Attention mechanisms in contextual anxiety and cued fear and their influence on processing of social cues

Aufmerksamkeitsmechanismen bei kontextueller Angst und reizspezifischer Furcht und deren Einfluss auf die Verarbeitung von sozialen Reizen

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Abstract

Anxiety is an affective state characterized by a sustained, long-lasting defensive response, induced by unpredictable, diffuse threat. In comparison, fear is a phasic response to predictable threat. Fear can be experimentally modeled with the help of cue conditioning. Context conditioning, in which the context serves as the best predictor of a threat due to the absence of any conditioned cues, is seen as an operationalization of sustained anxiety.

This thesis used a differential context conditioning paradigm to examine sustained attention processes in a threat context compared to a safety context for the first time. In three studies, the attention mechanisms during the processing of contextual anxiety were examined by measuring heart rate responses and steady-state-visually evoked potentials (ssVEPs). An additional focus was set on the processing of social cues (i.e. faces) and the influence of contextual information on these cues. In a last step, the correlates of sustained anxiety were compared to evoked responses by phasic fear, which was realized in a previously established paradigm combining predictable and unpredictable threat.

In the first study, a contextual stimulus was associated with an aversive loud noise, while a second context remained unpaired. This conditioning paradigm created an anxiety context (CTX+) and a safety context (CTX-). After acquisition, a social agent vs. an object was presented as a distractor in both contexts. Heart rate and cortical responses, with ssVEPs by using frequency tagging, to the contexts and the distractors were assessed. Results revealed enhanced ssVEP amplitudes for the CTX+ compared to the CTX− during acquisition and during presentation of distractor stimuli. Additionally, the heart rate was accelerated in the acquisition phase, followed by a heart rate deceleration as a psychophysiological marker of contextual anxiety.

Study 2 used the same context conditioning paradigm as Study 1. In contrast to the first study, persons with different emotional facial expressions were presented in the anxiety and safety contexts in order to compare the differential processing of these cues within periods of threat and safety. A similar anxiety response was found in the second study, although only participants who were aware of the contingency between contexts and aversive event showed a sensory amplification of the threat context, indicated by heart rate response and ssVEP activation. All faces irrespective of their emotional expression received increased attentional resources when presented within the anxiety context, which suggests a general hypervigilance in anxiety contexts.

In the third study, the differentiation of predictable and unpredictable threat as an operationalization of fear and anxiety was examined on a cortical and physiological level. In the predictable condition, a social cue was paired with an aversive event, while in the unpredictable condition the aversive event remained unpaired with the respective cue. A fear response to the predictable cue was found, indicated by increased oscillatory response and accelerated heart rate. Both predictable and unpredictable threat yielded increased ssVEP amplitudes evoked by the context stimuli, while the response in the unpredictable context showed longer-lasting ssVEP activation to the threat context.

To sum up, all three studies endorsed anxiety as a long-lasting defensive response. Due to the unpredictability of the aversive events, the individuals reacted with hypervigilance in the anxiety context, reflected in a facilitated processing of sensory information and an orienting response. This hypervigilance had an impact on the processing of novel cues, which appeared in the anxiety context. Considering the compared stimuli categories, the stimuli perceived in a state of anxiety received increased attentional resources, irrespective of the emotional arousal conveyed by the facial expression. Both predictable and unpredictable threat elicited sensory amplification of the contexts, while the response in the unpredictable context showed longer-lasting sensory facilitation of the threat context.

Zusammenfassung

Angst wird als ein langanhaltender Zustand, induziert durch eine unvorhersehbare, diffuse Bedrohung, gesehen. Furcht hingegen wird als eine kürzere Reaktion auf einen spezifischen Bedrohungsreiz definiert. Diese phasische Reaktion kann durch Furchtkonditionierung induziert werden. Bei der Kontextkonditionierung hingegen wird durch die Abwesenheit vorhersagender Hinweisreize der Kontext zum besten Prädiktor für den aversiven Reiz und induziert dadurch eine chronische Erwartung der Bedrohung und einen langanhaltenden Angstzustand. Diese Promotionsarbeit präsentiert ein neu angepasstes differentielles Kontextkonditionierungsparadigma, welches implementiert wurde, um ein kontinuierliches Maß langanhaltender Angst im Bedrohungskontext zu erhalten. In drei Studien wurden Aufmerksamkeitsmechanismen mittels Erhebung von Herzrate und steady-state visuell evozierte Potentiale (ssVEPs) untersucht. Ein zusätzlicher Fokus lag in der Verarbeitung von sozialen Reizen (d.h. Gesichtern) und dem Einfluss von kontextuellen Informationen. Zusätzlich wurden mittels eines bereits etablierten Paradigma, welches die Vorhersagbarkeit von Bedrohungsreizen moduliert, die elektrokortikalen und physiologischen Korrelate von Angst mit Furchtreaktionen verglichen.

In der ersten Studie wurde ein Kontextstimulus mit einem aversiven lauten unvorhersagbaren Geräusch assoziiert, während ein zweiter Kontextstimulus ungepaart blieb. In diesem differentiellen Paradigma entstanden so ein Angstkontext (CTX+) und ein Sicherheitskontext (CTX-). Nach der Akquisition wurden ein sozialer Agent und ein Objekt als Distraktoren in beiden Kontexten präsentiert. Die Herzrate und die kortikale Aktvierung mittels ssVEPs in Reaktion auf beide Kontexte und beide Distraktoren wurden gemessen. Die Ergebnisse zeigten erhöhte ssVEP-Amplituden in Reaktion auf den CTX+ im Vergleich zum CTX- während der Akquisitionsphase und der simultanen Präsentation der Distraktoren. Diese langanhaltende Angstreaktion wurde unterstützt durch Befunde von einer Akzeleration der Herzrate während der Konditionierungsphase und einer darauffolgenden Dezeleration im Angstkontext.

Studie 2 verwendete dasselbe Kontextkonditionierungsparadigma wie die erste Studie, allerdings wurden hier Personen mit unterschiedlichen emotionalen Gesichtsausdrücken als Distraktoren

im Angst- und Sicherheitskontext präsentiert, um die differentielle Verarbeitung von emotionalen Reizen innerhalb von Phasen der Angst und Sicherheit zu untersuchen. Es konnte eine ähnliche Angstreaktion wie in der ersten Studie nachgewiesen werden, allerdings zeigte sich diese nur bei den kontingenzbewussten Probanden, die den Zusammenhang zwischen den aversiven Konsequenzen und den beiden Kontexten richtig wiedergeben konnten. Sie zeigte sich in einer sensorischen Verstärkung des CTX+, abgeleitet durch Herzrate und ssVEP-Aktivierung. Alle Gesichter, unabhängig ihres emotionalen Gehalts, evozierten verstärkte Aufmerksamkeitsressourcen im CTX+, was auf eine generelle Hypervigilanz in Angstkontexten hindeutet.

In der dritten Studie wurde die Differenzierung von vorhersagbarer und unvorhersagbarer Bedrohung, als Operationalisierung von Furcht und Angst, auf kortikaler und physiologischer Ebene untersucht. In der vorhersagbaren Bedingung wurde ein sozialer Reiz mit einem aversiven Ereignis gepaart; in der unvorhersagbaren Bedingung wurde dieses aversive Ereignis zufällig präsentiert. Eine Furchtreaktion auf den vorhersagbaren Reiz konnte mit erhöhten ssVEP-Amplituden sowie einer erhöhten Herzrate gezeigt werden. Sowohl die vorhersagbare als auch die unvorhersagbare Bedrohung lösten eine sensorische Verstärkung der Kontexte gegenüber der Sicherheitsbedingung aus, wobei die Reaktion auf den unvorhersagbaren Kontext eine länger andauernde ssVEP-Aktivierung beinhaltete.

Die Ergebnisse von den drei Studien konnten Angst als eine langanhaltende defensive Reaktion bestätigen. Aufgrund der Unvorhersagbarkeit der aversiven Ereignisse reagieren Individuen mit einer erhöhten Wachsamkeit im Angstkontext, gezeigt in einer erleichterten Verarbeitung von sensorischer Information und einer Orientierungsreaktion. Diese erhöhte Wachsamkeit hatte auch einen Einfluss auf die Verarbeitung von neuen Reizen, welche im Angstkontext erschienen. Abhängig von den Vergleichsstimuli, erhielten Stimuli die im Angstkontext wahrgenommen wurden, erhöhte Aufmerksamkeitsressourcen, unabhängig vom emotionalen Gehalt der Gesichter. Sowohl vorhersagbare als auch unvorhersagbare Bedrohungen förderten eine verstärkte sensorische Verarbeitung der Kontexte, während diese im Angst- im Gegensatz zum Furchtkontext länger andauerte.

Abbreviations

ACC	Anterior cingulate cortex
ANOVA	Analysis of variance
BNST	Bed nucleus of the stria terminalis
CeA	Central nucleus of the amygdala
CS	Conditioned stimulus
CS+	Conditioned fear cue
CS-	Conditioned safety cue
CTX+	Conditioned anxiety context
CTX-	Conditioned safety context
ECG	Electrocardiogram
EEG	Electroencephalogram
ERP	Event-related potential
fMRI	Functional magnetic resonance imaging
GAD	Generalized anxiety disorder
HR	Heart rate
NPU-threat test	Threat of the predictable and unpredictable aversive events test; Conditions: N=neutral, P=predictable; U=unpredictable
PANAS	Positive and Negative Affect Schedule
PD	Panic disorder
PTSD	Posttraumatic stress disorder
SCR	Skin conductance response
SPAI	Social Phobia and Anxiety Inventory
STAI	State-Trait Anxiety Inventory
ssVEP	Steady-state visually evoked potential
US	Unconditioned stimulus

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1 Theoretical background

In everyday life, the terms fear and anxiety are commonly used interchangeably, while in the scientific literature it is agreed that these two are distinct affective states. These differences manifest themselves in subjective experience as well as neural and psychophysiological correlates. The Diagnostic and Statistical Manual of Mental Disorders (4th ed., text rev.; DSM-IV-TR; American Psychiatric Association, 2000) summarizes all disorders related to excessive fear, worrying, anxiety and panic attacks into 'anxiety disorders', while there are no 'fear disorders', originating from the classification that fear describes an adaptive behavior, and anxiety a maladaptive state (Blanchard & Blanchard, 2008). However, with the definition of fear as a phasic reaction in response to a specific aversive stimulus, disorders like phobias are much closer linked to fear than anxiety. Anxiety, on the other hand, is a sustained response, characterized by a chronic apprehension of more ambiguous, threatening events without any clear evidence of real occurrence. Anxiety disorders that have excessive worrying as a key characteristic, such as generalized anxiety disorder (GAD), panic disorder (PD), and posttraumatic stress disorder (PTSD), are commonly associated with sustained anxiety. Hereby, the exposure to unpredictable and uncontrollable events represents an important factor for the development and maintenance of these anxiety disorders (Mineka & Zinbarg, 2006).

With a lifetime prevalence of 14.7 % in the EU being the second most common mental disorder in Europe (Alonso & Lepine, 2007), anxiety disorders are a fundamental strain on our health care system, our society, and for each suffering patient. This highlights the importance of research that helps to shed more light on the etiology of anxiety disorders. Researchers agree on the important role of associative learning processes to understand anxiety disorders. Mechanisms of fear learning on the behavioral and neural level have already been the focus of extensive research. However, more research considering the interplay of cue conditioning and contexts benefits this understanding and complements the learning approach towards anxiety disorders.

In classical fear conditioning, a cue is paired with an aversive event and subsequently elicits a fear response in the absence of the aversive event (LeDoux, 1995; Pavlov, 1927). At the same time, the enclosing context during conditioning, e.g. the cage in animal research, was found to be involved in and modulates the learning process (Fanselow, 1990). The context creates an environment which is also, although more diffuse, associated with the aversive event. It serves as an occasion setter and, after the learning experience, creates a chronic expectation of the negative event due to the unavailability of any predictive cue. The incapability to predict the threatening event leads to a more sustained anxiety response compared to the phasic fear reaction elicited by a conditioned cue (Baas, Nugent, Lissek, Pine, & Grillon, 2004). The ability to create associations between previously neutral and irrelevant cues and aversive stimuli is an adaptive behavior that is essential for survival for each organism. While some stimuli are inherently aversive and automatically elicit defensive responses, even random, initially fear-irrelevant stimuli can activate the fear system with sufficient environmental input (Öhman & Mineka, 2001). Being in a state of negative apprehension changes the way how organisms perceive their environment. Potential dangers need to be rapidly identified in order to show defensive behavior in time. Therefore, the attention mechanisms towards novel and threatening cues can change when organisms are in a state of anxiety. As faces represent highly relevant stimuli for humans, the influence of a threatening environment on the perception and processing of faces is very helpful in understanding the impact of contextual anxiety.

In this thesis, a theoretical approach to the topic of context conditioning and its ways to measure contextual anxiety experimentally is given. Moreover, I give a conceptual demarcation on fear and anxiety, and discuss their neural and physiological correlates. A paradigm, the NPU-threat test, is introduced as a possibility to compare contextual anxiety and cued fear directly. In the second part of the thesis, three studies are reported, which were conducted in order to examine contextual anxiety in comparison to a safety context on both explicit (i.e. ratings) and implicit measures (i.e. electrocortical oscillations and heart rate). A second focus was set on the processing of novel cue stimuli when perceived in a state of anxiety or safety. Thereby, the processing of faces, in comparison to other non-social objects (Study 1, Chapter 2) and the differential influence of faces containing emotional expressions (Study 2, Chapter 3) were examined under the influence of contextual threat. Study 3 (Chapter 4) applied a previously established paradigm to compare contextual anxiety and cued fear, based on a manipulation of predictability, which was adapted for the current methodological requirements. In the general discussion, the findings of the three studies are discussed and overall conclusions are drawn. Finally, I discuss some limitations and an outlook is given.

1.1 Cued fear conditioning

Cued fear conditioning is one form of classical conditioning (Pavlov, 1927). Thereby, one previously neutral stimulus is associated with an aversive event (unconditioned stimulus; US), such as an electric shock, which leads to a fear response in the animal. The neutral stimulus is learned to be associated with the threat, becomes a conditioned stimulus (CS), and elicits a fear response (conditioned response; CR) even in the absence of the US, which is similar to the unconditioned response. In animal research, this could mean that a single light serves as CS and is paired with an electric shock, serving as US. After some pairings of the light and the shock, the light alone leads to a freezing response in the animal, and thereby signals fear.

In experimental settings, differential fear conditioning is often applied in order to compare a cued fear response with an uncued stimulus. Instead of a single CS, two neutral stimuli serve as CS during the learning process. Only one of these stimuli is paired with the US (CS+), while the second stimulus remains unpaired (CS-). In order to create associative learning between CS+ and US, the US needs to be presented together or shortly after the CS+, hence becoming a predictor of the aversive event. Thus, the CS+ serves as a predictor to signal the threat occurrence and elicits a fear response, while the unpaired CS- signals safety and does not prompt any fear reaction (Grillon, 2002b; Seligman & Binik, 1977).

Extinction processes occur when the CS+ is repeatedly presented without the presentation of the US. Instead of erasing the existing associative memory, a new memory trace is formed, which stands in competition with the previously learned experience (Bouton, 2004; Myers & Davis, 2007). During conditioning, not only the respective cue but also the context in which the setting is embedded becomes associated with the aversive experience and has an influence on the fear response (Bouton, 1994). In the following chapter, context conditioning as another form of conditioning is presented.

1.2 Context Conditioning

In learning theory, the concept of a context describes the surrounding environment of a stimulus, meaning the background stimuli in which the learning takes place (Bouton, 2010). Contexts are separable into interoceptive and exteroceptive contexts (Bouton, 2002). While interoceptive contexts comprise internal states of the organism such as drug, hormonal or mood state, the exteroceptive context describes the external setting such as the room and environment in which the learning takes place. Maren, Phan, and Liberzon (2013) list next to spatial, and temporal contexts also cognitive contexts and social and cultural contexts as possible contexts forms. Importantly, contexts form a complex representation of multiple features binding together to create a complex unified compound. The context itself can elicit a certain behavior or evoke certain emotions. Moreover, it signals information about contingencies and supports memory retrieval. And finally, contexts frame the perception and experience of an event by including information of time and creating expectations about future experiences (Bouton, 2010; Maren et al., 2013). One important context characteristic is the long duration (Bouton, 2010). The temporal dimensions of a stimulus may determine the type of response elicited during conditioning (Bouton, 2010; Bouton, Mineka, & Barlow, 2001). Bouton et al. (2001) discuss in their review the consequences of a stimulus signaling proximal occurrence of an US as eliciting fear or panic, while cues which are more distant to an aversive event evoke anxiety.

1.2.1 Paradigms of context conditioning

Initially, context conditioning was observed during fear conditioning, when the context in which the learning experience occurred elicited a freezing response in the rat even without the presence of the CS which signaled actual threat (Blanchard, Fukunaga, & Blanchard, 1976; Fanselow, 1990). Grillon and colleagues implemented a first human context study following previous animal studies using a natural threatening context (e.g. bright light for rodents) (Grillon, Pellowski, Merikangas, & Davis, 1997). For humans, darkness constitutes the equivalent threatening context, which elicits an anxiety response within the threat context. Experimentally, background context conditioning is translatable from animal studies to human studies using virtual reality (Baas et al., 2004). Two rooms serve as experimental contexts. In one context, a CS is paired with a shock, while the other room remains unpaired with the US and therefore served as safety room. After the acquisition trials, defensive physiological responses were observed in the threat context compared to the safe context even in the absence of the CS.

This background context conditioning occurs in addition to the associative learning of the CS-US contingencies, as an external signal that frames the learning experience. Other paradigms implemented explicit context conditioning without any CS presentation but the aversive event occurred unsignaled within the context (Alvarez, Biggs, Chen, Pine, & Grillon, 2008; Andreatta et al., 2015; Glotzbach, Ewald, Andreatta, Pauli, & Mühlberger, 2012). In these studies, the US was presented unpredictably within one context (CTX+) and a second context was not associated with the aversive event and signaled safety (CTX-). Compared to the safety context, enhanced reactions on explicit (ratings), implicit (physiological reactions) levels were observed; additionally the anxiety context was avoided. Next to virtual rooms serving as contexts, other studies used a long lasting visual stimulus or light changes of the experimental room as contextual cues, which also elicited anxiety responses in the threat context compared to the safety context. (Mol, Baas, Johanna M P, Grillon, van Ooijen, & Kenemans, 2007; Shechner, Pelc, Pine, Fox, & Bar-Haim, 2012; Vansteenwegen, Iberico, Vervliet, Marescau, & Hermans, 2008).

The safety signal hypothesis described by Seligman and Binik (1977) serves as a theoretical basis for these findings. According to the safety signal hypothesis, learning the association between a CS and an aversive events leads to the ability to predict the aversive event. Consequently, the absence of the CS is identified as safety periods, during which no US will occur. However, when these predictions cannot be made, and thus the US is unpredictable, the organism is not able to identify any safety periods. The result is an experience of chronic anxiety since it does not know when to expect the aversive event (Seligman & Binik, 1977).

The unpredictability of the aversive event within the context is an essential feature in context conditioning. Thereby, both the uncertainty about whether a cue occurs at all (cue unpredictability) and the temporal unpredictability needs to be included in the anxiety model (Davies & Craske, 2015). Sustained anxiety responses were found for contexts in which the aversive event was not predictable compared to context in which a predictable threat occurred (Vansteenwegen et al., 2008). When no cues signal when to expect the shock, the context itself consequently becomes the best predictor of the aversive event and creates a chronic expectation. This sustained apprehension is referred to as anxiety in comparison to the phasic fear response elicited by predictable threat through cue conditioning. A paradigm combining both cue and context conditioning in order to compare fear and anxiety responses directly is described in Chapter 1.3.

1.2.2 Psychophysiological and neural correlates of sustained anxiety

An advantage of the use of virtual reality is that active behavior within contexts can easily be recorded and measured. Hereby, two natural contexts, such as two office rooms, serve as CTX+ and CTX-. Studies which applied virtual reality in context conditioning paradigms, showed that experiencing threat in a certain context room led to subsequent avoidance of this room (Glotzbach et al., 2012; Grillon, Baas, Cornwell, & Johnson, 2006). Evaluation of the context revealed reports of increased arousal and anxiety during the CTX+, which was also perceived as more unpleasant. Next to the behavioral level, multiple physiological measures of contextual anxiety such as the fear potentiated startle response and skin conductance response (SCR) were obtained. The anxiety context (in the absence of a predicting cue) is related to potentiated startle responses (Baas et al., 2004). The startle response, a defensive eye blink reflex elicited by a loud noise, is known to be potentiated during processing of threatening stimuli and reflects activation of the defense system (Bradley, Codispoti, Cuthbert, & Lang, 2001; Lang, 1995). Again, predictability was the important factor, as a potentiated startle response was found in the unpredictable context compared to a predictable context (Vansteenwegen et al., 2008). Moreover, a larger SCR for the onset of the CTX+ and larger skin conductance level during the threat context were found, showing an initial and sustained response of increased arousal in the threat context (Alvarez et al., 2008).

The neural basis of context conditioning could be examined in various fMRI studies, utilizing the advantages of virtual rooms and different lights as contexts. Although the learning process might appear to be similar between cued fear conditioning and contextual anxiety conditioning as two associative learning processes that change the motivational significance of certain (contextual) stimuli, distinct neural networks with different brain structures are involved. Chapter 1.3.3 will explicitly focus on the differential neural activation of fear and anxiety.

Two key structures in context conditioning, the amygdala and the hippocampus are numerously reported to be activated by threat contexts (Alvarez et al., 2008; Davis, Walker, Miles, & Grillon, 2010; Maren et al., 2013; Marschner, Kalisch, Vervliet, Vansteenwegen, & Büchel, 2008). A context which is associated with an aversive event elicits the fear network around the amygdala similar to fear conditioning (LeDoux, 2000). Fear relevant information, which comprises inherently arousing stimuli as well as fear conditioned cues, are processed on both the subcortical thalamo-amygdala road which allows fast and subconscious processing, and a slower route involving the cortex (LeDoux, 1995). Lesion studies in rats showed impaired cue and context conditioning after amygdala lesions, but hippocampal damage only interfered with contextual learning (Phillips & LeDoux, 1992). This supports the idea of an important role of the hippocampus during context conditioning (Alvarez et al., 2008; Marschner et al., 2008; Phillips & LeDoux, 1992; Rudy, Barrientos, & O'Reilly, 2002). The hippocampus is seen as an essential structure for the representation of temporal-spatial information of a context in both animals and humans (Rudy, 2009). Its role in learning and remembering contexts is shown by lesions of the hippocampus in rodents, which yielded reduced freezing behavior in conditioned contexts (Phillips & LeDoux, 1992; Selden, Everitt, Jarrard, & Robbins, 1991). Importantly, the hippocampus was found to be active during unpredictable compared to predictable threat (Marschner et al., 2008). The importance of the hippocampus could be additionally supported by a study showing a linear positive correlation between hippocampal volume and contextual anxiety (Pohlack et al., 2012). Interestingly, differential neural activation for the initial reaction to a conditioned context and the succeeding sustained response associated with anxiety were observed, with amygdaloid-hippocampal activation as the sustained conditioned response (Andreatta et al., 2015). Taken together, the amygdala seems to play a general role during aversive conditioning showing involvement of the fear network, while the hippocampus plays an additional role during contextual anxiety conditioning.

Another key structure during sustained anxiety is the “extended amygdala”, namely the bed nucleus of the stria terminalis (BNST), which mediates anxiety responses (Alvarez, Chen, Bodurka, Kaplan, & Grillon, 2011; Davis & Shi, 1999; Davis et al., 2010; Sullivan et al., 2004). A detailed discussion of its influence on sustained anxiety in comparison to cued fear is described in Chapter 1.3.3.

A broad neural network is activated during context conditioning next to the already mentioned prominent brain structures, involving the parieto-occipital cortex, anterior cingulate cortex (ACC), insula, medial prefrontal cortex, and inferior and orbito-frontal cortex (Alvarez et al., 2008; Andreatta et al., 2015; Hasler et al., 2007; Marschner et al., 2008). Visual contexts that signaled the occurrence of threat showed increased activity of the parietal cortex, which is interpreted as increased attention to the threat-associated environment by the authors (Armony & Dolan, 2001). An increased activation in the attention network reflected by involvement of extended visual areas, ACC, insula and parietal cortex support the idea of context conditioning as model for anxiety with increased vigilance towards potential, but unpredictable threat (Maren et al., 2013).

In order to extend the previous findings of fMRI studies regarding the processing of contextual anxiety, a measure of electrocortical activation during context conditioning is applied in this thesis with the intention to examine attentional resources drawn by the anxiety context compared to the safety context. The steady-state visually evoked potentials (ssVEPs), recorded by electroencephalogram (EEG), allows us to continuously delineate cortical activation in extended visual areas in response to threat and safety. The ssVEP amplitudes are thought to reflect involvement of attention networks (Moratti, Keil, & Stolarova, 2004), and therefore represent an ideal measure of sustained anxiety. Chapter 1.5 gives an elaborate overview of this method.

1.2.3 Context conditioning as model for anxiety disorders

Trait anxiety, as a marker of risk for anxiety disorders, was previously shown to influence fear conditioned responses. Trait anxiety is defined as a as a general tendency to react negatively to stressful situations (Bados, Gómez-Benito, & Balaguer, 2010; Spielberger, Gorsuch, & Lushene, 1970) and is assessed by the Spielberger’s State-Trait-Anxiety-Inventory (STAI) (Spielberger et al., 1970). Most studies investigating learning differences depending on anxiety levels focused on fear conditioning paradigms. Different influences of trait anxiety were found on the conditioned fear response. While some studies reported that trait anxiety did not have any influence on startle amplitudes, skin conductance response or subjective reports of fear during fear conditioning (Torrents-Rodas et al., 2013), others did indeed show a relationship between fear conditioning and trait anxiety. High trait anxious individuals showed higher fear responses assessed by startle response and distress ratings to the CS- than low anxious individuals did. Moreover, extinction learning were found to be impaired for high anxious individuals (Gazendam, Kamphuis, & Kindt, 2013). These findings are underscored by a recent meta-analysis, which reported a reduced capability to differentiate between the fear related and the safety cue in differential conditioning paradigm, with an increased fear response to the CS- in anxiety patients (Duits et al., 2015). These increased fear responses to the safety stimulus are also discussed as exaggerated stimulus generalization (Haddad, Pritchett, Lissek, & Lau, 2012). The deficient safety learning, together with impaired extinction learning in high anxious individuals as well anxiety patients leads to an increased chronic expectation of the US and therefore generates and maintains sustained anxiety (Baas, van Ooijen, Goudriaan, & Kenemans, 2008; Grillon, 2002a).

There are various studies discussing the importance of conditioning for the development and maintenance of anxiety disorders. In their review, Mineka and Oehlberg (2008) argue for the influence of contextual associations, and the role of unpredictable threat in disorders which are commonly associated with a tonic and more diffuse state of anxiety such as GAD, PTSD and PD. Especially unpredictable threatening events leading to increased contextual anxiety may play a major role in PTSD and PD. Thus, panic attacks perceived as uncontrollable and especially unpredictable by exteroceptive or interoceptive cues lead to a stronger agoraphobic avoidance, which is often found in patients with PD (Bouton et al., 2001; Mineka & Oehlberg, 2008; Mineka & Zinbarg, 2006). Several studies reported increased contextual anxiety to unpredictable events in PTSD and panic patients, while there was no difference found in the fear response to predictable cues (Gorman, 2000; Grillon et al., 2008; Grillon et al., 2009). That is, both patients diagnosed with PTSD and PD exhibited increased potentiated startle responses in a context of anticipatory anxiety compared to healthy controls. In PTSD patients, impaired fear inhibition, as increased startle responses to a safety signal, is additionally discussed as a marker (Jovanovic et al., 2009; Jovanovic et al., 2010). The inability to predict a potential threat (and detect periods of safety) leads to long lasting apprehension and chronic expectations of negative events, which foster the development and maintenance of anxiety disorders such as PTSD, GAD or PD.

