups and both phases anxiety ratings were higher for CXT+ compared to CXT-. Error bars represent standard errors of the mean (SEM).

*3.6.7.6. US-expectancy rating*

There were significant main effects of context, $F(1, 76) = 55.00$, $p < .001$, $\eta_p^2 = .42$, and phase, $F(1, 76) = 19.00$, $p < .001$, $\eta_p^2 = .20$, and a significant interaction Phase × Context, $F(1, 76) = 4.53$, $p = .036$, $\eta_p^2 = .06$, suggesting higher expectancy ratings for CXT+ compared to CXT- after both re-extinction phases, Re-Extinction 1: $F(1, 79) = 43.24$, $p < .001$, $\eta_p^2 = .35$, Re-Extinction 2: $F(1, 79) = 39.75$, $p < .001$, $\eta_p^2 = .34$, but the differences between ratings for CXT+ and CXT- declined from Re-Extinction 1 to Re-Extinction 2 as indicated by the significant interaction Phase × Context. There were no effects involving any genotype (all $ps > .13$), see Figure 23.

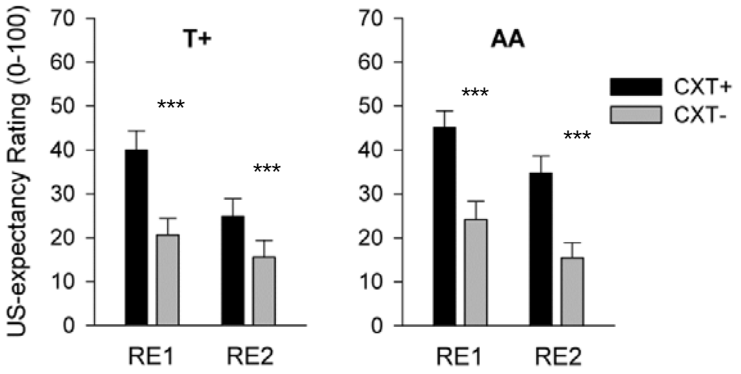


*Figure 23. Study 1: US-expectancy ratings after re-extinction phases depending on NPSR1 genotype.* US-expectancy ratings ranged from 0 (*no expectancy at all*) to 100 (*definitely expected*) and were collected after Re-Extinction 1 (RE1) and Re-Extinction 2 (RE2). Results are shown separately for NPSR1 genotype groups: T+ (left) vs. AA carriers (right). Error bars represent standard errors of the mean (SEM). *** $p \leq .001$.

## 3.7. Discussion

Contextual fear conditioning and its modulation by 5-HTTLPR and NPSR1 polymorphisms were investigated in a virtual reality paradigm with two offices serving as conditioned contexts. Human research suggested that both the S+ allele of the 5-HTTLRP polymorphism and the T+ allele of the NPSR1 polymorphism are associated with heightened fear and anxiety (Canli & Lesch, 2007; Domschke et al., 2011; Lonsdorf et al., 2011; Norrholm & Ressler, 2009; Raczka et al., 2010), presumably as a result of facilitated fear conditioning (Mineka & Oehlberg, 2008; Orr et al., 2000). Therefore, I expected that carriers of these two risk alleles would exhibit enhanced contextual fear conditioning. Additionally, the S allele of the 5-HTTLPR polymorphism has been associated with extinction deficits in rodents as well as in humans (Dai et al., 2008; Lonsdorf et al., 2009; Wellman et al., 2007). Thus, I hypothesized that S+ allele carriers would show extinction deficits during extinction learning and extinction recall. Additionally, an interaction between genotypes and environmental stress on the acquisition and extinction of contextual anxiety was assumed.

Most important, the first hypothesis could be confirmed. I found that the effects of contextual fear conditioning as measured with the "non-cognitive" implicit behavioral measure of the anxiety-potentiated startle reflex were modulated by a gene × gene interaction of 5-HTTLPR and NPSR1 polymorphisms. Only participants carrying both risk alleles, the S allele of the 5-HTTLPR and the T allele of the NPSR1 polymorphism, exhibited potentiated startle responses in the anxiety compared to the safety context during conditioning on Day 1. Since this effect was especially clear in the later acquisition phase this might reflect *learning* of anxiety. This result confirms and extends previous findings

on enhanced fear and anxiety in S allele (Klucken et al., 2013; Klumpers et al., 2012; Lonsdorf et al., 2009) and T allele carries (Dannlowski et al., 2011; Raczka et al., 2010). The fear- or anxiety-potentiated startle reflex is used cross-species as a translational measurement for the activation of the innate defensive system which is especially relevant for implicit and automatic fear responses after learning (Hamm & Weike, 2005; Mineka & Öhman, 2002). Therefore, the gene × gene interaction on the fear-potentiated startle reflex further underscores the importance of both polymorphisms and transmitter systems in amygdala-dependent fear learning. Furthermore, this heightened behavioral expression of conditioned contextual anxiety in carriers of the S+ and the T+ allele might function as an endophenotype of anxiety disorders, particularly those characterized by sustained anxiety levels. Supporting this view, firstly, studies revealed that panic disorder and PTSD are characterized by enhanced contextual anxiety, as indicated by fear-potentiated startle (Grillon et al., 2008; Grillon, Pine, et al., 2009). Secondly, disease-specific genetic associations between 5-HTTLPR and PTSD (Kolassa et al., 2010; Wang et al., 2011) and between NPSR1 and panic disorder (Domschke et al., 2011) were reported.

However, the conditioning effect in S+ and T+ carriers could have emerged due to higher baseline startle responses, as there were higher raw startle magnitudes during startle habituation on Day 1 in S+/T+ carriers. Grillon and Baas (2002) showed that the difference between CS+ and CS- in fear-potentiated startle was dependent on the baseline startle (as indicated in the raw startle magnitude during CS-): the higher the baseline startle was, the more pronounced the conditioning effect (difference between CS+ and CS-) was. However, an explorative analysis of raw startle magnitudes in the present study during conditioning revealed neither genotype main effects nor an interaction 5-HTTLPR × NPSR1 (all *p*s > .18), suggesting that absolute startle magnitudes during conditioning did not differ between genotype groups. Moreover, in the Grillon and Baas study (2002), 'low startlers' exhibited raw startle magnitudes lower 100 µV during CS- and 'high startlers' had raw startle magnitudes higher than 300 µV. In the present study, raw startle magnitudes during conditioning were lower than 100 µV. Thus, it seems implausible that differences in absolute startle reactivity accounted for conditioning effects in the high risk subgroup in the present study.

Interestingly, after consolidation of the fear memory 24 h later, during the first extinction phase only participants of intermediate genetic risk, i.e. carriers of only one risk allele (S+/AA, T+/LL), exhibited conditioned startle discrimination, whereas anxiety-potentiated startle was already extinguished in carriers of both risk alleles. Carrying both risk alleles seems to fasten anxiety learning but also to speed up extinction on an implicit behavioral level. In contrast, carrying one risk allele seems to delay the expression of

learned anxiety. There were no conditioning effects in anxiety-potentiated startle in any genotype group during the second extinction phase indicating successful extinction in all participants. Finally, during extinction recall anxiety-potentiated startle response was not affected by any genotype and still extinguished in all participants, suggesting successful consolidation of the extinction memory. Taken together, the second hypothesis regarding extinction deficits in S+ allele carriers was not supported for startle data. However, it could be speculated that the T+ allele of the NPSR1 polymorphism might have compensated the extinction deficit of S allele carriers. Moreover, the test for extinction recall could have been not sensitive enough to detect between-group differences.

The results clearly indicate successful contextual fear conditioning as reflected in enhanced physiological arousal (SCL) triggered by the anxiety context compared to the safety context. SCL effects are frequently interpreted as an objective indicator of successful learning in cue (Olsson & Phelps, 2004; Schiller et al., 2010; Tabbert et al., 2011) as well as contextual fear conditioning (Glotzbach-Schoon, Tadda, et al., 2013; Tröger et al., 2012). Thus, the SCL results confirm successful contextual fear conditioning, which extinguished during Day 2 and this extinction effect was also recalled on Day 3. Interestingly, results did not reveal any modulation of the differential conditioning and extinction effects by the examined genetic polymorphisms. This lack of SCL modulation by the genetic polymorphisms is in line with other studies on cue conditioning which also failed to find a modulation of conditioned SCR by 5-HTTLPR or NPSR1 polymorphisms (Hermann et al., 2012; Klucken et al., 2013; Lonsdorf et al., 2009; Raczka et al., 2010). However, there is one study on observational fear learning, which found higher SCR to a CS+ in S+ carriers compared to LL carriers (Crisan et al., 2009). Based on these and the present results it might be concluded that the *learning* of conditioned fear and anxiety as reflected in higher skin conductance is rarely influenced by genetic variations.

As already mentioned SCL is a measure for autonomic arousal independent of stimulus valence and is discussed to be influenced by contingency awareness, i.e. the explicitly learned knowledge about the association between CXT and US. That is, conditioned SCL is only obvious in aware participants, whereas startle potentiation is not influenced by contingency awareness (Hamm & Vaitl, 1996). Possibly, the genotype effects in SCL might be covered by a subsample of participants who were unaware. However, there were no true unaware participants in this sample. There were only nine participants who were uncertain about the CXT+US-contingency, because they stated that in both contexts they had received electric stimuli, and these uncertain participants were equally distributed over genotype subgroups. Also US-expectancy ratings revealed that all participants were very well aware of the contingencies. Excluding the uncertain

participants did not reveal genotype effects on differential conditioning in SCL,[6] leaving it unlikely that contingency uncertainty really affected the SCL in this study. Furthermore, US-expectancy ratings and SCL revealed contextual fear conditioning effects already in the first acquisition phase indicating that participants cognitively apprehended contingencies quite early. Therefore, it seems reasonable to conclude that genetic influences on SCL cannot be expected, at least if contingencies are clear and easily apprehended, as in the present study.

In contrast, extinction of SCL was affected by 5-HTTLPR genotype with reduced overall SCL in S+ compared to LL carriers and this effect was even obvious during extinction recall, although not significant. Why was there an influence of genotype on extinction and extinction recall, but not on the acquisition of conditioned SCL? During extinction and extinction recall electrodes for US-administration were attached, but no US was delivered. This might have caused less clear contingencies between CXT+ and US, because the US was unexpectedly omitted. Furthermore, during extinction new inhibitory learning was required about the new contingency between CXT+ and no-US (Bouton, 2002). This unclear situation and new learning might have led to differences between genotype groups, i.e. less physiological arousal to both CXT+ and CXT- in S+ compared to LL carriers during extinction and extinction recall. Notably, conditioned SCR correlated with amygdala activity (Cheng et al., 2006), and therefore reduced SCL during extinction in S+ carries could reflect reduced amygdala activity. In line with this suggestion, Herrmann et al. (2012) reported reduced amygdala activity during extinction in S+ carriers with a high number of traumatic life events compared to LL carriers. They interpret their finding as a neural endophenotype linked to an altered extinction process in S+ carriers, because normally the amygdala would have been involved during extinction learning. However, in the Herrmann study this effect was observed regarding the contrast CS+ vs. CS-, whereas in the present study S+ carriers responded to both CXT+ and CXT- with reduced SCL. Nevertheless, the reduced SCL during extinction in S+ carries in the present study[7] might still reflect reduced amygdala involvement during extinction, possibly showing altered neuronal and physiological responding in S+ carriers. Despite of that, reduced SCL in S+ carriers during extinction might also be interpreted as a habituation effect, showing a stronger decline of arousal.

[6] The AVONA on conditioning data of only aware participants ($n$ = 71) revealed no significant main effects and interactions involving any factor of genotype (all $p$s > .11).

[7] There was neither a significant correlation between the number of life events and SCL in S+ ($r$ = -.213, $p$ = .186) nor in LL carriers ($r$ = -.214, $p$ = .184) during extinction.

On an explicit cognitive level, conditioning effects were obvious in all four rating types (valence, arousal, anxiety and US-expectancy) after acquisition on Day 1 and all these effects were resistant to extinction, indicated by different ratings for CXT+ and CXT- after extinction learning and even after extinction recall, consistent with previous results (e.g., Vansteenwegen et al., 1998). Interestingly, the conditioning effects on the explicit ratings level were influenced by the NPSR1 polymorphism, but there was no interaction with the 5-HTTLPR polymorphism. In detail, AA allele carriers (no risk allele) reported more negative valence and higher anxiety in CXT+ compared to CXT- after contextual fear conditioning on Day 1, whereas T+ allele carriers (risk allele) did not. In contrast, contingency awareness (US-expectancy ratings) was influenced by both polymorphisms, i.e. S+/AA carriers showed the highest difference between CXT+ and CXT- ratings. These results stand in contrast to the conditioning effects of anxiety-potentiated startle in S+ and T+ carriers only. Extinction of explicit ratings was also affected by NPSR1 polymorphism. As an effect of conditioning, being only obvious in AA carriers, higher anxiety in CXT+ compared to CXT- was only reported in AA but not T+ carriers after the first extinction phase. Additionally, anxiety and US-expectancy ratings for CXT+ were higher in AA compared to T+ carriers. Furthermore, AA carriers reported general higher arousal (for both CXT+ and CXT-) compared to T+ carriers after extinction on Day 2, which persisted until extinction recall on Day 3. Therefore, all these data suggest enhanced acquisition of contextual anxiety and extinction deficits in AA carriers on an explicit cognitive level.

The contrary effects of NPSR1 polymorphism on implicit (anxiety-potentiated startle) and explicit (ratings) responses might be explained with the two level account of human fear conditioning with different response output systems of conditioned fear in humans, i.e. an explicit/cognitive level versus an implicit level (Hamm & Weike, 2005). The explicit (rating, SCR) level is regarded to be dependent on contingency knowledge, whereas the implicit level (fear-potentiated startle) is considered to be independent on contingency knowledge. Diverging explicit and implicit responses have previously been reported in the fields of fear extinction and pain relief learning. As stated already in the Introduction, resistance to extinction could be found in explicit ratings but not in the fear-potentiated startle response (Vansteenwegen et al., 1998). Applying backward conditioning, the startle magnitude was diminished by a stimulus signaling US offset, whereas explicit valence of this stimulus was rated as negative, which is discussed as a risk factor for psychopathology (Andreatta et al., 2010). The present results show that it is important to measure different fear levels.

It should also be considered that AA carriers reported higher arousal for the US than T+ carriers. This difference in the explicit evaluation of the US might have

contributed to the differential conditioning effects in explicit anxiety ratings in AA vs. T+ carriers. To prove whether US-arousal was associated with anxiety ratings but not startle data, I exploratively correlated differential conditioning effects in anxiety ratings and startle data with US-arousal. Interestingly, I found a significant correlation between US-arousal and the amount of differential conditioning in anxiety ratings but not with differential conditioning effects in anxiety-potentiated startle.[8] This might be a hint that US-arousal had a greater impact on the explicit level than on the implicit anxiety-potentiated startle response.

Furthermore, explicit conditioning but not extinction effects were influenced by an interaction between the NPSR1 genotype and environmental stress. Recently, a study by Klauke et al. (2012) found similar results, namely an interaction between childhood maltreatement and NPSR1 polymorphism on anxiety sensitivity, another endophenotype for anxiety disorders. Moreover, the NPS system is involved in the regulation the of stress response in animals (Jüngling et al., 2012) and humans (Kumsta et al., 2013). In detail, I found a negative correlation between the context conditioning effect in explicit anxiety ratings and the number of stressful life events. This negative association could only be found in T+ carriers of the NPSR1 polymorphism but not in AA carriers. The higher the number of stressful life events reported by T+ carriers was, the weaker the conditioning effect was. T+ risk allele carriers with many life events even tended to rate the safety context as more anxiety inducing than the anxiety context. Therefore, the absent explicit conditioning effect in T+ carriers might be also due to an additional modulation by the variable life stress. Notably, not only faster and higher fear conditionability is discussed as an endophenotype for anxiety disorders (Orr et al., 2000), but also the failure to inhibit fear responses in the presence of safety (Lissek et al., 2005, 2009). Carrying the T risk allele in addition to a high number of life-stress might impair subsequent safety learning on a cognitive explicit level. However, this is very speculative as participants were not pre-selected on the basis of life events and this negative association has to be replicated in larger samples.

In contrast to previous studies (Herrmann et al., 2012; Klucken et al., 2013), I did not find an interaction between the 5-HTTLPR polymorphism and life stress on contextual fear conditioning and extinction. It is suggest that particularly early developmental periods are most susceptible to life stress and that a gene × environment interaction is

[8] Differential conditioning effects in anxiety ratings and startle data were assessed as the difference score between anxiety ratings/ startle response in CXT+ and CXT- at Day 1. Anxiety ratings with US-arousal: $r = .268$, $p = .017$; startle response with US-arousal: $r = .054$, $p = .631$.

most active during this early life period when the brain matures (Leonardo & Hen, 2008). Especially, the serotonin system seems to be crucial for the normal development of anxiety-related circuits (Kriegebaum et al., 2010b; Leonardo & Hen, 2006, 2008). In the present study environmental stress was assessed with a questionnaire adopted from the Life History Calendar of Caspi et al. (1996), which assesses life events during the whole life and according to a wide range of events differing in severity (e.g. marriage, work stress, diseases, relocation, abuse etc.). Therefore, life stress was not restricted to early severe environmental stress, which would have had a greater impact and would possibly be associated with the 5-HTTLPR genotype. Supportively, Herrmann et al. (2012) and Klucken et al. (2013), who found an interaction between 5-HTTLPR and stressful life events on neuronal correlates of fear conditioning, used a different questionnaire (Life Events Checklist, LCE; Gray, Litz, Hsu, & Lombardo, 2004), which was especially developed for assessing traumatic experiences associated with PTSD. Therefore, the absent interaction between 5-HTTLPR and life events in the present study might be due to the assessment type of a broader range of life events including less severe events.

A limitation of this study might be that anxiety-potentiated startle effects were not very strong and could not be seen across all 80 participants but only in the high risk subgroup. There are several reasons for this discrepancy. Firstly, it might be that there were not enough learning trials, especially for the no-risk subgroup (LL/AA) who might have needed more learning trials to show differential conditioning. Therefore, it is not clear, whether acquisition of contextual anxiety could not be established in LL/AA carriers or whether it was only slowed. Secondly, the US could have been not aversive enough. Human participants always have the opportunity to interrupt the experiment and to control the intensity of the electric stimulus (US), so that it might not be highly aversive (see Mineka & Öhmann, 2002). Thirdly, the virtual situation was anxiety irrelevant (offices) and could have been too artificial to evoke significant amygdala-driven anxiety. According to Mineka and Öhman (2002) fear irrelevant stimuli might be associated with an aversive US only on a pure cognitive level without significant emotionality. Therefore, stronger differential startle potentiation could be expected using anxiety relevant contexts, like height, open-spaces or darkness. However, this anxiety-irrelevant paradigm was effective enough to evoke contextual anxiety in carriers of the two risk alleles for anxiety disorders. Fourthly, I should point out that the participants of this study were healthy, highly educated, and non-anxious individuals (mostly students). Stronger conditioning effects in anxiety-potentiated startle have to be expected in a more anxious sample, as a study employing the same virtual contextual fear conditioning design found in highly trait anxious individuals (Glotzbach-Schoon, Tadda, et al., 2013). Therefore, further studies investigating genetic polymorphisms should examine a more anxious sample

perhaps revealing stronger conditioning effects on the implicit level (anxiety-potentiated startle reflex) and on the explicit cognitive level, especially in T+ allele carriers. Presumably, T+ allele carriers would report enhanced anxiety in a threatening context compared to a safe one, if they were more anxious.