One might therefore expect increased anxiety responses to the conditioned context during conditioning for high anxious individuals. Indeed, subjects who failed to learn the association between cue and shock were the ones with higher trait anxiety (Baas et al., 2008). Moreover, an enhanced discrimination between CTX+ and CTX- reflected in faster startle response during acquisition for the threat context was found in high anxious individuals during context conditioning (Glotzbach-Schoon et al., 2013). An increased amygdala activity during the threat context in the extinction phase was related to higher trait anxiety (Barrett & Armony, 2009).

However, so far the influence of trait anxiety on contextual anxiety has not been investigated in a comparable extent to the influence on cue conditioning.

1.3 Cued fear vs. contextual anxiety

1.3.1 Differentiation between fear and anxiety

While there is no consensus on a precise demarcation between the two concepts of fear and anxiety in the relevant research literature, it is now comprehensively acknowledged that fear (phasic) and anxiety (sustained) are different affective states (Sylvers, Lilienfeld, & LaPrairie, 2011). In some works, anxiety is defined as an affective state, which is oriented towards future events and associated with the preparation for possible negative events, while fear is defined as an alarm response in reaction to imminent, present danger (Barlow, 2002). It meets a broad consent when characterizing fear as an aversive reaction to a specific threat stimulus, whereas anxiety as a prolonged vigilance to a diffuse, unspecific threat (Davis & Shi, 1999; Davis et al., 2010; Lang, Davis, & Öhman, 2000; MacLeod & Rutherford, 1992). Especially the factor predictability of upcoming aversive events during anticipation is considered a key feature for the distinction between transient phasic and sustained tonic fear (Davis et al., 2010).

Fear and anxiety are also classified into different stages of the defense cascade model (Blanchard, Yudko, Rodgers, & Blanchard, 1993; Bradley et al., 2001; Davis et al., 2010; Lang et al., 2000). The defense cascade model initially describes defensive behavior as a sequential organization in animals (Fanselow, 1994). According to the defense cascade model, the distance to the source of threat as well as the imminence of threat is relevant for the type of response. Lang, Bradley, and Cuthbert (1997) proposed a similar organization of defensive behavior in humans referring to psychophysiological responses during picture processing (Figure 1).

The defensive behavior system describes three modes: the pre-encounter phase, the post-encounter phase, and the circa-strike event or overt action. During the post-encounter phase, animals as well as humans show an orienting response, with an interplay of both parasympathetic and sympathetic nervous system (Bradley et al., 2001; Cacioppo & Berntson, 1994; Cacioppo, Gardner, & Berntson, 1999). A shrinking distance to a source of threat, however, leads to an immediate mobilization of all resources, which is dominated by activity of the sympathetic nervous system, culminating in a fight-or-flight response (Blanchard, Hynd, Minke, Minemoto, & Blanchard, 2001). While anxiety is linked to the pre- and post-encounter stage of the defense cascade model, fear is associated with the circa-strike zone in which the animal is directly threatened by the predator (Blanchard et al., 1993; Bradley et al., 2001; Davis et al., 2010). The different stages of the defense cascade model are associated with quantitatively and qualitatively different physiological responses, as depicted in Figure 1. According to Bradley et al. (2001), in the post-encounter phase, moderately increased skin conductance and startle response are observed together with a heart rate deceleration. With increasing threat, physiological reactions increase. During the post-encounter phase, physiological responses suggest a hyperalertness, which leads to orient towards the sensory input in a state of anxiety. This hyperalertness helps to process further contextual details in order to receive more information from the environment regarding the threat and to prepare for possible action (Bradley et al., 2001).

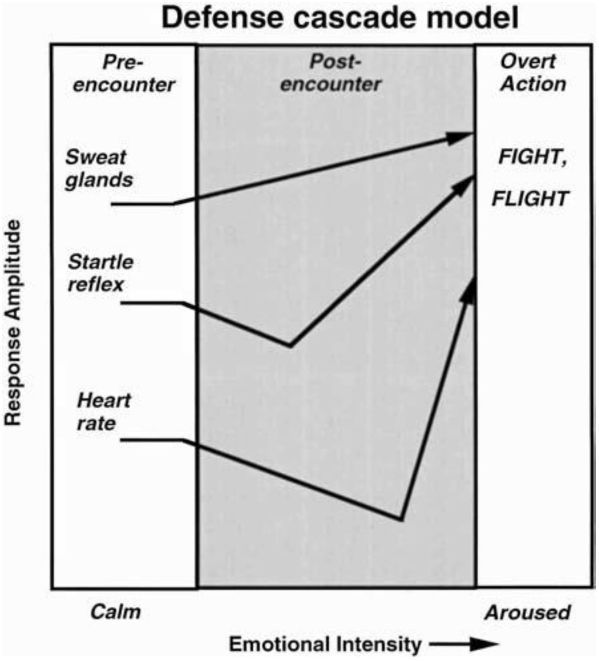

Figure 1: The defense cascade model and the physiological responses associated with the different phases. The autonomic defense reaction is dependent on the imminence of threat. Reprinted and adapted from "Emotion and motivation: Defensive and appetitive reactions in picture processing by M.M. Bradley, 2001, Emotion 1 (3), p.279. Copyright 2001 by the American Psychological Association.

1.3.2 Paradigms comparing fear and anxiety

A prominent study design which allows comparing fear and anxiety on physiological correlates is the threat of the predictable and unpredictable aversive events test (NPU-threat test; Schmitz & Grillon, 2012). This paradigm is based on the idea of fear and anxiety developing due to a different extent of predictability of a threatening event. As mentioned above, the experience of an aversive event that can be predicted by an accompanying cue leads to a phasic fear response. Unpredictable aversive events, on the other hand, are thought to be associated with the embedding context and create a state of sustained anxiety due to the chronic expectation of threat. Next to the nature of threat (predicable vs. unpredictable), a second important demarcation of fear and anxiety is their duration. While fear is considered a phasic response, anxiety is a long-lasting, sustained reaction. These two factors were used as an operational definition to design the NPU-threat test (Schmitz & Grillon, 2012). The NPU-threat test itself is based on three different conditions: a neutral condition without any US occurrence (N), a predictable condition (P), in which an aversive event, e.g. a shock, loud noise, or airblast to the throat is predicted by a cue, and an unpredictable condition (U), in which the US is presented without any predicting cue. Each condition comprises a specific context and a corresponding cue. While the N-condition serves as a control condition, in which the participants experience safety, the P-condition should elicit a phasic fear response, while in the U-condition a sustained anxiety response should be observed. Mostly simple geometric figures serve as cues in the conditions, while the background consists of a simple depiction of the condition (i.e., showing the words “predictable” or “unpredictable” on the screen) and therefore creates a specific context (Grillon, Baas, Pine et al., 2006; Grillon et al., 2008; Grillon, Baas, Lissek, Smith, & Milstein, 2004). However, there are also studies using virtual reality as a possibility to create environments of high external validity (Alvarez et al., 2011; Baas, 2013; Grillon, Baas, Cornwell et al., 2006; Haaker, Lonsdorf, Thanellou, & Kalisch, 2013; Lonsdorf, Haaker, & Kalisch, 2014). In this case, three specific rooms serve as contextual stimuli. In each room, certain cues appear and either predict the occurrence of the US or are presented without any informative character about the aversive event. Participants are informed about the nature of the three conditions beforehand. A fear response is defined as the response to the cue compared to the context in the predictable condition (Pcue > Pcontext), whereas anxiety is represented by the response to the context in the unpredictable compared to the neutral condition (Ucontext > Ncontext) (Schmitz & Grillon, 2012).

1.3.3 Psychophysiological and neural correlates of fear and anxiety

The neural and psychophysiological correlates of sustained anxiety have already been described in Chapter 1.2.2. The following chapter elaborates on the differentiation between the fear and anxiety circuits in the brain and any physiological responses elicited differentially by fear and anxiety as measured by the NPU-threat test.

There is support for distinct neural circuits for predictable and unpredictable threat, with differential involvement of the amygdala vs. the extended part of the amygdala (BNST) during fear and anxiety conditioning (Davis & Shi, 1999; Davis et al., 2010). While the central nucleus of the amygdala (CeA) plays a key role for short termed fear responses, the BNST mediates the sustained responses seen during contextual anxiety (Alvarez et al., 2011; Davis et al., 2010). The role of the amygdala in fear conditioning was highlighted already early in time, supported by findings of amygdala lesions that led to impaired fear conditioning and extinction (Hitchcock & Davis, 1986). On the other hand, sustained anxiety responses in rats, elicited by bright light, were shown to be impaired by inhibition of the BNST, revealing a double dissociation between the involvement of the CeA and the BNST (Davis & Shi, 1999; Walker & Davis, 1997). The different involvement of the CeA and the BNST with their associated circuitries is depicted in Figure 2. Sensory information regarding the fear stimulus enters the basolateral amygdala, which then prominently projects to the medial division of the CeA and subsequently elicits a phasic fear response by targeting hypothalamic and brainstem areas. Regarding contextual information, the basolateral amygdala also projects lightly to the lateral division of the CeA, resulting in strong corticotropin-releasing factor (CRF) containing projections to the BNST, which controls the long-lasting sustained anxiety reaction. The lateral division of the CeA also receives input from cortical regions, suggesting the mediation of the cognitive aspects of apprehension, and the stress-sensitive paraventricular nucleus of the hypothalamus (PVT) (Davis et al., 2010). Both areas project to similar hypothalamic and brain-stem areas, which are involved in specific symptoms and reactions of fear and anxiety (Davis & Shi, 1999).

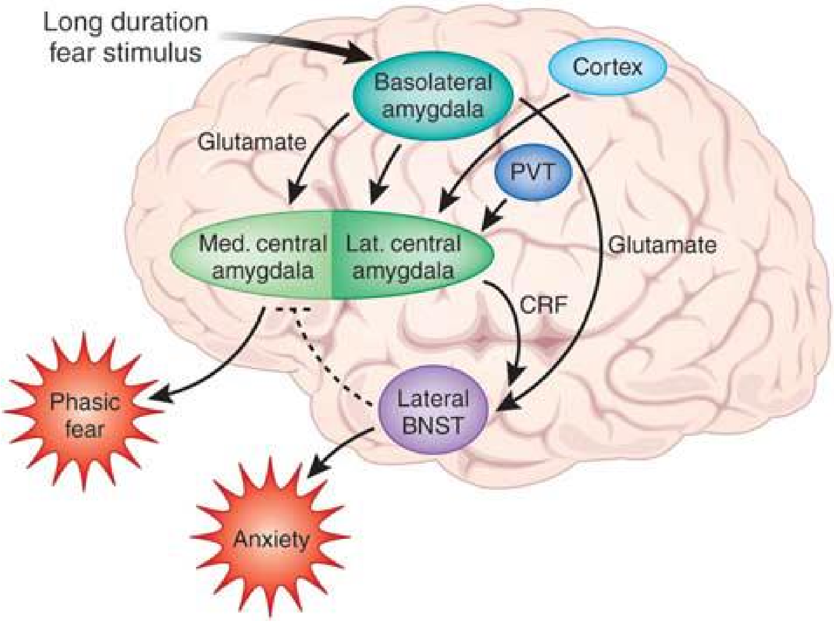

Figure 2: Schematic illustration of the involvement of the central nucleus of the amygdala and the BNST during phasic short and long-term startle responses.
Reprinted by permission from Macmillan Publishers Ltd: Neuropsychopharmacology “Phasic vs Sustained Fear in Rats and Humans: Role of the Extended Amygdala in Fear vs Anxiety” M. Davis, 2010, Neuropsychopharmacology 35 (1), p.119, copyright (2015)

Additionally, a broad network of brain areas is reported to be activated in unpredictable compared to predictable threat (Alvarez et al., 2011; Somerville et al., 2013). While both predictable and unpredictable threat activates the amygdala (as depicted in Figure 2), this is only a transient response to threat (Somerville et al., 2013). As a sustained response, unpredictable threat additionally activates the BNST as well as the anterior insula and a fronto-parietal cortical network associated with hypervigilance. Moreover, unpredictable threat compared to predictable threat lead to activity in the hippocampus, whose important role for context conditioning was already discussed in Chapter 1.2.2 (Alvarez et al., 2011).

As a psychophysiological marker of fear and anxiety, the startle response is assessed during predictable and unpredictable threat. Schmitz and Grillon (2012) define a fear potentiated startle as the difference in the startle magnitude elicited during the cue presentation compared to during context-only presentation within the predictable condition. An anxiety potentiated startle is defined as the difference between startle magnitudes elicited in the context of the unpredictable condition compared to in the context of the neutral condition. The unpredictable compared to the neutral and predictable context in the absence of a predicting cue elicited an anxiety potentiated startle response, while the predictable cue compared to the context of the predictable condition elicited fear potentiated startle response (Grillon, 2008; Grillon et al., 2009; Grillon et al., 2004).

So far, no studies assessed heart rate to predictable and unpredictable threat within one paradigm to obtain a psychophysiological differentiation between fear and anxiety, as it is described in the defense cascade model. The model predicts a heart rate deceleration as orienting response in a state of anxiety during post-encounter phase, whereas imminent threat leads to a heart rate acceleration as marker of phasic fear (Bradley et al., 2001). Moratti et al. (2004) reported a heart rate deceleration in response to longer viewing of unpleasant pictures. As picture viewing is suggested as being analogous to the post-encounter phase (Lang et al., 1997), this finding supports the assumption of a heart rate deceleration during anxiety. Conversely, imminent threat posed by proximity to the participant changed the heart rate response from a deceleration to an accelerative heart rate (Löw, Lang, Smith, & Bradley, 2008), thereby supporting the association of an increased heart rate within the circa-strike event as a psychophysiological marker of fear. Previous studies already confirmed a heart rate acceleration for fear cues following conditioning (Moratti & Keil, 2005).

Support for the concept of two distinct emotional states is also given by pharmacological studies. Anxiety related responses were found to be sensitive to anxiolytic drugs, while fear related response were not (Blanchard et al., 1993). This could be also shown in modulation of the fear potentiated startle within the NPU-threat test, only affecting the responses elicited during the unpredictable threat (Grillon, Baas, Pine et al., 2006).

1.4 Context-dependent stimulus processing

While it is important to understand the processes involved in the acquisition and processing of sustained anxiety, this is just one part contributing to the big picture. A context contains environmental information that sets other cues in relation to each other. So when talking about anxiety contexts and the importance of associative learning processes for the development and maintenance of anxiety disorders, one also has to think of novel stimuli and new experiences that an individual encounters when being that state of anxiety. As discussed in the previous chapters, anxiety can change behavioral and physiological responses and may alter sensory processing, therefore the perception and processing of objects appearing within this context, may be altered as well. Maren et al. (2013) uses the example of an office room which elicits feelings of stress and anxiety in an employee after he was signed off by his boss in this office. Apart from the question of these associative learning processes and how the anxiety response is expressed on different levels, we should also ask ourselves how this employee perceives a novel person, which comes into this negatively associated room and whether it makes a difference if this new person frowns or looks happy. While I aim at examining the associative learning processes during context conditioning and the acquisition of contextual anxiety on a neuronal and physiological level in this thesis, I also focus on this second question regarding the context-dependent processing of novel cues after the acquisition of contextual anxiety.

1.4.1 Faces in context: Generalized and selective hypervigilance

A context forms the predictions of objects which are expected to be found in this certain context and can modulate our attention to these (Bar, 2004). As already criticized by Barrett, Mesquita, and Gendron (2011), in the last decades studies focusing on emotion perception and processing of faces often used context-less, disembodied faces in their designs. However, in real life faces as all other stimuli are embedded in a context and do not appear in isolation. The context surrounding the face has an influence on its perception and processing (Wieser & Brosch, 2012).

The integration of context and face are based on automatic and involuntary processes (Aviezer, Bentin, Dudarev, & Hassin, 2011). The multiple forms of contextual influences are classified in external and internal contexts, similarly to context definitions in learning theories (see Chapter 1.2). In their review, Wieser and Brosch (2012) list within-face features such as gaze or emotional expression but also within-perceiver features which could be a state of anxiety as internal contextual characteristics. External features comprise the visual scenes the face is embedded in. This chapter focuses on threatening or emotionally arousing scenes as contextual information and their influence on the processing of faces conveying emotional expressions. While it is without controversy that contextual information influences the perception and processing of faces, the direction of this influence is still under debate. In general, congruent contexts foster the processing of objects fitting into this context, while unexpected objects reduce and impair the processing (Bar, 2004; Davenport & Potter, 2004; Ganis & Kutas, 2003). Moreover, the neural responses to auditory cues change when perceived in a context associated with threat (Armony & Dolan, 2001).

A general hypervigilance hypothesis promotes the enhanced processing of all facial stimuli when perceived in a threatening environment, irrespective of the emotional load. Studies supporting this claim reported that objects which were presented within emotional contexts were processed more thoroughly than objects perceived in neutral contexts (Smith, Dolan, & Rugg, 2004; Smith, Henson, Dolan, & Rugg, 2004). No differences in the potentiation of the cortical response between emotional and neutral cues were observed when perceived in an acute defense phase; but the original difference in ssVEP amplitudes due to the emotional content was still maintained (Keil et al., 2010). During threat of shock, pleasant, unpleasant, and neutral pictures similarly elicited potentiated startle responses compared to within neutral contexts (Dunning, DelDonno, & Hajcak, 2013). Enhanced reactivity to faces presented in a threatening compared to a neutral context could be also shown in an EEG study (Righart & de Gelder, 2005). Anticipatory anxiety was also found to lead to enhanced early and late event-related potential (ERP) components and increased startle amplitudes for all cues within threat of shock, irrespective of picture valence (Bublatzky, Guerra, Pastor, Schupp, & Vila, 2013; Bublatzky & Schupp, 2012). Both neutral and ambiguous faces elicited enhanced neural responses when presented in a threatening context, consisting of inhalation of stress sweat (Rubin, Botanov, Hajcak, & Mujica-Parodi, 2012).

Likewise, there is evidence showing angry or fearful faces, both transferring threat information, benefit exponentially, and receive enhanced processing compared to neutral faces, when perceived in a threatening context, (selective hypervigilance). Supporting this assumption, although both neutral and emotional faces elicited an augmented N170 in a threat context, this effect was potentiated for fearful faces (Righart & de Gelder, 2005). When encoding emotionally arousing and neutral stimuli in a state of anticipatory anxiety, enhanced neural correlates of memory were only reliably found for emotionally arousing stimuli (Weymar, Bradley, Hamm, & Lang, 2013, 2014). Moreover, enhanced defensive reactions, meaning fear potentiated startle responses, were specifically to fearful but not neutral faces when perceived in a threatening context (Grillon & Charney, 2011). While most studies report increased reactivity due to threatening faces within threatening contexts, avoidance of angry faces as measured by a dot-probe paradigm, when perceived in contextual anxiety could be also found (Shechner, Pelc et al., 2012).

Taken the evidence of context conditioning as an effective paradigm to induce contextual anxiety, this method creates a good opportunity of using anxiety-conditioned contexts to examine the context-dependent processing of faces. This allows using contexts that combine internal and external context features, such as visual features surrounding the faces and anxiety as within-perceiver feature.

1.4.2 Influence of social anxiety on face processing during threat

When tackling the processing of faces in scientific research one always should take social anxiety as a potential influence on face processing into account, as face processing differs between high and low socially anxious individuals (e.g. Mogg & Bradley, 2002). There is a tendency for individuals with higher anxiety levels to show an attentional bias towards threatening stimuli (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007). Specifically, subjects reporting high social anxiety show increased attention allocation towards faces depicting threatening information, such as angry expressions (Garner, Mogg, & Bradley, 2006; Mogg et al., 2000; Mogg & Bradley, 2002). Next to these behavioral evidences, studies also showed increased cortical activation reflecting enhanced attentional resources in response to angry faces in high socially anxious compared to low socially anxious individuals (Wieser, McTeague, & Keil, 2011, 2012). When socially anxious individuals perceive faces in threatening contexts these attention biases can change (Adolph, Meister, & Pause, 2013; Amir et al., 1996; Garner et al., 2006; Helfinstein, White, Bar-Haim, & Fox, 2008; Schwarz, Wieser, Gerdes, Mühlberger, & Pauli, 2013). Enhanced late ERP amplitudes were observed for faces when they were presented in the context of chemosensory anxiety signals for high socially anxious compared to low socially anxious individuals (Adolph et al., 2013). On the other hand, studies using spatial cueing tasks, stroop tasks, or eye tracking showed a reduced bias to faces under situations of stress (Amir et al., 1996; Garner et al., 2006; Helfinstein et al., 2008). The influence of threatening contextual information on attention towards emotional faces was found to be differentially affected for high compared to low socially anxious individuals (Garner et al., 2006). Therefore, social anxiety needs to be considered in the studies that examine context (threat) dependent influence on face processing.

1.5 Steady-state visually evoked potentials

1.5.1 Method

The steady-state visually evoked potential (ssVEP) is a large-scale oscillatory field potential over visual areas assessed by EEG. It is elicited by a stimulus modulated in luminance or contrast (i.e., flickering). The frequency of the oscillatory response equals the one of the driving stimulus (Regan, 1989; Vialatte, Maurice, Dauwels, & Cichocki, 2010). The oscillatory response has the advantage that the signal is exactly defined in frequency as well as time-frequency and can therefore be thoroughly separated from all ongoing noise (Wang, Clementz, & Keil, 2007). Generators of the activity are located in the extended visual cortex together with contributions from the striate cortex and higher order cortices (Di Russo et al., 2007; Müller, Teder, & Hillyard, 1997; Wieser & Keil, 2011). Next to the involvement of lower tiers of the visual cortex, the stimulus driven ssVEP activation also reflects re-entrant, top down modulation of sensory processing indicating higher order processes. Augmented ssVEP amplitudes indicate an increase in attention resources towards stimuli, such as explicitly paying attention to a stimulus (Keil, Gruber, & Müller, 2001). Likewise, high arousing stimuli evoke increased ssVEP amplitudes in a free viewing paradigm (Keil et al., 2003). This facilitated sensory processing due to motivational significance is found to be linearly increased with spatial attention (Keil, Moratti, Sabatinelli, Bradley, & Lang, 2005). No explicit task or event is necessary in order to evoke this sensory amplification of arousing stimuli. It is even found that task irrelevant, but highly arousing stimuli withdraw processing resources away from an explicit task (Müller, Andersen, & Keil, 2008). Source localization identified a fronto-parietal network next to activation in the extrastriate region during arousing picture viewing, which supports the assumption of the involvement attention related networks, interacting with visual emotion processing and leading to facilitated sensory processing (Moratti et al., 2004).

Due to the exact definition of the driving frequency, ssVEPs allow simultaneously presenting multiple stimuli flickering in different frequencies and separating the electrocortical responses to each stimulus from each other (Wang et al., 2007). This method is known as frequency tagging. The evoked responses to simultaneously presented stimuli can be quantified and competition between different stimuli can be tracked (Wieser et al., 2012).

1.5.2 Steady-state VEP amplitudes during conditioning

The amygdala as a crucial structure of emotional expression projects to the visual pathways and simultaneously receives preprocessed sensory information from these networks (Amaral, Behniea, & Kelly, 2003). Cue conditioning studies applying fMRI confirmed the involvement of the visual occipital and temporal areas (Cheng, Knight, Smith, Stein, & Helmstetter, 2003; Hugdahl et al., 1995). The feedback loop leads to an enhanced stimulus processing in the visual system, which is reflected in enhanced ssVEP amplitudes with activation in the occipital areas. This facilitated sensory processing of threat-associated visual stimuli has been described before in cue conditioning (Miskovic & Keil, 2012, 2013a, 2013b; Moratti & Keil, 2009; Moratti, Keil, & Miller, 2006; Song & Keil, 2014; Wieser, Miskovic, Rausch, & Keil, 2014). The threat related sensory amplification, indexed by increased oscillatory occipital responses was reported in EEG and MEG studies (Miskovic & Keil, 2012). Namely, cues predicting the occurrence of an aversive stimulus elicit increased ssVEP amplitudes compared to an unpaired control stimulus (Miskovic & Keil, 2013a; Moratti & Keil, 2005, 2009; Moratti et al., 2006). Thereby, not the expectancy of US occurrence, but the actual associative strength between CS and US is related to the ssVEP response (Moratti & Keil, 2009). Moreover, the timing of the CS-US contingency seems to be of importance as only with temporally contiguous CS-US associations increased oscillatory responses in visual areas were observed, but not when CS und US presentations were temporally separated (Miskovic & Keil, 2013b).

Although the use of ssVEPs as a measure of fear conditioning increased, as far as known no studies applied continuous cortical recordings during context conditioning yet. Particularly in context conditioning, one may assume that a hypervigilance due to the uncertainty of threat is induced (Davis et al., 2010) which should lead to increased sensory processing, reflected by augmented ssVEP amplitudes. While some studies already confirmed the involvement of the visual cortex in the processing of fear conditioned cues (Cheng et al., 2003; Hugdahl et al., 1995), Hasler et al. (2007) also showed an involvement of visual brain areas together with subcortical structures during unpredictable threat. During context conditioning, the context gains motivational significance during the acquisition phase in which one context is paired unpredictably with an aversive noise. Due to the acquired motivational significance of the threat context, it evokes an increased ssVEP signal compared to the safe contextual stimulus, similarly to high arousing pictures and cued stimuli during fear conditioning (Keil et al., 2003).

An advantage of the ssVEPs is the fact of exactly knowing the driving frequency and being able to quantify the ssVEP signal in to a time-varying measure. Therefore, it is a perfectly suited method for tracking sustained anxiety responses such as contextual anxiety. Applying this technique, neural and psychophysiological findings of sustained anxiety are corroborated and extended by adding a continuous neural correlate of anxiety, which provides more information about one essential feature of anxiety as a long-lasting response.

1.6 Goals and main hypotheses of the thesis

In this thesis, context conditioning as a model of anxiety was investigated to understand the change of attention mechanisms between anxiety and safety contexts. It was planned to confirm anxiety as a long-term affective response, in which sustained facilitating processing resources are recruited. Moreover, it aimed at differentiating activations in the psychophysiological correlates between contextual anxiety and cued fear. As a second objective, the thesis aimed at answering the question concerning the influence of contextual anxiety on the processing of social cues and vice versa. Therefore, a newly adapted paradigm of differential context conditioning as a model of sustained anxiety was realized. This paradigm was designed to successfully implement contextual anxiety, as reflected in explicit, i.e. ratings of valence, arousal and anxiety, and implicit measures, i.e. heart rate and ssVEPs.

I hypothesized the following:

(1) A context associated with an aversive event (CTX+) elicits sustained anxiety responses compared to a safety context. This should reveal itself on all assessed response levels throughout the three studies:

   a. On explicit measures, the CTX+ is perceived as more unpleasant, arousing, and anxiogenic than the CTX-.

   b. These conditioning effects manifest themselves in a sensory amplification of the threat context, as indicated by increased ssVEP amplitudes while being in the anxiety context compared to responses in the safety context.

   c. Sustained anxiety is also indicated by a heart rate deceleration as an orienting response in the anxiety context.

   d. Importantly, augmented ssVEP amplitudes and decreased cardiovascular responses show a temporal specificity and are reflected during the whole time the anxiety context is present in order to validate the concept of anxiety as a sustained, tonic response.

   e. Additionally, it was expected that the sensory amplification indicated by heightened electrocortical responses to the CTX+ is not attenuated by simultaneously presented distractor stimuli.

(2) A second research line of this thesis focused on the processing of social cues within a threatening, anxiogenic context compared to a safety environment. First, the idea was to clarify whether social cues, as inherently more relevant to humans, and non-social cues are differentially affected by anxiety contexts (Study 1). In a second step, persons conveying different emotional expressions were presented in both threatening and safe contexts in order to explore the reciprocal influences of contexts and facial cues (Study 2).

   a. As a non-directional hypothesis, I expected either enhanced responses to all stimuli presented within the anxiety contexts, irrespective of the semantic category of the stimuli or emotional facial expression, due to a general hypervigilance during a state of anxiety.

   b. On the other hand, a preferential enhancement to faces compared to objects and angry faces compared to neutral faces might be expected in accordance with a selective hypervigilance.

   c. As social anxiety has an influence on the processing of faces and environmental stress influences this preferential processing, social anxiety was taken into account.

(3) The third study aimed at a demarcation between phasic fear and sustained anxiety as results of cue and context conditioning, by modulating the predictability of the aversive event.

   a. These differences are reflected in time varying cortical responses. Specifically, the context associated with the unpredictable threat elicits long-lasting enhanced ssVEP amplitudes compared to the other contexts, reflecting sustained anxiety. On the other hand, responses to the predictable cue elicits heightened short-term electrocortical responses, indicating cued fear.

   b. Moreover, differential heart rate patterns of fear and anxiety were expected, according to the earlier presented defensive cascade model: During anxiety, i.e. unpredictable threat, which is thought to represent the pre- and post-encounter phase of the model, a deceleration of heart rate is expected. Phasic fear, as the state of ultimate defensive reactions in the circa-strike phase, is associated with a heart rate acceleration.

(4) As described earlier, trait anxiety is a potential influence on associative learning processes, while scientific studies about its influence on context conditioning are still scare. Therefore, trait and state anxiety were assessed in order to examine the influence on contextual anxiety and phasic fear in all three studies. This influence was expected to be represented either by increased anxiety responses to the conditioned context during conditioning or by an impaired safety learning for high anxious individuals.

2 Study 1: Sustained attention in context conditioning

This study has been partly published in the *International Journal of Psychophysiology* (Kastner, Pauli, & Wieser, 2015).

2.1 Introduction

Fear and anxiety are two emotional states, which prepare the organism for adaptive behavior (Davis et al., 2010; Lang, 1995). They help to prioritize cues for further processing, e.g., emotional cues receive more attention than non-threatening cues (LeDoux, 1995). Whereas cue conditioning is supposed to be a good research model for anxiety disorders characterized by phasic fear (e.g., specific phobias), context conditioning is assumed to be relevant for anxiety disorders characterized by sustained anxiety (e.g., PD or PTSD) (Bouton et al., 2001; Craske et al., 2009; Davis et al., 2010; Mineka & Zinbarg, 2006). In human and animal research, paradigms of cue fear conditioning are realized with repeated pairings of an aversive event (unconditioned stimulus; US) with a predictive stimulus (conditioned stimulus; CS+). After few learning trials, the CS+ elicits fear-related responses. In differential cue conditioning paradigms, a second stimulus remains unpaired with the US and therefore becomes a safety signal (CS-).

Importantly, the context in which the conditioning takes place becomes also associated with the aversive stimulus (Davis et al., 2010). Animal studies report a defensive freezing response when rats were placed in the context (e.g. cage) in which they previously received shocks (Rudy et al., 2002). This context needs to be encoded by the animal, and represents then, in the absence of any predicting CS, the best predictor of the aversive US (Fanselow, 1990). Various animal studies examined the importance of context and its role in learning and extinction (Bouton, 1993, 1994; Maren et al., 2013; Rudy, 2009). This context conditioning occurs both during cue conditioning and in the absence of a predictive cue. Compared to cue conditioning which leads to transient fear responses which quickly resolve after removing the CS, context conditioning is used for modeling sustained anxiety associated with a chronic anticipation of the threatening event (Davis et al., 2010; Vansteenwegen et al., 2008). A lack of predictability of the aversive events fosters these effects. When an aversive stimulus is presented unpredictably within a certain context, the whole context later elicits a sustained state of apprehension of an unknown threat (Davis et al., 2010). Similarly, individuals who subjectively fail to learn a CS-US association exhibit more sustained anxiety responses compared to individuals who explicitly learn to predict the aversive events (Baas et al., 2008; Grillon, 2002a). Thus, unpredictable aversive events lead to a sustained state of anxiety and a chronic expectation of the US.

On a neuronal level, fear and anxiety are attributed to different neuronal circuits. The amygdala is considered as a key structure during phasic fear reactions while sustained anxiety responses are additionally mediated by the hippocampus and the BNST (Alvarez et al., 2011; Andreatta et al., 2015; Marschner et al., 2008). Lesions of the hippocampus in rodents were shown to reduce freezing behavior in conditioned contexts (Phillips & LeDoux, 1992; Selden et al., 1991). Studies in humans using a differential context conditioning paradigm also found a greater physiological indicator of an enhanced motivational significance elicited by the threatening context compared to the safe context. Hence the context associated with the US (CTX+) compared to the safe context (CTX-) elicited enhanced startle amplitudes (Baas et al., 2004; Grillon, 2002b; Vansteenwegen et al., 2008) as well as enhanced skin conductance responses (Shechner, Pelc et al., 2012). Additionally, conditioning effects were seen on the explicit level with enhanced fear and arousal ratings for the threat compared to the safety context as well as avoidance of the threat context (Glotzbach et al., 2012; Grillon, Baas, Cornwell et al., 2006). A functional imaging study showed that sustained threat (unpredictability of shocks) increased activity in fronto-parietal areas in concert with visual brain areas, which points toward a hypervigilance while being in the anxiety context (Hasler et al., 2007). Further research on the attentional dynamics of sustained tonic anxiety might be best investigated by using EEG together with steady-state visually evoked potentials methodology since this methods allows continuous tracking of the temporal dynamic of attention allocation (Wieser & Keil, 2011).

Therefore, the present study aimed at assessing continuous visuocortical and psychophysiological responses during context conditioning. To this end, a differential context conditioning paradigm was developed in which pictures of different rooms served as context stimuli (Glotzbach et al., 2012), and steady-state visually evoked potentials (ssVEPs) and heart rate were recorded during the acquisition and the subsequent test phase. The ssVEP, as a large-scale oscillatory field potential in visual areas, which is elicited by a stimulus modulated in luminance or contrast (i.e., flickering), has the advantage that the signal is exactly defined in frequency as well as time-frequency and can therefore be thoroughly separated from all ongoing noise. Moreover, this allows presenting simultaneously multiple stimuli flickering in different frequencies and separating the electrocortical responses to each stimulus from each other (frequency tagging) (Wang et al., 2007). The evoked responses can be quantified and competition between different stimuli can be tracked (Wieser et al., 2012). The stimulus driven ssVEP activation also reflects re-entrant, top down modulation of sensory processing reflecting higher order processes. Augmented ssVEP amplitudes indicate an increase in attention resources towards a stimulus, such as explicitly paying attention to a stimulus (Keil et al., 2001). Emotional arousing pictures evoke higher ssVEP amplitudes compared to neutral pictures in visual cortices (Keil et al., 2010), a similar response can be seen for emotional facial expressions compared to neutral facial expressions (McTeague, Shumen, Wieser, Lang, & Keil, 2011). On the other hand, task irrelevant emotional stimuli capture attention resources and withdraw them from an ongoing detection task (Müller et al., 2008). In sum, this methods seems well suited to track attention allocation, however, until now was not applied to context anxiety conditioning. Previous studies examined attentional processes during cue conditioning only by using oscillatory electrocortical responses registered via EEG or MEG. Results showed increased ssVEP amplitudes for the CS+ compared to CS- revealing enhanced attention resources drawn by the threat cue compared to the safety cue (Miskovic & Keil, 2012, 2013a; Moratti & Keil, 2009; Moratti et al., 2006). Heart rate is seen as one indicator of defensive reactions to stimuli and reflects the threat imminence. Thereby, the state of anxiety is associated with the post-encounter phase within the defense cascade model (Blanchard et al., 1993; Davis et al., 2010). As threat cannot be predicted and appears diffuse in that phase, it leads to a heart rate deceleration indicating an orienting response, before a fight-or-flight response seems necessary, which is associated with a heart rate acceleration (Bradley et al., 2001). Previous studies showed a decelerated heart rate during stimuli signaling threat (Bradley et al., 2001; Bradley, Moulder, & Lang, 2005; Löw et al., 2008).

Additionally, there is the question of how context conditioning as well as the resulting anxiety levels are affected when a novel neutral distractor stimulus is presented within a context. Maren et al. (2013) defines a context as a complex representation consisting of multiple stimuli all binding together. The present study therefore used a complex visual stimulus consisting of multiple features, presented for a longer duration, such that the definition of a context stimulus are fulfilled, and at the same time, the requirements of ssVEP recordings can be met. So far, even contexts with a duration of 8.5 seconds (when compared to short-term presentation times of cues within those) were shown to evoke contextual anxiety (Mol et al., 2007), while the present study presented each context for 20 seconds to make it qualify as a context. Thereby, the unpredictability of the US, as another important factor regarding contextual anxiety as seen in various studies using unpredictable aversive events in an NPU-threat test to elicit sustained anxiety (Grillon, Baas, Cornwell et al., 2006; Schmitz & Grillon, 2012), is also realized.

As in real life, a context actually never stands by itself but always contains multiple, exchangeable cues which might be unrelated to the threat association learned before. Therefore, the novel context conditioning paradigm used in the present study was designed to examine also how novel cues might change processing of the threat and the safety context and vice versa how novel cues are processed in a threatening compared to safe environment. On the one hand, a state of sustained anxiety could lead to a prioritized processing of some stimuli over others (selective hypervigilance). Previous studies already showed that the neural responses to cues changed by the visual context predicting an aversive event (Armony & Dolan, 2001). This assumption is also supported by findings revealing enhanced defensive reactions specifically to fearful faces but not for neutral faces when perceived in a threatening context. (Grillon & Charney, 2011). So far, mainly emotional versus neutral stimuli were studied within threatening contexts. However, it would be interesting to start one step further back and to look at different categories of neutral stimuli first. There is, for example, evidence that faces, which have more evolutionary significance, capture attention more easily than objects (Langton, Law, Burton, & Schweinberger, 2008) and are more frequently and longer looked at compared to non-social stimuli (Garner et al., 2006). Due to their ambiguity, neutral faces might receive enhanced processing when perceived in a threatening environment compared to an irrelevant object. On the other hand, an anxiogenic context might enhance sensitivity indiscriminately to all cues (general hypervigilance). This assumption is supported by findings of enhanced reactivity to cues presented in a threatening compared to a neutral context (Dunning et al., 2013; Righart & de Gelder, 2005). Similarly, anticipatory anxiety was found to lead to enhanced early and later ERP components and increased startle amplitudes for all cues indicating threat, irrespective of picture valence (Bublatzky et al., 2013; Bublatzky & Schupp, 2012). When encoding stimuli in a state of anticipatory anxiety, neural correlates of memory were enhanced during both encoding and recognition phase (Weymar et al., 2013, 2014). Also, both neutral and ambiguous faces elicited enhanced neural responses when presented in a threatening context, consisting of inhalation of stress sweat (Rubin et al., 2012).

The ssVEP amplitude due to the time and frequency logged signal seems perfectly suited to track conditioned responses even over a longer time period. Moreover, frequency tagging – as mentioned above – has ideal requirements to unravel the reciprocal influence between contexts and cues (Miskovic & Keil, 2013a; Wieser & Keil, 2014). Using this method, studies examining faces embedded in a neutral or a threatening context showed enhanced ssVEP amplitudes for the arousing context. Moreover, fearful faces also led to heightened processing of the arousing context (Wieser & Keil, 2014). In contrast to these previous studies, context conditioning was used to create threatening and safe contexts. This allows combining the external features of a visually surrounding context with the within-perceiver feature of sustained anxiety while perceiving novel cues. Additionally, heart rate response allows to track a continuous marker of defensive response mechanisms.

Anxiety is defined as a state of chronic apprehension, with hypervigilance to possible unpredictable future aversive events. As such, in a state of conditioned anxiety through context conditioning, increased attentional resources reflected by increased ssVEP activity, seems a plausible hypothesis. For example, it was demonstrated in a functional imaging study that sustained threat by unpredictability of shocks, increased activity in fronto-parietal areas in concert with visual brain areas, which points toward the proposed hypervigilance (Hasler et al., 2007). Increased ssVEP signal does not only reflect initial visual attention, but involvement of a widespread affective network with re-entrant modulation of visual processing by distributed networks, including neocortical and subcortical structures (Keil et al., 2003). It is expected that during unpredictable threat, the hypervigilance is reflected by prolonged heightened ssVEP signal. An explicit task or specific events are not necessary in order to show this facilitated processing, but freely viewing arousing stimuli already draw to increased attentional resources, even from an ongoing explicit task, when viewing as irrelevant distractor stimuli (Müller et al., 2008). Supporting the expectation of prolonged heightened ssVEP signal, supposedly reflecting sustained anxiety, is the expectation of a tonic, progressive heart rate deceleration associated with hypervigilance during the threat context compared to the safety context. This is supported by the idea of associating the state of anxiety with the post-encounter phase within the defense cascade model (Blanchard et al., 1993; Davis et al., 2010). Previous studies showed a decelerated heart rate during stimuli signaling threat (Bradley et al., 2001; Bradley et al., 2005).

Based on previous studies on cue conditionings (Miskovic & Keil, 2012; Moratti & Keil, 2005) I expected increased ssVEP amplitudes for the threatening context compared to the safe context for the whole context presentation during the acquisition phase. Moreover, this differential processing of contexts should be maintained during the test phase when novel distractors were presented in both contexts (Miskovic & Keil, 2013a). This sustained response should be similarly reflected in a continuous heart rate deceleration. Regarding the responses to distractors, two different results would be possible. On the one hand, responses to distractors might be enhanced when perceived in the threatening context, as they gain affective value and potential relevance to the observer, which would be in line with a general hypervigilance hypothesis. On the other hand, following a selective hypervigilance, the distinction between social and non-social distractor, as mentioned before, with faces being more relevant to us (Darwin, 1872), might lead to that the social cue receives increased attentional resources when perceived in the threat contexts compared to the object. Moreover, the design allows me to examine competition effects between context and distractor processing. In previous studies, task-irrelevant stimuli with high personal significance were found to elicit increased ssVEP amplitudes at the cost of processing simultaneously task-relevant superimposed grating (Wieser et al., 2012). A threatening context should be of similar personal importance and therefore may elicit competition effects when simultaneously presenting a distractor within this context. However, there might be a difference for a social distractor, as this is generally considered as an evolutionary significant stimulus, and therefore diminish competition effects.

2.2 Methods

2.2.1 Participants

Participants were 31 undergraduate students from the University of Würzburg, who received course credits for participation. One participant had to be excluded due to a psychiatric disorder (self-report), resulting in 30 participants (25 females). The participants' age ranged from 18 to 25 years (M = 21.67, SD= 2.0) and all reported normal or corrected-to-normal vision. Included participants did not report any psychiatric or neurological disorder. Before participating, all subjects gave their written informed consent. The study was approved by the ethics committee of the University of Würzburg.

2.2.2 Stimuli and apparatus

Screenshots from two virtual offices used in previous context conditioning studies (Ewald et al., 2014; Glotzbach-Schoon et al., 2013) were used as context stimuli. The virtual environment was created with the Source Engine from the Valve Corporation (Bellevue, USA). The two offices differed in layout, window style, furniture arrangement, and view to outside (Figure 3), but were balanced for luminance and complexity. This was achieved by controlling the luminance values returned by the Image Manipulation Program GIMP 2.8.14 (159.3 for the context with the red carpet and 158.9 for the context with the brown carpet). Moreover, visual inspection aimed at a similar distribution of all single features within both rooms. As a quantitative measure of complexity, entropy for both contexts was calculated with the respective MATLAB function, which revealed comparable values of 6.68 and 6.79. Cortical activation of both contexts and of the distractors did not differ before conditioning, as confirmed in a small pre-study. In this pre-study four subjects were passively viewing both context stimuli for 20 seconds as well as the distractor stimuli for 4 seconds, all presented in both frequencies (12 and 15 Hz). Paired Samples t-test revealed no difference between the two context stimuli ($t[3] = 1.21$, p = .315) and the two distractor stimuli ($t[3] = 0.67$, p = .549).

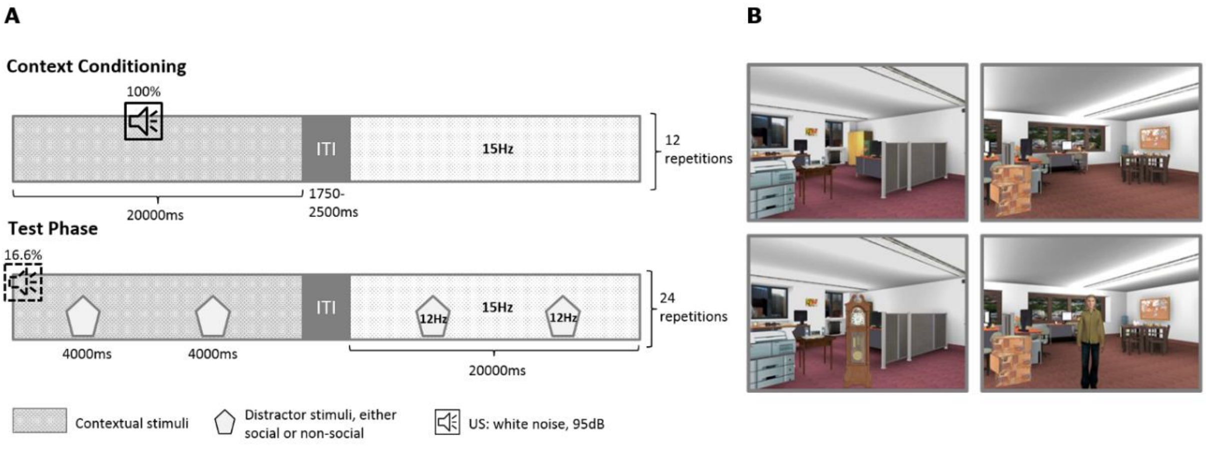

Figure 3: The schematic design of the study for context conditioning and test phase.
(A) Two screenshots from a virtual environment, created with the Source Engine from the Valve Corporation, which were used as context stimuli during both phases, and two stimuli consisting of context and distractor, which appeared during the test phase. All possible combinations of context and distractor were presented within the test phase (B).

A loud startle-probe (white noise) presented at 95 dB for 500 ms served as an unpleasant unconditioned stimulus (US). In the second part of the experiment, two objects of either social or non-social category of the same size, a female person vs. a floor clock, were presented in these context stimuli. Both distractor stimuli were also derived from the same Source Engine as the context stimuli. Stimuli subtended a horizontal visual angle of 33.38° and 4.17° and a vertical visual angle of 23.90° and 13.21° for context stimuli and distractors, respectively. They were presented on a screen with a 60 Hz refresh rate, using the presentation software. The distance between the stimuli and the subjects' eyes was about 100 cm. A white fixation cross was presented in the center of the screen for the whole time. In order to evoke steady-state visually evoked potentials, stimuli were presented in flickering mode in either 12 or 15 Hz for contexts and objects, respectively. This frequency tagging enabled to differentiate between the electrocortical response to the context and the object.

All stimuli were rated for valence, arousal, and anxiety on a 9-point rating scale. Ratings for context stimuli took place at the beginning of the experiment, after the conditioning phase and after the test phase to assess the effect of the conditioning on the explicit level. Ratings for the distractor stimuli, (without the contexts), were taken before test phase (as they did not appear beforehand) and after the test phase, using the same ratings scales as for the context stimuli.

EEG was continuously recorded using a HydroCel Geodesic Sensor Net (Electrical Geodesics, Inc., Eugene, OR) with 128 channels. Data was acquired with the software NetStation on a Macintosh computer. Sampling rate was set at 250 Hz and the vertex sensor (Cz) was used as a reference channel. Data was on-line band pass filtered at 0.1 – 100 Hz. Impedance of each sensor was kept below 50 kΩ as recommended for the Electrical Geodesics high-impedance amplifiers.

2.2.3 Procedure and design

After arriving at the laboratory, participants signed an informed consent form and filled out a sociodemographic questionnaire, as well as the State and Trait Anxiety Inventory (STAI) (Spielberger et al., 1970) and the Social Phobia and Anxiety Inventory (SPAI) (Turner, Beidel, Dancu, & Stanley, 1989) in their German versions (Fydrich, 2002; Laux, Glanzmann, Schaffner, & Spielberger, 1981). After the EEG sensor net was attached, they were seated in a chair in a small, dimly lit chamber where the impedance was checked. Participants received the instructions to simply look at the presented pictures with their eyes focused on the fixation cross. They were also told that from time to time at certain pictures they would hear a loud noise.

The experiment itself consisted of two different parts: a context conditioning phase and a test phase. A schematic sequence is depicted in Figure 3. The context conditioning consisted of an acquisition phase using a differential conditioning paradigm. Participants learned to associate the presentation of one context with the occurrence of the US (CTX+), while the second context was never presented together with the noise and therefore served as a safety context (CTX-). The contexts were presented for 20,000 ms and were always followed by an inter-trial interval, consisting of a gray screen, for 1,750 to 2,500 ms. During the presentation of CTX+, the US was randomly presented once between 1,000 and 12,000 ms after stimulus onset without any predicting cue. All CTX+ presentations were combined with one US presentation, which represents a reinforcement rate of 100 %. Each context was presented for twelve times in randomized order. The context stimuli were presented in flickering mode at either 12 or 15 Hz, counterbalanced across subjects.

In the test phase the processing of distractors was investigated depending on the context and vice versa. Therefore, two distractor stimuli of either social (a person) or objective nature (a floor clock) were presented in the center of each context. Again, the context was presented for 20 sec, and during this time, one distractor appeared twice for 4,000 ms each with an interval of 4,000 to 4,500 ms. In order to maintain the conditioning effect acquired in the first phase, a reinforcement rate of every sixth CTX+ was used. The reinforced trials were excluded from analysis. Using the same frequency mode as in the first part for the contexts for each subject, the distractor stimuli were presented in the respective other frequency, again counterbalanced across subjects. Each context was presented 24 times, always combined with a social or objective distractor twice, resulting in four conditions with 24 trials each. The order of the trials was pseudo-randomized. After each phase, subjects were asked about the perceived contingency of loud noise and both contexts. Participants were told to indicate the likeliness of each context being presented together with the noise on a 10-point scale ranging from 0 % (never associated) to 100 % (always associated).

2.2.4 Data analysis

Rating data

The context stimuli were rated before the context conditioning, after the conditioning and after the test phase for valence, arousal, and anxiety. Statistics were performed for each rating dimension separately in 3 (pre, post conditioning, post test phase) x 2 (CTX+, CTX-) repeated measures ANOVAs. The ratings for the distractor stimuli were analyzed in 2 x 2 repeated measures ANOVAs including the within subject factors of context and phase (before and after the test phase).

EEG data

Data reduction was performed with the MatLab-based software emegs (Peyk, De Cesarei, & Junghöfer, 2011). The continuous EEG data was low-pass filtered at 40 Hz off-line before preprocessing. The event-related epochs were extracted from 600 ms before until 20,600 ms after stimulus onset (context pictures). Artifact detection was performed on the preprocessed data in a two-step method as described in (Junghöfer, Elbert, Tucker, & Rockstroh, 2000). First, contaminated sensors and sensors with artifact activity were identified and rejected. The rejection was based on thresholds for some statistical parameters (maximum absolute amplitude, standard deviation, and gradient). Secondly, sensors with artifact activity were replaced by interpolation on the basis of all remaining sensors. The epochs were averaged separately for each condition. The raw ssVEP signal averaged over all participants and all conditions for a representative electrode (sensor 75, Oz) is depicted in Figure 4, together with the Fast Fourier Transformation on this ssVEP signal, showing the 12 and 15 Hz driving frequencies.

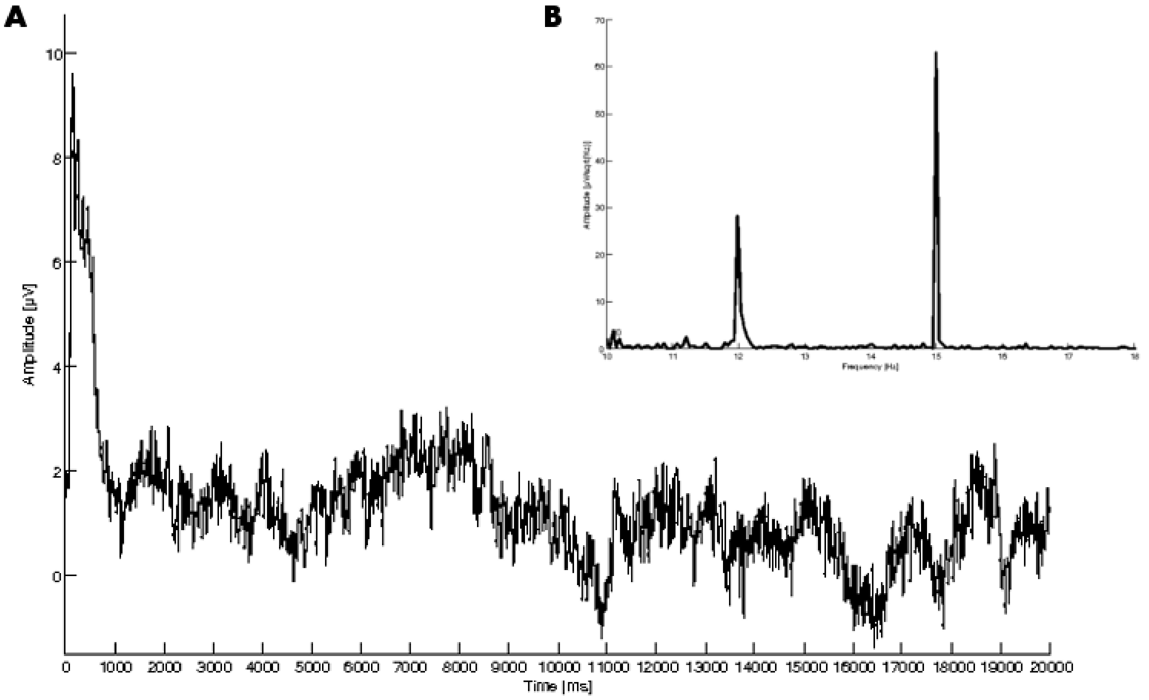

Figure 4: Grand mean and the Fast Fourier Transformation of ssVEP amplitudes.
(A) SsVEP signal was averaged across all participants and all conditions, shown for sensor 75 (Oz); (B) the Fast Fourier Transformation on this ssVEP signal, showing the two driving frequencies of 12 and 15 Hz

Afterwards, a Hilbert transform using a MATLAB script (see Miskovic and Keil (2013a) for detailed description) was performed in order to gain time-varying ssVEP amplitudes in the specified frequency across two clusters of occipital electrodes, where ssVEP activity was maximal (left: 63, 64, 68, 69, 73; right: 88, 89, 94, 95, 99) (see Figure 5 for the sensor configuration). For the analysis, five time windows of 4,000 ms each, dividing the whole trial length of 20 sec, were included. In a second step, a time window of 13,000 to 20,000ms after stimulus onset was chosen. This was done to exclude any possible effect driven by US presentation, as the US was only presented in the first half of context presentation. Due to a higher artifact appearance during the acquisition phase (likely due to US presentation and resulting eye movements), only 25 subjects could be included in the analysis of the conditioning phase.