In sum, I found a gene × gene interaction, namely risk allele carriers of the 5-HTTLPR and NPSR1 polymorphisms (S+/T+) exhibited anxiety-potentiated startle during a contextual fear conditioning paradigm. By contrast, 5-HTTLPR polymorphism had no effect on the explicit anxiety level, but only no risk allele carriers of the NPSR1 genotype (AA) showed differential contextual fear learning and extinction deficits. The serotonin system might only modulate amygdala-dependent anxiety learning but not the explicit evaluation of a threatening context, whereas the NPS system seems to have opposing effects on explicit and implicit anxiety responses. Further studies are definitely needed to elucidate the role of NPSR1 in explicit and implicit contextual fear conditioning. However, both genetic polymorphisms play an important role in contextual fear conditioning which is a model for unpredictable threat and chronic and sustained anxiety characteristic for panic disorder or PTSD (Grillon et al., 2008; Grillon, Pine, et al., 2009). In conclusion, contextual fear conditioning may function as an endophenotype for these anxiety disorders. Furthermore, extinction recall of anxiety-potentiated startle was not affected by any genotype, even not by the 5-HTTLPR, although previous studies indicated extinction deficits in S allele carriers (Lonsdorf et al., 2009). Possibly, the test for prolonged extinction effects (presenting additional extinction phases during extinction recall) was not sensitive enough to detect between group differences in anxiety-potentiated startle. Another paradigm to study extinction deficits and the return of fear is the reinstatement paradigm. The next study aimed at adopting this paradigm for contextual fear conditioning.

# 4. Study 2: Reinstatement of contextual anxiety

## 4.1. Introduction

Reinstatement is a mechanism of relapse after extinction. It is defined as the return of fear (CR) to an extinguished fear cue (CS) after US-only or unsignaled US presentation (Bouton, 2002). This mechanism can to some extend explain relapse in anxiety disorder patients after successful exposure therapy. Accordingly, encountering a traumatic event after successful therapy can lead to a return of fear. For example, a study in spider phobics showed that phobic encounters after exposure therapy predicted the return of fear at follow-up (Rodriguez, Craske, Mineka, & Hladek, 1999) and chronic stress after the treatment of agoraphobia resulted in less improvement and higher symptom levels (Wade, Monroe, & Michelson, 1993).

The first demonstration of reinstatement of fear in rats was described by Rescorla and Heth (1975). After extinction training rats received the US without any CS presentation, thus the US was presented unsignaled. One day later, rats were again exposed to the CS without any US, i.e. they received an additional extinction session. During this test phase, the formerly extinguished CS again evoked fear responses. Moreover, the authors also showed that reinstatement of fear can be achieved with a US different from the one used during acquisition. Furthermore, they pointed out that a reinstatement procedure would be more sensitive to evoke a return of fear than spontaneous recovery (Rescorla & Heth, 1975).

Bouton (2002) suggested context conditioning during the US presentation being the underlying mechanism of reinstatement. He showed that reinstatement of a fear cue in rats was context-dependent. Fear responses only returned, if the CS was presented in the same context where the unsignaled US was previously presented, but not if the US was presented in a different context (Bouton & Bolles, 1979). Additionally, extinction of the context after the US presentation diminished subsequent reinstatement of the CS (Bouton & Bolles, 1979). Therefore, Bouton (2002) assumed that the US-only presentation would lead to contextual fear conditioning which in turn would influence the reactions to the CS presented afterwards in this context, possibly due to the expectation of the US elicited by the background context.

The context dependency of the reinstatement of fear was also confirmed by studies demonstrating a crucial involvement of the hippocampus, BLA and BNST in this phenomenon. Hippocampal lesions before conditioning in rats led to an impaired reinstatement of fear, but did not affect the initial acquisition and extinction of cued fear

(Frohardt, Guarraci, & Bouton, 2000). Possibly, during US-only presentation after extinction, the hippocampus conveyed a contextual representation to the BLA. Therefore, hippocampal lesions prevented the establishment of a contextual representation (Frohardt, Guarraci, & Bouton, 2000). Additionally, lesions of the BLA prior to US-only exposure also resulted in reduced reinstatement of fear (Laurent & Westbrook, 2010). The BLA is normally responsible for associating the context representation with the US (Kim & Jung, 2006), therefore lesions to the BLA disrupted the context-US association and reinstatement of fear to an extinguished CS could not be observed (Laurent & Westbrook, 2010). As mentioned in the Introduction (2.4.2.), the BNST is also important for mediating sustained anxiety (Davis et al., 2010) and contextual fear conditioning (Alvarez et al., 2011; Luyten, van Kuyck, et al., 2011; Sullivan et al., 2004). Supportively, BNST lesions in rodents reduced reinstatement of cued fear. Furthermore, reduced fear during the US presentation in BNST lesioned animal was observed (Waddell et al., 2006). As reinstatement of fear seems to depend on the BLA, hippocampus and BNST, it seems plausible that contextual fear conditioning is a critical mechanism involved in this phenomenon.

In humans, reinstatement of cued fear has successfully been demonstrated using fear ratings, US-expectancy ratings, SCR and the fear-potentiated startle response as dependent variables (Dirikx et al., 2004, 2007; Hermans et al., 2005; LaBar & Phelps, 2005; Norrholm et al., 2006). In analogy to animal studies (Kim & Richardson, 2007; Rescorla & Heth, 1975), the return of fear in humans could also be achieved using different USs during the acquisition and the reinstatement procedure (Sokol & Lovibond, 2012). Additionally, in humans the reinstatement of fear seems also to be context-dependent (LaBar & Phelps, 2005). Reinstatement was only observed in participants who received the unsignaled US in the same context where the CS during test was presented. In contrast, participants who received the unsignaled US in a different context showed no reinstatement of fear to the CS. Interestingly, patients with hippocampal damage were not able to show a return of fear after reinstatement (LaBar & Phelps, 2005), further demonstrating that the hippocampus and the context play a critical role in the reinstatement of fear. Furthermore, negative stimulus valence is considered as a possible pathway to the return of fear in humans. It has been shown that the more negative the fear stimulus was evaluated after extinction, the higher the reinstatement of fear was measured with a secondary reaction-time task (Dirikx et al., 2004, 2007). The authors assumed, that evaluative conditioning would be less affected by extinction, but physiological arousal would decline during extinction (see also Vansteenwegen et al., 1998). The US presentation during the reinstatement procedure would lead to an increased arousal triggered by the context.

Therefore, presenting a still negatively valenced stimulus in this arousing context would increase the fear response evoked by this stimulus.

However, the return of fear might not only depend on the physical context but also on the general emotional state and mood of the individual, which also constitutes an interoceptive context (Bouton, 2002). In fact, it has been suggested that the internal context during extinction training might be crucial for extinction recall (see Huff, Hernandez, Blanding, & LaBar, 2009). Therefore, changing the emotional state from extinction to extinction recall could facilitate a return of fear.

In sum, reinstatement has been evidenced in cued fear conditioning paradigms in animals (e.g., Bouton & Bolles, 1979; Laurent & Westbrook, 2010; Ledgerwood, Richardson, & Cranney, 2004) well as in humans (e.g., Dirikx et al., 2004; Norrholm et al., 2006). However, attempts aiming at employing the reinstatement procedure for contextual fear conditioning have been rare. To my knowledge, there are two different procedures which tested the reinstatement of conditioned contextual anxiety in rodents. Firstly, after extinction the rats were re-exposed to the conditioned context for 3 min and then one footshock (US) was presented in this context. Reinstatement of anxiety was tested 24 h later by re-exposing the rats to this conditioned context, which led to an increased freezing compared to the 3 min exposure session conducted before the footshock (Yamada, Zushida, Wada, & Sekiguchi, 2009). However, while applying this procedure it remains unclear whether it is really a test for the reinstatement of anxiety or only a procedure for rapid re-acquisition with only one learning-trial, because the conditioned context was again paired with the US. In a second protocol, after extinction the rats received one footshock in a context which was different from the conditioned context. One day later reinstatement of contextual anxiety was tested in the original conditioned context, showing that freezing was increased compared to the last extinction session (Bertotto, Bustos, Molina, & Martijena, 2006; Stern, Gazarini, Takahashi, Guimarães, & Bertoglio, 2012). This protocol seems to be more elegant, but it may be possible that the footshock presented in the second context established contextual fear conditioning to this context. Therefore, a return of anxiety in the original conditioned context may also be due to a fast generalization process from the second context to the formerly conditioned context.

However, there are no studies demonstrating reinstatement of contextual anxiety in humans. Probing a reinstatement paradigm for contextual fear conditioning has the advantage to provide a sensitive test for the return of contextual anxiety after extinction (Rescorla & Heth, 1975). There are some hints that extinction of contextual anxiety develops faster during extinction learning compared to the extinction of cued fear (Grillon

et al., 2006; Phillips & LeDoux, 1992). But it is unsolved whether this fast extinction is more successful in preventing a return of anxiety after extinction. Furthermore, in Study 1 there were no differences between the different genotype groups during extinction recall one day after extinction training. Possibly, group differences would have been more obvious with a more sensitive procedure to test extinction recall or the return of contextual anxiety. Additionally, it is not clear whether similar cognitive mechanisms are involved in the return of contextual anxiety compared to cued fear, like the internal emotional state (Huff et al., 2009) or the negative stimulus valence (Dirikx et al., 2004, 2007).

## 4.2. Goals and hypotheses of Study 2

The goal of this study was to develop and test a reinstatement protocol of contextual anxiety in humans. I used the same differential contextual fear conditioning paradigm as described in Study 1, but the experimental group underwent a reinstatement procedure. One unsignaled US was presented one day after extinction i.e., before the re-extinction sessions of Day 3. In contrast to the animal studies, in which the US was either presented in the conditioned context or in a second context, I decided to deliver the US while the display of the HMD was turned black, so that no virtual context was visible. Therefore, neither rapid re-acquisition nor generalization of contextual anxiety from a second context should be possible. After the US presentation participants were again exposed to the conditioned contexts (CXT+ and CXT-) without further US presentations (re-extinction). Reinstatement of contextual anxiety was tested during the first trial of re-extinction. Importantly, a second group of participants acted as control group and received no US on Day 3, thus they experienced the same experimental procedure as in Study 1. Additionally, possible variables that could influence the reinstatement of contextual anxiety were exploratively investigated by assessing the emotional state and mood before extinction and the reinstatement procedure on Days 2 and 3.

I hypothesize that:

1) The US-only presentation 24 h after extinction results in a return of differential anxiety as reflected in elevated anxiety responses in CXT+ compared to CXT-. This effect should be obvious in the first re-extinction trial, but is not expected in the later trials, because of fast re-extinction effects. The control group displays no return of contextual anxiety, meaning they show no difference in anxiety responses between CXT+ and CXT- on Day 3.

2) Mood influences the reinstatement of contextual anxiety. According to Huff et al. (2009), I expected that a change of mood from extinction to the reinstatement test results in a return of contextual anxiety. In detail, if the difference between mood on Day 3 and Day 2 increases, the return of contextual anxiety on Day 3 will also be higher (difference in startle responses and anxiety ratings regarding the first re-extinction trial). This association will be more pronounced in the reinstatement group compared to the control group.

3) In analogy to studies by Dirikx et al. (2004, 2007), I expected that the more negatively valenced the context after extinction is, the higher the return of contextual anxiety will be as reflected in startle response after the reinstatement procedure on Day 3. In detail, I expected that the difference in valence ratings (CXT+ - CXT-) after extinction correlates positively with the difference in startle response (CXT+ - CXT-) during the first trial of re-extinction in the reinstatement group, but not in the control group.

## 4.3. Materials and Methods

### 4.3.1. Participants

Sixty-seven participants participated in this study: 39 in the reinstatement group and 28 in the control group. Within the reinstatement group 18 participants were excluded because of technical problems ($n$ = 6), simulator sickness ($n$ = 3), startle non-responding ($n$ = 1), current psychotherapy ($n$ = 1), unawareness of the contingency between context and US ($n$ = 1), not returning to the second session on Day 2 ($n$ = 1), and not rating the US intensity during the reinstatement procedure on the third day as painful ($n$ = 5; rating < 4; with 4 as 'just noticeable painful'). In the control group a total of seven participants were excluded because of similar reasons: simulator sickness ($n$ = 2), startle non-responding ($n$ = 2), unawareness of the contingency between context and US ($n$ = 2), and not returning to the second session on Day 2 ($n$ = 1). The final sample consisted of 42 participants with 21 participants in each group. All participants gave their written informed consent. Participants gained 30 € for their participation. The study of was approved by the Ethics Committee of the Medical Faculty of the University of Würzburg.

### 4.3.2. Procedure and Design

Questionnaires, unconditioned stimulus, conditioned contextual stimuli, recording of physiological data and design were the same as in Study 1 except the reinstatement procedure on Day 3. The reinstatement group received one electric stimulus with the

individual current intensity, which was determined on Day 1 during the shock workup procedure, while seeing a black screen. Afterwards they rated its intensity, valence and arousal. The instruction was: 'You now will receive the electric stimulus. Please indicate how painful it was on the scale from 0 to 10.' The scale which was used on Day 1 was again presented on the screen. Five participants were excluded from further analysis because they rated the US during the reinstatement procedure as not painful (rating < 4; with 4 as 'just noticeable pain'), because habituation of the US was found to attenuate the fear response (Rescorla, 1973). The control group did not receive any instruction or US, but underwent the same procedure on Day 3 as done in Study 1.

In addition to the ratings obtained after each phase (pre-acquisition, Acquisition 1, Acquisition 2, Extinction 1, Extinction 2, Re-Extinction 1, Re-Extinction 2) which referred to valence, arousal, anxiety and US-expectancy regarding the *whole phase*, ratings were also obtained for the *last trial of extinction* on Day 2 and the *first trial of re-extinction* on Day 3. These additional ratings were obtained after ratings for the whole phases which took place after the second extinction phase (Extinction 2) and after the first re-extinction phase (Re-Extinction 1) respectively. These additional ratings were collected to compare the last trial of extinction with the first trial of re-extinction in analogy to physiological data. Figure 24 displays the experimental design at the end of extinction and at the beginning of re-extinction in the reinstatement group.

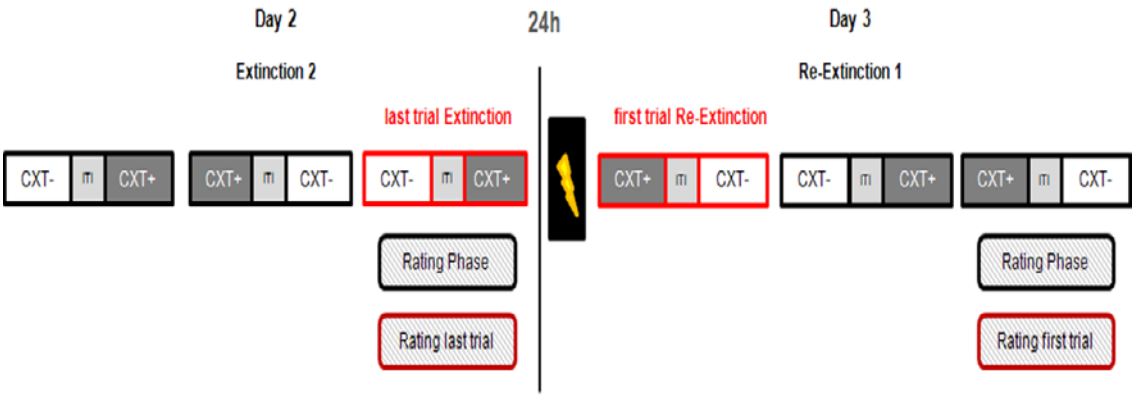


*Figure 24. Study 2: Reinstatement procedure.*
Before the re-extinction phases on Day 3 the reinstatement group received one unsignaled US (electric stimulus) while the display of the HMD was turned black. Ratings were obtained regarding the whole phase at the end of extinction on Day 2 and after the first re-extinction phase on Day 3 (Extinction 2 vs. Re-Extinction 1) and afterwards additional ratings were collected especially for the last trial of extinction and the first trial of re-extinction. To test a reinstatement of anxiety on Day 3, the last trial of the extinction phase on Day 2 (highlighted in red on the left side) was compared to the first trial of the re-extinction phase (highlighted in red on the right side) 24 h later on Day 3.

### 4.3.3. Data reduction

*4.3.3.1. Startle response and skin conductance level*

See Study 1. Two participants in the control group had to be excluded from SCL analysis because of technical problems during physiological recording. Additionally, pre-acquisition SCL data of one participant of the reinstatement group were not saved.

*4.3.3.2. Statistical analysis*

State anxiety, negative affect, positive affect, daily sleep quality measured before each experimental session[9], IPQ, and initial startle reactivity (during habituation) were analyzed with 3 (Day: 1, 2, 3) × 2 (Group: reinstatement, control) ANOVAs. If the assumption of sphericity for within-factors with three levels was violated ($p < .20$) Greenhouse-Geisser correction was applied and Greenhouse-Geisser Epsilon (GG-ε) was reported. Group differences between trait questionnaire data (age, ASI, STAI-Trait, BIS, BAS, PSQI, MEQ) were analyzed with independent *t* tests. In all analyzes the alpha level was set at $p \leq .05$. Effect sizes were calculated using the partial eta ($\eta_p^2$).

Prior to statistical analysis physiological data were first averaged for each phase (Acquisition 1, Acquisition 2, Extinction 1, Extinction 2, Re-Extinction 1, Re-Extinction 2) across the three runs per phase. Anxiety-potentiated startle was determined as the difference score between the mean startle response during contexts and ITI (CXT+ – ITI, or CXT- – ITI).

Startle, SCL and rating data were analyzed separately for each day. The different phases of the experiment were also analyzed with separate ANOVAs. During pre-acquisition startle responses were not collected as in Study 1. Therefore, only SCL and ratings data were analyzed with 2 (Context: CXT+, CXT-) × 2 (Group: reinstatement, control) ANOVAs during pre-acquisition. Acquisition, extinction and re-extinction data were analyzed separately with 2 (Context: CXT+, CXT-) × 2 (Phase: 1, 2) × 2 (Group: reinstatement, control) ANOVAs. To test for a return of anxiety after the reinstatement procedure on Day 3, the last trial of extinction against the first trial of re-extinction were analyzed, since strongest extinction effects have to be expected at the end of extinction and a strongest return of anxiety has to be expected during the first trial after the reinstatement procedure, resulting in a 2 (Context: CXT+, CXT-) × 2 (Time: last trial of extinction, first trial of re-extinction) × 2 (Group: reinstatement, control) ANOVA (see also Dirikx et al., 2004; Norrholm et al., 2006).

[9] STAI-State, PANAS and sleep quality data assessed on Day 1 of one participant (reinstatement group) were missing.

A change of mood from extinction to extinction recall (Huff et al., 2009) was determined as the difference between state scores (positive affect, negative affect, state anxiety) of Day 3 and Day 2 (Day 3 – Day 2). The higher this change index was, the higher the change of mood from Day 2 to Day 3 was assumed. Correlations were calculated between the change index and the return of contextual anxiety in startle responses and anxiety ratings (difference between CXT+ and CXT- in the first re-extinction trial). Correlations were calculated for positive affect, negative affect, and state anxiety separately. An association between negative stimulus valence after extinction and the return of anxiety in startle responses was also tested via a correlation analysis. The difference in valence ratings (CXT+ - CXT-) regarding the last extinction trial of Day 2 was correlated with the difference in startle responses (CXT+ - CXT-) in the first re-extinction trial of Day 3.

## 4.4. Results

### 4.4.1. Sample characteristics

Both groups did not differ in gender distribution, age, ASI, BIS, BAS, MEQ, PSQI, STAI-Trait, US current intensity, US pain rating and US valence rating on Day 1 (see Table 7). There were no significant effects for daily state anxiety, negative affect and sleep quality (all *p*s > .17), but changes in US arousal ratings differed between groups, Time × Group interaction, $F(1, 40) = 12.33$, $p = .001$, $\eta_p^2 = .24$. The reinstatement group reported higher US arousal prior to conditioning compared to the control group, but not after conditioning, see Table 7. For positive affect there was a significant main effect of day, $F(2, 78) = 3.46$, $p = .036$, $\eta_p^2 = .08$, with higher positive affect on Day 1 ($M = 29.10$, $SD = 5.72$) compared to Day 2 ($M = 27.17$, $SD = 6.03$), $F(1, 39) = 6.65$, $p = .014$, $\eta_p^2 = .15$. Finally, the analysis of IPQ data revealed a significant main effect of day, $F(2, 80) = 15.28$, $p < .001$, $\eta_p^2 = .28$, with higher presence on Day 1 ($M = 2.57$, $SD = 13.99$) compared to Day 2 ($M = -2.67$, $SD = 14.69$), $F(1, 40) = 19.96$, $p < .001$, $\eta_p^2 = .33$.