Statistical analysis was performed using IBM SPSS Statistics 19. In order to assess the effect of conditioning during the acquisition phase, repeated-measures ANOVAs were performed with the within-subject factors context (CTX+, CTX-), occipito-lateral sensor clusters (left, right) and time

(5 time windows a 4,000ms). For the analysis of the test phase, repeated measures ANOVAs were conducted separately for the ssVEP amplitudes in response to the context and ssVEP amplitudes in response to the distractors. In order to assess the conditioning effect in the test phase, a first analysis of the ssVEP amplitudes elicited by the context for the whole 20 sec included the factors context (CTX+, CTX-), distractor (social, objective), and time (5 time intervals a 4,000 ms). In a second step, the mean ssVEP activity was taken from onset to offset of the distractors with a length of 4,000 ms to assess the effect of the distractors within both contexts. The ssVEP amplitudes in response to the distractors were tested as a function of context in a central occipital cluster around sensor Oz (70, 71, 74, 75, 76, 82, 83) (see Figure 5 for the sensor configuration). This centrally located occipital cluster was chosen as the distractor stimuli were presented centrally in the visual field and smaller compared to the context stimuli and therefore elicited a more centrally located electrocortical response. Using these short 4,000 ms trials, repeated measures ANOVAS were calculated for the response of the distractors by using the ssVEP signal elicited by the frequency of the distractors, and including the factors context (CTX+, CTX-), distractor (social, objective) and time (4 time intervals a 1,000 ms). As reinforcement rate was drastically reduced during the test phase, it could be expected that results may be only visible during the first half of the test phase, which was more approximate in time to the acquisition phase. In order to account for this possibility, analysis for distractor associated ssVEPs was also conducted with an additional within-factor part (first half, second half) for the test phase.

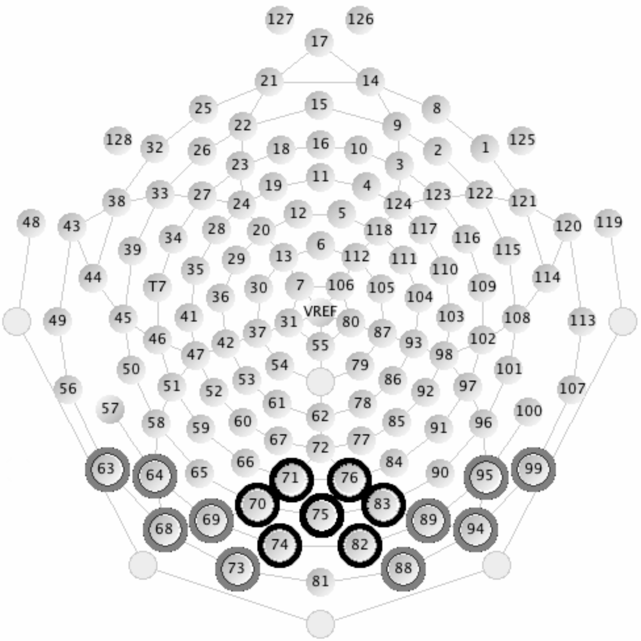

Figure 5: Sensor layout of the HydroCel Geodesic Sensor Net.
Locations of the sensors included in analysis are marked in gray for the ssVEP amplitudes elicited by the context stimuli and in black for the ssVEP amplitudes elicited by the distractor stimuli.

Competition analysis. As an additional analysis, competition indices between contexts and distractors were calculated and statistically compared. Following the competition analysis performed by Wieser et al. (2012), ssVEP amplitudes evoked by contexts and distractors in a time window of 0 to 4,000ms in the occipital sensor cluster were T-transformed across all six conditions for each participants. To obtain the relative cortical activity evoked by the contexts in relation to distractor stimuli, T-transformed ssVEP amplitudes evoked by the context were divided by the sum of T-transformed ssVEP amplitudes evoked by the distractor and context: T(context)/[T(distractor)+T(context)]. While a competition index of > 0.5 reflects a processing bias for the context, an index of < 0.5 shows a signal biased towards the novel distractor. Competition indices were analyzed using repeated measures ANOVA with the within-subjects factors context and distractor.

Heart rate

Processing of heart rate was performed with the software Vision Analyzer 2.0 (Brain Products Inc., Munich, Germany). R-spikes were automatically marked and continuous heart rate was calculated (Koers, Mulder, & van der Veen, 1999). Change scores were achieved by using a baseline of 1,000 ms before stimulus onset.

For the conditioning phase, the whole trial of 20 seconds was included in the analysis and a 2 (CTX+, CTX-) x 10 (10 time windows a 2,000 ms) was conducted. For the test phase, segments of the first 4,000 ms of the trial during context-only presentations and segments of 4,000 ms including the cue presentation were cut out. Repeated measures ANOVAs including the within-factors of context (CTX+, CTX-) and time (4 time windows a 1,000 ms) for the context-only segments and including the within-factors of context (CTX+, CTX-), distractor (social, objective) and time (4 time windows a 1,000 ms) for the cue segments were conducted. Due to bad data quality, two subjects had to be excluded from the analysis for the condition phase (resulting in 27 subjects) and one subject from the analysis for the test phase (resulting in 28 subjects).

In all analyses, the alpha level was set at $p < .05$. If the assumption of sphericity was violated, Greenhouse-Geisser correction was applied for effects involving factors with more than two levels and the Greenhouse-Geisser Epsilon (GG-ε) was reported. Effect sizes were calculated with the partial eta (η_p^2).

2.3 Results

2.3.1 Sample characteristics

The participants filled out the SPAI (Turner et al., 1989), STAI-State, and STAI-Trait (Spielberger et al., 1970) before the experimental phase. The mean social anxiety of the sample was M = 1.93 (SD = 0.79), mean trait anxiety was M = 40.73, (SD = 9.94), and mean state anxiety was M = 37.17 (SD = 5.86).

2.3.2 Rating data

Ratings of the context stimuli were collected before and after the conditioning phase and after the test phase. 2 x 3 repeated measures ANOVAs (within-subject factors context and phase) revealed strong conditioning effects, as illustrated in Figure 6.

Anxiety ratings showed a main effect for context ($F[1, 29] = 30.67$, $p < .001$, $\eta_p^2 = .51$), and a main effect for phase ($F[2, 58] = 30.42$, $p < .001$, $\eta_p^2 = .54$), as well as an interaction ($F[2, 58] = 24.15$, $p < .001$, $\eta_p^2 = .45$). The CTX+ elicited more anxiety after conditioning ($t[29] = 6.73$, $p < .001$), and after the test phase ($t[29] = 7.36$, $p < .001$) compared to pre-conditioning. Anxiety levels for CTX+ were also higher compared to CTX- after the conditioning phase ($t[29] = 4.37$, $p < .001$), and the test phase ($t[29] = 6.09$, $p < .001$), however they did not differ before conditioning ($t[29] = 1.00$, $p = .326$).

Similar patterns were found for ratings for arousal ratings with a significant main effect for context ($F[1, 29] = 27.99$, $p < .001$, $\eta_p^2 = .49$) and phase ($F[2, 58] = 33.48$, $p < .001$, $\eta_p^2 = .54$) as well as an interaction ($F[2, 58] = 21.97$, $p < .001$, $\eta_p^2 = .43$). This indicated increased arousal ratings for the CTX+ after the conditioning phase and the test phase compared to pre-conditioning ($t[29] = 6.41$, $p < .001$ and $t[29] = 8.12$, $p < .001$, respectively). Both contexts did not differ before conditioning ($t[29] = 1.0$, $p = .326$), but after the conditioning ($t[29] = 4.12$, $p < .001$) and after the test phase ($t[29] = 6.04$, $p < .001$).

In terms of valence, subjects rated the CTX+ overall as more unpleasant than the CTX- ($F[1, 29] = 17.11$, $p < .001$, $\eta_p^2 = .37$) and valence ratings were found to change between phases ($F[2, 58] = 12.79$, $p < .001$, $\eta_p^2 = .31$). These main effects were further specified by their interaction ($F[2, 58] = 13.59$, $p < .001$, $\eta_p^2 = .32$, $GG\text{-}\varepsilon = .75$), showing that subjects perceived the CTX+ as more unpleasant after the conditioning phase ($t[29] = 4.63$, $p < .001$) and after the test phase ($t[29] = 6.98$, $p < .001$) compared to pre-conditioning, while the ratings for CTX- did not change between phases. Both contexts differed after the conditioning ($t[29] = 3.33$, $p = .002$) and after the test phase ($t[29] = 4.79$, $p < .001$), but did not differ before conditioning ($t[29] = .72$, $p = .475$).

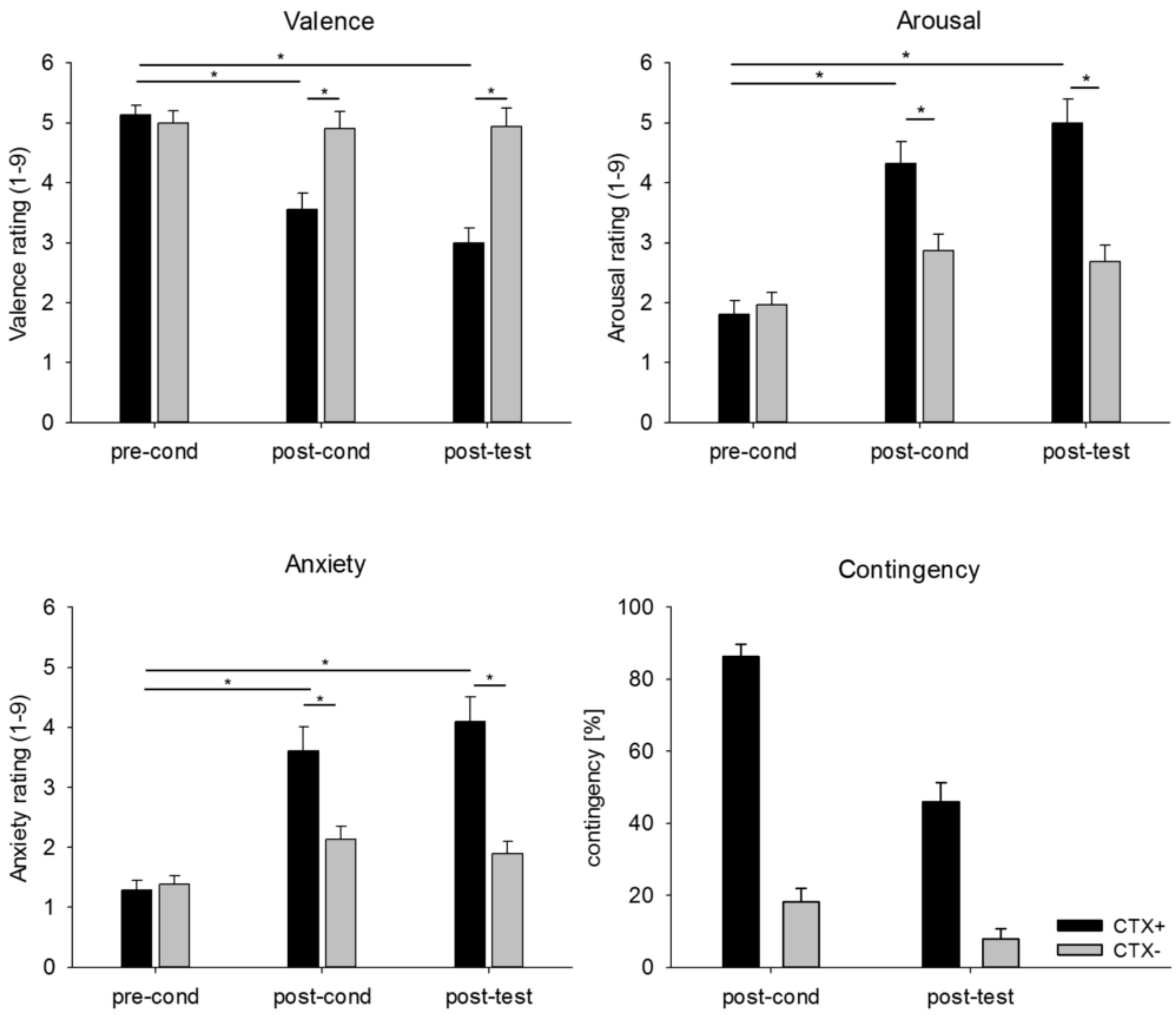

Figure 6: Mean subjective ratings of the anxiety and safety context.
Mean ratings and standard errors of the mean are displayed. Ratings were collected on valence (top left), arousal (top right), and anxiety (bottom left) for the anxiety (CTX+) and safety (CTX-) context before conditioning, after the conditioning phase and after the test phase. Contingency ratings were collected after the conditioning and after the test phase.

Most subjects seemed to be aware of the contingency between US and context. From 30 subjects, 24 reported a perceived probability of 80 to 100 % for the CTX+ being presented with the loud noise (M = 86.33, SD = 18.29), while 18 subjects reported a chance of 20 % or less to hear the US during CTX- presentation (M = 18.33, SD = 19.67).

Ratings for the distractor stimuli were analyzed within 2 x 2 repeated measures ANOVAs (within-subject factors distractor and phase: before and after test phase). Mean ratings are shown in Table 1. For arousal ratings, the main effects of distractor ($F[1, 29] = 5.75$, $p = .023$, $\eta_p^2 = .17$) and phase ($F[1, 29] = 27.99$, $p < .001$, $\eta_p^2 = .49$), indicate that the object distractor was rated as less arousing than the social distractor before the test phase ($t[29] = 3.17$, $p = .004$), but not after the test phase ($t[29] = 1.17$, $p = .252$). Arousal ratings increased after the test phase for both distractors ($t[29] = 4.08$, $p < .001$ and $t[29] = 5.32$, $p < .001$, respectively). However, there was no significant difference in anxiety ratings between the object and the social distractor ($F[1, 29] = 2.83$, $p = .103$, $\eta_p^2 = .09$) but an increase in perceived anxiety from before the test phase to after the test phase ($F[1, 29] = 22.06$, $p < .001$, $\eta_p^2 = .43$) for both types of distractors ($t[29] = 3.94$, $p < .001$ and $t[29] = 4.14$, $p < .001$, respectively). Ratings of valence showed a main effect for distractor ($F[1, 29] = 6.69$, $p = .015$, $\eta_p^2 = .19$) and phase ($F[1, 29] = 16.13$, $p < .001$, $\eta_p^2 = .36$), further explained by an interaction ($F[1, 29] = 9.38$, $p = .005$, $\eta_p^2 = .24$). There was only a significant difference between distractor types before the test phase ($t[29] = 4.06$, $p < .001$) but not after ($t[29] = .66$, $p = .517$) and a general decrease for both distractor types over the phases ($t[29] = 2.80$, $p = .009$ and $t[29] = 4.38$, $p < .001$, respectively).

Table 1: Mean ratings (+ SD) for ratings of social and object distractors before and after test phase.

	Anxiety		**Arousal**		**Valence**	
Phase	Person	Object	Person	Object	Person	Object
pre-conditioning	1.48(1.06)	1.16 (0.37)	2.00 (1.37)	1.39 (0.67)	4.87 (1.15)	5.77 (1.18)
after conditioning	2.71 (1.74)	2.45 (1.67)	3.35 (1.98)	3.03 (1.82)	4.13 (1.48)	4.32 (1.14)

2.3.3 EEG data

Conditioning Phase

Figure 7 depicts the scalp topography of ssVEP amplitude to the threat and the safe context across the 20 seconds context presentation. For the mean ssVEP amplitudes across the 20 seconds context presentation, there was a significant main effect for context ($F[1, 24] = 6.69$, $p = .016$, $\eta_p^2 = .22$) and time ($F[4, 96] = 3.48$, $p = .025$, $\eta_p^2 = .13$, $GG\text{-}\varepsilon = .67$), indicating enhanced ssVEP amplitudes for the threat context compared to the safe context and a significant increase in ssVEP signal from the first to the second time window (0 - 4,000ms) ($t[24] = 3.27$, $p = .003$), from the first to the fourth time window ($t[24] = 2.44$, $p = .022$), and a significant decrease from the second to the third time window ($t[24] = 3.07$, $p = .005$).

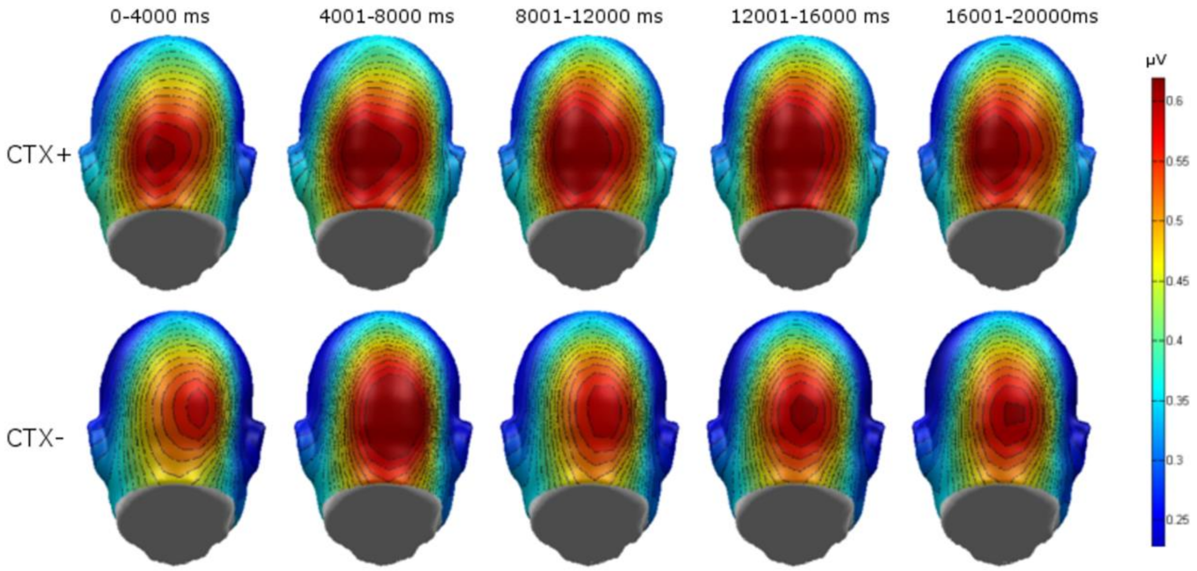

Figure 7: Scalp topographies of ssVEP amplitudes to CTX+ and CTX- during conditioning
Grand average scalp topographies of ssVEP amplitudes to CTX+ and CTX- during conditioning over the whole context presentation time of 20 seconds, divided into five time windows.

In order to exclude artifacts contamination of the overall ssVEP amplitude, a separate analysis was conducted, only using a time window in which the US was never presented (from 13 to 20 seconds after stimulus onset). Figure 8 shows the grand average scalp topography of ssVEP amplitude to the threat and the safe context separately. Repeated-measures ANOVA including the factor context and cluster side confirmed the main effect for context ($F[1, 24] = 7.68$, $p = .011$, $\eta_p^2 = .24$), while cluster side was not significant ($F[1, 24] = .423$, $p = .522$, $\eta_p^2 = .02$).

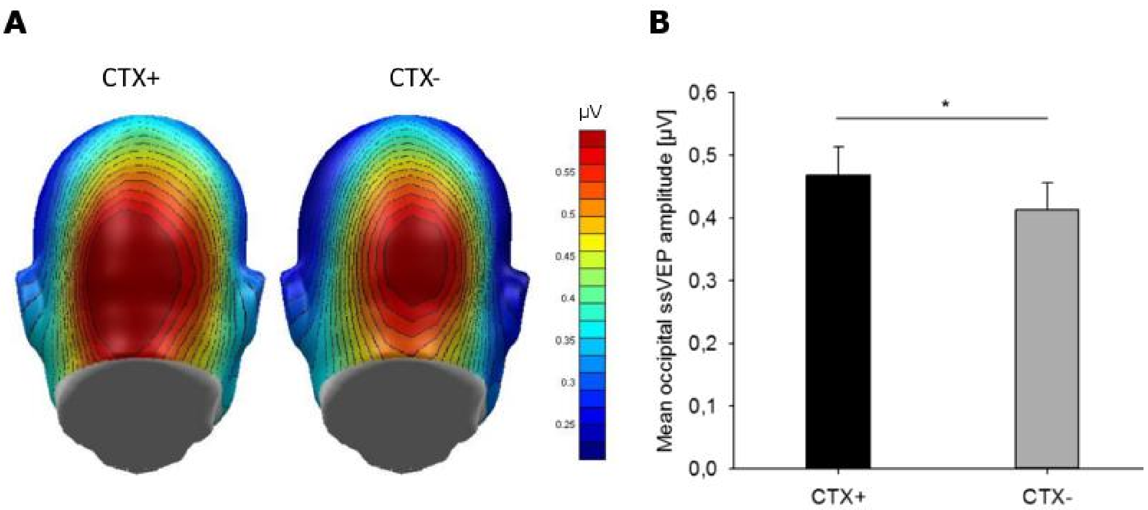

Figure 8: Scalp topography and grand mean of ssVEP amplitudes to CTX+ and CTX- for the time window without any US presentation.
(A) Grand average scalp topography and (B) mean ssVEP amplitude and standard error of the mean in occipito-lateral sensor region during conditioning for the time window without any US presentation (13,000 to 20,000) ms after stimulus onset in response to CTX+ and CTX-

Test phase

Context effects. A repeated-measures ANOVA (2 x 2 x 5) was conducted for the mean ssVEP amplitude across the whole 20 seconds during context presentation with the factors context, distractor, and time. The factor laterality was not included in further analysis, as it did not reveal any effects during the conditioning phase. Analysis revealed a main effect for context (F[1, 29] = 5.09, p = .032, η_p^2 = .15) with higher amplitudes for CTX+ (M = 0.33, SD = 0.13)compared to CTX- (M = 0.30, SD = 0.11), as well as a main effect for time (F[4, 116] = 33.57, p < .001, η_p^2 = .54, *GG-ε* = .30). Post-hoc tests showed a significant decrease in ssVEP signal from the first to the second time window (t[29] = 6.34, p < .001) as well as to all following time windows (t[29] = 6.01, p < .001, t[29] = 6.01, p < .001, t[29] = 5.70, p < .001, respectively). Moreover, a significant increase followed from the second (4,000 - 8,000ms) to the third (8,000 - 12,000ms) time window (t[29] = 2.74, p = .010) and from the fourth (12,000 - 16,000ms) to the fifth (16,000 - 20,000ms) time window (t[29] = 3.96, p < .001), while there was an amplitude decrease from the second to the fourth window (t[29] = 2.29, p = .030) and from the third to the fourth time window (t[29] = 3.27, p = .003). However, there was no context by distractor interaction (F[1, 29] = .32, p = .574, η_p^2 = .01) indicating that processing of contexts was not influenced by distractors.

Cue effects. The ssVEP amplitudes triggered by the distractors showed a main effect for distractor (F[1, 29] = 13.91, p = .001, η_p^2 = .32), and time (F[3, 87] = 4.59, p = .014, η_p^2 = .14, GG-ε = .68), but there was no influence of context (F[1, 29] = 1.50, p = .23, η_p^2 = .05). When repeating the analysis with the within-factor part (first half, second half), next to the main effect of distractor (F[1, 29] =13.59, p = .001, η_p^2 = .32) and time (F[3, 87] = 3.20, p = .027, η_p^2 = .14, GG-ε = .59), a marginally significant three-way interaction of context, distractor and part (F[1, 29] = 3.89, p = .058, η_p^2 = .12) was found, as well as an interaction of context, part and time (F[3, 87] = 7.19, p < .001, η_p^2 = .12). Looking only at the first half of the test phase, follow-up analysis revealed increased ssVEP amplitude for distractors in CTX+ compared to CTX- (F[1, 29] = 4.59, p = .041, η_p^2 = .14), and higher amplitudes in response to the object compared to the social distractor (F[1, 29] = 9.29, p = .005, η_p^2 = .24). These main effects were further qualified by a marginal significant interaction for context and distractor (F[1, 29] = 3.39, p = .076, η_p^2 = .11). The ssVEP amplitude in response to the distractor in the threat context relative to the safety context was only enhanced when an object was present (t[29] = 2.59, p = .015) but not when the social distractor was present (t[29] = 0.07, p = .994) (Figure 9). Also, an interaction of context and time (F[3, 87] = 3.92, p = .020, η_p^2 = .12, GG-ε = .76) revealed that differentiation between the threat and the safe context in the ssVEP response to the distractors, independent of the type of distractor, only started after 1,000ms after stimulus onset and lasted for the remaining 3 seconds (t[29] = 1.07, p = .292; t[29] = 2.07, p = .048; t[29] = 2.75, p = .01; t[29] = 2.10, p = .045 for 0-1s, 1-2s, 2-3s, 3-4s time interval, respectively). When looking at the second half of the test phase, only the main effect distractor (F[1, 29] = 10.38, p = .003, η_p^2 = .26) remained, while the interaction of context and cue did not return significant (F[1, 29] = 0.11, p = .748, η_p^2 = .004). An interaction of context and time (F[3, 87] = 4.07, p = .009, η_p^2 = .12) revealed a marginally significant differentiation between threat and safe context in the ssVEP response to the distractor in the first two seconds, but not in the following two seconds (t[29] = 1.91, p = .066; t[29] = 1.95, p = .061; t[29] = 1.16, p = .255; t[29] = 0.87, p = .393 for 0-1s, 1-2s, 2-3s, 3-4s time interval, respectively).

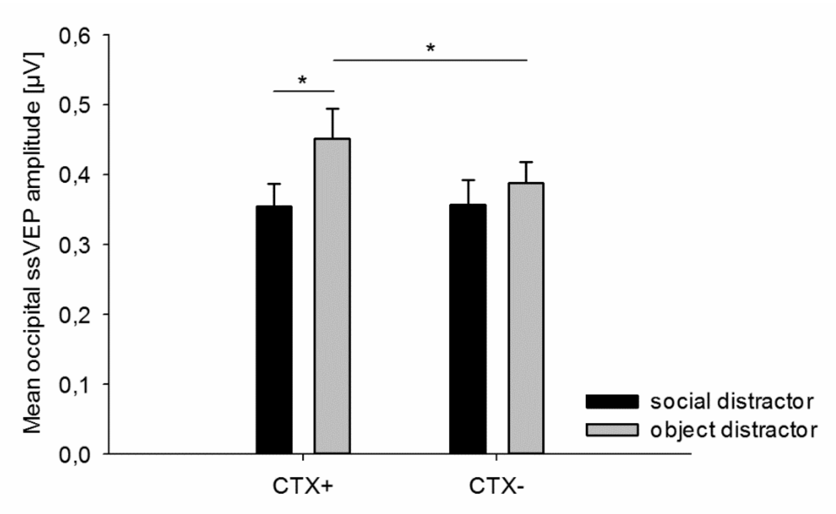

Figure 9: Mean ssVEP amplitudes in response to the distractors.
Mean ssVEP amplitudes and standard errors of the mean for the first half of the test phase in occipital region in response to the distractor, depending on the context the distractor was presented in.

Influence of awareness

In order to control for the influence of awareness on cortical activation an additional analysis was performed only including aware subjects. All subjects were excluded who did not reported a perceived contingency of 80 % or more for the CTX+ and 20 % contingency or less for the CTX- during the conditioning phase, resulting in 17 remaining subjects (13 subjects during the conditioning phase). Concerning the results during conditioning phase for the mean ssVEP amplitudes across the 20 seconds context, the main effect for context (F[1, 12] = 1.76, p = .210, η_p^2 = .13) did not return significant anymore. The same was true when only looking at the later US-free time window (F[1, 12] = 1.85, p = .199, η_p^2 = .13). Regarding the test phase, separate analysis for the ssVEP signal elicited by the frequency of the context and the ssVEP signal elicited by the frequency of the distractors were performed. For the mean ssVEP amplitude of the context, across the whole 20 seconds during context presentation, with the factors context, distractor and time, no significant context main effect was found anymore (F[1, 16] = 2.64, p = .124, η_p^2 = .14). The distractor main effect effects for the analysis regarding the amplitudes elicited by the distractors was still found, but test values decreased in significance (F[1, 16] = 5.55, p = .032, η_p^2 = .26). As a conclusion, all context effects were found to miss statistical significance and the distractor effect was diminished when excluding all subjectively unaware subjects. However, we assume that this may not actually due to the awareness, but it could rather be attributed to the decreased power of the analysis, as sample size was nearly cut in half.

Correlations between ratings and electrocortical activity

Here, we analyzed the association between the cortical activation to both contexts during the conditioning and the test phase and the ratings of these contexts. For the conditioning phase, a significant positive correlation between ssVEP activation in response to the CTX+ and the anxiety rating was found (r = .417, p = .038), revealing a stronger cortical response to the threatening context when rated as more anxiogenic. In the test phase, subjects with higher anxiety ratings in the CTX+ showed reduced ssVEP activation in response to both distractors presented in the CTX+ (r = -.372, p = .043), while the correlation between the ssVEP activation in response to the CTX+ and the anxiety ratings was not significant any more. In other words, the more anxiogenic the threat context, the less processing resources were devoted to distractors presented in this context.

Competition analysis

The analysis of the competition indices, which report the relative ssVEP signal elicited by the distractor compared to the overall signal from context and distractor, revealed a significant main effect of distractor (F[1, 29] = 12.31, p = .001, η_p^2 = .30). The ssVEP signal showed a stronger bias towards both contexts at the cost of the social distractor (M = 0.56, SD = 0.06) but no competition effects during object presentation (M = 0.51, SD = 0.07).

2.3.4 Heart rate

Conditioning phase

The CTX+ was found to elicit an increased heart rate response compared to the CTX- (F[1, 27] = 6.95, p = .014, η_p^2 = .21). Moreover, an interaction of context and time was found

(F[9, 243] = 5.38, $p < .001$, $\eta_p^2 = .17$, GG-$\varepsilon = .46$), showing a marginal difference between the two contexts between 6,000 - 8,000 ms (t[27] = 1.76, $p = .089$) and 8,000 - 10,000 ms (t[27] = 1.93, $p = .064$) and a subsequently significantly increased heart rate for the following time windows (ts > 2.58, ps < .016). Within the threat context, a marginal deceleration at the first two seconds (t[27] = 1.92, $p = .065$) was followed by a significant acceleration between 8,000 to 12,000 ms (t[27] = 2.29, $p = .030$) and another deceleration between 14,000 and 18,000 ms (t[27] = 2.51, $p = .018$). Within the safety context, only significant decrease of the heart rate was found between 8,000 and 12,000 ms (t[27] = 2.62, $p = .014$). The mean heart rate for CTX+ and CTX- across the conditioning trials is depicted in Figure 10A.

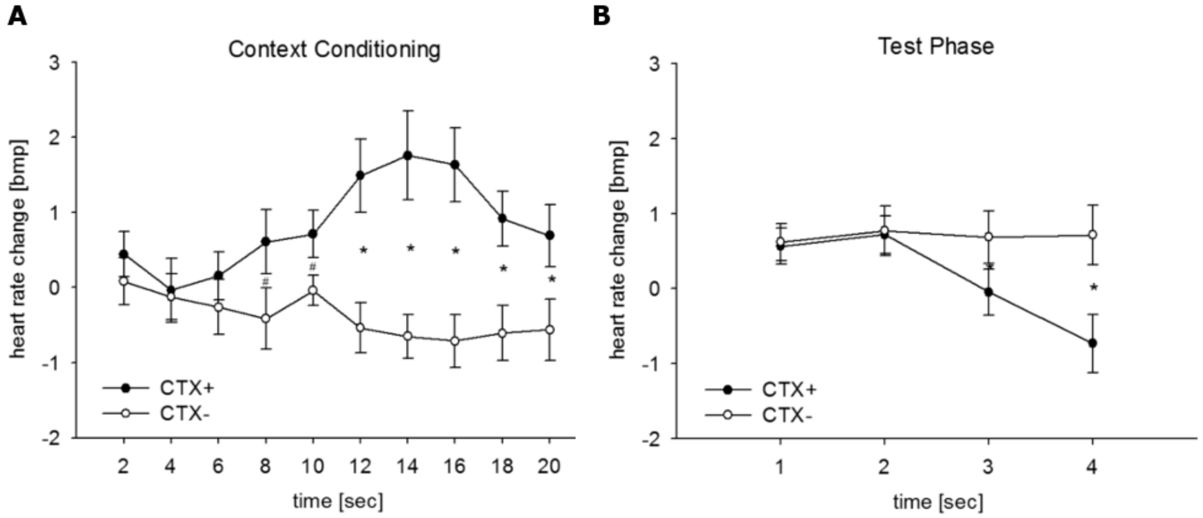

Figure 10: Heart rate changes for CTX+ and CTX- during the conditioning and the test phase.
(A) Averaged heart rate changes during context conditioning averaged over 2,000 ms time windows.
(B) Averaged heart rate changes during the test phase for the first 4,000 ms of each trial, without any distractor presentation.

Test phase

Analysis of the first 4 seconds of each trial revealed a main effect of context (F[1, 28] = 4.90, $p = .035$, $\eta_p^2 = .15$), a marginal main effect of time (F[3, 84] = 2.78, $p = .085$, $\eta_p^2 = .10$, GG-$\varepsilon = .52$) as well a significant interaction of these two factors (F[3, 84] = 7.00, $p = .005$, $\eta_p^2 = .19$, GG-$\varepsilon = .53$) (Figure 10B). Post-hoc tests showed a decreased heart rate during the CTX+ compared to the CTX- starting after 2 seconds for the following two seconds (t[28] = 1.97, $p = .059$; t[28] = 3.47, $p = .002$). The deceleration of the CTX+ started after the first second from 1,000 to 2,0000 ms and was maintained between all following time windows until 4,000 ms ($t[28] = 2.17$, $p = .039$; $t[28] = 2.93$, $p = .007$). The heart rate in response to the CTX- did not change between the successive time windows (ts < 0.72, ps $> .476$). During the cue presentation, neither the factor context ($F[1, 28] = 0.35$, $p = .559$, $\eta_p^2 = .01$) nor distractor ($F[1, 28] = 0.76$, $p = .39$, $\eta_p^2 = .03$) nor time ($F[3, 84] = 1.64$, $p = .212$, $\eta_p^2 = .05$, $GG\text{-}\varepsilon = .62$) returned any significant effect.

Additionally, the relationship between differential heart rate change to the anxiety context and the differential cortical response between CTX+ and CTX- were examined. However, no significant correlations were found ($r < .28$, $p > .147$).

2.3.5 Influence of anxiety

Ratings

No influence of anxiety (trait and state anxiety, social anxiety) was found in the differential ratings of CTX+ and CTX- after the conditioning phase and after the test phase (rs $< .24$, ps $> .201$).

EEG data

The differential ssVEP amplitudes between the threat and the safe context did not show any correlation with participants' anxiety level during conditioning or test phase (rs $< .24$, ps $> .212$).

For the electrocortical activation elicited during the distractor presentation, several significant correlations with participants' anxiety were found. With higher trait anxiety the difference between CTX+ and CTX-of the ssVEP elicited by the context when a social distractor was present decreased ($r = -.41$, $p = .025$). The ssVEP amplitudes elicited by the cue showed a similar relationship with trait anxiety with a negative correlation between CTX+ and CTX- when the social distractor was present ($r = -.36$, $p = .049$) and when the object was present ($r = -.38$, $p = .041$). A similar pattern evolved for the influence of social anxiety with decreasing ssVEP difference between the contexts elicited only by the social distractor ($r = -.38$, $p = .037$) (Figure 11A).

Heart rate

No correlation between trait or state anxiety and heart rate response during the context conditioning was found (*r*s < .06, *p* > .77). A negative correlation was observed between state anxiety and the differential heart rate between CTX+ and CTX- in the test phase during distractor presentation only for the social distractor (*r* = -.40, *p* = .031) but not for the object. A similar correlation was found for social anxiety (*r* = -.48, *p* = .008) (Figure 11B).

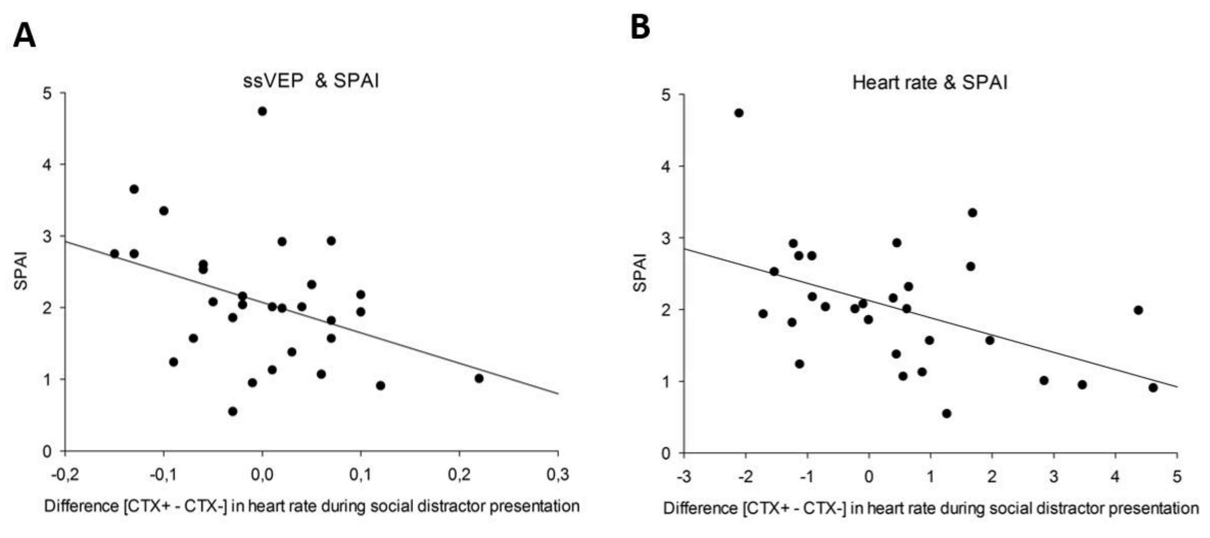

Figure 11: Scatterplots depicting the correlation between social anxiety measured by the SPAI and conditioning responses.
(A) Correlation between social anxiety and differential ssVEP activation; (B) correlation between social anxiety and differential heart rate change of the contexts during the social distractor presentation.

2.4 Discussion

In the present study a differential context conditioning paradigm was realized, in which sustained cortical activations to the threatening and the safe context were assessed. In addition, it was investigated how novel neutral distractors presented within contexts were processed and how they influenced the processing of these contexts. Two pictures of different office rooms served as contexts, pictures of an object and a person served as distractor stimuli. In order to measure continuous electrocortical responses during context conditioning and assessing the reciprocal influence of distractors and context, stimuli were presented in flickering mode to evoke ssVEPs. After repeated pairings of one of the contexts with an unpleasant loud noise in the absence of a predictive cue, this context gained threatening value and induced anxiety. The change in context processing due to conditioning was reflected on the explicit level in differential ratings of anxiety, valence, and arousal, and on the implicit level in differential electrocortical activations.

Most important, ssVEP amplitudes were increased for the CTX+ compared to the CTX- during the conditioning phase for the whole presentation time of the context. These new findings revealed sustained cortical activations related to context conditioning and corroborates and extends previous cue conditioning studies showing increased ssVEP amplitudes in response to threat conditioned cues (Miskovic & Keil, 2013a; Moratti & Keil, 2005, 2009) or in response to inherently emotionally arousing pictures (Keil et al., 2010). Heightened ssVEP amplitudes indicate cortical facilitation and are also seen during cued shifts of spatial attention (Morgan, Hansen, & Hillyard, 1996; Müller, Teder-Sälejärvi, & Hillyard, 1998), possibly reflecting attention mechanisms facilitating discriminative processing of stimuli (Müller & Hillyard, 2000). During conditioning, two stimuli are learned to be discriminated in order to identify the relevant context. This is reflected in increased recruitment of lower-tier visual cortical regions during presentation of the threat context (Miskovic & Keil, 2012), accompanied by the engagement of relevant cortical networks including the amygdala and frontal cortices, which engage in a re-entrant modulation of sensory cortical networks (Keil et al., 2009; Sabatinelli, Lang, Bradley, Costa, & Keil, 2009). This re-entrant amplification, resulting in facilitation of sensory processing of the threat context is reflected by increased ssVEP amplitudes for the threat context as shown in the present study. I could also show that this facilitation is sustained over the whole time of context. Consequently, the learning history of individuals, associated with the subjective evaluation of the context stimulus, shapes the processing of all incoming information and amplifies the context, which gained high motivational significance. The specific finding in this study, that this enhanced processing is seen even over a longer period of time, reflects a state of hypervigilance during threat, similarly to a study by Michalowski et al. (2009), in which subjects with spider phobia showed a state of hypervigilance just due to knowledge of fear relevant pictures being presented.

Further analysis revealed that neutral distractor stimuli presented within the anxiety or the safe context did not attenuate the ssVEP context conditioning effects. This result again extends findings of cue to context conditioning. Specifically, previous cue conditioning research also observed that a simultaneously presented novel stimulus does not dampen fear responses to a threat cue (Jovanovic et al., 2005; Miskovic & Keil, 2013a), while a safety signal presented together with a threat cue does (Jovanovic et al., 2005). It can be concluded that the anxiety-related cortical changes induced by the conditioned contexts are stable over time, supporting the idea that context conditioning reflects a model of sustained anxiety in comparison to the phasic fear reactions induced by cue conditioning (Davis et al., 2010).

Moreover, rating data confirmed the conditioning effect displayed by the differential neural response to the threat versus the safe context with increased anxiety and arousal rating as well as reduced valence ratings for the CTX+ compared to the CTX-, thus replicating previous work from our lab (Ewald et al., 2014; Glotzbach et al., 2012). The explicit negative evaluation of a conditioned context is associated with subsequent avoidance behavior of this threatening environment (Glotzbach et al., 2012). This study extends these findings, revealing a relationship between the reported anxiety experienced in the conditioned context and increased cortical activation. Higher reports of anxiety in the threat context were associated with an amplified neural response elicited by this context. The facilitated sensory processing, i.e., enhanced ssVEP amplitudes, of the threat context may reflect declarative cognitive processes of explicit evaluation of this context stimulus. This interpretation is in line with the idea that motivational relevance of a stimulus changes and facilitates sensory processing of this stimulus (Lang, Bradley, & Cuthbert, 1990).

The motivational significance of the context, acquired by the unpredictability of a potential threat in this context, is reflected in an enhanced cortical response and an acceleration of heart rate, followed by a heart rate deceleration in these context stimuli. The enhanced cortical response is similar for the activation in response to emotionally arousing pictures (Keil et al., 2003) or cues perceived during threat, leading to a mobilization of defense mechanism (Keil et al., 2010).

Accelerated heart rate response in the threat context compared to the safety context was observed. Passive viewing of aversive and highly arousing stimuli is associated with heart rate deceleration, mediated by parasympathetic and sympathetic activation (Bradley et al., 2001). However, when the stimulus reaches an unusual high level of threat, an increased physiological mobilization in order to prepare for action, is seen with a heart rate acceleration mediated by the sympathetic activation (Hamm, Cuthbert, Globisch, & Vaitl, 1997; Löw et al., 2008). Within the test phase, a heart rate deceleration were observed for the anxiety context compared to the safety context. It needs to be mentioned, that the heart rate acceleration observed during conditioning might be driven by the US presentation. However, as the last eight seconds of the anxiety context remained unpaired in each trial but heart rate was still significantly accelerated in these time windows, this restriction might be weakened.

The conditioned context elicited a sustained anticipatory anxiety, due to the unpredictability of the aversive event. Due to the simultaneous presentation of the distractor stimuli and the reduction of US presentation, the threat was probably perceived as more diffuse than in the acquisition phase, resulting in a heart rate pattern from an acceleration to a subsequent deceleration. This deceleration, as it is also described in animal literature as bradycardia, (Campbell, Wood, & McBride, 1997) reflects an orienting response showing increased vigilance (Lang et al., 2000) and is observed in participants viewing unpleasant pictures (Bradley et al., 2001; Moratti et al., 2004).

In both implicit measures (ssVEP and heart rate), a similar pattern was found for the influence of anxiety as personality trait on processing of contextual anxiety. That is, the difference between the threat and safety context diminished with increasing participants' anxiety. This was also prominent for social anxiety but mainly when the social distractor was present. Obviously, a fear relevant cue in social anxiety, e.g. a person, leads to an impaired ability to differentiate between external threat and safety. The impaired safety learning in anxiety patients was described before (Duits et al., 2015; Lissek et al., 2005; Peri, Ben-Shakhar, Orr, & Shalev, 2000). It could now be shown within a context conditioning paradigm and supports a recent finding of reduced discriminative amygdala activation between anxiety and safety context with increasing trait anxiety (Andreatta et al., 2015). Interestingly, in the present study it was only present when a potentially relevant cue – compared to a completely irrelevant object, - was visible within both contexts.

Interestingly, the object (clock) compared to the social distractor (human) received more attentional resources within both contexts. The increased attentional resources the object received compared to the social cue in general and particularly within the threat context could also mean that the clock – nowadays maybe an object less seen – popped out within a modern office. As an oddball, it receives facilitated sensory processing compared to a social cue, which might semantically be expected within an office. In line with the created hypervigilance associated within sustained anxiety, which was induced by the context conditioning, this oddball draws even more attentional resources when perceived in a threat context. This is consistent with findings from an ERP-study, which showed increased attention to oddball stimuli when perceived in a threatening affective context in high state anxious individuals (Mercado, Carretié, Tapia, & Gómez-Jarabo, 2006). Also, anxiety patients showed enhanced amplitudes in response to oddball stimuli, explained by the authors by a higher alertness in anxiety (Bruder et al., 2002). Moreover, this explanation would also support the finding of competition effects found in favor for the context processing when the social cue is present, but an equal distribution of attentional resources when the clock – as potential oddball – is present. An alternative explanation might be a semantic matching, which occurred between the clock and the loud noise and resulted in increased cortical activity elicited by the object compared to the social cue.

Only few participants reported the exact relationship between the aversive sound and contexts. Nevertheless, this did not affect necessarily differential processing of threat and safety contexts. Emotional aversive learning can occur without any conscious representation of the threat (Öhman, Flykt, & Lundqvist, 2000). Moratti and Keil (2009) could show that enhanced processing of conditioned cues actually decreased with increasing expectancy of participants, while the associative strength, meaning the number of pairings of conditioned cue and aversive stimulus, was linearly related. Therefore, limited awareness in participants should not be a concern when mapping conditioned responses using cortical activation.

To sum up, the first study successfully implemented context conditioning and demonstrated facilitated cortical processing of the threat context across the whole viewing time, accompanied by an initial heart rate acceleration to threat, followed by a deceleration, as expected as an anxiety response. This prioritized processing was shown to be immune to distraction by novel neutral objects and accompanied by a heart rate change due to the presence of threat. Moreover, I could show a relationship between increased processing of a threatening context, indicated by the heightened ssVEP amplitude, with the explicit evaluation of the context. One conclusion is that an unpredictable threat shapes further processing of the threat context, and that the resulting facilitated processing is associated with the experience of anxiety within this context.

3 Study 2:Influences of contextual anxiety on face processing

3.1 Introduction

Context conditioning serves as a paradigm to model sustained anxiety responses. Pairings of a previously neutral context with an aversive event leads to increased hypervigilance and a sustained anxiety response. Thereby, the unpredictability of the US is of special importance, which makes the context as its best predictor. Due to the nature of a context as being of long duration (Bouton, 2010), the context associated with the US elicits a chronic expectation of an aversive event and elicits a prolonged anxiety response. In order to compare the response elicited by the anxiety context, a second neutral context is used in differential context conditioning paradigms, which is never associated with the aversive event and therefore serves as a safety context (Glotzbach et al., 2012; Glotzbach-Schoon et al., 2013; Grillon, Baas, Cornwell et al., 2006; Maren et al., 2013). In previous studies, a threat context was found to be perceived as more anxiogenic and arousing and was subsequently avoided (Glotzbach et al., 2012; Grillon, Baas, Cornwell et al., 2006). Sustained anxiety was represented with increased activity in the amygdala and hippocampus during the initial anxiety response as well as during the prolonged anxiety response (Andreatta et al., 2015). In contrast to fear conditioning where an involvement of the amygdala was found contextual anxiety leads to additional activity of the BNST (Alvarez et al., 2011). The involvement of visual brain areas together with subcortical structures during unpredictable threat (Hasler et al., 2007) could be supported by findings of the first study of this thesis, which revealed enhanced ssVEP amplitudes in occipital areas. This oscillatory response was found to be increased for stimuli with high emotionally arousing content (Keil et al., 2010), as well as when explicitly paying attention to a stimulus (Keil et al., 2001). The increased attentional resources facilitated perceptual processing of relevant information. Thus, sustained increased ssVEP amplitudes during the anxiety context reflect a preferential processing of the anxiety context. In the first study, this response was found to be stable over time, supporting the idea that context conditioning reflects a model of sustained anxiety in comparison to the phasic fear reactions induced by cue conditioning (Davis et al., 2010). As to a successful context conditioning paradigm implementing sustained anxiety responses reflected in both cortical activation and heart rate change, the question regarding cue processing within contextual anxiety still remains unclear. Coming back to the example given in the introduction, we still do not know how the employee who was recently fired in his office perceives persons, smiling or frowning, who are coming into his office. A question with more relevance is about the perception of other persons in an anxiety patient in situations of sustained anxiety. The special nature of faces in humans, particularly when conveying emotional expressions could be shown in numerous studies before (Darwin, 1872; Öhman & Dimberg, 1978). Especially angry faces seem to be of special importance, such that conditioned responses to facial stimuli with angry expression show impaired extinction responses (Dimberg, 1987; Öhman & Dimberg, 1978). Moreover, angry faces are easier and faster detectable than faces with happy facial expressions (Fox et al., 2000; Tipples, Atkinson, & Young, 2002). Faces do, however, in real life never appear alone, but are embedded in a contextual setting, consisting of within-face and -sender features but also external features such as the visual scene (Wieser & Brosch, 2012). While congruent contexts foster the processing of objects perceived within these contexts, unexpected contexts lead to a reduced processing of these objects (Bar, 2004; Davenport & Potter, 2004; Ganis & Kutas, 2003).

Previously, objects embedded in emotional contexts showed enhanced processing of all objects compared to objects presented in neutral contexts (Smith, Dolan et al., 2004; Smith, Henson et al., 2004). Both aversive and neutral cues elicited heightened ssVEP amplitudes when perceived in an acute defense phase, while the difference between emotional and neutral cues was still maintained (Keil et al., 2010). This idea of a general hypervigilance in threat contexts is supported by findings of enhanced late positive potentials to both angry and neutral faces when perceived in chemosensory anxiety signals (Rubin et al., 2012). On the other hand, there is evidence that faces showing angry or fearful expression receive particularly enhanced processing when perceived within a threatening context (selective hypervigilance). Especially emotional faces elicited an augmented N170 in a threat context (Righart & de Gelder, 2005). Study 1 showed enhanced processing of objects, which were probably perceived as oddballs compared to the social cues, and the processing was heightened especially in the threat context due to a hypervigilance in contextual anxiety.

The context conditioning paradigm established in Study 1 was used again in Study 2 in order to create a sustained anxiety response. In the acquisition phase, in one of the contexts the aversive sound occurred unpredictably (CTX+), while the second context remained unpaired (CTX-). In the subsequent test phase, distractor stimuli were presented in the anxiety context and the safety context. A female person, showing either a happy, neutral, or an angry facial expression appeared in both contexts. As in the previous study, both the contextual stimuli as well as the distractors were presented in flickering mode in order to evoke an oscillatory response in the visual areas of the brain (Vialatte et al., 2010). The context and distractor stimuli were associated with different frequencies, allowing to differentiate the cortical response to the context and the stimuli in the foreground (Wang et al., 2007).

The study aimed at repeating the findings of the first study with a sustained anxiety response for the anxiety compared to the safety context during context conditioning and the test phase. This should reveal itself in increased ssVEP amplitudes in response to the CTX+ compared to the CTX- for the whole presentation time of the context. Additionally, I expected a heart rate acceleration during the anxiety context, followed by a deceleration in the threat context in the test phase, thereby replicating the findings of the first study. As shown before, this facilitated and sustained processing of the anxiety context should be maintained during the distractor presentation. However, in the second study distractor stimuli of higher motivational significance and higher personal relevance were used relative to the first study and a potential reciprocal influence of emotional stimuli and threat context might be observed. I expected that the contexts influence face perception, possibly with an enhanced sensitivity indiscriminately to all cues (general hypervigilance). Alternatively, an increased activation in response to an angry face when perceived in a threatening context (selective hypervigilance) would be possible. Also, as previously reported of fearful faces heightening ssVEP amplitudes in response to the context (Wieser & Keil, 2014), angry faces similarly might lead to increased context processing. Moreover, social anxiety and trait anxiety might influence the perception of facial expressions within different contexts. The discussion regarding processing of threatening faces in high (socially) anxious individuals is highly controversial: whether it is a hypervigilance towards, or avoidance of these angry faces (Bar-Haim et al., 2007; Shechner, Britton et al., 2012). One potential factor could be the context which the face is perceived in (Garner et al., 2006). That is, either a potentiated processing of an angry face might be seen when perceived in the CTX+ compared to the CTX- for high socially anxious individuals. On the other hand, an increased threat from an external source (e.g. a contextual threat through context conditioning) could lead to an avoidance of the angry face (Shechner, Pelc et al., 2012).

3.2 Methods

3.2.1 Participants

For this study, 30 subjects (16 females) were recruited with the help of an advertisement in a local online platform (www.wuewowas.de). Only participants without any psychiatric or neurological disorder were included in the study. The participants' age ranged between 19 and 35 years (M = 25.53, SD = 4.08). They all reported normal or corrected-to-normal eye vision. Before participating, all subjects gave their written informed consent. The study was approved by the ethics committee of the University of Würzburg.