*Table 7. Study 2: Demographic and psychometric data of both groups.*

| | **Control group** $N = 21$ | **Reinstatement group** $N = 21$ | $\chi^2, t$ | $p$ |
|---|---|---|---|---|
| Gender | 10 female | 12 female | 0.38 | .537 |
| Age (years) | 24.05 (2.85) | 23.62 (2.97) | 0.48 | .636 |
| US current intensity | 2.21 mA (0.91) | 2.12 mA (1.05) | 0.28 | .778 |
| US pain rating Day 1 | 5.17 (1.09) | 5.19 (1.25) | 0.07 | .984 |
| US valence (pre) | 34.05 (15.13) | 37.14 (13.47) | 0.70 | .448 |
| US valence (post) | 34.76 (15.61) | 34.76 (22.05) | 0.70 | .448 |
| US arousal (pre) | 37.62 (27.23) | 64.05 (16.93) | 3.78 | .001 |
| US arousal (post) | 57.38 (26.91) | 52.38 (26.30) | 0.00 | 1.00 |
| STAI Trait | 38.05 (9.27) | 37.76 (8.10) | 0.11 | .916 |
| ASI | 16.57 (8.72) | 16.24 (6.63) | 0.14 | .890 |
| BIS | 2.76 (0.60) | 2.89 (0.55) | 0.73 | .470 |
| BAS | 3.24 (0.32) | 3.24 (0.29) | 0.25 | .980 |
| MEQ | 46.24 (8.01) | 48.57 (10.29) | 0.82 | .417 |
| PSQI | 5.33 (2.35) | 5.86 (3.24) | 0.60 | .553 |
| STAI State Day 1 | 34.62 (8.66) | 35.30 (6.12) | 0.29 | .774 |
| STAI State Day 2 | 34.95 (7.34) | 36.30 (5.06) | 0.51 | .613 |
| STAI State Day 3 | 34.00 (8.37) | 36.20 (10.14) | 0.73 | .471 |
| NA Day 1 | 12.29 (3.27) | 12.25 (2.57) | 0.04 | .969 |
| NA Day 2 | 11.67 (2.52) | 13.05 (3.44) | 1.34 | .189 |
| NA Day 3 | 11.24 (1.87) | 12.90 (3.77) | 1.68 | .102 |
| PA Day 1 | 28.76 (5.94) | 29.45 (5.61) | 0.38 | .705 |
| PA Day 2 | 27.90 (6.50) | 27.25 (5.66) | 0.23 | .821 |
| PA Day 3 | 27.90 (7.62) | 27.15 (5.73) | 0.18 | .857 |
| Sleep quality Day 1 | 0.76 (0.70) | 0.80 (0.52) | 0.20 | .845 |
| Sleep quality Day 2 | 0.86 (0.85) | 0.90 (0.64) | 0.21 | .838 |
| Sleep quality Day 3 | 0.95 (0.67) | 0.70 (0.73) | 1.11 | .273 |
| IPQ Day 1 | 5.00 (11.18) | 0.14 (16.25) | 1.23 | .266 |
| IPQ Day 2 | -0.81 (10.75) | -4.52 (17.87) | 0.82 | .419 |
| IPQ Day 3 | -0.57 (12.77) | -7.43 (18.64) | 1.39 | .172 |

*Note:* Frequencies and means (SD) are displayed. ASI = Anxiety Sensitivity Index; BAS = Behavior Avoidance Scale; BIS = Behavior Inhibition Scale; IPQ = Igroup Presence Questionnaire; MEQ = Morningness-Eveningness-Questionnaire; NA = negative affect; PA = positive affect; PSQI = Pittsburgh Sleep Quality Index; STAI = State-Trait-Anxiety-Inventory.

### 4.4.2. Baseline measurements

*4.4.2.1. Initial startle reactivity*

The ANOVA revealed a significant main effect of day, $F(2, 80) = 9.10$, $p < .001$, $\eta_p^2 = .19$, GG-$\varepsilon = .92$, with higher startle magnitudes during habituation on Day 1 ($M = 84.06$, *SD*

= 40.36) compared to Day 2 ($M$ = 67.14, $SD$ = 33.49), $F(1, 40) = 19.47$, $p < .001$, $\eta_p^2 = .33$, but there was no difference between Day 2 and Day 3 ($M$ = 70.51, $SD$ = 47.21), $F(1, 40) = < 1$, suggesting habituation from Day 1 to Day 2. There were no effects of group (all $ps > .25$).

*4.4.2.2. Pre-acquisition*

There was a significant main effect of context for valence ratings, $F(1, 40) = 4.934$, $p = .032$, $\eta_p^2 = .10$, indicating in both groups more positive valence for CXT- ($M$ = 63.57, $SD$ = 16.05) compared to CXT+ ($M$ = 55.48, $SD$ = 19.06) before conditioning. Additionally, the control group displayed higher overall baseline skin conductance level ($M$ = 0.678, $SD$ = 0.210) compared to the reinstatement group ($M$ = 0.445, $SD$ = 0.177) before conditioning, $F(1, 37) = 14.30$, $p = .001$, $\eta_p^2 = .28$. There were no significant differences between groups or contexts for arousal and anxiety ratings before conditioning (all $ps > .08$).

### 4.4.3. Acquisition (Day 1)

Successful contextual fear conditioning was obvious in all dependent measurements across both groups. Detailed results are described below.

*4.4.3.1. Anxiety-potentiated startle*

The ANOVA revealed a significant main effect of context, $F(1, 40) = 4.91$, $p = .032$, $\eta_p^2 = .11$. Startle magnitudes were potentiated in CXT+ ($M$ = 3.99, $SD$ = 4.35) compared to CXT- ($M$ = 2.43, $SD$ = 3.73). There was neither a main effect nor any interaction with group, suggesting that all participants showed successful contextual fear conditioning (all $ps > .20$), see Figure 25.

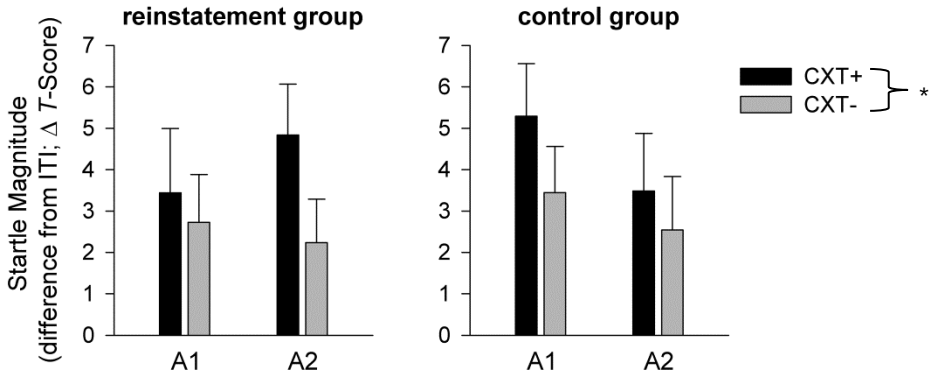


*Figure 25. Study 2: Anxiety-potentiated startle during acquisition phases.*
Only the main effect of context reached significance (* $p < .05$), meaning that across both groups and both phases startle magnitudes were potentiated in CXT+ compared to CXT-. However, results are shown separately for Acquisition 1 (A1) and Acquisition 2 (A2) and for each group: reinstatement (left) vs. control group (right). Error bars represent standard error of the mean (SEM).

*4.4.3.2. Skin conductance*

Successful contextual fear conditioning was reflected in SCL, because there was a significant main effect of context, $F(1, 38) = 8.37$, $p = .006$, $\eta_p^2 = .18$. SCL in CXT+ was significantly higher compared to CXT-, see Figure 26. In addition, SCL habituated from Acquisition 1 to Acquisition 2 as the main effect of phase indicated, $F(1, 38) = 12.05$, $p = .001$, $\eta_p^2 = .24$. Finally, participants of the control group had overall higher SCL than those of the reinstatement group, main effect of group: $F(1, 38) = 13.79$, $p = .001$, $\eta_p^2 = .27$.

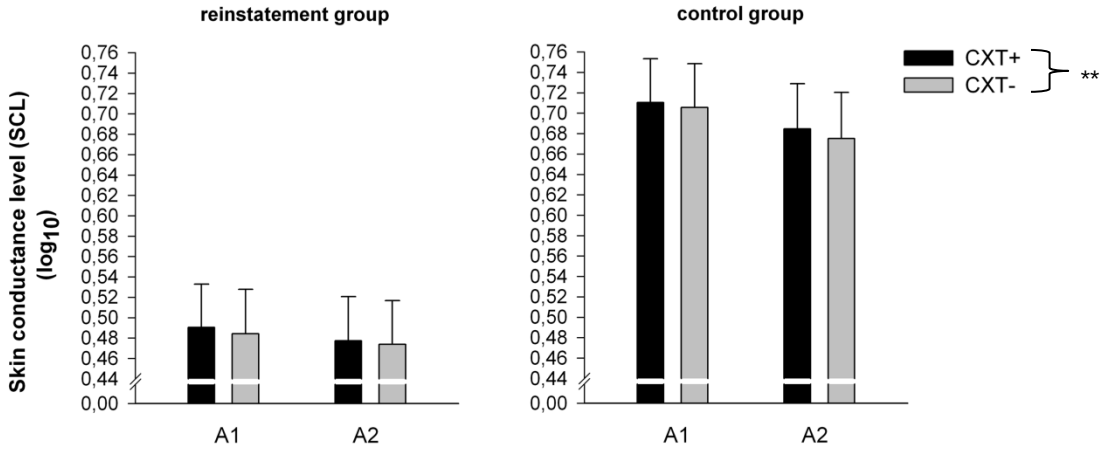


*Figure 26. Study 2: SCL during acquisition phases on Day 1.*
Only the main effect of context reached significance (** $p < .01$), meaning that across both groups and both phases startle magnitudes were potentiated in CXT+ compared to CXT-. However, results are shown separately for Acquisition 1 (A1) and Acquisition 2 (A2) and for each group: reinstatement (left) vs. control group (right). Error bars represent standard error of the mean (SEM).

*4.4.3.3. Valence rating*

There were significant effects of context, $F(1, 40) = 24.82$, $p < .001$, $\eta_p^2 = .38$, indicating that CXT+ was rated as more negative ($M = 36.57$, $SD = 18.61$) as CXT-, and Phase × Context, $F(1, 40) = 7.39$, $p = .010$, $\eta_p^2 = .16$., showing that the difference between contexts increased from Acquisition 1 to Acquisition 2. Additionally, the Context × Group interaction reached significance, $F(1, 40) = 6.79$, $p = .013$, $\eta_p^2 = .15$. Both groups reported significantly more negative valence for CXT+ compared to CXT-, control group: $F(1, 20) = 18.66$, $p < .001$, $\eta_p^2 = .48$, reinstatement group: $F(1, 20) = 6.17$, $p = .022$, $\eta_p^2 = .24$, but as the interaction indicates the difference between both contexts was greater in the control compared to the reinstatement group, see Figure 27.[10]

[10] Because there have been differences in valence ratings between contexts before conditioning (see pre-acquisition), a difference score (Acquisition – Pre-Acquisition) was calculated. So it was possible to consider effects of the acquisition phase corrected for baseline differences. The ANOVA conducted with these difference scores also revealed a main effect of context, $F(1, 40) = 4.98$, $p =$

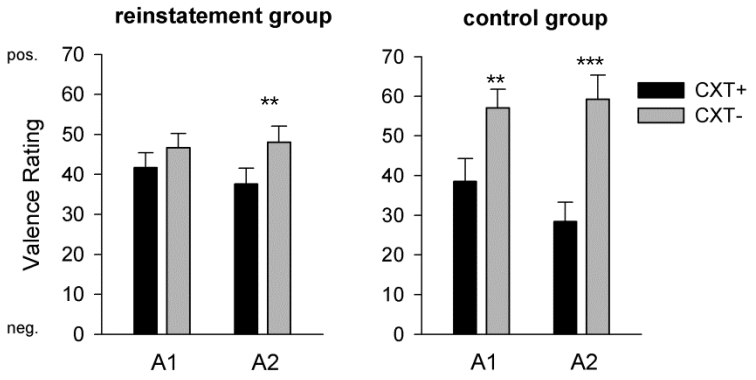


*Figure 27. Study 2: Valence ratings after acquisition phases.*
Valence ratings ranged from 0 (*very negative*) to 100 (*very positive*) and were collected after Acquisition 1 (A1) and Acquisition 2 (A2). Results are shown separately for the reinstatement (left) and the control group (right). Error bars represent standard error of the mean (SEM). ** $p < .01$, *** $p < .001$.

### *4.4.3.4. Arousal rating*

CXT+ ($M$ = 49.40, $SD$ = 22.78) was rated with higher arousal compared to CXT- ($M$ = 34.82, $SD$ = 19.99), as the significant main effect of context indicated, $F(1, 40) = 30.35$, $p < .001$, $\eta_p^2 = .43$, see Figure 28. No other effects reached significance (all $ps > .36$).

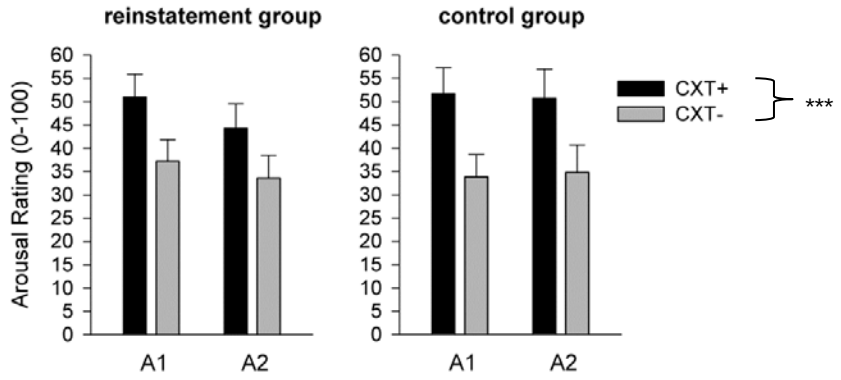


*Figure 28. Study 2: Arousal ratings after acquisition phases.*
Only the main effect of context reached significance (*** $p < .001$), meaning that across both groups and both phases arousal ratings were higher for CXT+ compared to CXT-. Arousal ratings ranged from 0 (*very calm*) to 100 (*very excited*) and were collected after Acquisition 1 (A1) and Acquisition 2 (A2). Results are shown separately for the reinstatement (left) and the control group (right). Error bars represent standard error of the mean (SEM).

.031, $\eta_p^2 = .11$, and an interaction Phase × Context, $F(1, 40) = 7.39$, $p = .010$, $\eta_p^2 = .16$, suggesting similar conditioning effects as in the standard analysis. The interaction Context × Group was only marginally significant, $F(1, 40) = 3.76$, $p = .060$, $\eta_p^2 = .09$.

*4.4.3.5. Anxiety rating*

All participants reported higher anxiety for CXT+ ($M$ = 33.55, $SD$ = 27.65) compared to CXT- ($M$ = 23.39, $SD$ = 20.77), as the main effect of context revealed, $F(1, 40) = 17.55$, $p < .001$, $\eta_p^2 = .31$, see Figure 29. No other effects were significant (all $ps > .39$).

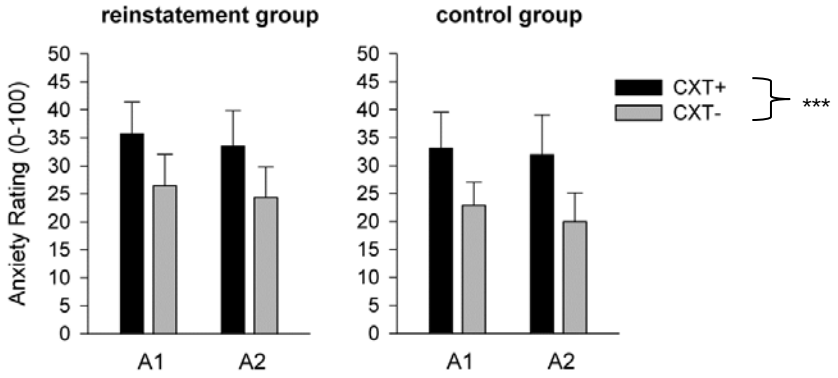


*Figure 29. Study 2: Anxiety ratings after acquisition phases.*
Only the main effect of context reached significance (*** $p < .001$), meaning that across both groups and both phases anxiety ratings were higher for CXT+ compared to CXT-. Anxiety ratings ranged from 0 (*no anxiety at all*) to 100 (*very high anxiety*) and were collected after Acquisition 1 (A1) and Acquisition 2 (A2). Results are shown separately for the reinstatement (left) and the control group (right). Error bars represent standard error of the mean (SEM).

*4.4.3.6. US-expectancy rating*

Results are displayed in Figure 30. US-expectancy ratings were significantly higher for CXT+ ($M$ = 90.12, $SD$ = 12.90) compared to CXT- ($M$ = 33.81, $SD$ = 29.83), main effect of context, $F(1, 40) = 116.55$, $p < .001$, $\eta_p^2 = .74$, and this difference increased from Acquisition 1 to Acquisition 2, significant interaction Phase × Context, $F(1, 40) = 15.99$, $p < .001$, $\eta_p^2 = .29$. The main effect of group just failed to reach significance, $F(1, 40) = 3.59$, $p = .065$, $\eta_p^2 = .08$.

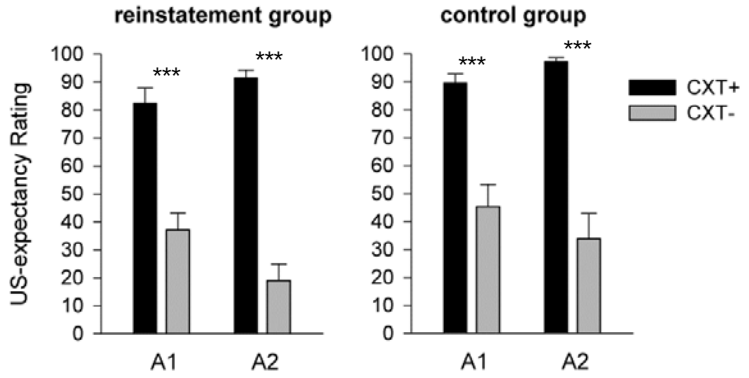


*Figure 30. Study 2: US-expectancy ratings after acquisition phases.*
US-expectancy ratings ranged from 0 (*no expectancy at all*) to 100 (*definitely expected*) and were collected after Acquisition 1 (A1) and Acquisition 2 (A2). Results are shown separately for the reinstatement (left) and the control group (right). Error bars represent standard error of the mean (SEM). *** $p < .001$.

### 4.4.4. Extinction (Day 2)

*4.4.4.1. Anxiety-potentiated startle*

The ANOVA revealed a significant main effect of context, $F(1, 40) = 4.52$, $p = .040$, $\eta_p^2 = .10$. Startle magnitudes were potentiated in CXT+ compared to CXT-. There was also a marginal significant interaction Phase × Context, $F(1, 40) = 3.72$, $p = .061$, $\eta_p^2 = .09$, indicating higher startle responses in CXT+ compared to CXT- during the first extinction phase, $F(1, 40) = 7.85$, $p = .008$, $\eta_p^2 = .16$, while this difference disappeared during the second extinction phase, $F(1, 40) < 1$, suggesting successful extinction in all participants, see Figure 31. Again, there was neither a main effect nor any interaction involving the factor group (all *p*s > .40).

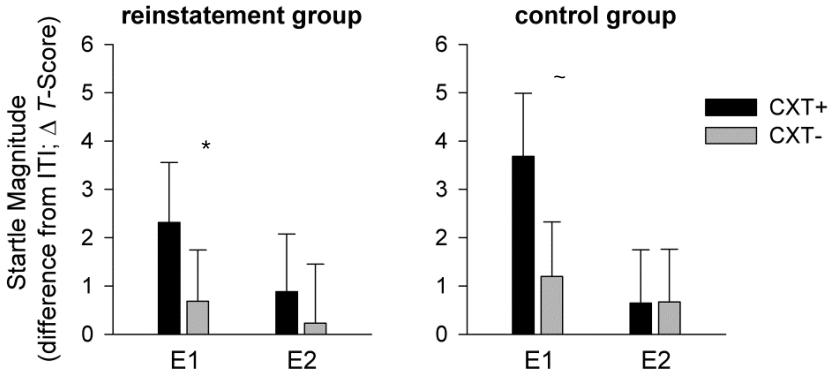


*Figure 31. Study 2: Anxiety-potentiated startle during extinction phases.*
Results are shown separately for Extinction 1 (E1) and Extinction 2 (E2) and for each group: reinstatement (left) vs. control group (right). Error bars represent standard error of the mean (SEM). ~ $p < .07$, * $p < .05$.

*4.4.4.2. Skin conductance*

There were neither significant main nor interaction effects involving the factor context, indicating successful extinction (all *p*s > .27). But, as in the pre-acquisition and in the acquisition phase the significant main effect of group, $F(1, 38) = 4.27$, $p = .046$, $\eta_p^2 = .10$, indicated that the control group exhibited higher overall SCL than the reinstatement group, see Figure 32.

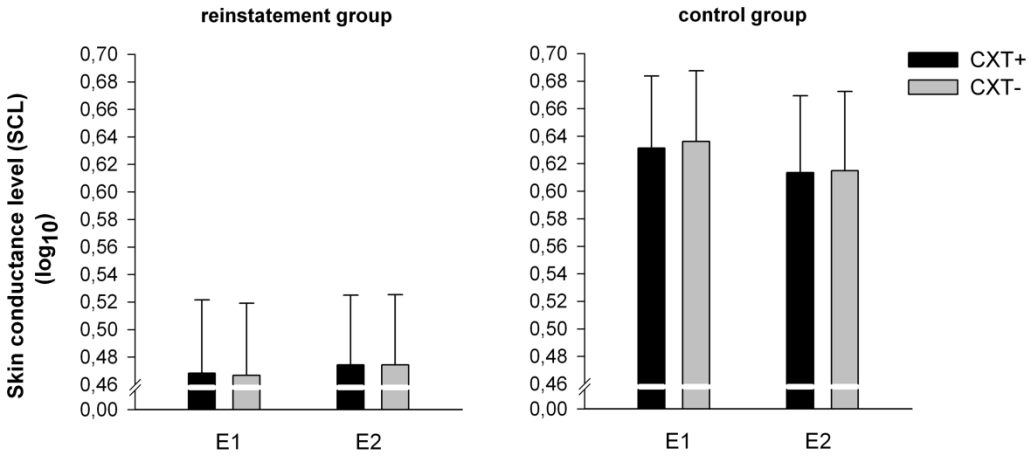


*Figure 32. Study2: SCL during extinction phases on Day2.*
Results are shown separately for Extinction 1 (E1) and Extinction 2 (E2) and for each group: reinstatement (left) vs. control group (right). Error bars represent standard error of the mean (SEM).

*4.4.4.3. Valence rating*

A main effect of context, $F(1, 40) = 5.81$, $p = .021$, $\eta_p^2 = .13$, and an interaction Context × Group, $F(1, 40) = 4.94$, $p = .032$, $\eta_p^2 = .11$, turned out significant. The three-way interaction Phase × Context × Group just failed to reach significance, $F(1, 20) = 3.73$, $p = .060$, $\eta_p^2 = .09$. Post hoc tests regarding the Context × Group interaction indicated that the control group still rated CXT+ as more negative as CXT-, $F(1, 20) = 7.62$, $p = .012$, $\eta_p^2 = .28$, across both extinction phases. However, as shown in Figure 33, this effect was only obvious in the first extinction phase, $F(1, 20) = 11.97$, $p = .002$, $\eta_p^2 = .37$, whereas in the second extinction phase differential valence ratings were extinguished, $F(1, 20) < 1$. The reinstatement group showed extinction of valence ratings across both extinction phases, both $F(1, 20) < 1$.

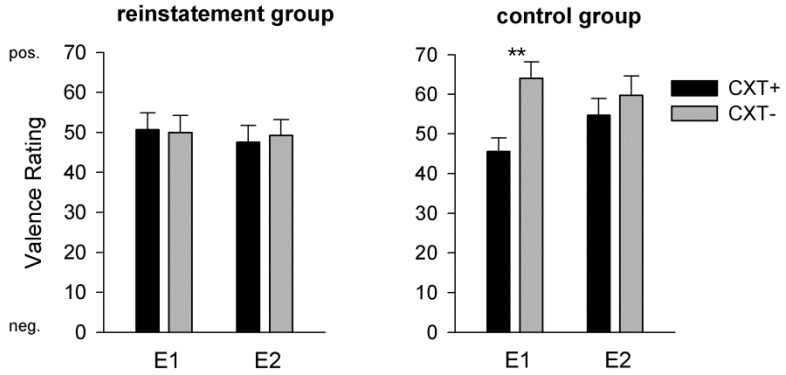


*Figure 33. Study 2: Valence ratings after extinction phases.*
Valence ratings ranged from 0 (*very negative*) to 100 (*very positive*) and were collected after Extinction 1 (E1) and Extinction 2 (E2). Results are shown separately for the reinstatement (left) and the control group (right). Error bars represent standard error of the mean (SEM).

*4.4.4.4. Arousal rating*

Even after both extinction blocks, CXT+ ($M$ = 29.76, $SD$ = 21.09) was rated as more arousing than CXT- ($M$ = 24.46, $SD$ = 19.29), as indicated by the significant main effect of context, $F(1, 40) = 6.96$, $p = .012$, $\eta_p^2 = .15$. Additionally the main effect of phase, $F(1, 40) = 10.68$, $p = .002$, $\eta_p^2 = .21$, and the interaction Phase × Group, $F(1, 40) = 5.18$, $p = .028$, $\eta_p^2 = .12$, turned significant. Arousal ratings for both CXT+ and CXT- in the control group habituated from Extinction 1 ($M$ = 36.43, $SD$ = 23.74) to Extinction 2 ($M$ = 21.79, $SD$ = 21.41), $F(1, 20) = 13.56$, $p = .001$, $\eta_p^2 = .40$, and reached the same arousal level as in the reinstatement group after Extinction 2 ($M$ = 23.81, $SD$ = 19.13), $F(1, 40) < 1$, see Figure 34.

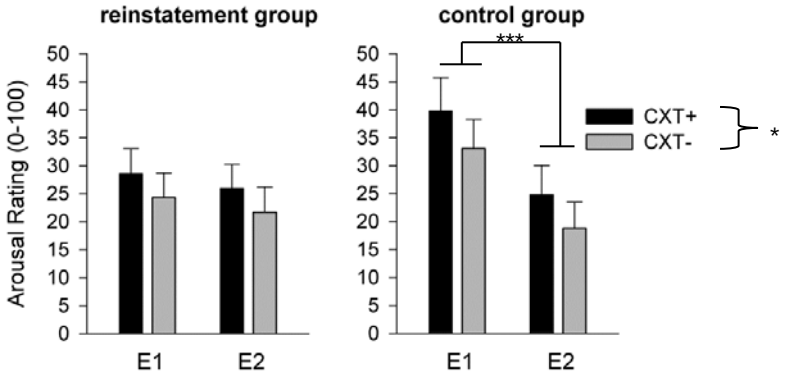


*Figure 34. Study 2: Arousal ratings after extinction phases.*
The main effect of context reached significance (* $p < .05$), meaning that across both groups and both phases arousal ratings were higher for CXT+ compared to CXT-. Arousal ratings ranged from 0 (*very calm*) to 100 (*very excited*) and were collected after Extinction 1 (E1) and Extinction 2 (E2). Results are shown separately for the reinstatement (left) and the control group (right). Error bars represent standard error of the mean (SEM).

*4.4.4.5. Anxiety rating*

There was only a marginal main effect of phase, $F(1, 40) = 3.78$, $p = .059$, $\eta_p^2 = .09$, indicating that anxiety ratings declined from Extinction 1 ($M$ = 17.80, $SD$ = 19.03) to Extinction 2 ($M$ = 13.87, $SD$ = 18.55), suggesting extinction, see Figure 35.

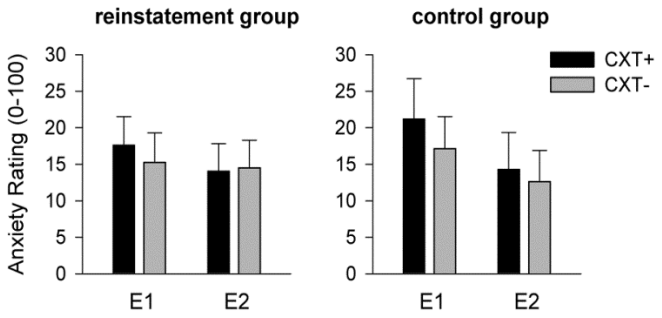


*Figure 35. Study 2: Anxiety ratings after extinction phases.*
Anxiety ratings ranged from 0 (*no anxiety at all*) to 100 (*very high anxiety*) and were collected after Extinction 1 (E1) and Extinction 2 (E2). Results are shown separately for the reinstatement (left) and the control group (right). Error bars represent standard error of the mean (SEM).

*4.4.4.6. US-expectancy rating*

The ANOVA revealed significant main effects of context, $F(1, 40) = 37.29$, $p < .001$, $\eta_p^2 = .48$, and phase, $F(1, 40) = 19.60$, $p < .001$, $\eta_p^2 = .33$, as well as significant interactions Phase × Context, $F(1, 40) = 6.55$, $p = .014$, $\eta_p^2 = .14$, and Phase × Group, $F(1, 40) = 7.03$, $p = .011$, $\eta_p^2 = .15$. In both groups the US-expectancy ratings were higher for CXT+ compared to CXT- after both extinction phases, Extinction 1: $F(1, 40) = 39.18$, $p < .001$, $\eta_p^2 = .50$, Extinction 2: $F(1, 40) = 11.90$, $p = .001$, $\eta_p^2 = .23$, but the interaction also indicated that the difference between CXT+ and CXT- ratings decreased from Extinction 1 to Extinction 2. Post-hoc tests regarding the Phase × Group interaction revealed that in the control group US-expectancy ratings for both CXT+ and CXT- decreased from Extinction 1 to Extinction 2 , $F(1, 20) = 18.41$, $p < .001$, $\eta_p^2 = .48$, and reached the same level as in the reinstatement group after Extinction 2, $F(1, 40) = 2.09$, $p = .156$, $\eta_p^2 = .05$, see Figure 36.

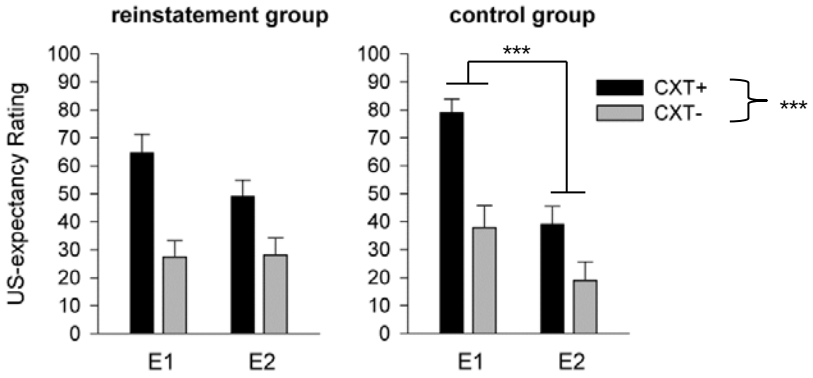


*Figure 36. Study 2: US-expectancy ratings after extinction phases.*
The main effect of context reached significance (*** $p < .001$), meaning that across both groups and both phases US-expectancy ratings were higher for CXT+ compared to CXT-. US-expectancy ratings ranged from 0 (*no expectancy at all*) to 100 (*definitely expected*) and were collected after Extinction 1 (E1) and Extinction 2 (E2). Results are shown separately for the reinstatement (left) and the control group (right). Error bars represent standard error of the mean (SEM).

### 4.4.5. Test for reinstatement of anxiety

*4.4.5.1. Anxiety-potentiated startle*[11]

To test a reinstatement of contextual anxiety after the unsignaled US at the beginning of Day 3, a 2 (Context: CXT+, CXT-) × 2 (Time: last trial of extinction, first trial of re-extinction) × 2 (Group: reinstatement, control) ANOVA was conducted. The main effect of time reached significance, $F(1, 35) = 5.66$, $p = .023$, $\eta_p^2 = .14$, reflecting a general increase of startle magnitudes during the first trial of re-extinction on Day 3 compared to the last trial of the extinction phase on Day 2. There was also a significant interaction Time × Context, $F(1, 35) = 9.99$, $p = .003$, $\eta_p^2 = .22$, suggesting higher startle magnitudes in CXT+ compared to CXT- during the first re-extinction trial, $F(1, 35) = 7.98$, $p = .008$, $\eta_p^2 = .19$, while there was no difference during the last extinction trial, $F(1, 35) < 1$. The three-way interaction Time × Context × Group was not significant, $F(1, 35) < 1$, which would have been the crucial interaction to further test for a context-specific return of anxiety within the reinstatement group on Day 3. However, based on the directed hypothesis that only for the reinstatement group the difference between CXT+ and CXT- should be significant during the first re-extinction trial, but not for the control group, 2 × 2 (Time × Context) ANOVAs were conducted separately for each group. A Time × Context interaction was found in the reinstatement group, $F(1, 18) = 4.86$, $p = .041$, $\eta_p^2 = .21$, as well as in the control group, $F(1, 17) = 5.10$, $p = .037$, $\eta_p^2 = .23$. Next, *F* contrasts conducted separately for both groups revealed that during the last extinction trial differential anxiety-potentiated startle responses were extinguished in both groups (reinstatement group: $F(1, 18) < 1$; control group: $F(1, 17) = 2.19$, $p = .157$, $\eta_p^2 = .11$). Interestingly, during the first trial of the test phase, startle responses were significantly potentiated for CXT+ compared to CXT- in the reinstatement group, $F(1, 18) = 7.33$, $p = .014$, $\eta_p^2 = .29$, but not in the control group, $F(1, 17) = 1.46$, $p = .244$, $\eta_p^2 = .08$, confirming the first hypothesis, see Figure 37. [12]

[11] Note, that for the reinstatement test, only the last trial of extinction and the first trial of re-extinction were considered (per trial: 2-3 startle probes per CXT, 1-2 startle probes in the ITI). Therefore, five participants had to be excluded because they did not have enough valid responses for ITI (at least one response), CXT+ (at least two responses) or CXT- (at least two responses). A difference score (CXT – ITI) was calculated to account for baseline differences and to keep constant with the presentation of startle data during the other experimental phases. Finally, the analysis of startle data was conducted in 37 participants: 19 were in the reinstatement group and 18 in the control group.

[12] Explorative analyses were carried out which considered group differences between the return of differential anxiety on Day 3. It was defined as the difference between CXT+ and CXT- (CXT+ - CXT-)

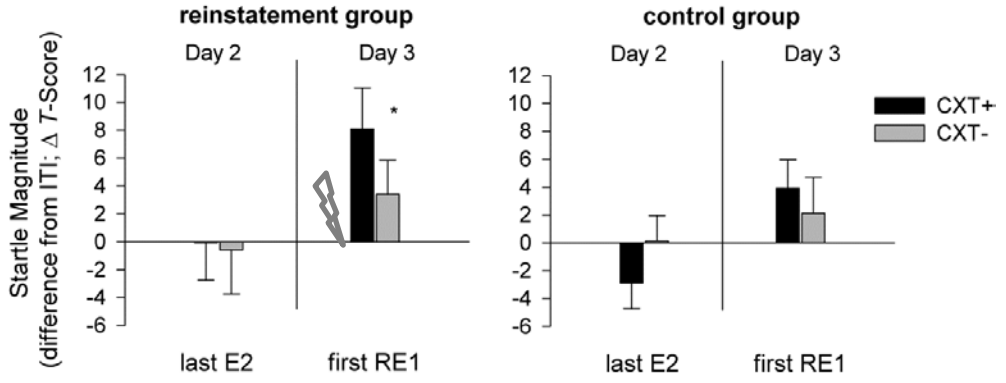


*Figure 37. Study 2: Anxiety-potentiated startle during the reinstatement test.*
Results are shown separately for the last extinction trial (last E2) on Day 2 and the first re-extinction trial (first RE1) on Day 3. The reinstatement group ($n$ = 19; left) received one unsignaled US before the first re-extinction trial on Day 3, whereas the control group ($n$ = 18; right) did not. Error bars represent standard error of the mean (SEM). * $p$ < .05.

### *4.4.5.2. Skin conductance*

There were no significant effects (all $p$s > .09), suggesting prolonged extinction effects and no return of contextual anxiety, see Figure 38.

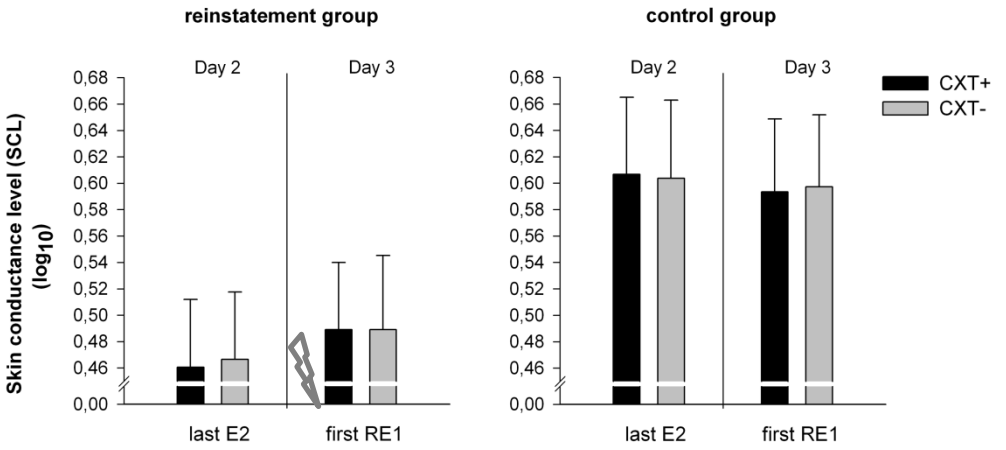


*Figure 38. Study2: SCL during extinction the reinstatement test.*
Results are shown separately for the last extinction trial (last E2) on Day 2 and the first re-extinction trial (first RE1) on Day 3. The reinstatement group (left) received one unsignaled US before the first re-extinction trial on Day 3, whereas the control group (right) did not. Error bars represent standard error of the mean (SEM).

### *4.4.5.3. Valence rating*

In analogy to startle data, the interaction Time × Context, $F(1, 40) = 8.11$, $p = .007$, $\eta_p^2 = .17$, turned significant, but no interactions involving the factor group (all $p$s > .61). Again, separate 2 × 2 (Time × Context) ANOVAs were carried out separately for both

in the first trial of re-extinction on Day 3. $F$ contrast revealed no significant difference between the reinstatement and the control group, $F(1, 35) = 1.58$, $p = .217$, $\eta_p^2 = .04$.

groups, with no significant effects in the control group (all *p*s > .16), but a significant interaction Time × Context in the reinstatement group, $F(1, 20) = 9.42$, $p = .006$, $\eta_p^2 = .32$. The absence of any effect in the control group indicated prolonged extinction effects, whereas the post-hoc analysis of the significant interaction in the reinstatement group showed no difference in valence ratings between CXT+ and CXT- for the last trial of extinction, $F(1, 20) < 1$, but CXT+ was rated more negative than CXT- concerning the first trial after the reinstatement procedure, $F(1, 20) = 5.53$, $p = .029$, $\eta_p^2 = .22$, see Figure 39.