3.2.2 Stimulus materials and apparatus

Two screenshots of two virtual offices, created with the Source Engine from the Valve Corporation (Bellevue, USA) were used as contextual stimuli. These stimuli, taken from the previous study, differed in layout, furniture arrangement, carpet color, window layout, and view to the outside. As mentioned above, the stimuli were balanced for luminance and complexity. In contrast to the previous study, only social distractors were used in the current paradigm during the test phase, with three different screenshots of a female agent taken from the Source Engine from the Valve

Corporation (Bellevue, USA). The screenshots conveyed the facial expressions happy, neutral, and angry. The distractor stimuli were superimposed on the context, with the face in the center of the screen so that the person was visible up to the waistline and the impression of standing in close distance to the person was created. Stimuli subtended a horizontal visual angle of 33.38° and 6.18° and a vertical visual angle of 23.90° and 11.07° for context stimuli and distractors, respectively. They appeared on a computer screen, with a distance of 100 cm to the participant and a refresh rate of 60 Hz. The contextual stimuli were presented in flickering mode in 12 or 15 Hz, with the superimposed distractor stimulus flickering in the respective other frequency. The frequency tagging was counterbalanced across subjects. A loud startle noise (white noise) presented for 500 ms with 95 dB served as US. A white fixation cross was presented in the center of the screen for the whole time.

EEG was continuously recorded using a HydroCel Geodesic Sensor Net (Electrical Geodesics, Inc., Eugene, OR) with 128 channels. The vertex sensor (Cz) served as a reference channel. Impedance of each sensor was kept below 50 kΩ as recommended for the Electrical Geodesics high-impedance amplifiers. An electrocardiogram with two adhesive Ag/AgCl electrodes were used to assess heart rate. One electrode was fixed on the middle of the chest on the sternum and the second electrode on the left side on the lower ribs. All physiological data was collected using the software NetStation on a Macintosh Computer with a sampling rate of 250 Hz and an online band pass filter of 0.1 - 100 Hz.

3.2.3 Procedure and design

The same paradigm and the same procedure used in the previous study was applied. After signing the informed consent and filling out a demographic questionnaire and the SPAI (Fydrich, 2002; Turner et al., 1989), STAI-State and STAI-Trait (Laux et al., 1981; Spielberger et al., 1970) in their German translations, an EEG net was attached and participants were seated in a small dimly lit room. Instructions were to simply look at the screen and focus the eyes on the fixation cross. They were also informed that during certain pictures a loud noise would be presented.

All stimuli were rated for valence, arousal, and anxiety on a 9-point rating scale. These ranged from 1 “very unpleasant”, “very calm” and “not anxious” to 9 “very pleasant”, “very arousing” and “very anxious”, respectively. Participants were asked how (pleasant, aroused, anxious, respectively) they felt when looking at the respective picture. Ratings for context stimuli took place at the beginning of the experiment, after the conditioning phase and after the test phase to assess the effect of the conditioning on the explicit level. Ratings for the distractor stimuli, (without the contexts), were taken before test phase (as they did not appear beforehand) and after the test phase, using the same ratings scales as for the context stimuli. Moreover, participants answered an emotion recognition question to each distractor stimulus, regarding the facial expression the person was showing. The perceived valence of the US was retrieved at the beginning of the experiment.

The experiment consisted of two different parts: a context conditioning phase and a test phase. During the context conditioning, a differential conditioning paradigm was applied. Participants learned to associate the presentation of one context with the occurrence of the US (CTX+), while the second context was never presented together with the noise, and therefore served as a safety context (CTX-). A trial consisted of a context stimulus, which was presented for 20,000 ms and was always followed by an inter-trial interval of a gray screen for 1,750 to 2,500 ms. During the presentation of the CTX+, the US was randomly presented once between 1,000 and 12,000 ms after stimulus onset and occurred unpredictably. All CTX+ presentations were combined with one US presentation, which represents a reinforcement rate of 100 %. Each context was presented twelve times in randomized order. The context stimuli were presented in flickering mode at either 12 or 15 Hz, counterbalanced across subjects.

In the test phase, the distractor stimuli appeared in the center of each context. Again, the context was visible for 20 seconds, and during this time, one distractor appeared twice for 4,000 ms each with an interval of 4,000 to 4,500 ms between them. Within one trial (i.e., context presentation) always the same distractor stimuli was presented twice. In order to maintain the conditioning effect acquired in the first phase, every sixth CTX+ was reinforced in the test phase. These reinforced trials were excluded from analysis. Applying the same frequency mode as in the first part for each subject, the distractor stimuli were presented in the respective other frequency, again counterbalanced across subjects. Each context was presented 36 times, always combined with two presentations of a distractor showing either a happy, neutral or angry expression, resulting in six conditions with 12 trials each for the context and 24 trials for the distractors (when considering the repetitions). The order of the trials was pseudo-randomized. After each phase, subjects were asked about the likeliness of each context being presented together with the noise. They indicated the perceived contingency on a 10-point scale ranging from 0 % (never associated) to 100 % (always associated).

3.2.4 Data analysis

Rating data

Ratings of the contextual stimuli regarding valence, arousal and anxiety, collected before the conditioning after the conditioning and after the test phase, were analyzed in 3 x 2 repeated measures ANOVAs including the within-subject factors of phase (pre-, post-conditioning, post test phase) and context (CTX+, CTX-). Ratings of the distractor stimuli were collected before and after the test phase. Repeated measures ANOVAs for each rating dimension were conducted including the within-subject factors of phase (before and after test phase) and facial expression (happy, neutral, angry).

EEG data

Data reduction was performed with the MatLab-based software emegs version 2.4 (Peyk et al., 2011) and similar data processing to the previous study was applied. The continuous EEG data was low-pass filtered at 40 Hz off-line before preprocessing. Afterwards, the event-related epochs were extracted from 600 ms pre-stimulus until 20,600 ms after stimulus onset, meaning the contextual stimuli. Artifact detection was performed on the preprocessed data in a two-step method as described in Junghöfer et al. (2000). First, contaminated sensors and sensors with artifact activity were identified and rejected. The rejection was based on thresholds for the statistical parameters maximum absolute amplitude, standard deviation, and gradient. Secondly, sensors with artifact activity were replaced by interpolation based on all remaining sensors. Following the artifact rejection, the epochs were averaged separately for each condition. A Hilbert transform using a MATLAB script (see Miskovic and Keil (2013a) for detailed description) was performed in order to gain time-varying ssVEP amplitudes in the specified frequency across two occipital sensor clusters. These were chosen according to the previous study, in which these clusters showed a maximal conditioning effect (left: 63, 64, 68, 69, 73; right: 88, 89, 94, 95, 99) (see Figure 5).

Statistical analysis was performed using IBM SPSS Statistics 22. In order to assess the effect of conditioning during the acquisition phase, repeated-measures ANOVAs were performed with the within-subject factors context (CTX+, CTX-), and time (5 time windows a 4,000ms), thereby covering the whole trial length of 20 seconds per context. In a second step, a time window of 13,000 to 20,000ms after stimulus onset was analyzed separately in order to exclude any possible effect driven by US presentation, which was only presented between 1,000 and 12,000 ms after stimulus onset. Due to a higher artifact appearance during the acquisition phase (possibly due to US presentation and resulting eye movements), only 28 subjects could be included in the analysis of the conditioning phase. In order to assess the conditioning effect in the test phase, a first analysis of the ssVEP amplitudes elicited by the context for the whole 20 seconds included the factors context (CTX+, CTX-), distractor (happy, neutral, angry), and time (5 time intervals a 4,000 ms). In a second step, the mean ssVEP activity was taken from onset to offset of the distractors with a length of 4,000 ms to assess the effect of the distractors within both contexts. The ssVEP amplitudes in response to the distractors were averaged over repetitions and tested as a function of context in a centrally occipital cluster around Oz (sensors 70, 71, 74, 75, 76, 82, 83), similarly to the previous study. Using these short 4,000 ms trials, repeated measures ANOVAS were calculated for the response of the distractors by using the ssVEP signal elicited by the frequency of the distractors and including the factors context (CTX+, CTX-), distractor (happy, neutral, angry) and time (4 time intervals a 1,000 ms).

Influence of participants' awareness was taken into account and the sample was split into an aware and an unaware group. Repeated measures ANOVAs for the ssVEP signal elicited by the context in the acquisition and test phase and for the ssVEP signal during distractor presentation were conducted with awareness as between-subject factor. Corrected degrees of freedom and *t*-values were reported if the assumption of equality of variances was violated for independent samples *t*-tests.

Competition analysis. As in the previous study, competition indices between contexts and distractors were calculated and statistically compared. The ssVEP amplitudes evoked by contexts and distractors in a time window of 0 to 4,000ms in the occipital sensor cluster were T-transformed across all six conditions for each participants. To obtain the relative cortical activity evoked by the contexts in relation to distractor stimuli, T-transformed ssVEP amplitudes evoked by the context were divided by the sum of T-transformed ssVEP amplitudes evoked by the distractor and context: T(context)/[T(distractor)+T(context)]. While a competition index of > 0.5 reflects a processing bias for the context, an index of < 0.5 shows a signal biased towards the novel distractor. Competition indices were analyzed using repeated measures ANOVA with the within-subjects factors context and distractor.

Heart rate

Processing of heart rate was performed with the software Vision Analyzer 2.0 (Brain Products Inc., Munich, Germany). R-spikes were automatically marked and continuous heart rate was calculated (Koers et al., 1999). Change scores were achieved by using a baseline of 1,000 ms before stimulus onset. Heart rate response during conditioning was examined with a 2 (CTX+, CTX-) x 10 (10 time windows a 2,000 ms) repeated measures ANOVA for the 20 seconds of context presentation. During the test phase, the first 4,000 ms of each trial (before the onset of the first distractor presentation) were analyzed for possible context effects. Subsequently, the heart rate during 4,000 ms of distractor presentation were evaluated in a repeated measures ANOVA including the within-subject factors of context (CTX+, CTX-), expression (happy, neutral, angry) and time (4 time intervals a 1,000 ms).

Additionally, participants' awareness was taken into account and repeated measures ANOVA including the between-subject factor awareness were conducted for the heart rate response during conditioning and test phase.

In all analyses, alpha level was set at $p < .05$. If the assumption of sphericity was violated, Greenhouse-Geisser correction was applied for effects involving factors with more than two levels and the Greenhouse-Geisser Epsilon (GG-ε) was reported. Effect sizes were calculated with the partial eta (η_p^2).

3.3 Results

3.3.1 Sample characteristics

The mean social anxiety of the sample, assessed by the SPAI, was M = 1.89 (SD = 1.04). Participants' mean state anxiety was M = 34.03 (SD = 7.71) and mean trait anxiety M = 35.63 (SD = 9.40). From 30 subjects, 15 reported a perceived probability of 80 to 100 % for the CTX+ being presented with the loud noise (M = 69.00, SD = 27.84), while 18 subjects reported a chance of 20 % or less to hear the US during CTX- presentation (M = 26.33, SD = 27.85), as reported after the conditioning phase. When splitting the sample into aware and unaware subjects, with awareness defined as a perceived contingency for the CTX+ of at least 80 % and for the CTX- of 20 % or less, 12 participants could be labeled as 'aware' and 18 participants as 'unaware'. The two groups did not differ in state or trait anxiety (t[28] = 1.40, p = .174 and t[28] = 0.61, p = .546) nor in social anxiety (t[28] = 0.39, p = .700) or gender distribution (X^2[1, N = 30] = 1.43, p = .232). However, the aware group was significantly younger than the unaware group (t[28] = 2.41, p = .023) (Table 2).

Table 2: Sample characteristics and psychometric measures (mean and standard deviation) for aware and unaware subjects and the statistical values

	Aware (SD) (N=12)	Unaware (SD) (N=18)	*t*, X^2	*p*
Age (years)	23.50 (3.68)	26.89 (3.83)	2.41	.023
Gender	8 female	8 female	1.43	.232
SPAI	1.80 (1.02)	1.95 (1.08)	0.39	.700
STAI-Trait	34.33 (8.70)	36.50 (10.00)	0.61	.546
STAI-State	33.95 (7.52)	37.29 (6.45)	1.40	.174

3.3.2 Rating data

Explicit ratings of anxiety, arousal, and valence for the contextual stimuli, measured before and after the conditioning and test phase revealed strong conditioning effects (Figure 12). The repeated measures ANOVA of anxiety ratings revealed a significant main effect of context ($F[1, 29] = 11.66$, $p = .002$, $\eta_p^2 = .29$), a main effect of phase ($F[2, 58] = 12.17$, $p < .001$, $\eta_p^2 = .30$, $GG\text{-}\varepsilon = .69$), which was further qualified by an interaction of context and phase ($F[2, 58] = 10.44$, $p = .001$, $\eta_p^2 = .27$, $GG\text{-}\varepsilon = .69$). Post-hoc tests revealed increased anxiety ratings for the CTX+ after the conditioning ($t[29] = 3.60$, $p = .001$) and after the test phase ($t[29] = 3.94$, $p < .001$) compared to the baseline rating, while the anxiety ratings for the CTX- only increased marginally from before to after conditioning ($t[29] = 1.96$, $p = .059$) and did not show any difference to after the test phase ($t[29] = 1.56$, $p = .129$). While the two contexts did not differ before the learning phase ($t[29] = 0.49$, $p = .625$), the CTX+ was significantly perceived as more anxiogenic compared to the CTX- after the conditioning ($t[29] = 3.16$, $p = .004$) and after the test phase ($t[29] = 3.75$, $p = .001$).

A similar pattern evolved for the arousal ratings, showing a main effect of context ($F[1, 29] = 14.59$, $p = .001$, $\eta_p^2 = .34$), a main effect of phase ($F[2, 58] = 14.56$, $p < .001$, $\eta_p^2 = .33$, $GG\text{-}\varepsilon = .83$) as well as an interaction ($F[2, 58] = 10.14$, $p = .001$, $\eta_p^2 = .26$, $GG\text{-}\varepsilon = .72$). The CTX+ elicited increased self-reported arousal after the conditioning ($t[29] = 4.63$, $p < .001$) and after the test phase ($t[29] = 5.11$, $p < .001$) compared to pre-conditioning. No increase was found for the CTX- from pre-conditioning to after conditioning (t[29] = 1.16, p = .255), and only a marginal increase to after the test phase (t[29] = 1.76, p = .090). The contexts did not differ before the conditioning phase (t[29] = 0.31, p = .758), but the CTX+ was perceived as more arousing than the CTX- after the conditioning phase (t[29] = 3.44, p = .002) and after the test phase (t[29] = 4.27, p < .001).

Reported valence of the contexts showed a main effect of context (F[1, 29] = 5.07, p = .032, η_p^2 = .15), a main effect of phase (F[2, 58] = 14.35, p < .001, η_p^2 = .33), which were further qualified by an interaction (F[2, 58] = 8.36, p = .001, η_p^2 = .22). The anxiety context showed a significant decrease in valence after the conditioning (t[29] = 4.95, p < .001) and after the test phase (t[29] = 5.63, p < .001) compared to pre-conditioning, while the safety context did not change in perceived valence (t[29] = 0.12, p = .905 and t[29] = 0.59, p = .557, respectively). The two contexts did not differ in valence before the conditioning (t[29] = 1.73, p = .094), but the CTX+ was perceived as more unpleasant than the CTX- after the conditioning (t[29] = 2.51, p = .018) and after the test phase (t[29] = 2.71, p = .011).

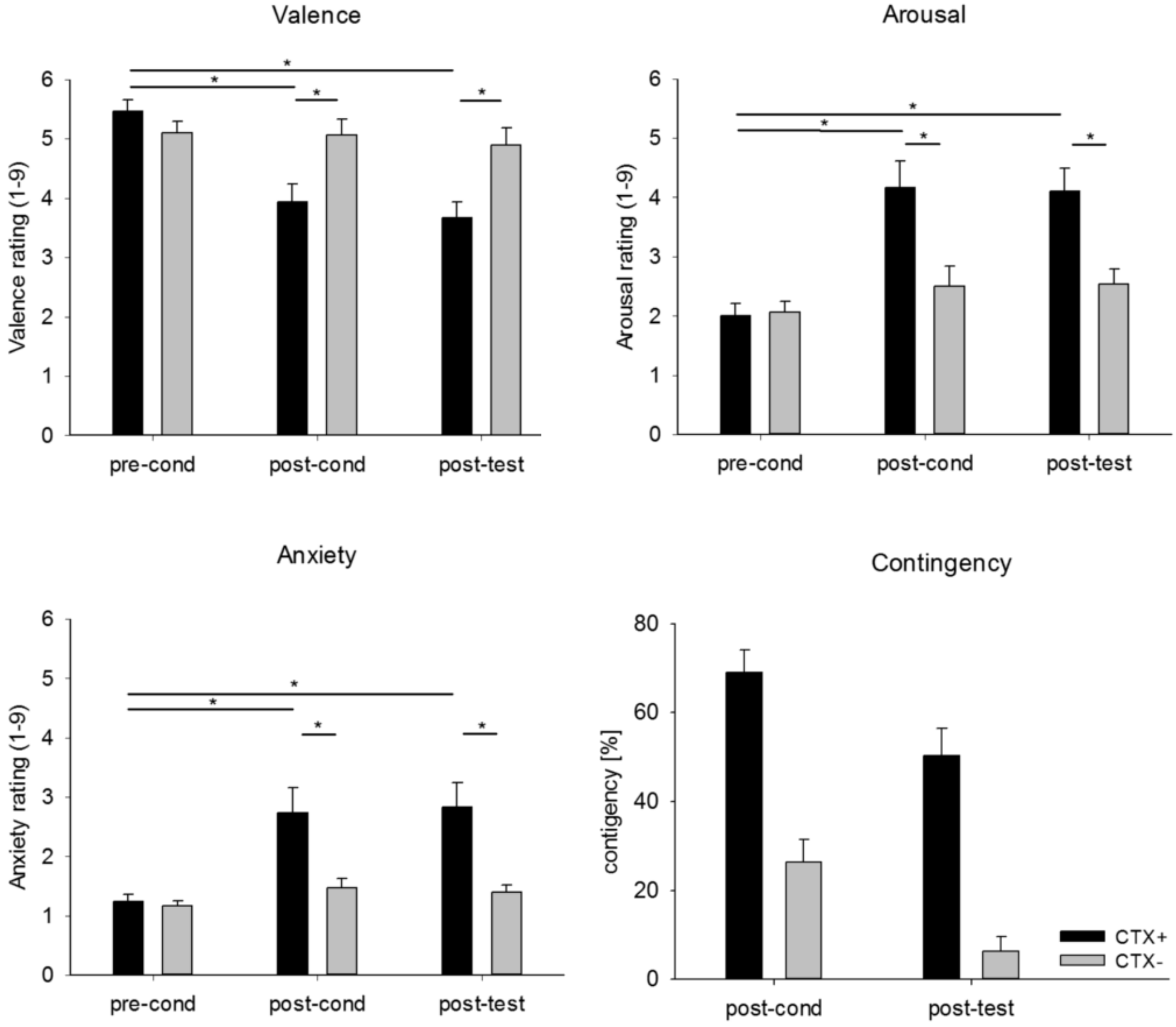

Figure 12: Mean subjective ratings of the anxiety and safety context.
Mean ratings and standard errors of the mean are displayed. Ratings were collected on valence (top left), arousal (top right), and anxiety (bottom left) for the anxiety (CTX+) and safety (CTX-) context before conditioning, after the conditioning phase and after the test phase. Contingency ratings were collected after the conditioning and after the test phase.

The aversive sound serving as US was rated at the beginning of the experiment as unpleasant (M = 2.13 SD = 1.04), with a significant difference from neutral (test value = 5) (t[29] = 15.07, p < .001).

The distractor stimuli were rated before and after the test phase outside the contexts for valence, arousal, and anxiety and were analyzed with 2 (pre-test, post-test) x 3 (happy, neutral, angry) repeated measures ANOVAs (for means see Table 3). The faces were perceived significantly differently in anxiety regarding the emotional expression, (F[2, 58] = 7.63, p = .002, η_p^2 = .21, GG-

ε = .82), while ratings did not differ between phases (F[1, 29] = 2.23, p = .146, η_p^2 = .07). The angry face was perceived as more anxiogenic than the happy face (t[29] = 3.68, p = .001) and the neutral face (t[29] = 2.23, p = .034), while the happy and neutral face only differed on a marginal level (t[29] = 1.94, p = .063). Arousal ratings revealed a main effect of expression (F[2, 58] = 4.07, p = .022, η_p^2 = .12), a main effect of phase (F[2, 58] = 5.58, p = .025, η_p^2 = .16) as well as an interaction (F[2, 58] = 4.07, p = .031, η_p^2 = .12, GG-ε = .81). The happy face showed an increase in arousal from the first to the second time point of rating (t[29] = 2.28, p = .030), while the angry and neutral face did not show any differences between phases (t[29] = 0.65, p = .522 and t[29] = 0.67, p = .506, respectively). Before the test phase, the angry face was perceived as more arousing than the happy face (t[29] = 2.37, p = .024), while it was not different to the neutral face (t[29] = 0.57, p = .576) nor was there a difference between happy and neutral (t[29] = 1.41, p = .170). After the test phase, the angry face was perceived as more arousing compared to the happy face (t[29 = 2.16, p = .039) and the neutral face (t[29] = 4.01, p < .001). Regarding the valence ratings, analysis showed a main effect of expression (F[2, 58] = 18.00, p < .001, η_p^2 = .38, GG-ε = .82), a main effect of phase (F[1, 29] = 7.25, p = .012, η_p^2 = .20) and an interaction of expression and phase (F[2, 58] = 4.97, p = .024, η_p^2 = .15, GG-ε = .64). The happy face was perceived as significantly more unpleasant after the test phase compared to before the test phase (t[29] = 2.89, p = .007), while valence ratings did not change for the angry and neutral face between phases (t[29] = 0.37, p = .71 and t[29] = 0.66, p = .514, respectively). Before the test phase, the angry face and the neutral face were rated as more unpleasant compared to the happy face (t[29] = 8.61, p < .001 and t[29] = 5.41, p < .001), while the neutral and angry face did not differ (t[29] = 1.68, p = .103). After the test phase, the angry face was still perceived as more unpleasant compared to the happy face (t[29] = 2.23, p = .034) and more unpleasant compared to the neutral face (t[29] = 1.99, p = .056), while the happy and neutral face (t[29] = 1.59, p = .122) did not differ.

Table 3: Mean ratings (+ SD) of social and object distractors before and after test phase.

	Anxiety			Arousal			Valence		
	Happy	Neutr	Angry	Happy	Neutr	Angry	Happy	Neutr	Angry
Pre-test	1.23 (1.10)	1.70 (1.34)	2.33 (1.94)	2.07 (1.20)	2.53 (1.72)	2.73 (1.91)	6.40 (1.19)	4.73 (1.23)	4.20 (0.10)
Post-test	2.33 (1.94)	1.90 (1.61)	2.43 (1.91)	2.70 (1.77)	2.37 (1.85)	3.37 (2.13)	5.40 (1.83)	4.87 (1.14)	4.27 (1.44)

Influence of awareness

It was additionally examined whether the participants' awareness influenced the explicit measures of the contextual stimuli. Two groups split the sample into 'aware' and 'unaware' subjects. The ratings of the contexts for valence, arousal, and anxiety for the aware and unaware group are separately reported in Table 4. Repeated measures ANOVAs including the factor context (CTX+, CTX-) and phase (post-conditioning, post-test phase) with the additional between-subject factor awareness were conducted for anxiety, arousal, and valence ratings of the contexts. For the anxiety ratings, a main effect of awareness (F[1, 28] = 4.22, p = .049, η_p^2 = .14) was found, further qualified by an interaction of context and awareness (F[1, 28] = 4.13, p = .052, η_p^2 = .13). Anxiety ratings for the CTX+ after both conditioning and test phase were increased in the aware group compared to the unaware group (t[28] = 2.16, p = .040). While anxiety ratings within the unaware group differed only slightly between contexts (t[17] = 1.75, p = .098), the aware group showed highly increased anxiety ratings for the CTX+ compared to the CTX- (t[11] = 3.53, p = .005). Regarding arousal ratings, an interaction of context and awareness (F[1, 28] = 4.85, p = .036, η_p^2 = .15) and an interaction of context, phase and awareness was found (F[1, 28] = 4.83, p = .036, η_p^2 = .18). The CTX+ elicited marginally higher arousal after the conditioning phase reported by the aware group compared to the unaware group (t[28] = 1.73, p = .095). While in the unaware group, the conditioning effect was only visible after the test phase (t[17] = 2.99, p = .008), but not after conditioning (t[17] = 1.49, p = .154), in the aware group the increased arousal rating for the CTX+ could be seen at both rating points (t[11] = 3.68, p = .004 and t[11] = 3.12, p = .010). A similar pattern was found for the valence ratings with a marginal interaction of context and awareness

($F[1, 28] = 3.44$, $p = .074$, $\eta_p^2 = .11$) and a significant interaction of context, phase and awareness ($F[1, 28] = 4.72$, $p = .038$, $\eta_p^2 = .14$). The two contexts showed stronger conditioning effects after the acquisition phase for the aware compared to the unaware participants, with marginally decreased valence for the CTX+ ($t[28] = 1.90$, $p = .068$) and increased valence ratings for the CTX- ($t[28] = 2.19$, $p = .037$). No differentiation between threat and safety context was found in the unaware group ($t[17] = 0.47$, $p = .646$ and $t[17] = 1.63$, $p = .122$). With awareness of the US contingency, the CTX+ was rated as more unpleasant than the CTX- at both rating times ($t[11] = 3.41$, $p = .006$ and $t[11] = 2.25$, $p = .046$).

Table 4: Mean ratings (+ SD) of the context after the conditioning and after test phase, separately for aware and unaware subjects

	Aware (N=12)				Unaware (N=18)			
	After cond		After test		After cond		After test	
Rating	CTX+	CTX-	CTX+	CTX-	CTX+	CTX-	CTX+	CTX-
Valence	3.25 (1.82)	5.75 (1.60)	3.25 (1.60)	4.92 (1.51)	4.39 (1.46)	4.61 (1.24)	3.94 (1.39)	4.89 (1.68)
Arousal	5.08 (2.50)	2.00 (1.54)	4.67 (2.31)	2.50 (1.78)	3.56 (2.28)	2.83 (2.09)	3.72 (2.02)	2.56 (1.25)
Anxiety	3.83 (2.48)	1.50 (1.00)	3.75 (2.56)	1.58 (0.79)	2.00 (1.94)	1.44 (0.86)	2.22 (1.80)	1.28 (0.58)

3.3.3 EEG data

Conditioning Phase

During context conditioning, analysis only revealed a trend towards increased ssVEP amplitudes for the CTX+ compared to the CTX- ($F[1, 27] = 2.61$, $p = .118$, $\eta_p^2 = .09$) in the occipito-lateral sensor clusters. Figure 13 depicts the grand mean as well as the topography during the whole 20 seconds during conditioning for the CTX+ and the CTX-. The electrocortical activation did not change over the 20 seconds of context presentation ($F[4, 108] = 1.03$, $p = .394$, $\eta_p^2 = .04$). A significant interaction of context and time ($F[4, 108] = 4.78$, $p = .001$, $\eta_p^2 = .15$) revealed a significant increase of the cortical activation in response to the CTX+ compared to the CTX- for the first 4,000 ms

($t[27] = 3.22$, $p = .003$). For the following 8,000 ms this differentiation was only at trend level ($t[27] = 1.53$, $p = .138$ and $t[27] = 1.33$, $p = .194$).