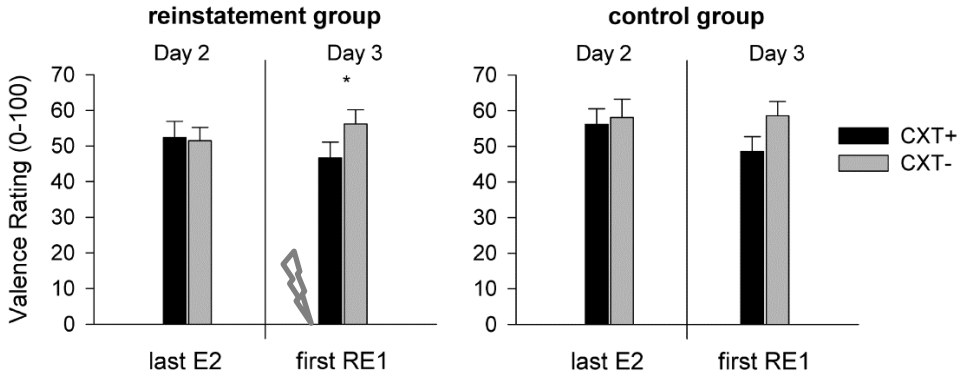


*Figure 39. Study 2: Valence ratings during the reinstatement test.*
Valence ratings ranged from 0 (*very negative*) to 100 (*very positive*) and were collected concerning the last extinction trial (last E2) on Day 2 and the first re-extinction trial (first RE1) on Day 3. Results are shown separately for the reinstatement (left) and the control group (right). Error bars represent standard error of the mean (SEM). * $p < .05$.

*4.4.5.4. Arousal rating*

The main effects of time, $F(1, 40) = 7.86$, $p = .008$, $\eta_p^2 = .16$, and context, $F(1, 40) = 10.45$, $p = .002$, $\eta_p^2 = .21$, reached significance as well as the interaction Time × Context, $F(1, 40) = 5.00$, $p = .031$, $\eta_p^2 = .11$, but no interactions involving the factor group (all *p*s > .24). Nevertheless, 2 × 2 (Time × Context) ANOVAs separately for both groups yielded no significant effects for the control group (all *p*s > .10), but significant main effects of time, $F(1, 20) = 14.64$, $p = .001$, $\eta_p^2 = .42$, context, $F(1, 20) = 7.55$, $p = .012$, $\eta_p^2 = .27$, and a significant interaction Time × Context in the reinstatement group, $F(1, 20) = 7.04$, $p = .015$, $\eta_p^2 = .26$. Again, the absence of any significant effect in the control group indicated prolonged extinction effects, whereas the post-hoc analysis of the significant interaction in the reinstatement group showed similar effects as for the valence ratings. Thus, there was no difference in arousal ratings between CXT+ and CXT- for the last trial of extinction, $F(1, 20) = 1.84$, $p = .190$, $\eta_p^2 = .08$, but a clear reinstatement effect. CXT+ was rated as more arousing as CXT- concerning the first trial after the reinstatement procedure, $F(1, 20) = 10.57$, $p = .004$, $\eta_p^2 = .35$, see Figure 40.

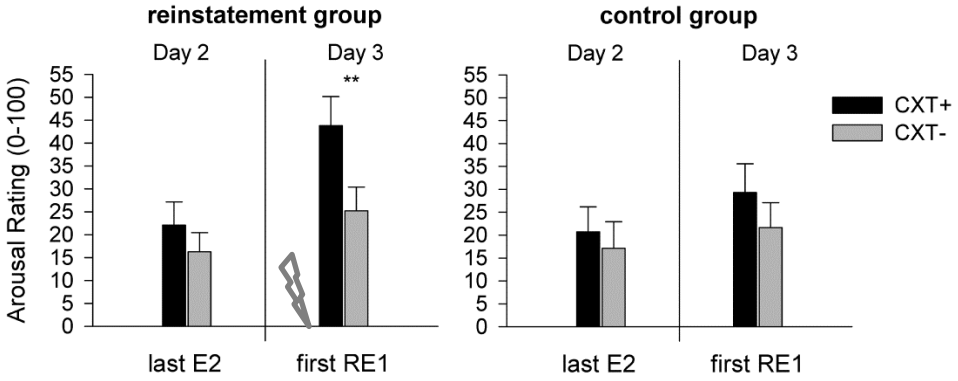


*Figure 40. Study 2: Arousal ratings during the reinstatement test.*
Arousal ratings ranged from 0 (*very calm*) to 100 (*very excited*) and were collected concerning the last extinction trial (last E2) on Day 2 and the first re-extinction trial (first RE1) on Day 3. Results are shown separately for the reinstatement (left) and the control group (right). Error bars represent standard error of the mean (SEM). ** $p < .01$.

*4.4.5.5. Anxiety rating*

Again, the main effects of time, $F(1, 40) = 4.56$, $p = .039$, $\eta_p^2 = .10$, and context, $F(1, 40) = 6.05$, $p = .018$, $\eta_p^2 = .13$, and the interaction Time × Context, $F(1, 40) = 12.49$, $p = .001$, $\eta_p^2 = .24$, were significant, but no interactions involving the factor group (all $p$s > .13). However, 2 × 2 (Time × Context) ANOVAs separately for both groups showed no significant effects for the control group (all $p$s > .15), indicating prolonged extinction effects and no return of anxiety. On the contrary, in the reinstatement group there were significant effects of time, $F(1, 20) = 11.51$, $p = .003$, $\eta_p^2 = .29$, context, $F(1, 20) = 4.21$, $p = .054$, $\eta_p^2 = .17$, and Time × Context, $F(1, 20) = 7.04$, $p = .015$, $\eta_p^2 = .37$. Similar to the results of valence and arousal ratings, the reinstatement group showed clear extinction effects at the end of Day 2, $F(1, 20) < 1$, but increased anxiety for CXT+ compared to CXT- after the reinstatement procedure, $F(1, 20) = 9.12$, $p = .007$, $\eta_p^2 = .31$, see Figure 41.

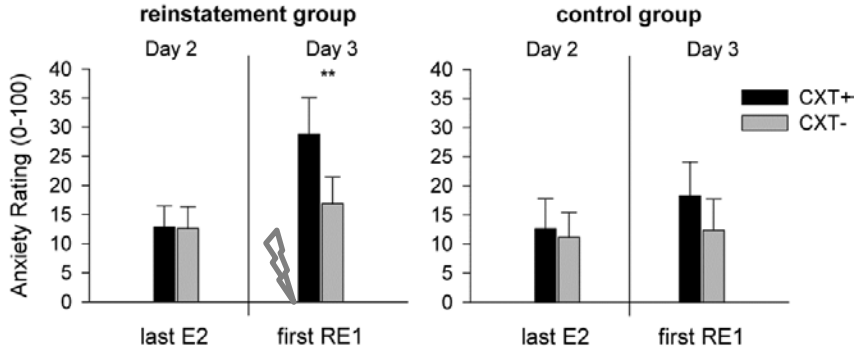


*Figure 41. Study 2: Anxiety ratings during the reinstatement test.*
Anxiety ratings ranged from 0 (*no anxiety at all*) to 100 (*very high anxiety*) and were collected concerning the last extinction trial (last E2) on Day 2 and the first re-extinction trial (first RE1) on Day 3. Results are shown separately for the reinstatement (left) and the control group (right). Error bars represent standard error of the mean (SEM). ** $p < .01$.

*4.4.5.6. US-expectancy rating*

The ANOVA revealed significant effects of time, $F(1, 40) = 8.30$, $p = .006$, $\eta_p^2 = .17$, context, $F(1, 40) = 18.70$, $p < .001$, $\eta_p^2 = .32$, and Time × Context, $F(1, 40) = 5.94$, $p = .019$, $\eta_p^2 = .13$, and only a marginal significant main effect of group, $F(1, 40) = 3.68$, $p = .062$, $\eta_p^2 = .08$, but no interactions involving the factor group (all *p*s > .21). However, 2 × 2 (Time × Context) ANOVAs separately for both groups showed a significant main effect of context for the control group, $F(1, 40) = 6.14$, $p = .022$, $\eta_p^2 = .24$, indicating higher US-expectancy ratings for CXT+ compared to CXT- after the last trial of extinction as well as the for the first trial of re-extinction on Day 3. In the reinstatement group there were significant effects of time, $F(1, 20) = 12.87$, $p = .002$, $\eta_p^2 = .39$, context, $F(1, 20) = 12.62$, $p = .002$, $\eta_p^2 = .39$, and Time × Context, $F(1, 20) = 6.18$, $p = .022$, $\eta_p^2 = .24$. In the reinstatement group, the US-expectancy ratings for CXT+ and CXT- did not differ significantly at the end of extinction on Day 2, $F(1, 20) = 3.200$, $p = .089$, $\eta_p^2 = .14$, but US-expectancy ratings were higher for CXT+ compared to CXT- after the reinstatement procedure, $F(1, 20) = 19.87$, $p < .001$, $\eta_p^2 = .50$, see Figure 42.

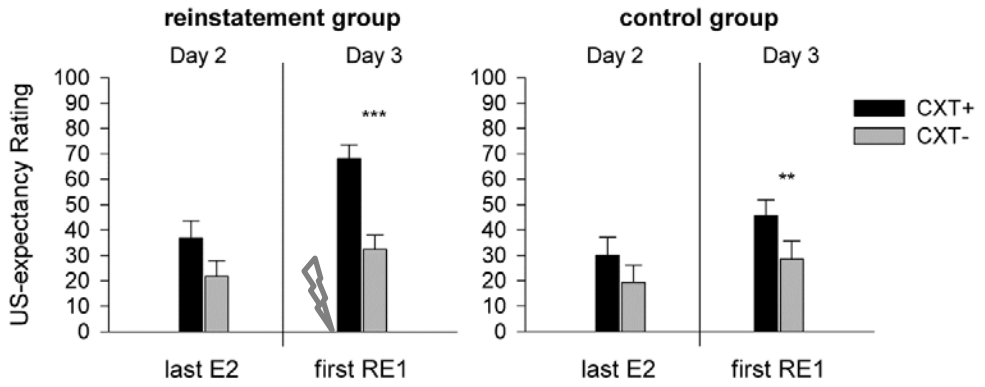


*Figure 42. Study 2: US-expectancy ratings during the reinstatement test.*
US-expectancy ratings ranged from 0 (*no expectancy at all*) to 100 (*definitely expected*) and were collected concerning the last extinction trial (last E2) on Day 2 and the first re-extinction trial (first RE1) on Day 3. Results are shown separately for the reinstatement (left) and the control group (right). Error bars represent standard error of the mean (SEM). ** $p < .01$, *** $p < .001$.

### 4.4.6. Re-Extinction (Day 3)

*4.4.6.1. Physiological data*

Physiological data were averaged across three runs (one phase), but the ANOVA revealed no significant effects (SCL: all *p*s > .18; Startle: all *p*s > .20). Startle data are shown in Figure 43 below.

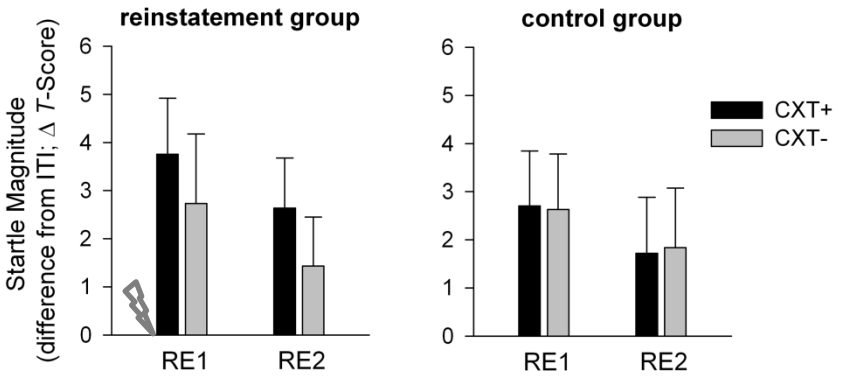


*Figure 43. Study 2: Anxiety-potentiated startle during re-extinction phases.*
Results are shown separately for Re-Extinction 1 (RE1) and Re-Extinction 2 (RE2) and for each group: reinstatement (left) vs. control group (right). Error bars represent standard error of the mean (SEM).

### *4.4.6.2. Valence rating*

The main effect of context was significant, $F(1, 40) = 8.54$, $p = .006$, $\eta_p^2 = .18$, meaning that both groups reported more negative valence for CXT+ ($M = 53.87$, $SD = 17.15$) compared to CXT- ($M = 60.12$, $SD = 18.17$), see Figure 44. There were no significant effects involving the factor group (all *p*s > .28).

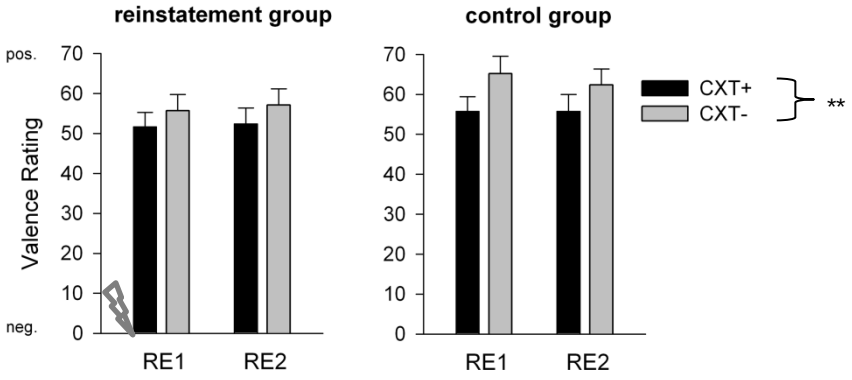


*Figure 44. Study 2: Valence ratings after re-extinction phases.*
Only the main effect of context reached significance (** $p < .01$), meaning that across both groups and both phases valence ratings were more negative for CXT+ compared to CXT-. Valence ratings ranged from 0 (*very negative*) to 100 (*very positive*) and were collected after Re-Extinction 1 (RE1) and Re-Extinction 2 (RE2). Results are shown separately for the reinstatement (left) and the control group (right). Error bars represent standard error of the mean (SEM).

### *4.4.6.3. Arousal rating*

The main effects of context, $F(1, 40) = 22.86$, $p < .001$, $\eta_p^2 = .36$, and phase, $F(1, 40) = 5.54$, $p = .024$, $\eta_p^2 = .12$, were significant, meaning that both groups reported higher arousal for CXT+ ($M = 26.85$, $SD = 21.51$) compared to CXT- ($M = 16.90$, $SD = 17.14$), but arousal declined from Re-Extinction 1 ($M = 24.76$, $SD = 21.60$) to Re-Extinction 2 ($M =$

18.99, *SD* = 17.91), see Figure 45. There were no significant effects involving the factor group (all *p*s > .10).

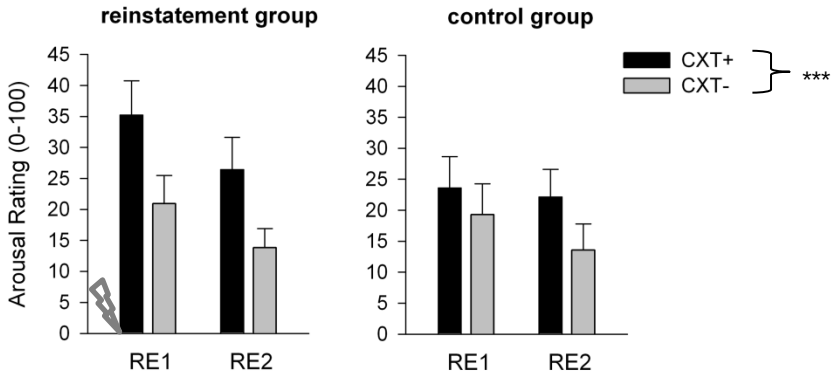


*Figure 45. Study 2: Arousal ratings after re-extinction phases.*
The main effect of context reached significance (*** $p < .001$), meaning that across both groups and both phases valence ratings were more negative for CXT+ compared to CXT-. Arousal ratings ranged from 0 (*very calm*) to 100 (*very excited*) and were collected after Re-Extinction 1 (RE1) and Re-Extinction 2 (RE2). Results are shown separately for the reinstatement (left) and the control group (right). Error bars represent standard error of the mean (SEM).

*4.4.6.4. Anxiety rating*

The analysis revealed a significant main effect of phase, $F(1, 40) = 4.97$, $p = .032$, $\eta_p^2 = .11$; anxiety ratings declined from Re-Extinction 1 ($M = 12.68$, $SD = 17.60$) to Re-Extinction 2 ($M = 9.64$, $SD = 14.82$). Additionally, the main effect of context, $F(1, 40) = 6.67$, $p = .014$, $\eta_p^2 = .14$, as well as a significant interaction Context × Group, $F(1, 40) = 5.20$, $p = .028$, $\eta_p^2 = .12$, turned out significant. The reinstatement group reported higher anxiety regarding CXT+ compared to CXT- across both phases, $F(1, 20) = 7.48$, $p = .013$, $\eta_p^2 = .27$, but not the control group, $F(1, 20) < 1$, see Figure 46.

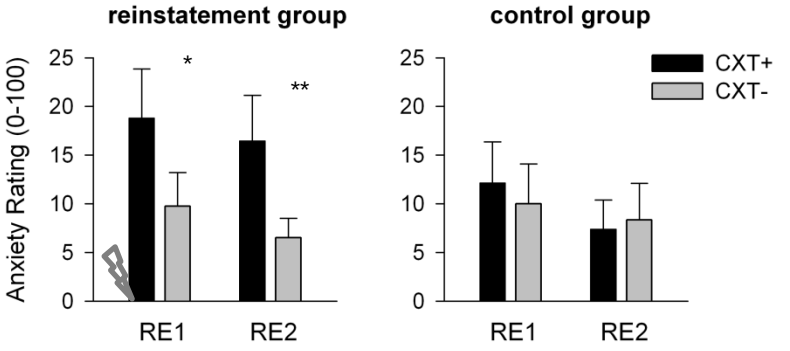


*Figure 46. Study 2: Anxiety ratings after re-extinction phases.*
Anxiety ratings ranged from 0 (*no anxiety at all*) to 100 (*very high anxiety*) and were collected after Re-Extinction 1 (RE1) and Re-Extinction 2 (RE2). Results are shown separately for the reinstatement (left) and the control group (right). Error bars represent standard error of the mean (SEM). * $p < .05$, ** $p \leq .01$.

*4.4.6.5. US-expectancy rating*

Again, there were significant main effects of context, $F(1, 40) = 36.73$, $p < .001$, $\eta_p^2 = .48$, and phase, $F(1, 40) = 9.98$, $p = .003$, $\eta_p^2 = .20$, and an additional main effect of group, $F(1, 40) = 10.69$, $p = .002$, $\eta_p^2 = .21$. US-expectancy ratings were higher for CXT+ ($M = 36.61$, $SD = 23.31$) than for CXT- ($M = 16.43$, $SD = 17.57$), but ratings for CXT+ and CXT- declined from Re-Extinction 1 ($M = 33.10$, $SD = 24.96$) to Re-Extinction 2 ($M = 19.94$, $SD = 18.75$). Interestingly, the reinstatement group reported higher US-expectancy for both contexts ($M = 34.46$, $SD = 15.94$) compared to the control group ($M = 18.57$, $SD = 15.56$), see Figure 47.

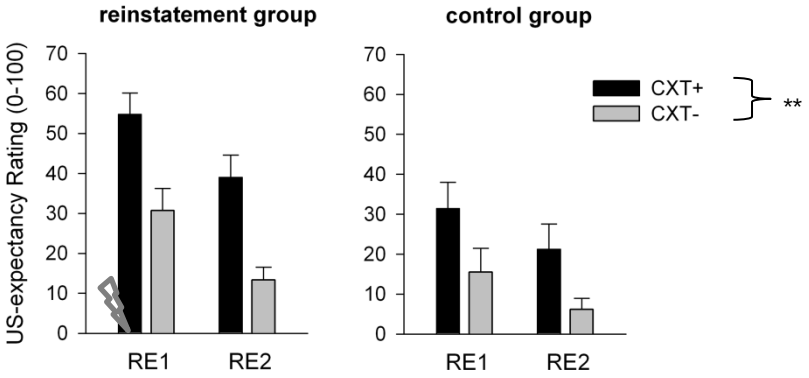


*Figure 47. Study 2: US-expectancy ratings after re-extinction phases.*
The main effect of context reached significance (** $p < .01$), meaning that across both groups and both phases US-expectancy ratings were higher for CXT+ compared to CXT-. US-expectancy ratings ranged from 0 (*no expectancy at all*) to 100 (*definitely expected*) and were collected after Re-Extinction 1 (RE1) and Re-Extinction 2 (RE2). Results are shown separately for the reinstatement (left) and the control group (right). Error bars represent standard error of the mean (SEM).

### 4.4.7. Correlation analyses with the return of contextual anxiety

According to Huff et al. (2009), a change of the internal context i.e., mood and state anxiety, from extinction to extinction recall should favor a return of anxiety. Additionally, Dirikx et al. (2004, 2007) showed that the more negative the stimulus valence was after extinction, the higher the return of fear was. To prove these suggestions, correlation analyses were carried out between the return of contextual anxiety in startle response on Day 3 (difference score between startle responses during the first trial of re-extinction; CXT+ - CXT-) and (1) the change of mood (positive affect, negative affect, state anxiety) from extinction to re-extinction (difference scores between Day 3 and Day 2: Day 3 – Day2), and (2) the difference in valence ratings (CXT+ - CXT-) after the last extinction trial. The reinstatement effect in differential anxiety ratings (difference score between anxiety ratings concerning the first trial of re-extinction; CXT+ - CXT-) was also correlated with the change of mood as described above.

*4.4.7.1. Change of mood from extinction to re-extinction*

There were no significant correlations for the control group, but for the reinstatement group (Table 8). Importantly, the change of mood from extinction to re-extinction was equal for both groups, because *F* contrasts comparing both groups were not significant (for state anxiety, negative affect, and positive affect all *F*s < 1).

*Table 8. Study 2: Correlations between reinstatement effects and change of mood.*

| | **Reinstatement of anxiety (first trial of re-extinction; CXT+ - CXT-)** | | | |
|---|---|---|---|---|
| | **Startle response** | | **Anxiety rating** | |
| **Change of mood (Day 3 – Day2)** | Reinstatement group (*n* = 19) | Control group (*n* = 18) | Reinstatement group (*n* = 21) | Control group (*n* = 21) |
| State anxiety | ***r* = .503, *p* = .028*** | *r* = .025, *p* = .921 | ***r* = .594, *p* = .004**** | *r* = -.053, *p* = .819 |
| Negative affect | *r = .443, p = .057* | *r* = -.105, *p* = .679 | ***r* = .621, *p* = .003**** | *r* = .097, *p* = .677 |
| Positive affect | ***r* = -.591, *p* = .008**** | *r* = .335, *p* = .174 | *r* = -.136, *p* = .556 | *r* = .064, *p* = .782 |

Note: Significant correlations are displayed in bold. * $p < .05$, ** $p < .01$.

The positive correlations with state anxiety indicated that the greater the state anxiety on Day 3 compared to Day 2 (indicated by positive difference scores) was, the greater the return of contextual anxiety on Day 3 in both implicit (startle response) and explicit (rating) measures turned out. Figure 48 depicts the scatterplot of the correlation between the change of state anxiety and the return of anxiety in startle responses (left) and anxiety ratings (right), respectively.

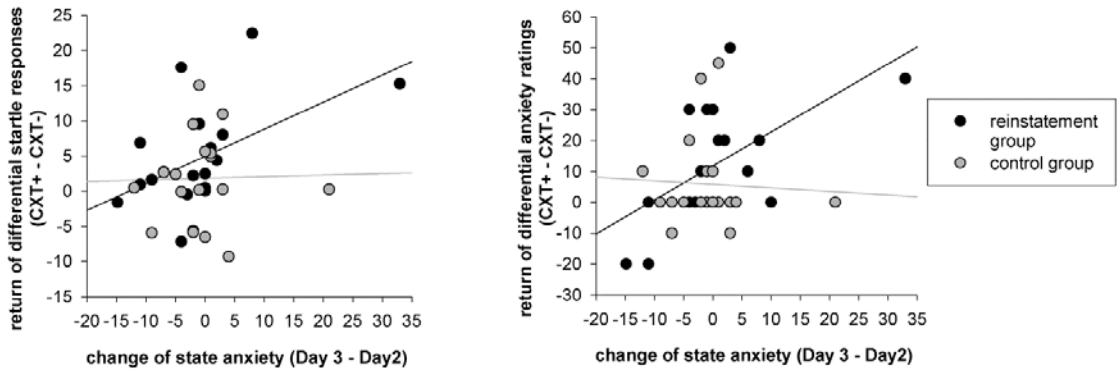


*Figure 48. Study 2: Scatterplots depicting the correlation between the reinstatement effects and the change of state anxiety.*
The correlations between the change of state anxiety (Day 3 – Day 2) and the reinstatement effect in anxiety-potentiated startle (CXT+ - CXT- during the first trial of re-extinction) is shown in the left graph, whereas the correlation with the reinstatement effect in anxiety ratings (CXT+ - CXT- concerning the first trial of re-extinction) is shown in the right graph. Black circles represent the reinstatement group, gray circles the control group.

Similarly, the less the positive affect on Day 3 compared to Day2 (indicated by negative difference scores) was, the greater the return of anxiety-potentiated startle on Day 3 was, which was depicted in a negative correlation, see Figure 49 (left). There were no significant correlations with anxiety ratings (see Figure 49, right).

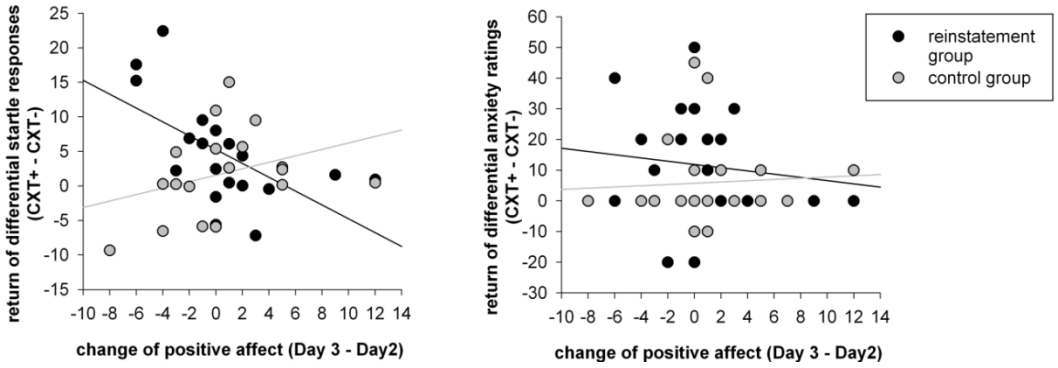


*Figure 49. Study 2: Scatterplots depicting the correlation between the reinstatement effects and the change of positive affect.*
The correlations between the change of positive affect (Day 3 – Day 2) and the reinstatement effect in anxiety-potentiated startle (CXT+ - CXT- during the first trial of re-extinction) is shown in the left graph, whereas the correlation with the reinstatement effect in anxiety ratings (CXT+ - CXT- concerning the first trial of re-extinction) is shown in the right graph. Black circles represent the reinstatement group, gray circles the control group.

The positive correlation between the return of anxiety-potentiated startle and the change of negative affect in the reinstatement group was only marginally significant, but points into the same direction, see Figure 50 (left), but the positive correlation with the return of differential anxiety ratings turned significant (Figure 50, right).

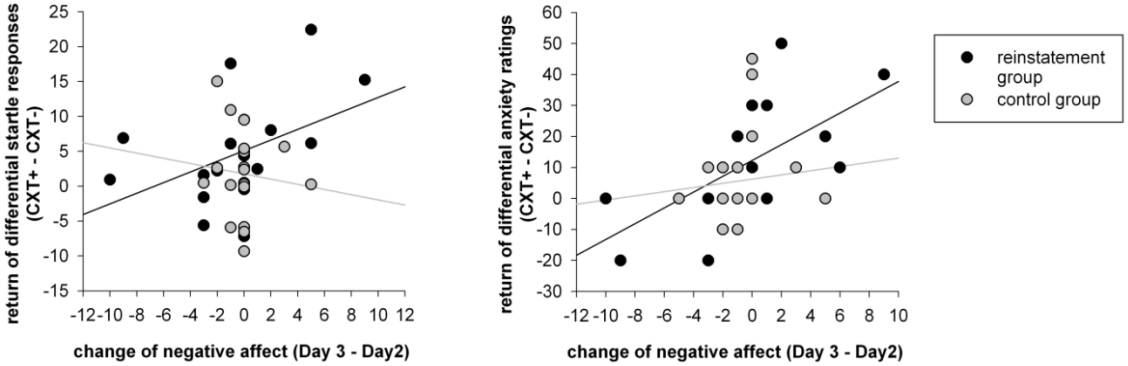


*Figure 50. Study 2: Scatterplots depicting the correlation between the reinstatement effects and the change of negative affect.*
The correlations between the change of negative affect (Day 3 – Day 2) and the reinstatement effect in anxiety-potentiated startle (CXT+ - CXT- during the first trial of re-extinction) is shown in the left graph, whereas the correlation with the reinstatement effect in anxiety ratings (CXT+ - CXT- concerning the first trial of re-extinction) is shown in the right graph. Black circles represent the reinstatement group, gray circles the control group.

*4.4.7.2. Valence ratings after extinction*

A difference in valence ratings after extinction did neither correlate with the return of contextual anxiety in startle responses in the reinstatement group ($r$ = .061, $p$ = .805) nor in the control group ($r$ = -.047, $p$ = .853).

## 4.5. Discussion

Reinstatement of cued fear has been demonstrated in animal (e.g., Bouton & Bolles, 1979; Laurent & Westbrook, 2010) as well as in human studies (e.g., Dirikx et al., 2004; Norrholm et al., 2006). However, reinstatement of contextual anxiety has only been studied in animals (Stern et al., 2012; Yamada et al., 2009). The present study aimed at investigating a new reinstatement paradigm for contextual anxiety in humans. To this end, two groups underwent contextual fear conditioning in VR on Day 1 and extinction training on Day 2. The reinstatement group received one unsignaled US at the beginning of Day 3 while the HMD was turned black, and afterwards re-experienced the conditioned contexts without any US again. In contrast, the control group received no US, but re-experienced only the conditioned contexts. Thus, the two groups performed the same experimental sessions like in Study 1, except for the unsignaled US in the reinstatement group. I expected that participants in the reinstatement group would show a return of anxiety in the first trial of the re-extinction phase on Day 3 as indicated by higher anxiety responses in CXT+ compared to CXT-. Additionally, I expected no return of contextual anxiety in the control group.

Firstly, results demonstrated successful contextual fear conditioning on Day 1. In detail, all participants showed higher anxiety in CXT+ compared to CXT- in implicit (anxiety-potentiated startle reflex) and explicit anxiety responses (SCL, ratings). Secondly, during the second extinction phase on Day 2, anxiety responses were no longer higher for CXT+ vs. CXT- in anxiety-potentiated startle, SCL, valence and anxiety ratings in all participants. Thirdly, with regard to physiological responses and ratings for the last trial of extinction there were no differences between CXT+ and CXT- in any group and/or in any dependent variable, thus demonstrating successful extinction.

Importantly, at the beginning of Day 3 a return of differential contextual anxiety, i.e. higher anxiety responses in CXT+ compared to CXT-, could only be observed in the reinstatement group in all dependent variables, except for SCL, but not in the control group confirming the first hypothesis. Thus, this newly developed paradigm seems to be suitable to study reinstatement of contextual anxiety in humans. To my knowledge, this is also the first study which proved a reinstatement effect for the implicit behavioral level as

measured with the startle reflex, as well as in a variety of explicit ratings level (valence, arousal, anxiety ratings, US-expectancy) in the same study. Previous cue conditioning studies only reported reinstatement of fear as measured with explicit ratings alone (Dirikx et al., 2004, 2007; Hermans et al., 2005), or with startle and US-expectancy ratings only (Norrholm et al., 2006; Sevenster, Beckers, & Kindt, 2012).

The present study found no reinstatement effect for SCL. Previous studies which investigated reinstatement of cued fear as indexed by SCR provided mixed results. On the one hand, LaBar and Phelps (2005) demonstrated significantly higher SCR to the CS+ compared to the CS- after four unsignaled US presentations. On the other hand, others reported no differential reinstatement effect for SCR after two (Milad, Orr, et al., 2005) or three unsignaled USs (Kull, Müller, Blechert, Wilhelm, & Michael, 2012; Sevenster et al., 2012). These studies reported a generalized reinstatement of conditioned SCR to both CS+ and CS-. Notably, LaBar and Phelps (2005) used a 100 dB white noise as US, whereas in the other studies (Kull et al., 2012; Milad, Orr, et al., 2005; Sevenster et al., 2012) an electric stimulus served as US, like in the present study. These results demonstrate that it is challenging to produce a differential reinstatement effect in SCR or SCL, and possibly, the quality of the US (white noise vs. electric stimulus) as well as the amount of unsignaled US presentations (four vs. three/two/one) might be crucial to induce a differential reinstatement of conditioned SCR/SCL.

It is important to note that the reinstatement effects in anxiety-potentiated startle and anxiety ratings in the reinstatement group were associated with the change in mood from extinction to extinction recall, supporting the second hypothesis. Specifically, a change to more negative mood (as indexed by both, the positive correlation with state anxiety and negative mood, and the negative correlation with positive affect) was associated with a return of anxiety on Day 3 in the reinstatement group, but not in the control group. Therefore, the reinstatement of anxiety seems to be influenced by the negative mood of the participants as well as their current anxiousness. This assumption can be explained by the mood-congruent memory effect. It is defined as “a phenomenon in which emotional material is remembered more reliably in moods that match the emotional content of the memories” (Lewis & Critchley, 2003, p. 431). Accordingly, the results of the present study suggest that on Day 3 the retrieval of the anxiety memory (which was established during conditioning on Day 1) was facilitated because the participant experienced an anxious mood. Thus, emotions or moods may function as important retrieval contexts for fear memories (Bouton & Swartzentruber, 1991). Therefore, the mood-congruent memory effect could be a possible pathway for the return

of anxiety during a reinstatement procedure, on both anxiety levels – the implicit behavioral level (startle response) and the explicit verbal level (anxiety rating).

Nevertheless, why did this mood-congruent memory effect only work in the reinstatement group but not in the control group? One can argue that the change of mood to a more anxious state could have been higher in the reinstatement group than in the control group and therefore the mood-congruent effect would be stronger. However, this was not the case: both groups did not differ in their change of mood in all three measures. Therefore, it seems plausible that the return of anxiety was influenced by the combination of the increased negative and anxious mood *and* the post-extinction shock in the reinstatement group. As proposed by the associative-network theory (Bower, 1981), human memory is organized as an associative network of semantic concepts and schemata represented as nodes that describe events. Similarly, each emotion is represented as a distinct node that is associated with its physiological arousal, behavioral expression, and verbal labels, and of course the emotion is connected to specific events during which the emotion was experienced. Thus, increased state anxiety and could have activated the "anxiety node" which in turn could have activated the learned connection between the anxiety context and the US established during contextual fear conditioning on Day 1 and thus, leading to the retrieval of the anxiety memory. However, it is also assumed that the activation of the semantic network must reach a suprathreshold level to achieve the memory retrieval (Bower, 1981). The activation elicited by the congruent mood alone could have been too weak to retrieve the anxiety memory and anxiety responses in both groups. Possibly, the US given during the reinstatement procedure could have activated an additional node for the US and together with the state anxiety node the activation, which was spread out through the associative network, was strong enough to retrieve the anxiety memory in the reinstatement group only.

However, these interpretations are speculative as they are based on correlational analyses which cannot be interpreted causally. To prove a causal relationship between mood and reinstatement of conditioned anxiety, mood has to be manipulated experimentally before the reinstatement procedure on Day 3. A positive or negative mood can be induced by emotional film scenes or imagination of self-experienced positive or negative events. Negative mood before the reinstatement procedure should result in a higher reinstatement of conditioned fear or conditioned anxiety, whereas a positive mood before the reinstatement procedure should lead to a reduced reinstatement effect. Especially, if the latter assumption would be confirmed, this could have relevant clinical implications in a way that enhanced positive mood after exposure therapy might help to reduce a return of fear and anxiety.

Another limitation of the present study resembles the non-significant three-way interaction Time × Context × Group, which would have been the crucial test for the differential return of anxiety on Day 3 in the reinstatement group, suggesting that the reinstatement effect is not really strong. Moreover, some other studies which investigated the reinstatement of cued fear also reported a non-significant interaction Time × Context × Group for the reinstatement test (Dirikx, Vansteenwegen, Eelen, & Hermans, 2009; Kull, Müller, Blechert, Wilhelm, & Michael, 2012), but post-hoc tests revealed a non-differential return of fear (increased fear responses to CS+ and CS-), indicating that a differential return of fear after might not be easy to establish. The absent three-way interaction in the present study might be due to a lack of power because of the small sample size ($n$ = 21 per group). Additionally, only one unsignaled US was delivered whereas most other studies used two to four US (e.g., Dirikx et al., 2004; Hermans et al., 2005; Norrholm et al., 2006) which might result in stronger reinstatement effects. Furthermore, the reinstatement effect in the implicit behavioral measure (anxiety-potentiated startle) was not persistent but was extinguished quickly, because the analysis of both re-extinction phases on Day 3 showed no significant difference between CXT+ and CXT- anymore. Maybe, a stronger and more persistent reinstatement effect would be observed, if the state anxiety and negative affect before the reinstatement procedure became even higher,[13] and possibly, if anxiety disorder patients were investigated, who were discussed to have deficits in extinction learning (e.g., Blechert et al., 2007; Milad & Quirk, 2012).

In this study, it was also tested, if negative stimulus valence after extinction is associated with a return of anxiety after the reinstatement procedure (Hypothesis 3). In contrast to previous studies (Dirikx et al., 2004, 2007), the reinstatement of anxiety-potentiated startle did not correlate with valence ratings after extinction. However, Dirikx et al. (2004, 2007) only reported an association between valence and reaction-time measurements as an index of reinstated fear and did not investigate an association with startle response. It could be possible, that the change of mood has a stronger influence on the return of anxiety than the stimulus valence after extinction.

[13] However, a significant three-way interaction Time × Context × Group could also not be observed, if only participants were considered, who experienced a high change in state anxiety, $F(1, 18) < 1$, negative affect, $F(1, 21) < 1$, and positive affect, $F(1, 17) < 1$, before the reinstatement procedure (determined by a median split). Maybe, a very strong change of state anxiety and mood (upper 25% quartile) would result in a stronger effect, but due to the small sample size it was not possible to determine this effect in the present study.

In sum, this study aimed at probing a new paradigm to study reinstatement of contextual anxiety. Presenting an unsignaled US in VR but without showing a spatial context successfully induced a differential return of anxiety on implicit (anxiety-potentiated startle) and explicit (verbal ratings) levels. Crucially, this effect was associated with a change of the internal context from extinction to the reinstatement test, namely the change to more anxious, less positive and more negative mood. An anxious mood together with the presentation of the US could have resulted in a mood-congruent memory effect which facilitated the retrieval of the original "anxiety memory". Thus, an anxious state could function as a trigger for the return of anxiety after extinction and could possibly conform to a relapse mechanism of clinical anxiety after successful exposure therapy. Therefore, further studies should experimentally test, whether induced high state anxiety will reduce the return of fear and positive mood will diminish a return of fear, but not only in a reinstatement paradigm but also in other tests (renewal, rapid re-acquisition).