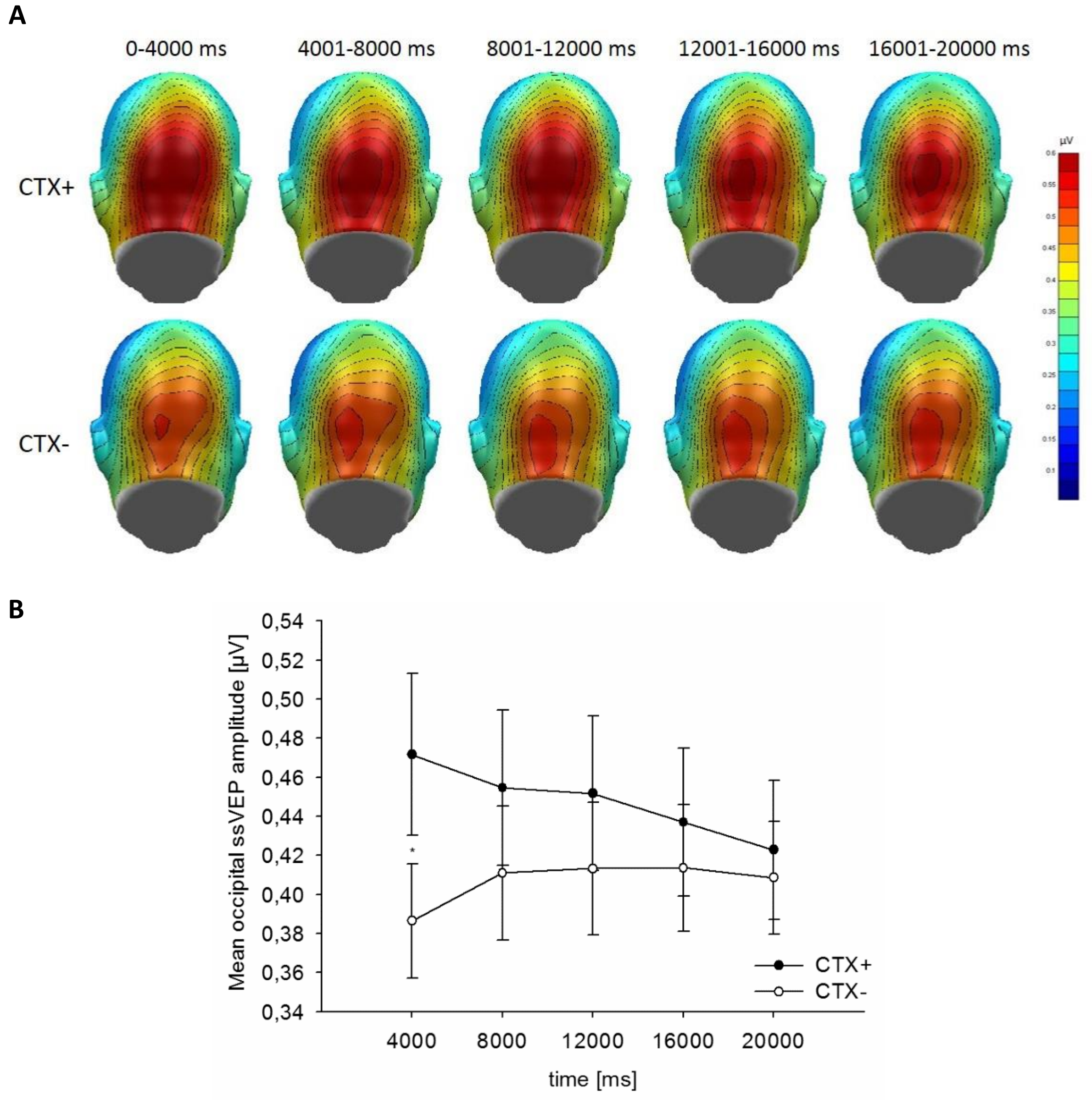

Figure 13: Scalp topographies and grand mean of ssVEP amplitudes to the anxiety and safety context.
(A) Grand average scalp topographies and (B) the grand mean with standard errors of the mean of ssVEP amplitudes to CTX+ and CTX- over occipito-lateral sensor clusters during conditioning over the whole context presentation time of 20 seconds, divided into five time windows.

Between 12,000 and 16,000 ms and 16,000 and 20,000 ms no effect was found anymore ($t[27] = 0.92$, $p = .365$ and $t[27] = 0.49$, $p = .631$). When only examining the last 7,000 ms of the trials, where no US was presented, the main effect context was not significant ($F[1, 27] = 0.36$, $p = .555$, $\eta_p^2 = .01$). As the factor laterality did not show any effects, ($F[1, 27] = 0.05$, $p = .821$, $\eta_p^2 < .01$), it was excluded in any further analyses.

Test phase

Context effects. In order to examine the context effects during the test phase, the whole trial of context presentation (20 seconds) was analyzed in a 2 (CTX+, CTX-) x 3 (happy, neutral, angry) x 5 (5 time windows a 4,000 ms) repeated measures ANOVA. Neither the factor context (F[1, 29] = 1.71, p = .202, η_p^2 = .06) nor the factor expression (F[2, 58] = 0.37, p = .964, η_p^2 < .01) was significant, while a main effect of time was found (F[4, 116] = 34.04, p < .001, η_p^2 = .54, GG-ε = .29). In a second step, separate analyses were conducted for the 4,000 ms segments during distractor presentations for the ssVEP signal elicited by the distractor and for the ssVEP signal elicited by the context surrounding the distractor, averaged over a centrally occipital sensor cluster. Regarding the ssVEP signal elicited by the context during distractor presentation, neither the main effect context (F[1, 29] < 0.01, p = .986, η_p^2 < .01) nor the main effect of expression (F[2, 58] = 0.12, p = .884, η_p^2 < .00) was significant. A main effect of time (F[3, 87] = 4.88, p = .013, η_p^2 = .14, GG-ε = .61) showed a general increase of the ssVEP signal from 1,000 to 2,000 ms (t[29] = 2.21, p = .035), while no changes were seen for the other subsequent time windows (2,000 to 4,000 ms; t[29] = 0.24, p = .236 and t[29] = 0.573, p = .571).

Cue effects. Regarding the signal elicited by the distractor, no main effect of context (F[1, 29] = 0.39, p = .537, η_p^2 = .01) or expression (F[2, 58] = 0.38, p = .963, η_p^2 < .01) was found, but a main effect of time (F[3, 87] = 3.62, p = .027, η_p^2 = .11, GG-ε = .75) as well as a marginal interaction of time and expression (F[6, 174] = 2.10, p = .077, η_p^2 = .07, GG-ε = .75). During the first 1,000 ms of distractor presentation, the angry face elicited increased electrocortical activation compared to the neutral face (t[29] = 1.94, p = .063). In the second time window from 1,000 to 2,000 ms, the angry face elicited marginally decreased ssVEP amplitudes compared to the happy face (t[29] = 1.81, p = .080). The angry face showed a strong decrease in the ssVEP signal from 1,000 to 2,000 ms (t[29] = 4.00, p < .001). The ssVEP signal elicited by the happy face showed a later decrease from 3,000 to 4,000 ms (t[29] = 3.06, p = .005).

Influence of awareness

The previously conducted analyses regarding ssVEP amplitudes evoked by context and cue stimuli during conditioning and test phase were repeated and the between-subject factor of awareness was included.

Context effects. No influence of awareness on context processing was found in the repeated measures ANOVA for the whole 20 seconds trial during the conditioning phase ($F[1, 26] = 2.162$, $p = .691$, $\eta_p^2 < .01$) nor during the test phase ($F[1, 28] < 0.01$, $p = .955$, $\eta_p^2 < .01$). The analysis of the segments during distractor presentations, however, revealed an influence of awareness. The ssVEP signal elicited by the context during distractor presentation showed a marginal interaction of context and awareness ($F[1, 28] = 3.85$, $p = .060$, $\eta_p^2 = .12$). As this interaction was of major interest, paired t-test within each awareness group and independent samples t-tests between the groups were calculated. No difference was found in the cortical activation for CTX+ or CTX- between the two groups (ts < 0.84, ps > .41). While the two contexts did not differ in the unaware group ($t[17] = 1.09$, $p = .293$), marginally increased cortical activation in response to the CTX+ compared to the CTX- was found in the aware group ($t[11] = 2.02$, $p = .068$).

Cue effects. A similar pattern was found for the ssVEP signal elicited by the distractor. Next to a marginal interaction of context and awareness ($F[1, 28] = 3.10$, $p = .089$, $\eta_p^2 = .10$), a highly significant three-way interaction of context, time and awareness was found ($F[1, 28] = 6.92$, $p < .001$, $\eta_p^2 = .20$). The ssVEP amplitudes of distractors in the CTX+ did not differ between groups in all four time windows (ts < 1.64, ps > .113). However, the aware group compared to the unaware group showed marginally decreased cortical activation in response to the distractors in the CTX- from 1,000 to 2,000 ms ($t[24.80] = 1.95$, $p = .063$) and the two following time windows up to 4,000 ms ($t[25.64] = 2.21$, $p = .036$ and $t[25.35] = 1.99$, $p = .058$). Within the unaware group, differentiation between the threat and the safety context during distractor processing was found between 2,000 and 3,000 ms ($t[17] = 2.16$, $p = .046$), with increased ssVEP amplitudes in response to the CTX-. The aware group showed a differentiation between contexts in the same time window with increased cortical activation in response to the distractors in the CTX+ compared to the CTX-

($t[11] = 2.75$, $p = .019$). Figure 14 depicts the mean ssVEP amplitudes in response to the anxiety and the safety context depending on the participants' awareness.

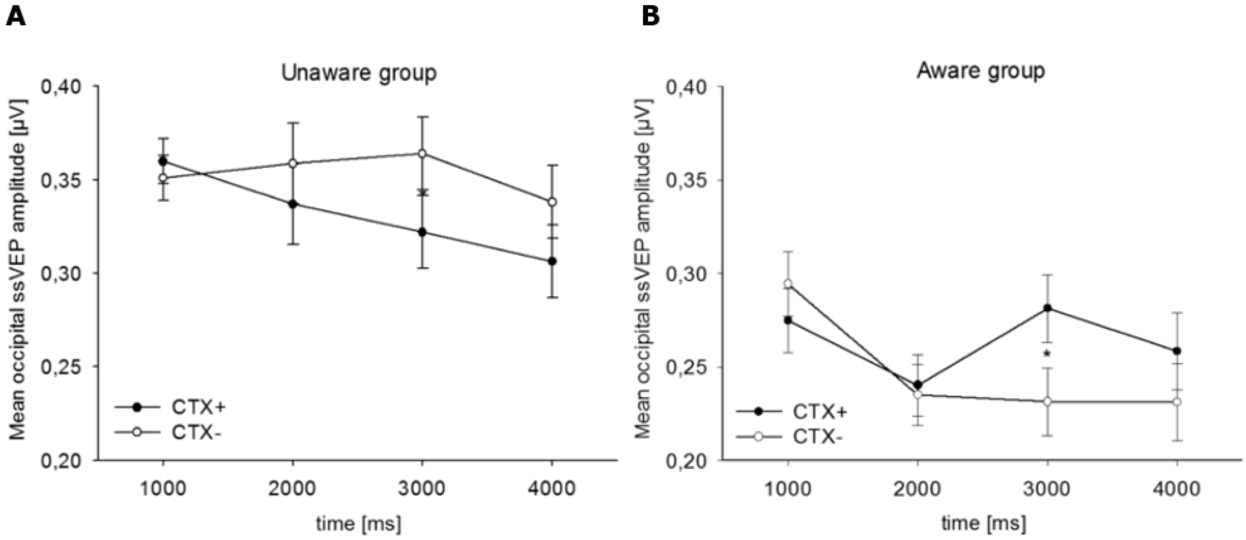

Figure 14: Mean ssVEP amplitudes in response to the distractors for the aware and unaware group. Grand mean and standard errors of sample mean of occipital ssVEP amplitudes during the test phase in response to the distractors in CTX+ and CTX-, averaged over the factor expression, and divided into 4 time windows with each 1,000 ms, separately for the unaware (left) and aware (right) group

Competition analysis

Competition indices indicating the relative processing of the context to the distractor did not show any differences between conditions (context: $F[1, 29] < 0.01$, $p = .956$, $\eta_p^2 < .01$; expression: $F[1, 28] = 0.51$, $p = .572$, $\eta_p^2 = .02$, $GG\text{-}\varepsilon = .83$).

3.3.4 HR data

The heart rate response during conditioning did not show any significant changes between contexts ($F[1, 28] = 0.22$, $p = .642$, $\eta_p^2 = .01$), but a general change in time ($F[9, 252] = 2.96$, $p = .020$, $\eta_p^2 = .10$, GG-ε = .48), with a decrease from 3,000 to 4,000 ms ($t[28] = 2.28$, $p = .030$) and an increase from 5,000 to 6,000 ms ($t[28] = 2.06$, $p = .049$). In the test phase, the first 4 seconds of context without distractor presentation revealed a marginal significant interaction of context and time (4 time windows a 1,000 ms) ($F[3, 84] = 3.01$, $p = .080$, $\eta_p^2 = .10$, $GG\text{-}\varepsilon = .45$). This showed a marginal significant deceleration of heart rate during the threat context compared to the safety context (t[28] = 1.88, p = .070) between 3,000 and 4,000 ms. Heart rate response during the distractor presentations did not reveal any main effect of context (F[1, 28] =0.22, p = .640, η_p^2 = .01), or expression (F[2, 56] = 1.75, p = .183, η_p^2 = .06).

Additionally, it was examined whether there was any relationship between the heart rate change to the anxiety context and its cortical response. A positive correlation of differential heart rate between CTX+ and CTX- and the differential processing of CTX+ and CTX+ reflected in ssVEP activation during the conditioning phase, averaged across the whole trial length (r = .34, p = .079) was observed.

Influence of awareness

Awareness did not influence heart rate response during conditioning (F[1, 27] = 2.20, p = .150, η_p^2 = .08). In the test phase, a significant three-way interaction of the within-subject factors context, time and the between-subject factor awareness was found for the heart rate response during the first 4,000 ms of context presentation (F[3, 81] = 6.72, p = .007, η_p^2 = .20, $GG\text{-}\varepsilon$ = .481). Post-hoc tests revealed that heart rate was decreased in the unaware compared to the aware group between 0 and 1,000 ms for the CTX+ (t[27] = 2.11, p = .044) and the CTX- (t[27] = 1.75, p = .092). While there were no differences between the two contexts in the unaware group during the 4 time windows (ts < 0.46, ps > .648), the aware group showed a decreased heart rate for the CTX+ compared to the CTX- between 2,000 and 4,000 ms (t[10] = 2.32, p = .043 and t[10] = 3.68, p = .004) (Figure 15).

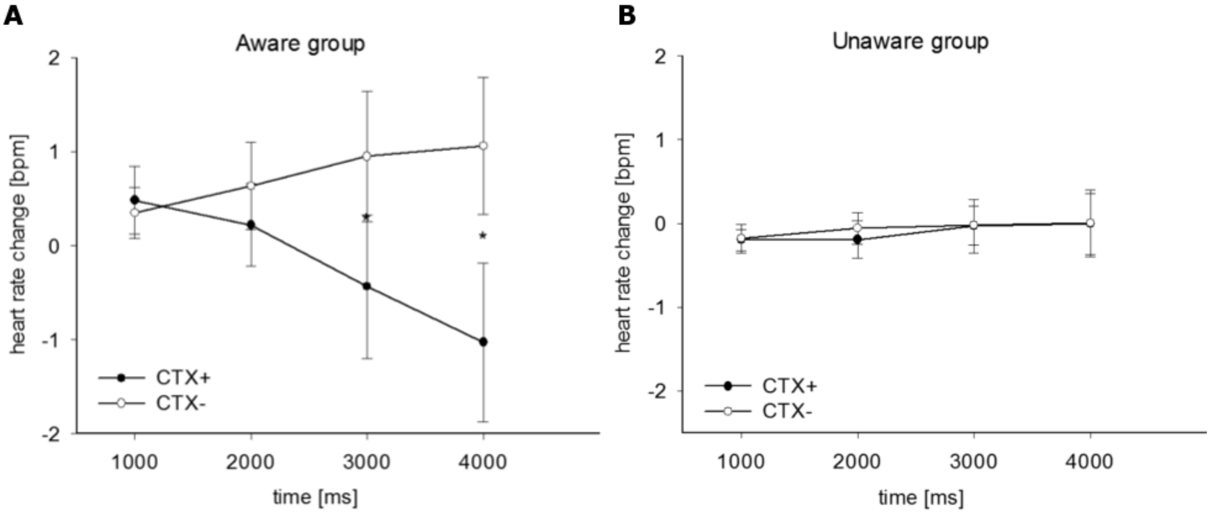

Figure 15: Heart rate changes for CTX+ and CTX- during the test phase
Averaged heart rate changes for CTX+ and CTX- for the first 4,000 ms of each trial during the test phase, without any distractor presentation, separately for aware and unaware subjects.

Also, analysis of the heart rate during distractor presentation revealed a context x awareness effect (F[1, 27] = 4.23, p = .050, η_p^2 = .14) as well as a marginal interaction of context, time and awareness (F[3, 81] = 2.71, p = .085, η_p^2 = .10, GG-ε = .57). No difference between groups was found for the heart rate during CTX+ (t[27] = 0.52, p = .611). However, heart rate response during CTX- was different between groups, with a slightly decreased heart rate for the aware, and increased heart rate for the unaware group (t[27] = 1.72, p = .097). No significant difference between contexts was found in the unaware group (t[17] = 1.22, p = .241) or in the aware group (t[10] = 1.43, p = .184).

3.3.5 Influence of anxiety

Ratings

Correlations between anxiety indices of trait anxiety, state anxiety, and social anxiety taken from the respective questionnaires and the conditioning effect in ratings (i.e., difference between CTX+ and CTX-) were calculated. Anxiety (trait and state anxiety, social anxiety) did not influence the differential ratings of CTX+ and CTX- after the conditioning phase and after the test phase (rs < .27, ps > .151).

EEG data

Correlations between the differential ssVEP amplitudes of CTX+ and CTX- and state anxiety, trait anxiety and social anxiety were calculated. The differential ssVEP amplitudes between the threat and the safety context did not show any correlation with participants' anxiety level during conditioning or test phase (*r*s < .19, *p*s > .345). Furthermore, anxiety had no influence on the differential ssVEP signal of CTX+ and CTX- during distractor presentation. However, a marginal significant correlation between social anxiety and the difference of angry minus neutral facial expression of the ssVEP signal elicited by the CTX+ was found (*r* = .34, *p* = .068). This correlation suggests an increased cortical activation for the threat context when an angry face compared to a neutral face was present with higher social anxiety level.

For the signal elicited by the distractors, anxiety had no influence on the difference between contexts (*r*s < .25, *p*s > .178) nor on the difference between facial expressions (*r*s < .24, *p*s > .195).

Heart rate

Correlations between state anxiety, trait anxiety, and social anxiety and differential heart rate of CTX+ and CTX- in conditioning and test phase were calculated. Anxiety had no influence on the conditioning effect for heart rate during both phases (*r*s < .27, *p*s > .177).

3.4 Discussion

A newly established context conditioning paradigm was used in order to model contextual anxiety and safety. One context was always paired with an aversive noise, while the second one remained unpaired and therefore served as safety context. Due to the inability to use cues to predict the occurrence of the US, the whole context is associated with the unpleasant event. The unpredictability of the US creates a chronic expectation of the US and sustained anxiety should be seen when the threat context is presented. Explicit measures show indeed a successfully implemented context conditioning. The threat context was rated as more anxiogenic and more arousing than the safety context, while at the same time, it was perceived as more unpleasant. This effect remained stable over time and the anxiety context was still perceived as more unpleasant, arousing and anxiogenic than the safety context after the test phase despite the drastic reduction of the US occurrence. However, a sustained increased cortical activation, being stable over time, as it was expected and observed in the first study, could not be found. Only an initial increase in oscillatory occipital activity was observed during the anxiety context. These heightened ssVEP amplitudes reflect increased attentional resources towards a stimulus of significance. This is, on the one hand, bottom-up modulated by inherently emotionally arousing stimuli (Keil et al., 2003). On the other hand, selective spatial attention towards a stimulus additionally enhance the evoked oscillatory response (Keil et al., 2005). As previous cue conditioning studies showed, a cue known to predict an electric shock elicited increased occipital ssVEP amplitudes (Miskovic & Keil, 2013a; Moratti & Keil, 2005). While an initial sensory facilitation with increased ssVEP amplitudes was observed, this facilitated perceptual processing was not maintained over the whole presentation time of the context in this study, against all expectations. In a recent fMRI study, brain response patterns discriminated between initial and sustained brain responses to contextual conditioned anxiety (Andreatta et al., 2015). The sustained anxiety response is classified by the activation of structures already previously associated with context conditioning: the amygdala and hippocampus (Alvarez et al., 2008; Alvarez et al., 2011; Lonsdorf et al., 2014; Maren et al., 2013). In contrast, activations of the initial response in the threat context suggest conditioned fear reactions, recall of US contingency and evaluation of threat (Andreatta et al., 2015). Similar to the ssVEP-results, the heart rate response to the contexts only yielded marginal conditioning effect. One possible explanation for the lack in increased processing of the anxiety context is the role of participants' contingency awareness, meaning whether learning of the relationship between stimulus and the aversive event on the cognitive level took place. In the literature, it is debated whether contingency awareness is necessary in order to show conditioned fear responses on different physiological markers. (Dawson, Rissling, Schell, & Wilcox, 2007; Hamm & Vaitl, 1996; Hamm & Weike, 2005; Tabbert et al., 2011). Indeed, in the present study, the contingency awareness as reported by the participants mediated all explicit and implicit measures of contextual anxiety. A differentiation between anxiety and safety during distractor presentation could be only observed for participants who reported the correct contingency between contexts and aversive sound for both ssVEP amplitudes and heart rate response. In the aware group, the context also elicited differential evaluations in valence, arousal, and anxiety, while conditioning effects were only weak or not present in the unaware group. Within the scope of the two-level account of conditioning, a dissociation between an implicitly learned, automatic fear response and learning on a cognitive level is discussed (Hamm & Weike, 2005). Fear potentiated startle is observed in cue conditioning without the influence of awareness. Skin conductance response and explicit ratings on the other hand are dependent on contingency awareness. It is argued that the hippocampus plays an important role in learning the contingency between a stimulus and the aversive event on a cognitive level (Hamm & Weike, 2005; Tabbert et al., 2011). Additionally, the hippocampus is reported to be involved during context conditioning in contrast to cue conditioning (Alvarez et al., 2008; Marschner et al., 2008). Having one important key neuronal structure in common could explain the importance of contingency awareness on expressing contextual anxiety on an implicit and explicit level in the present study. It still remains unclear, however, why contingency awareness did not mediate the results of the conditioning phase. Even when considering awareness, I did not find any significant conditioning effects in the acquisition phase, which would allow suggesting sustained anxiety during the CTX+.

Enhanced ssVEP amplitudes as observed in the contingency aware subjects reflect increased attention allocation facilitating discriminative processing of stimuli (Müller & Hillyard, 2000). The motivational significance of the threat context increased due to the unpredictable occurrence of the aversive noise, and therefore received sensory amplification. The extent of the contextual threat value shaped by participants' learning experience is also reflected by the decreased heart rate during the test phase. Within the test phase, both increased ssVEP amplitudes and the heart rate deceleration elicited by the threat context reflects an orienting response, suggesting heightened vigilance and a hyperalertness (Bradley et al., 2005; Lang & Bradley, 2010). This hypervigilance is in line with the definition of anxiety as a state sustained apprehension of negative events, although, as suggested by the present results, an explicit representation of the relationship between context and aversive event might be required in order to show these sustained anxiety responses on the explicit and implicit level.

A correlation between differential heart rate response during context conditioning phase and the difference in ssVEP activation between anxiety and safety context was found. Differential physiological activation in response to the anxiety context relative to the safety context seems to be related to the differential cortical activation in the extended visual areas between anxiety and safety. This is in line with previous work, showing peripheral correlates of fear predict the presence of visual facilitation of the conditioned fear stimuli (Moratti & Keil, 2005; Moratti et al., 2006).

In the present study, I additionally examined the reciprocal influence between context information and face processing with different emotional expressions. An influence of facial expression (for fearful faces) on context processing, as it was described elsewhere (Wieser & Keil, 2014), could not be observed in the present study. Here, one has to consider that fearful and angry faces have different effects on attention processes. While both expressions transfer threat information, angry faces attract and hold attention (Williams, Moss, Bradshaw, & Mattingley, 2005). On the other hand, it might be adaptive to widen the attentional focus to detect any environmental threat when we see fearful faces as potential threat warning (Palermo & Rhodes, 2007). Moreover, no threat-dependent effect of face processing was found when observing the whole sample. However, for the aware participants, an evidence for a general hypervigilance hypothesis was observed. All faces perceived in the anxiety context elicited increased oscillatory responses in the occipital area, compared to the safety context. This effect was irrespective of the emotional expressions of the faces. For the angry face, as a stimulus of specific threat value to humans (Öhman & Dimberg, 1978), facilitated processing when in the threat context, as seen before with increased N170 amplitudes, could have been expected (Righart & de Gelder, 2005). Possibly, the angry face stimuli in this study were too weak to elicit any strong responses in comparison to the context, as a highly relevant and imminent threat at this time point compared to the irrelevant face. This could be already shown with emotional faces, which did not show any differential processing depending on the emotionally arousing background scenes (Wieser & Keil, 2014). When using faces as cues, one has to keep in mind that threatening faces elicit weaker defensive engagement than emotionally arousing scenes do (Wangelin, Bradley, Kastner, & Lang, 2012). While this is especially true for low socially anxious individuals, high socially anxious participants show greater affective reactions towards faces. The influence of social anxiety on the processing of facial cues was therefore examined as well. Previous studies highlighted the importance of the context, in which the perceiver is situated for the processing of facial cues regarding social anxiety (Garner et al., 2006; Grillon & Charney, 2011). Here, only a marginal correlation between social anxiety and context processing depending on the presence of an angry face could be reported. With increasing social anxiety, the anxiety context received more processing resources only when an angry face was present. These results suggest that the threat context receives heightened resources in high socially anxious individuals when an additional threat cue, that is the angry face, is present. While Wieser and Keil (2014) showed a context amplification in the presence of a fearful face, the present study could show a similar finding, while at the same time restricting it to social anxiety as a mediating factor.

Taken together, while an initial increase in attention resources in the threat context could be described in the present study for all participants, a more sustained anxiety response could be only observed in participants with high contingency awareness. This response was indicated by a heightened cortical activation in extended visual areas, together with an orienting response indicated by a heart rate deceleration in the test phase during the distractor presentation. Reported experience of anxiety and increased arousal within this context supports the assumption of an increased vigilance in the anxiety context and a heightened alertness. Stimuli that appeared in the anxiety context received increased attentional resources compared to an appearance in the safety context. The emotional expression of the faces did not influence this amplification due to the contextual threat, which supports the idea of a general hypervigilance induced by anxiety.