# 5. General Discussion

Although it is assumed that, in contrast to cued fear conditioning, contextual fear conditioning is a better model to explain *sustained anxiety* and the development of complex *anxiety disorders*, like panic disorder, PTSD and GAD (Craske et al., 2009; Grillon, 2002), there is only limited research on contextual fear conditioning in humans, and to my knowledge, even no research on extinction learning and extinction recall of contextual anxiety (using a foreground contextual fear conditioning paradigm). Therefore, this thesis aimed at extending previous research on contextual fear conditioning, extinction learning and extinction recall of contextual anxiety. Especially, inter-individual risk factors on the basis of genetic variants (Study 1), and the reinstatement as a mechanism of the return of contextual anxiety after extinction was tested (Study 2). To this end, two contextual fear conditioning studies were conducted and acquisition, extinction and return of contextual anxiety or extinction recall were investigated on three consecutive days. Anxiety was measured on three response levels: behavior (anxiety-potentiated startle), physiology (SCL), and the emotional-cognitive level (verbal ratings). A VR paradigm was used with two virtual offices serving as conditioned contexts (CXT+ vs. CXT-). For the first time, a gene × gene interaction of 5-HTTLPR and NPSR1 polymorphisms was studied (Study 1), and a reinstatement protocol for contextual anxiety was applied (Study 2). Results of Study 1 showed a facilitated acquisition of anxiety-potentiated startle in carriers of both risk alleles (S+ allele carriers of the 5-HTTLPR polymorphism *and* T+ allele carriers of the NPSR1 polymorphism). It was concluded that facilitated contextual fear conditioning on the implicit behavioral level (anxiety-potentiated startle) might function as an endophenotype for anxiety disorders, especially those which are characterized by symptoms of sustained anxiety (panic disorder, PTSD, GAD). In contrast, the explicit-verbal anxiety level was only influenced by the NPSR1 genotype in a way that only no risk allele carriers (AA) showed evaluative conditioning effects which persisted after extinction learning. Moreover, the absent conditioning effect in risk allele carriers (T+) was associated with a higher number of stressful life events, which can be regarded as a hint for a gene × environment interaction on an explicit-cognitive level. Deficits in extinction, as reflected in anxiety-potentiated startle response, could not be confirmed as an additional endophenotype, because extinction recall was not affected by any genotype. Notably, the mere presentation of the conditioned contexts during extinction recall might have been not sensitive enough to detect between-group effects. Therefore, in Study 2 a new reinstatement paradigm for contextual anxiety was applied. Supportively, the reinstatement group, who received one unsignaled US at the beginning of Day 3, showed a return of differential contextual anxiety in anxiety-potentiated startle and verbal ratings. Interestingly, the return of anxiety was associated with a change of mood from extinction

to extinction recall in the reinstatement group only. Thus, Study 2 revealed that a change of the internal context, namely in the direction to more negative mood and anxious state, could be an important and yet overlooked pathway to the return of (contextual) anxiety.

According to the results of Study 1, several issues have to be discussed. The 5-HTTLPR polymorphism only influenced the anxiety-potentiated startle response but not the explicit-verbal level. It was concluded that the serotonin system might be more relevant for the amygdala-dependent fear learning and expression rather than for the explicit evaluation of a threatening context (see 3.7.). However, heightened amygdala responding in S allele carriers seems not to be specific for anxiety-related stimuli. There is converging evidence for a general involvement of the 5-HTTLPR polymorphism in the modulation of the amygdala activity to *emotional stimuli*. In fact, S allele carriers showed higher amygdala activity to emotional stimuli than LL allele carriers, which is discussed to result from reduced inhibition of the PFC on the amygdala (for a review see Hariri & Holmes, 2006). However, this abnormality in the cortico-amygdala pathway is not specific for anxiety responses but has also been implicated in the etiology of depression (Caspi, Hariri, Holmes, Uher, & Moffitt, 2010; Hariri & Holmes, 2006; Lesch, 2007). Moreover, S allele carriers are discussed to be more prone to life stress and to react with a hyper-reactive HPA stress response (Alexander et al., 2012; Caspi et al., 2003). There is consensus about the interaction of environmental stress and the 5-HTTLPR polymorphism on emotional regulation and dysfunction in *emotional disorders*, i.e. anxiety disorders and depression (Caspi et al., 2010; Hariri & Holmes, 2006; Lesch, 2007). Therefore, enhanced contextual fear conditioning in S allele carriers may also be a hint for enhanced emotional reactivity in general, but may not to be attributable specifically to the acquisition and expression of anxiety. In the same vein, NPS is also discussed to play a role not only in anxiety, but also in stress, arousal, and wakefulness (Jüngling et al., 2012; Kumsta et al., 2013; Okamura & Reinscheid, 2007; Pape et al., 2010; Xu et al., 2007). However, NPS seems to be not involved in depression-related behavior in rats (Leonard et al., 2008), and in humans the NPSR1 modulated amygdala activity to threatening faces was not affected by depression level (Dannlowski et al., 2011). Therefore, the NPS system may be more specifically involved in anxiety rather than in general negative affect (Dannlowski et al., 2011).

Although it can be concluded that S allele carriers of the 5-HTTLPR and the T allele carriers of the NPSR1 polymorphism may be at higher risk for emotional disorders, like depression and anxiety disorders, why are these alleles more frequently distributed than the no risk alleles (LL and AA) in European populations (e.g., Dannlowski et al., 2011;

Lesch et al., 1996; Lesch, 2007)?[14] Logically, these mutations should also have advantages for survival. Indeed, S allele carriers of the 5-HTTLPR polymorphisms are better in cognitive tasks and show better social conformity than homozygous L allele carriers (for a review see Homberg & Lesch, 2011). Homberg and Lesch (2011) suggested that S allele carriers are hypervigilant for motivationally relevant environmental stimuli. If they are not distracted by other stimuli, they will react to the motivationally relevant stimuli with enhanced emotional responses. This can even lead to pathological emotional reactions, if there is no acute danger. However, if S allele carriers are distracted, their hypervigilance can have the advantage of monitoring the environment more precisely and of avoiding risks and selecting the best outcome. Also NPS is discussed to be involved in enhanced memory consolidation and in better long-term memory regardless of emotional content (Okamura et al., 2011). T+ allele carriers showed enhanced response inhibition and increased error monitoring (Beste et al., 2013). Speculatively, depending on the valence of a memory, increased long-term memory storage and error monitoring might be an advantage or disadvantage. For example, increased memory performance for object-recognition and spatial contexts can be very beneficial, whereas increased memory performance for fearful contents might increase the risk for anxiety disorders. In addition, Domschke et al. (2011) speculated that the increased arousal level in T+ allele carriers could have optimized the fight- or-flight reaction which might have been beneficial in predatory environments. Conclusively, enhanced contextual fear conditioning and fast extinction exhibited by S+ and T+ allele carriers (Study 1 of this thesis) could have also been a result of enhanced cognitive performance in this group. It could be argued that this genetic subgroup showed the most adaptive and flexible behavior with fast adaptation to changing situations, that is they showed enhanced anxiety in a dangerous context where threat was actually present (US during conditioning), but exhibited reduced anxiety when the threat was not present anymore (no US during extinction). Note, however, that participants in the present study were healthy and relatively low anxious, and although the S+/T+ subgroup showed fear conditioning effects, they were able to easily regulate their anxiety during extinction, which could be adaptive in this low anxious sample. In contrast, considering a more anxious sample or even anxiety disorder patients, it could be speculated the increased anxiety level could result in even stronger fear conditioning (see also Glotzbach-Schoon, Tadda et al., 2013; Orr et al., 2000; Grillon, Pine, et al., 2009) which

[14] In the whole Z2 sample there were 37.7 % (*n* = 187) LL and 30.8 % (*n* = 152) AA carriers, but 66.3 % S+ (*n* = 309; 225 S/L carries and 84 SS carriers) and 69.2 % T+ carriers (*n* = 341; 240 T/A carriers and 101 TT carriers).

could be more resistant to extinction (Orr et al., Michael et al., 2007). High anxious S+ allele carriers might be less distractible and therefore might be hypervigilant for fear conditioned stimuli. In a similar vein, increased response inhibition displayed by T+ allele carriers was positively correlated with anxiety sensitivity (Beste et al., 2013), and therefore, more anxious T+ allele carriers might be expected to react with increased anxiety responses. Moreover, T+ allele carriers might demonstrate increased memory consolidation for the fear memory acquired through contextual fear conditioning (although a memory enhancing effect of NPS has only been reported in rats, see Okamura et al., 2011). Based on these considerations, I would expect that S+/T+ carriers of a more anxious sample would show more elevated fear responses during acquisition compared to no risk allele carriers and healthy risk allele carriers. And as a result of enhanced fear acquisition, I would expect slowed extinction learning or even deficits in extinction recall (Mineka & Oehlberg, 2008; Orr et al., 2000) or an inability to inhibit fear responses during extinction (Milad & Quirk, 2012).

In a next step, risk allele carriers could also be tested in a reinstatement paradigm. According to the results of Study 1, genotype groups did not differ in extinction recall; all groups showed consolidation of the extinction memory at test on Day 3. However, the reinstatement paradigm developed in Study 2 might be more suitable to detect differences between the genotype groups. It could be hypothesized that after the reinstatement procedure the risk allele carriers (S+/T+) would show a greater return of anxiety-potentiated startle compared to the other groups. Therefore, proneness to exhibit a high amount of fear or anxiety after extinction learning could resemble an additional endophenotype for anxiety disorders because this would them predispose to suffer from relapse of clinical anxiety. Furthermore, it has been shown that panic disorder and PTSD patients are prone to contextual threat (Grillon et al., 2008; Grillon, Pine, et al., 2009). However, it has neither been studied, if extinction of contextual anxiety is also impaired in these patients in analogy to cue conditioning studies (Blechert et al., 2007; Michael et al., 2007), nor if they show a greater return of anxiety compared to healthy controls. Therefore, the reinstatement paradigm described in Study 2 could be used to compare the extinction recall or return of anxiety in different anxiety patients groups.

Importantly, the reinstatement of anxiety in Study 2 was associated with the change of state anxiety and mood from extinction to test: the more anxious the participants became, the higher the return of anxiety was. This effect was explained by the associative-network theory of Bower (1981): an anxious state together with the presentation of the unsignaled US might have activated the “anxiety node” in the associative network and this in turn might have activated the nodes which represented the

three response levels (physiological arousal, behavioral expression, and verbal labels). Therefore, a return of anxiety could be measured in anxiety-potentiated startle and ratings. It was concluded that an anxious state and negative mood could be an important pathway to the return of anxiety after extinction. However, I would further assume that events which are different from the US used during conditioning could also activate the "anxiety network". On the one hand, it has been demonstrated that a new US was able to reinstate fear in animals and humans (Rescorla & Heth, 1975; Sokol & Lovibond, 2012). It was concluded that this new US could build up a new fear memory and that this new fear memory in turn could reinstate old fear memories (Sokol & Lovibond, 2012). In analogy to the associative-network theory, it could be suggested that the "new fear" activated nodes that were also associated with the "old fear" and therefore, fear to previously extinguished stimuli was reinstated. On the other hand, I would assume that presenting a fear cue in an extinguished fear context could also reinstate the context-related anxiety because of the same mechanism as described above. Furthermore, as I have already noted in the Discussion of Study 2, the activation of the network has to reach a certain threshold to spread out and activate associated nodes (Bower, 1981). Presumably, on the one hand the suprathreshold activation could depend on the number of activated associated nodes, but on the other hand it could also depend on the strength of the activation of one node. For example, a less fearful stimulus could activate the network not strongly enough compared to a conditioned stimulus which evoked a strong CR or a fear-relevant stimulus. In addition, activation of the network could also be easier with a less extinguished stimulus or context because of residual anxiety on one response level like the cognitive-verbal level, or few extinction trials.

Regarding clinical implications, results of Study 2 can provide some interesting hints. It could be concluded that the inhibition of the anxiety memory during extinction alone is not sufficient enough to reduce anxiety. Moreover, it seems plausible that not only the anxiety memory itself has to be the target during interventions but also concepts and schemata which are associated with the "anxiety memory node", like events, moods and contexts. Conclusively, a general anxious apprehension and negative mood should also be considered during psychotherapy. Moreover, the contexts where the anxiety was experienced can become conditioned stimuli and trigger a return of anxiety, when exposed to it. Therefore, it might be useful to expose patients also to contexts associated with their anxiety. Moreover, extinction learning often reduces only the implicit-physiological anxiety level, but leaves anxious and fearful cognitions and evaluations (ratings) intact (Vansteenwegen et al., 1998). Logically, it would be very useful to reduce the explicit anxiety level during extinction training, because it seems that this emotional-cognitive anxiety level (state anxiety, mood) can have a boosting effect on the return of anxiety.

Furthermore, positive mood could have an inhibitory effect on the anxiety-related nodes in the associative network (see Bower, 1981). Therefore, psychotherapy should not only focus on anxiety symptoms and on reducing negative feelings, but should also emphasize positive emotions, possibly via positive feedback, increased self-efficacy, and a faithful and warm client-therapist relationship.

Moreover, it has been reported that pharmacological treatments reduced sustained anxiety (Grillon, Chavis, Covington, & Pine, 2009) and the return of cued fear (Das et al., 2013; Kindt, Soeter, & Vervliet, 2009; Soeter & Kindt, 2011). In detail, chronic SSRI administration reduced anxiety-potentiated startle during contextual fear conditioning, however effects on extinction have not been investigated (Grillon, Chavis, et al., 2009). In addition, cannabidiol administration after extinction training led to lower overall US-expectancy ratings (i.e., for both CS+ and CS-) during the reinstatement test (Das et al., 2013). Furthermore, propranolol administration, a beta-adrenergic receptor antagonist, before the reactivation of the fear memory (i.e., presenting a reminder CS before extinction training) disrupted the reinstatement of fear-potentiated startle to a cue (Kindt et al., 2009; Soeter & Kindt, 2011). Therefore, the new reinstatement paradigm for contextual anxiety can be used to test, whether these pharmacological treatments also block the return of contextual anxiety. Besides these pharmacological interventions, behavioral manipulations are under debate to successfully prevent a return of conditioned fear. Using a cue conditioning paradigm, Schiller et al. (2010) reported that the performance of extinction learning within the reconsolidation window (i.e., 10 min after a reminder CS was presented) reduced the reinstatement of conditioned SCR. However, this effect has been rarely replicated (Kindt & Soeter, 2013; Oyarzún et al., 2012; Soeter & Kindt, 2011) and has not been tested in a contextual fear conditioning paradigm. In conclusion, to prove, whether these pharmacological and behavioral manipulations are also successful in preventing the return of sustained anxiety, the paradigm examined in the present study seems to be useful.

## 5.1. Limitations

The strength of Study 1 of this thesis was that it investigated the interaction of *two* genotypes on contextual fear conditioning – an endophenotypes for anxiety disorders – rather than only one genotype which has been done in most studies. However, complex psychiatric diseases, like anxiety disorders, are likely to develop due to multiple genetic variants and their interaction with life stress (Leonardo & Hen, 2006). Therefore, further studies should account for more gene interactions, when investigating endophenotypes or genetic risks factor for anxiety disorders itself, which would also imply larger sample

sizes. Additionally, as also reported above (see Discussion, 3.7.) life stress should be measured carefully with a focus on early severe environmental stress, because early life stress could have a greater impact on neuronal development (Leonardo & Hen, 2008). Although, on a molecular level an influence of NPS on serotonin release in frontal cortex and amygdala has been described in mice (Gardella et al., 2013; Raiteri, Luccini, Romei, Salvadori, & Calò, 2009), it might also be useful to consider gene variants that are relevant for the same neurotransmitter system. For example, to investigate the role of the serotonin system in emotional behavior, not only the serotonin transporter gene polymorphism (5-HTTLPR) but also polymorphisms in the serotonin receptors genes (e.g., HTR1A) and tryptophan-hydroxylase[15] genes (TPH1, TPH2) should be examined (Nugent et al., 2011). Furthermore, the cannabinoid receptor gene (CNR1, rs2180619) could be an interesting candidate gene to study differences in extinction learning and contextual anxiety. Although, it has only been probed in one study which used a background contextual fear conditioning protocol, the authors found that AA allele carriers showed deficits in the extinction of conditioned fear to a CS+ and this was accompanied by higher contextual anxiety compared to G+ allele carriers (Heitland et al., 2012).

There are also some limitations regarding the second study of this thesis. The association between change of mood and reinstatement of contextual anxiety was based on correlation analyses. Therefore, to better control the influence of mood on the return of anxiety responses, mood should be manipulated before the reinstatement test, to prove a causal relationship between mood and the return of fear or anxiety (as discussed above, 4.5.). Furthermore, mood has only been measured *before* extinction training but not after extinction. Therefore, it is not totally clear, whether the change of mood from extinction to re-extinction was induced by the extinction training itself. Possibly, successful extinction induced a less anxious state and positive mood. The difference scores calculated in this thesis tested only the change from the beginning of the experimental session on Day 2 (before extinction training) to the beginning of Day 3 (before the reinstatement test), but did not take into account what might have caused the change. Therefore, also evaluating the change of mood induced by the extinction training, i.e. after extinction, might be very important. Additionally, Bouton (2006) proposed that the unsignaled US during the reinstatement procedure would evoke the same emotion that was prevalent during fear conditioning, and therefore a return of would be facilitated. This suggestion would speak in favor of the mood-dependent memory effect, i.e. “the facilitation of memory when mood at retrieval is matched to mood at encoding.” (Lewis & Critchley, 2003, p. 431). Therefore, the unsignaled US could have induced an anxious mood similar to the anxious mood

[15] Tryptophan-hydroxylase is essential for the biosynthesis of serotonin (Kriegebaum et al., 2010a).

during contextual fear conditioning. The congruency between both emotional states could have facilitated the retrieval of the anxiety memory on Day 3. However, to test this assumption it would have been necessary to measure state anxiety and mood directly *after* conditioning on Day 1 and directly *after* the unsignaled US on Day 3 and to compare both measurements. If both measurements had indicated an equally strong state anxiety and negative mood, then reinstatement of contextual anxiety could have been facilitated. In sum, further studies should also examine state anxiety and negative affect *after* conditioning, extinction and the unsignaled US during the reinstatement procedure.

Additionally, two methodological issues have to be considered. Firstly, contextual fear conditioning in this thesis was conducted with an US-only paradigm. However, a more valid contextual fear conditioning paradigm might be the CS-US unpaired procedure used by Grillon et al. (2006), because in real-life, contexts also contain specific cues. In animals these two paradigms did not differ in the amount of contextual fear conditioning (Luyten, Vansteenwegen, van Kuyck, Deckers, et al., 2011). However, this has still to be proven in humans and would be important to ascertain comparability between different studies and results. Secondly, as discussed above (see 3.7., Discussion) it has been suggested that the activation of the subcortical, automatic and reflex-like defensive system might be faster or better established using fear-relevant CS rather than fear-irrelevant CS (Mineka & Öhmann, 2002). Therefore, a contextual fear conditioning paradigm using anxiety-relevant contexts (heights, darkness, open spaces) would be more powerful in establishing robust fear conditioning and would maybe delay extinction.

It must also be considered that, besides contextual fear conditioning, there are other potential endophenotypes for anxiety disorders. For example, SCR habituation has been found to be heritable (Hettema et al., 2003). Habituation is a non-associative learning process and has been proposed as an additional pathway to the development of specific phobias (Poulton & Menzies, 2002). According to this non-associative model of fear acquisition, there are limited innate fears. Phobias can develop because of a lack of exposure to fear-relevant stimuli and situations and therefore habituation cannot occur (Poulton & Menzies, 2002). However, this model does not exclude associative learning as a pathway to pathologic anxiety, but the authors stress the division into evolutionary-relevant and evolutionary-neutral fears. Non-associative learning would better account for evolutionary-relevant fear, whereas fear conditioning would better account for the acquisition of evolutionary-neutral fears (Poulton & Menzies, 2002). Therefore, the question still remains open whether specific genetic variants would affect fear habituation, specifically habituation to evolutionary-relevant stimuli like heights or darkness.

## 5.2. Summary and outlook

In sum, this thesis used contextual fear conditioning as a model for sustained anxiety and found an influence of functional polymorphisms in the 5-HTT gene (5-HTTLPR) and in the NPSR1 gene on the acquisition of contextual anxiety. Therefore, facilitated contextual fear conditioning could be regarded as an endophenotype for anxiety disorders, which contributes to a better understanding of the etiology of anxiety disorders. Furthermore, reinstatement of contextual anxiety as a model for relapse after successful extinction training has been proven and was linked to a change of state anxiety and mood. This can have important clinical implications, namely that it might be crucial to target anxious and negative mood and to enhance positive mood during or after exposure therapy in order to improve the efficacy of psychotherapy by reducing relapses. Methodologically, the strength of the present studies was that virtual reality environments were used as conditioned contexts which were presented via HMD with simultaneous head-tracking. This allows for presenting realistic contexts and enhancing immersion and presence within these contexts Moreover, VR can be used in therapeutic settings, for example virtual reality exposure therapy (VRET) can successfully reduce fear in spider phobia (Shiban, Pauli, & Mühlberger, 2013) or in aviophobia (Mühlberger, Herrmann, Wiedemann, Ellgring, & Pauli, 2001). Therefore, the VR-based contextual fear conditioning paradigm seems to be a powerful tool to study sustained anxiety and contextual influences in a laboratory-based controlled setting. In a next step, it can be used to introduce specific cues into the contexts, or to create evolutionary-relevant contexts. Furthermore, VR has been used successfully to measure approach and avoidance behavior to conditioned contexts (Glotzbach et al., 2012; Grillon et al., 2006). Therefore, it would also be interesting to test, whether participants carrying risk alleles would also differ in their avoidance behavior from no risk allele carriers, or to measure approach and avoidance after a reinstatement manipulation. Moreover, an even more realistic and immersive virtual reality setting can be established with the help of a CAVE system (cave automated virtual environment), in which the virtual environment is projected three-dimensionally on the walls and the floor of a room.

Although contextual fear conditioning is only one mechanism which might contribute to the etiological model of anxiety disorders, and research has to be extended to additional mechanisms (e.g., mood and state anxiety, inter-individual differences in habituation), it is an important model to understand basic learning and neuronal mechanisms. Supportively, I would like to end with a statement of LeDoux and Phelps (2008, p. 161) on fear conditioning: “Fear conditioning may not be able to tell us everything we need to know about emotions and the brain, or even about fear and the brain, but it has been an excellent starting point.”.

# 6. References


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# 7. Annex

A Experimental procedures of Studies 1 and 2

B Information for participants

1. Study 1

2. Study 2

C Written informed consent of both studies

D Written instructions

E Questionnaires

1. Demographic data and exclusion criteria

2. Daily sleep quality

3. Determination of pain threshold

F Detailed trial order and pseudo-randomization

# A Experimental procedures of Studies 1 and 2

Day 1:

1. written informed consent
2. demographic data and exclusion criteria
3. trait questionnaires: MEQ, PSQI
4. state questionnaires: STAI X1, PANAS, sleep quality of the last night
5. written instructions
6. attachment of electrodes and HMD
7. training of joystick handling (in a different virtual environment)
8. determination of individual pain threshold and rating of the final US (intensity, valence, arousal)
9. pre-acquisition phase (actively exploring each context via joystick for 2 min each)
10. ratings of contexts (valence, arousal, anxiety)
11. startle habituation (4 startle probes)
12. acquisition phase 1 (3 trials in which each context was presented once)
13. ratings of contexts (valence, arousal, anxiety, US-expectancy)
14. acquisition phase 2 (3 trials in which each context was presented once)
15. ratings of contexts (valence, arousal, anxiety, US-expectancy)
16. ratings of US (valence, arousal)
17. detachment of electrodes and HMD
18. questionnaire: IPQ

Day 2:

1. state questionnaires: STAI X1, PANAS, sleep quality of the last night
2. written instructions
3. attachment of electrodes and HMD
4. startle habituation (4 startle probes)
5. extinction phase 1 (3 trials in which each context was presented once)
6. ratings of contexts (valence, arousal, anxiety, US-expectancy)
7. extinction phase 2 (3 trials in which each context was presented once)
8. ratings of contexts (valence, arousal, anxiety, US-expectancy)
9. *only Study 2:* ratings of contexts regarding the last trial of extinction (valence, arousal, anxiety, US-expectancy)
10. detachment of electrodes and HMD
11. questionnaire: IPQ

Day 3:

1. state questionnaires: STAI X1, PANAS, sleep quality of the last night
2. written instructions
3. attachment of electrodes and HMD
4. *only Study 2 in the reinstatement group*: 1x US and ratings (intensity, valence, arousal)
5. startle habituation (4 startle probes)
6. re-extinction phase 1 (3 trials in which each context was presented once)
7. ratings of contexts (valence, arousal, anxiety, US-expectancy)
8. *only Study 2:* ratings of contexts regarding the first trial of re-extinction (valence, arousal, anxiety, US-expectancy)
9. re-extinction phase 2 (3 trials in which each context was presented once)
10. ratings of contexts (valence, arousal, anxiety, US-expectancy)
11. detachment of electrodes and HMD
12. questionnaire: IPQ
13. *only Study 2*: trait questionnaires: STAI X2, ASI, BIS-BAS
14. reimbursement

# B Information for participants

## 1. Study 1

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Würzburg, Mai 2010

### Probandeninformation zur Studie

**Teilprojekt B01 „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 u. a. wichtige Angaben bezüglich Ihrer Person festgehalten werden. Dann wird der Versuchsleiter zur Messung Ihrer Schweißdrüsenaktivität und Ihrer Muskelspannung mehrere Messelektroden in Ihrem Gesicht 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 die 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.

## 2. Study 2

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**Probandeninformation zur Studie** **EG02**

**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 und Ihrer Muskelspannung mehrere Messelektroden in Ihrem Gesicht und auf Ihrer Hand anbringen. Dazu wird Ihre Haut mit Alkohol und einer speziellen Paste 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.

Während der Untersuchung werden wir Ihnen eine Virtuelle Welt, d. h. von einem Computer erzeugte Räume, zeigen. Sie sollen diese Räume 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.

Außerdem werden Sie manchmal über Kopfhörer ein kurzes, lautes Geräusch hören. Dieses Geräusch wird unangenehm für Sie sein und Sie werden sich erschrecken, es ist aber unschädlich. Dieses Geräusch ist wichtig für die physiologischen Messungen, 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 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 Written informed consent of both studies

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Würzburg, Mai 2010

### 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, Informationen 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

# D Written instructions

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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.

Das Experiment wird über drei Sitzungen an drei aufeinander folgenden Tagen durchgeführt. Bitte lesen Sie alle Instruktionen, um sich mit dem Ablauf vertraut zu machen. Sie erhalten dieses Informationsblatt an allen drei Tagen.

**Alle 3 Tage:**

Im Laufe des gesamten Experiments werden wir Sie über ein Head Mounted Display in eine virtuelle Welt versetzen. Diese virtuelle Welt besteht aus einem Flur, von dem 2 Türen abgehen. Hinter diesen Türen befinden sich verschiedene Büroräume, die Sie an allen 3 Tagen sehen werden. An allen 3 Tagen wird der Versuch dann aus mehreren Phasen bestehen. In jeder Phase gibt es mehrere Raumdurchgänge, d.h. Sie werden mehrmals durch die virtuellen Büroräume passiv geführt. Sie können nicht aktiv in den Verlauf eingreifen. Sie können aber **immer durch eigene Kopfbewegungen Ihr Blickfeld verändern**.

Nach jeder Phase werden Ihnen verschiedene Fragen gestellt. Diese Fragen beziehen sich entweder **allgemein auf alle Raumdurchgänge der vorangegangenen Phase** oder nur auf **einzelne Raumdurchgänge**. Im Folgenden sind Beispiele für diese Fragen aufgeführt mit den entsprechenden Skalen.

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

.....
0 50 100

**Wie stark war Ihre Aufregung in diesem Raum?**
Nennen Sie dann bitte eine Zahl von **0 (gar keine Aufregung)** bis **100 (sehr starke Aufregung)** auf der unten angegebenen Skala.

.....
0 50 100

2

**Wie stark war Ihre Angst in diesem Raum?**
Nennen Sie dann bitte eine Zahl von **0 (keine Angst)** bis **100 (sehr starke Angst)** auf der unten angegebenen Skala.

.....
**0** 50 **100**

**Haben Sie in diesem Raum einen elektrischen Reiz erwartet?**
Nennen Sie dann bitte eine Zahl von **0 (nicht erwartet)** bis **100 (sicher erwartet)** auf der unten angegebenen Skala, wobei **50 unsicher** bedeutet.

.....
**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.

<u>Zusätzlich am ersten Tag:</u>

**Am ersten Tag** können Sie zunächst die virtuelle Welt mit Hilfe des **Joysticks** selbst erkunden.
Außerdem wird am ersten Tag Ihre **persönliche Schmerzschwelle** bestimmt. Dies dient zum einen dazu, einen elektrischen Reiz zu bestimmen, der leicht schmerzhaft für Sie persönlich ist, zum anderen können Sie die Reize, die Sie im Experiment erhalten werden, kennenlernen. Über eine 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 gespürt** **eben wahrnehmbarer Schmerz** **sehr starker 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. Sie werden nach jedem Reiz mündlich dazu aufgefordert, den Reiz zu beurteilen. Sie werden die Skala nicht mehr sehen. Nennen Sie dann bitte die entsprechende Zahl.
Zum Schluss wird Ihnen der elektrische Reiz, der Ihrer persönlichen Schmerzschwelle entspricht, nochmals präsentiert.

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

# E Questionnaires

## 1. Demographic data and exclusion criteria

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

2. Daily sleep quality

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

**Datum:** **TAG:**

---

**Bitte beantworten Sie die folgenden Fragen:**

Wann sind Sie gestern Abend zu Bett gegangen? _____

Wann sind Sie heute Morgen aufgestanden? _____

Wie viele Stunden haben Sie letzte Nacht tatsächlich geschlafen? _____

Wie würden Sie insgesamt die Qualität Ihres Schlafes während der letzten Nacht beurteilen?
Bitte kreuzen Sie an:

| ☐ | ☐ | ☐ | ☐ |
|---|---|---|---|
| Sehr gut | ziemlich gut | ziemlich schlecht | sehr schlecht |

3. Determination of pain threshold

**Untersuchung:**

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

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**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:** _____

## F Detailed trial order and pseudo-randomization

Contexts were presented in one of four pre-defined pseudo-randomized orders. In Orders 1 and 2, the green office served as CXT+ and the red office served as CXT-. In Orders 3 and 4, the red office served as CXT+ and the green office served as CXT-. Order 2 mirrored the sequence of Order 1, and Order 4 mirrored the sequence of Order 3. The following table depicts the contexts which were entered first and second in a trial (note that each trial consisted of entering each context once: CXT1 – ITI – CXT2). Each phase of the experiment consisted of three trials, except pre-acquisition which consisted of only one trial.

| **Phase** | **Order 1**<br>*Green = CXT+*<br>*Red = CXT-* | **Order 2**<br>*Green = CXT+*<br>*Red = CXT-* | **Order 3**<br>*Red= CXT+*<br>*Green = CXT-* | **Order 4**<br>*Red = CXT+*<br>*Green = CXT-* |
|---|---|---|---|---|
| Pre-acquisition | CXT- CXT+ | CXT+ CXT- | CXT+ CXT- | CXT- CXT+ |
| Acquisition 1 | CXT- CXT+<br>CXT- CXT+<br>CXT+ CXT- | CXT+ CXT-<br>CXT+ CXT-<br>CXT- CXT+ | CXT+ CXT-<br>CXT+ CXT-<br>CXT- CXT+ | CXT- CXT+<br>CXT- CXT+<br>CXT+ CXT- |
| Acquisition 2 | CXT+ CXT-<br>CXT- CXT+<br>CXT+ CXT- | CXT- CXT+<br>CXT+ CXT-<br>CXT- CXT+ | CXT- CXT+<br>CXT+ CXT-<br>CXT- CXT+ | CXT+ CXT-<br>CXT- CXT+<br>CXT+ CXT- |
| Extinction 1 | CXT- CXT+<br>CXT+ CXT-<br>CXT+ CXT- | CXT+ CXT-<br>CXT- CXT+<br>CXT- CXT+ | CXT+ CXT-<br>CXT- CXT+<br>CXT- CXT+ | CXT- CXT+<br>CXT+ CXT-<br>CXT+ CXT- |
| Extinction 2 | CXT+ CXT-<br>CXT- CXT+<br>CXT- CXT+ | CXT- CXT+<br>CXT+ CXT-<br>CXT+ CXT- | CXT- CXT+<br>CXT+ CXT-<br>CXT+ CXT- | CXT+ CXT-<br>CXT- CXT+<br>CXT- CXT+ |
| Re-Extinction 1 | CXT- CXT+<br>CXT+ CXT-<br>CXT- CXT+ | CXT+ CXT-<br>CXT- CXT+<br>CXT+ CXT- | CXT+ CXT-<br>CXT- CXT+<br>CXT+ CXT- | CXT- CXT+<br>CXT+ CXT-<br>CXT- CXT+ |
| Re-Extinction 2 | CXT+ CXT-<br>CXT+ CXT-<br>CXT- CXT+ | CXT- CXT+<br>CXT- CXT+<br>CXT+ CXT- | CXT- CXT+<br>CXT- CXT+<br>CXT+ CXT- | CXT+ CXT-<br>CXT+ CXT-<br>CXT- CXT+ |

# Publications list

**Research articles in peer-reviewed journals:**

Domschke, K., Gajewska, A., Winter, B., Herrmann, M. J., Warrings, B., Mühlberger, A., Wosnitza, K., **Glotzbach, E.**, Conzelmann, A., Dlugos, A., Fobker, M., Jacob, C., Arolt, V., Reif, A., Pauli, P., Zwanzger, P., & Deckert, J. (2012). ADORA2A gene variation, caffeine, and emotional processing: A multi-level interaction on startle reflex. *Neuropsychopharmacology*, *37*(3), 759–769.

**Glotzbach, E.**, Ewald, H., Andreatta, M., Pauli, P., & Mühlberger, A. (2012). Contextual fear conditioning predicts subsequent avoidance behavior in a virtual reality environment. *Cognition & Emotion, 26*(7), 1256–1272.

**Glotzbach, E**., Mühlberger, A., Gschwendtner, K., Fallgatter, A. J., Pauli, P., & Herrmann, M. J. (2011). Prefrontal brain activation during emotional processing: a functional near infrared spectroscopy study (fNIRS). *The Open Neuroimaging Journal, 5,* 33-39.

**Glotzbach-Schoon, E.**, Andreatta, M., Reif, A., Ewald, H., Tröger, C., Baumann, C., Deckert, J., Mühlberger, A., & Pauli, P. (2013). Contextual fear conditioning in virtual reality is affected by 5HTTLPR and NPSR1 polymorphisms: effects on fear-potentiated startle. *Frontiers in Behavioral Neuroscience*, 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. (2013). Enhanced discrimination between threatening and safe contexts in high-anxious individuals. *Biological Psychology, 93*(1), 159-166.

Tröger, C., Ewald, H., **Glotzbach, E.**, Pauli, P., & Mühlberger, A. (2012). Does pre-exposure inhibit fear context conditioning? A virtual reality study. *Journal of Neural Transmission*, *119*(6), 709–719.

**Review articles:**

**Glotzbach-Schoon, E.**, Andreatta, M., Mühlberger, A., & Pauli, P. (in press). Kontextkonditionierung in virtueller Realität als Modell für pathologische Angst. *Neuroforum*. [German version]

**Glotzbach-Schoon, E.**, Andreatta, M., Mühlberger, A., & Pauli, P. (in press). Context conditioning in virtual reality as a model for pathological anxiety. e-*Neuroforum*. [English version]

**Published abstracts:**

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

Andreatta, M., **Glotzbach, E**., Mühlberger, A., & Pauli, P. (2012). The role of anxiety in backward and forward conditioning. *Psychophysiology, 49,* S110.

Andreatta, M., Mühlberger, A., **Glotzbach-Schoon, E**., & Pauli, P. (2013). Pain predictability reverses valence of a relief-associated stimulus. *Cognitive Neuroscience Society (CNS),* San Francisco, USA.

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

**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.

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

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.

**Oral conference presentations:**

**Glotzbach, E.,** Ewald, H., Andreatta, M., Mühlberger, A., Pauli, P. (2011). Neuronale Korrelate von Delay und Trace Furchtkonditionierung in virtueller Realität. *Psychologie und Gehirn*, Heidelberg, Germany.

**Glotzbach-Schoon, E.,** Andreatta, M., Mühlberger, A., Pauli, P. (2013). Wiederkehr von kontextueller Angst: ein Reinstatement-Paradigma. *Psychologie und Gehirn*, Würzburg, Germany.

# Curriculum Vitae

## Personal Details


| | |
|---|---|
| Name: | Evelyn Glotzbach-Schoon |
| Birth name: | Evelyn Glotzbach |
| Date of birth: | June 13, 1983 |
| Place of birth: | Bad Salzungen, Germany |
| Private address: | Ludwigstr. 26<br>90763 Fürth |
| Office address: | University of Würzburg<br>Department of Psychology I<br>Marcusstr. 9-11<br>97070 Würzburg |
| Phone: | 0049 (0)931 31 80176 |
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## Work Experience

| | |
|---|---|
| Since 01/2013 | Research Assistant in the project B01 “Context conditioning and generalization: processes and person variables“ within the DFG-funded SFB TRR 58 (Collaborative Research Center) “Fear, Anxiety, Anxiety Disorders” |
| 10/2008-12/2012 | Research Assistant in the project B01 “Structural/ functional correlates of contextual fear conditioning in humans“ within the DFG-funded SFB TRR 58 (Collaborative Research Center) “Fear, Anxiety, Anxiety Disorders” |
| 2005-2008 | Student Assistant at the Department of Psychology IV (Educational Psychology and Developmental Psychology), University of Würzburg |

## Education

| | |
|---|---|
| 11/2008-04/2013 | Member of the PhD program “Biopsychology of Pain and Emotion” of the Universities of Bamberg and Würzburg |
| 09/2008 | Diploma in Psychology<br>diploma thesis: "The influence of emotion regulation strategies on cortical activity: an ERP - fNIRS study" |
| 2007 | Internship at the Max Planck Institute for Human Cognitive and Brain Sciences, Department of Neuropsychology, Leipzig, Germany |
| 2006 | Internship at the rehab hospital for children and adolescents Charlottenhall, Bad Salzungen, Germany |
| 09/2005-03/2006 | Studies of Psychology at the University of Elche, Spain, within the ERASMUS program |
| 04/2003 – 09/2008 | Studies of Psychology at the University of Würzburg |
| 10/2002 – 03/2003 | Studies of Industrial Engineering and Management at the Technical University of Dresden |
| 2002 | High school diploma at Johann-Gottfried-Seume Gymnasium, Vacha |

## Teaching Responsibilities

| | |
|---|---|
| Summer semester 2013 | Seminar *Personality Disorders*<br>Seminar *Biopsychology* |
| Winter semester 2012/2013 | Seminar *Introduction into Clinical Psychology II*<br>Seminar *Affective Disorders* |

## Awards

| | |
|---|---|
| 11/2012 | 2nd poster award (German Society for Anxiety Research, GAF) |

## Affidavit

I hereby confirm that my thesis entitled

*Contextual fear conditioning in humans: The return of contextual anxiety and the influence of genetic polymorphisms*

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