4 Study 3: Differentiation of fear and anxiety: Influence of predictability

4.1 Introduction

The third study investigated both fear and anxiety, reflected by cue and context conditioning within one study. It aimed at comparing the previous findings of a continuous activation during sustained anxiety on an electrocortical and physiological level with the activation induced by phasic fear. As already mentioned, the predictability of the aversive event plays a crucial role during conditioning paradigms (Bouton, 1994; Maren et al., 2013). During phasic fear, responses that reflect defensive activation, such as an increased startle response, are evoked when a cue which predicts an aversive event is present (Bradley et al., 2005). On the other hand, a context which became associated with an aversive event without predicting the actual occurrence of it elicits anxiety related responses (Alvarez et al., 2011; Grillon et al., 2004) and avoidance of this context (Glotzbach et al., 2012; Grillon, Baas, Cornwell et al., 2006). Similarly, a failure to learn the predictable value of a cue leads to increased anxiety (Baas et al., 2008). The two concepts of fear and anxiety, operationalized by the manipulation of predictability of an aversive event, are also differentiated on a neuronal level. While for both predictable and unpredictable threat the amygdala is activated, the hippocampus and the BNST are additionally found to be involved during unpredictable threat (Alvarez et al., 2011; Hasler et al., 2007). The two neuronal systems underlying the state of phasic fear and sustained anxiety are two overlapping, but nevertheless distinct networks (Davis et al., 2010; Maren et al., 2013).

Fear and anxiety are also classified into different stages of the defense cascade model (Blanchard et al., 1993; Bradley et al., 2001; Davis et al., 2010; Lang et al., 2000). According to the defense cascade model, the distance to the source of threat is relevant for the type of response. During the post-encounter phase, animals as well as humans show an orienting response, with an interplay of the parasympathetic and sympathetic nervous system (Bradley et al., 2001; Cacioppo & Berntson, 1994; Cacioppo et al., 1999). A shrinking distance to a source of threat, however leads to immediate mobilization of all resources, which is regulated by activity of the sympathetic nervous system (Blanchard et al., 2001; Löw et al., 2008). This circa-strike zone is associated with the state of fear, in which the animal is directly threatened by the predator, while anxiety is linked to the pre- and post-encounter stages of the defense cascade model (Blanchard et al., 1993; Davis et al., 2010).

In order to directly compare predictable and unpredictable threat, a paradigm including neutral, predictable, and unpredictable events was previously developed (Grillon et al., 2004; Schmitz & Grillon, 2012). The NPU-threat test includes three different conditions, which vary in their predictability of an aversive event. In each condition, a context signaling the nature of the trial is presented for a longer duration, in which repetitions of a certain cue per condition occur. In the neutral condition (N), no US is presented, while in the predictable condition (P) the US only occurs during the presentation of the respective cue. In the unpredictable condition (U) the US is presented unpredictably within the context-only parts of the trial. Startle amplitudes measured during the cue presentation and in the context-only parts of the trials revealed that these were potentiated during the cue in the P-condition compared to all other conditions, i.e., a fear potentiated startle. For the context-only parts, startle amplitudes were increased during the unpredictable context compared to the two other conditions, which is defined as anxiety potentiated startle (Grillon et al., 2004; Vansteenwegen et al., 2008). Haaker et al. (2013) showed a differentiation in fear ratings, with the highest fear rating for the unpredictable context, as well as physiological arousal reflected by skin conductance response for the contextual stimuli. Using the same paradigm in virtual reality, unpredictable threat elicited increased activity in the amygdala, similarly to predictable threat, while unpredictable threat was additionally related to sustained activity in the forebrain region, associated with the BNST (Alvarez et al., 2011). A distinction between predictable threat eliciting a state of fear and sustained anxiety responses due to threat unpredictability could be seconded by showing that the benzodiazepine alprazolam did not affect phasic fear reactions to predictable threat cues but dampened sustained anxiety responses (Grillon, Baas, Cornwell et al., 2006). While the initial NPU test was performed with simple geometric figures as cues (Schmitz & Grillon, 2012), some studies already used more environmentally valid stimuli, such as rooms in a virtual environment (Alvarez et al., 2011; Grillon, Baas, Cornwell et al., 2006; Haaker et al., 2013; Lonsdorf et al., 2014).

In the present study, the NPU-threat test of Schmitz and Grillon (2012) was adapted to be able to measure steady-state visually evoked potentials during both cue and context conditioning, using more complex, realistic stimuli, such as office rooms and persons. The possibility to assess continuous visuocortical responses during the contextual stimuli and observe high-resolution changes in time, as well as being able to differentiate the responses of the context and the cues makes ssVEP amplitudes a perfectly suited measure for the NPU-threat test. Steady-state visually evoked potentials were measured while tagging context and cue stimuli with different frequencies, allowing to differentiate between context- and cue-related responses. Within each condition, an office room served as a long-lasting background stimulus, and repetitions of one specific person per condition occurred within these contexts. While a US never occurs during the N condition, in the P condition the presentation of the respective cue signals the occurrence of the US. During the U condition, the US is not predictable for the participant.

The ssVEP signal as oscillatory field potential elicited by the driving frequency of a flickering stimulus reflects activity of the extended visual cortex as well contributions from the primary visual cortex and involvement of higher order cortices (Di Russo et al., 2007; Müller et al., 1997; Wieser & Keil, 2011). Augmented ssVEP amplitudes reflect increased attentional resources towards a stimulus, driven by top-down processes such as explicitly paying attention to it (Keil et al., 2001), or by bottom-up processes guided by stimulus properties. An increased motivational significance of a stimulus like emotionally arousing pictures evokes heightened oscillatory cortical responses compared to neutral pictures (Keil et al., 2010; Wieser et al., 2011). Similarly, a stimulus which was learned to be of significance, as in conditioning, was shown to elicit higher ssVEP amplitudes in visual cortices (Miskovic & Keil, 2012, 2013a). This does not only apply to fear conditioning, but could be also found in a state of anticipatory anxiety (Gray, Kemp, Silberstein, &

Nathan, 2003). In the two previous studies, sustained anxiety responses induced by contextual associative learning were associated with increased oscillatory response in occipital areas.

Similar to results of the previous studies, heightened activation of the visual cortices elicited by the context of the unpredictable condition should exceed both the activation during the neutral and predictable context. During the cue presentation, however, the significance of conditions changes and the predictable cue is expected to draw increased attentional resources compared to the neutral and unpredictable cue, as w observed before during cue conditioning (Miskovic & Keil, 2012, 2013a). It is expected that this differential response pattern is also reflected in heart rate changes. As fear and anxiety are grouped into different defense stages according to Blanchard et al. (1993), a heart rate deceleration, as it is described for the post-encounter phase of the defense cascade model, is expected during the context of the unpredictable condition compared to the predictable and neutral condition as an index of anxiety. An acceleration of heart rate, on the other hand, is expected during the cue in the predictable condition compared to the neutral and unpredictable cue, as in this condition the threat is maximized and immediately present, thereby eliciting a fear related response (Bradley et al., 2001).

As the significance of context and cue vary between the predictable and unpredictable condition, one might expect differences in the relative processing of the cue at the cost of the context. Cues of high significance were shown to withdraw attentional resources from irrelevant cues (Hindi Attar, Andersen, & Müller, 2010; Müller et al., 2008; Wieser et al., 2012). Thus, competition effects for the cue in the predictable condition are expected, and possibly for the unpredictable context at the cost of the – in the unpredictable condition irrelevant – cue. Regarding the influence of anxiety as a trait, different influences were found previously on the processing of conditioned fear cues and contextual anxiety. As mentioned before, while some studies reported no effects of trait anxiety on various fear conditioning responses (Torrents-Rodas et al., 2013), others reported increased conditionability for high anxious individuals in fear conditioning (Orr et al., 2000; Peri et al., 2000). Other studies suggest impaired safety learning in anxiety patients, leading to a reduced differentiation between the fear and the safety cue (Duits et al., 2015; Gazendam et al., 2013). With the incapability to learn the correct association between cue and negative consequences, an increased chronic expectation of the US leads to sustained anxiety (Baas et al., 2008; Grillon, 2002a). So one might expect to find increased anxiety responses in conditioned contexts for higher anxious individuals. So far, subjects with high trait anxiety were found to show enhanced discrimination between threatening and safe context in a context conditioning paradigm (Glotzbach-Schoon et al., 2013), as well as higher activity in the amygdala during the threat context in the extinction phase (Barrett & Armony, 2009). This study aims at taking influences of trait anxiety (due to the nature of the cues social anxiety is considered, too) into account when mapping attention mechanisms in a combined cue-context-conditioning paradigm.

4.2 Methods

4.2.1 Subjects

Participants of the study were 45 females who were recruited with the help of an advertisement in a local online platform. Exclusion criteria were any psychiatric or neurological disorders. Participants received 9 Euros as study reimbursement for participation. Due to the failure to differentiate between the three context stimuli after the experimental session three participants had to be excluded from analysis, resulting in 42 datasets. Participants' age ranged from 18 to 34 (M = 23.14, SD = 3.35).

4.2.2 Stimulus materials and apparatus

According to the study design, three context stimuli and three cue stimuli were used. The context stimuli were screenshots from virtual office rooms, created with the Source Engine from the Valve Corporation (Bellevue, USA). The office room differed in layout and furniture but were balanced for luminance and complexity by controlling the luminance values returned by the Image Manipulation Program GIMP 2.8.14 (159, 160, 160 for the context stimuli) and the quantitative measure of entropy, calculated with the respective MATLAB function (6.68, 6.80 and 7.20 respectively).

The cue stimuli were taken from the Radboud faces database (RaFD) (Langner et al., 2010). Three female faces with frontal orientation and neutral expressions were chosen (Rafd090_02, Rafd090_32, Rafd090_58), with a comparable valence and intensity ratings (see Table 5). As the RaFD pictures only depict faces, the facial stimuli and a neutral female body were merged in order to create a valid context-cue combination with a person standing in an office room.

Table 5: Validation data for the used cue stimuli, taken from the support material for the article from Langner et al. (2010) regarding the Radboud faces database

Stimulus	Agreement (%)	Intensity	Clarity	Genuineness	Valence
Rafd090_02	100	3.61	3.78	4.22	3.04
Rafd090_32	100	3.58	3.96	3.96	3.08
Rafd090_58	86	3.36	4.00	4.23	3.05

A loud startle noise (white noise), presented at 95 dB for 500 ms served as unconditioned stimuli. Stimuli subtended a horizontal visual angle of 33.38° and 2.86° and a vertical visual angle of 23.90° and 5.32° for context and cue stimuli, respectively. To indicate which type of condition participants were confronted with next, each trial was preceded by a gray screen showing either the words “kein Geräusch” (no noise), “vorhersagbar” (predictable) or “unvorhersagbar” (unpredictable). The combination of context and cue stimuli and the assignment to the three conditions was counterbalanced across subjects.

In order to evoke ssVEPs, stimuli were presented in flickering mode in either 12 or 15 Hz for the context, while the superimposed cue stimuli were presented in the respective other frequency. This was counterbalanced across subjects. The stimuli were presented on a conventional computer screen with a refresh rate of 60 Hz, which was positioned at a distance of 100 cm from the participants’ eyes.

All stimuli were rated before and after the experimental session for valence, arousal, and anxiety on 9-point analogue scales. These ranged from 1 “very unpleasant”, “very calm” and “not anxious” to 9 “very pleasant”, “very arousing” and “very anxious”, respectively. An additional sympathy scale was used for the cues, ranging from 1 “not likeable” to 9 “very likeable”. Context and cue stimuli were rated separately, and as context-cue compound, similar to how they were appearing in the experimental session.

EEG was continuously recorded with a HydroCel Geodesic Sensor Net (Electrical Geodesics, Inc., Eugene, OR) with 128 sensors. The vertex electrode (Cz) was set as reference electrode. The impedance of each sensor was kept below 50 kΩ, as recommended for the Electrical Geodesics high-impedance amplifiers. Heart rate was assessed by recording an electrocardiogram with two adhesive Ag/AgCl electrodes. One electrode was fixed on the middle of the chest on the sternum, and the second electrode on the left side on the lower ribs. All physiological data was collected using the software NetStation on a Macintosh Computer with a sampling rate of 250 Hz and an online band pass filter of 0.1 – 100 Hz.

4.2.3 Procedure and design

Participants were asked to sign the informed consent form and to fill out a sociodemographic questionnaire, as well as the Positive and Negative Affectiveness Scale (PANAS) (Krohne, Egloff, Kohlmann, & Tausch, 1996; Turner et al., 1989), the STAI (Laux et al., 1981; Spielberger et al., 1970) as well as the SPAI (Fydrich, 2002; Turner et al., 1989) in their German versions before the EEG sensor net was attached and participants were seated in a small, dimly lit chamber. After the ratings of all stimuli (context stimuli, cue stimuli, cue-context compound), instructions were given on the screen. Participants were explained that they are about to learn when they can predict the occurrence of the noise and under which conditions it is not predictable. After seeing all stimuli in each condition and reading the instructions, participants performed a small test assigning each context and cue to the right condition and to rephrase the instructions in order to check their comprehension of instructions. A similar test was performed after the experimental session, when subjects had to assign each context and cue again to the correct condition. As mentioned above, three subjects had to be excluded as they mixed up the conditions. After the experimental session, another rating of valence, arousal, anxiety, as well as sympathy for the cues took place, and subjects filled out the state part of the STAI and the PANAS questionnaire.

The design of the study followed the procedure suggested by Schmitz and Grillon (2012) for the NPU paradigm. A schematic procedure is depicted in Figure 16. One trial comprised the presentation of one context for 60 seconds and three occurrences of the associated cue for 4 seconds each, with context-only intervals of 11 to 13 seconds. An instruction screen giving the information about the following condition preceded each trial with a duration of 2,000 ms. An inter-trial interval of 1,750 - 2,000 ms followed each trial. The US occurred once or twice within each trial for the predictable and unpredictable condition (50 % single occurrence within a trial, 50 % two occurrences within a trial) but was never presented in the neutral condition. In the predictable condition, the US was presented at the last 500 ms of cue presentation, with the cue and US terminating together. In the unpredictable condition, the US was presented randomly in the context-only intervals. The order of trials was determined following the protocol of Schmitz and Grillon (2012) with blocks of trials with a PNUNUNP or UNPNPNU order. The experimental session consisted of four of these blocks, with alternating order of PNUNUNP and UNPNPNU, while the beginning block was counterbalanced across subjects. After half the session, participants took a short break. Altogether, 8 predictable, 8 unpredictable and 12 neutral conditions were presented. The context stimuli were presented in flickering mode in either 12 or 15 Hz, with the cue stimuli being presented in the respective other frequency.

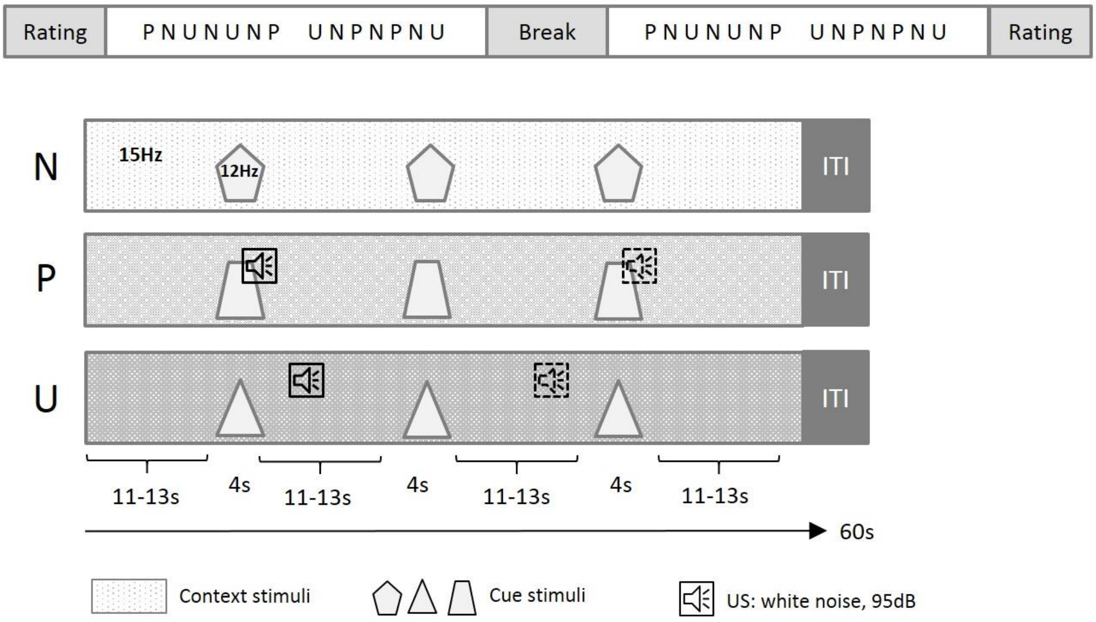

Figure 16: Schematic design of the NPU-threat test with the according timing in seconds, adapted from Schmitz and Grillon (2012).
N = neutral condition, P = predictable condition U = unpredictable condition. One possible run is depicted on top, another order started with the UNPNPNU block, followed by PNUNUNP. Each trial lasted for 60 seconds and was followed by an intertribal interval (ITI) between 1750 and 2500 ms. While in the N condition, no US was presented, one or two US occurred during the P condition at the last 500 ms of each cue, and one or two US were presented randomly in the context-only phases during U.

4.2.4 Data analysis

Rating data

Similar to previous studies (Grillon, Baas, Pine et al., 2006; Grillon et al., 2004; Haaker et al., 2013) ratings taken before the experimental phase were not included in the main analysis but were tested separately for equality in repeated measures ANOVAs each for the context and cue stimuli. The ratings of valence, arousal, and anxiety for the context stimuli collected after the experimental phase were analyzed in repeated measures ANOVAs including the within-subject factors of condition (N, P, U). Similarly, the ratings of valence, arousal, anxiety and sympathy for the cue-context compound stimuli as they were presented during the experiment, were tested in repeated measures ANOVAs.

EEG data

EEG data preprocessing was performed with the Matlab based software emegs 2.4 (Peyk et al., 2011). Data was low-pass filtered at 40 Hz and event-related epochs were extracted from 600 ms before until 60,600 ms after context stimulus onset. In order to assess cortical activation during cue processing separately, discrete epochs were extracted during cue presentation, from 600 ms pre-stimulus until 4,600 ms after cue presentation. Artifact detection on the preprocessed data was performed in a two-step method as described by Junghöfer et al. (2000). Contaminated sensors and sensors with artifact activity were identified and rejected, based on thresholds for some statistical parameters (maximum absolute amplitude, standard deviation, and gradient). Afterwards, the sensors with artifact activity were replaced by interpolation based on all remaining sensors. Due to the unusual trial length of 60 seconds for the context stimuli, the artifact detection was not performed on the whole trial analysis for context effects. All epochs were averaged separately for every condition (NCtx, PCtx, UCtx for the 60 seconds context epochs and NCue, PCue, UCue for the 4 seconds cue epochs). The raw ssVEP signal averaged over all participants and all conditions for the first 4 seconds of each trial for a representative electrode (sensor 75, Oz) is depicted in Figure 17, together with the Fast Fourier Transformation on this ssVEP signal, showing the 12 and 15 Hz driving frequencies. A Hilbert transformation using a MATLAB script (see Miskovic and Keil (2013a) for detailed description) was performed on the raw signal in order to gain time varying cortical potentials in the specified frequencies. Subsequently, separate analyses were conducted for the ssVEP signal elicited by the context using the context-only epochs of 60 seconds and the shorter epochs during cue presentation. During these cue related epochs, the ssVEP signal elicited by the cue and the ssVEP signal elicited by the context in the background, encoded by the respective other frequency were analyzed separately.

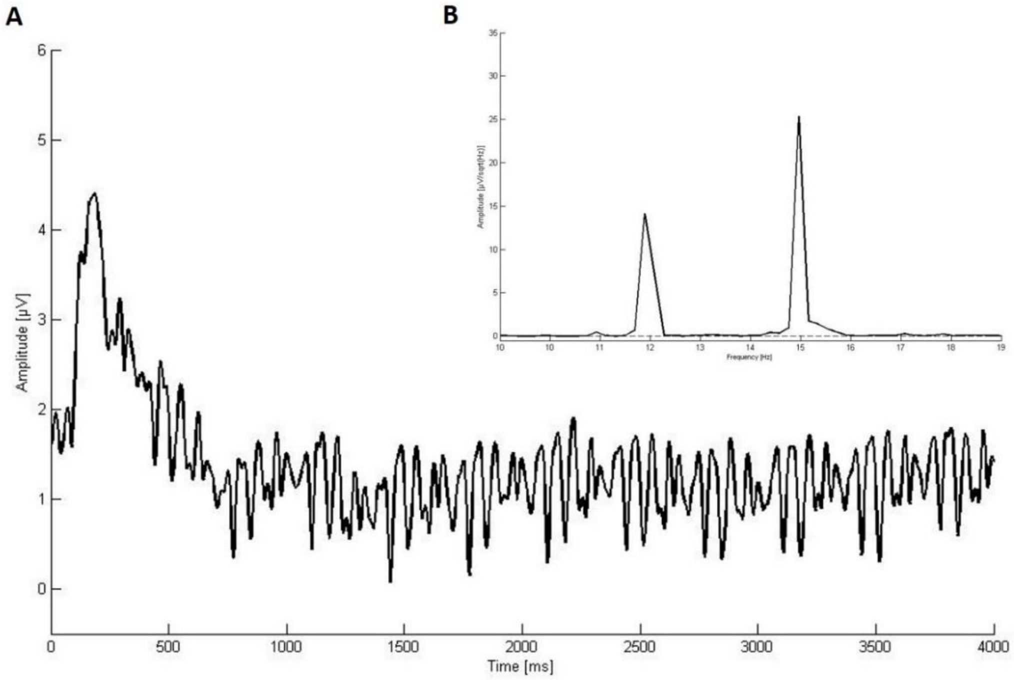

Figure 17: Grand mean and the Fast Fourier Transformation of ssVEP amplitudes.
(A) The ssVEP signal was averaged across all participants and all conditions for the first 4 seconds of each trial, shown for sensor 75 (Oz) (B) Fast Fourier Transformation of this ssVEP signal, showing the two driving frequencies of 12 and 15 Hz

Statistical analysis was performed with IBM SPSS Statistics 22. For context related responses during the whole trial length, a 3 x 5 repeated measures ANOVA including the within-subject factors condition (N, P, U) and five time windows a 12,000 ms was calculated, using the ssVEP signal averages across two occipito-lateral sensor clusters, (left: 63, 64, 68, 69, 73; right: 88, 89, 94, 95, 99) (see Figure 5 for the sensor configuration). Sensor clusters were chosen according to the first study in which context effects were found to be maximized. In a second step, four segments with equal length of 7,000 ms were cut out of the context-only interval between the cue presentations (0-7000, 17000- 24000; 34000- 41000, 51000- 58000 ms from context onset) to exclude any possible effects driven by the cue presentation. A repeated measures ANOVA including the within-factors condition (N, P, U) and phase (4 segments) was conducted.

Regarding the cue epochs, repeated measures ANOVAs including the factors condition (N, P, U) and time window (4 time windows a 1,000ms) were conducted for the ssVEP signal elicited by the cue and separately for the ssVEP signal elicited by the context during cue presentation.

Following the two previous studies, a centrally located occipital sensor cluster around sensor Oz (70, 71, 74, 75, 76, 82, 83) (see Figure 5 for the sensor configuration) was chosen for averaging the ssVEP signal, as the cue were presented more centrally located in the visual field and therefore stimulated a smaller, centrally located electrocortical response.

Competition analysis. In order to account for any possible competition effects, competition indices between cue and context were calculated. Therefore, the ssVEP amplitudes elicited by contexts and cues during cue presentation (0 - 4,000 ms after cue onset) were T-transformed across all three conditions for each participant. To obtain the relative processing of the cue to the context, T-transformed ssVEP amplitudes evoked by the cue were divided by the sum of T-transformed ssVEP amplitudes elicited by the cue and the context: T(cue)/[T(cue)+T(context)] for each condition. While a competition index of >.05 reflects a processing bias towards the cue, an index <.05 shows a bias for context processing at the expense of cue processing. Competition indices were analyzed in a repeated measures ANOVA including the factor condition.

Heart rate

Differences between the two ECG electrodes were computed and continuous heart rate was calculated by automatically marking R-spikes in the electrocardiogram (Koers et al., 1999). Change scores were calculated by subtracting a baseline of 1,000 ms before context stimulus onset. Due to technical problems during recording, only 39 subjects could be included in the analysis for context effects and 36 regarding cue effects. Segments of 4,000 ms during cue presentation were averaged for each condition as were the segments of the first 10 seconds of the trial during which only the context was presented. A 3 x 4 repeated measures ANOVA including the within factors condition (N, P, U) and 4 time windows a 1,000 ms was conducted for the cue segments, while the analysis for the context effects included 10 time windows a 1,000 ms.

In all analyses, the alpha level was set at $p < .05$. If the assumption of sphericity was violated, Greenhouse-Geisser correction was applied for effects involving factors with more than two levels and the Greenhouse-Geisser Epsilon (GG-ε) was reported. Effect sizes were calculated with the partial eta (η_p^2).

4.3 Results

4.3.1 Sample characteristics

The participants filled out the SPAI, STAI-State, STAI-Trait and the PANAS before the experimental phase and another STAI-State and PANAS after the experimental session. The mean questionnaire scores are displayed in Table 6. Paired sample t-tests were calculated for the difference of state anxiety, positive and negative affect from before to after the experimental phase. State anxiety and negative affect was significantly increased after experiment ($t[41] = 3.38$, $p = .002$ and $t[41] = 2.32$, $p = .025$, respectively), and the positive affect was diminished compared to before the experiment ($t[41] = 3.75$, $p = .001$).

Table 6: Psychometric data of all participants included in the analysis.
SPAI = Social Phobia and Anxiety Inventory; STAI = State Trait Anxiety Inventory; PANAS = Positive and Negative Affect Schedule, -PA = positive affect, -NA = negative affect

Questionnaire	Mean (SD) pre	Mean (SD) post	*t*	*p*
SPAI	1.74 (0.83)			
STAI-Trait	35.19 (8.49)			
STAI-State	33.95 (7.52)	37.29 (6.45)	3.38	.002
PANAS-PA	30.62 (5.45)	27.50 (7.38)	3.75	.001
PANAS-NA	11.93 (2.55)	12.71 (3.03)	2.32	.025

4.3.2 Rating data

Ratings for context and cue stimuli were obtained before the experimental phase, before instructions and right after the experimental phase. The pre-ratings were analyzed separately in repeated measures ANOVAs for the dimensions of valence, arousal, and anxiety regarding the context stimuli and for the cue stimuli additionally for sympathy. These analyses did not reveal any differences in valence ($F[2, 82] = 0.27$, $p = .761$, $\eta_p^2 = .01$), arousal ($F[2, 82] = 0.39$, $p = .681$, $\eta_p^2 = .01$) or anxiety ($F[2, 82] = 0.74$, $p = .482$, $\eta_p^2 = .02$) for the context stimuli. Similarly, the cue stimuli did not differ in valence ($F[2, 68] = 0.61$, $p = .545$, $\eta_p^2 = .02$), arousal ($F[2, 68] = 0.81$, $p = .922$, $\eta_p^2 < .01$), anxiety ($F[2, 68] = 0.88$, $p = .392$, $\eta_p^2 = .03$, $GG\text{-}\varepsilon = .73$) or sympathy ($F[2, 68] = 0.68$, $p = .508$, $\eta_p^2 = .02$).

Regarding the post-rating, analyzes of the context stimuli revealed a main effect of condition ($F[2, 82] = 26.05$, $p = < .001$, $\eta_p^2 = .39$, $GG\text{-}\varepsilon = .84$) with decreased valence ratings for the predictable ($t[41] = 5.88$, $p < .001$) and unpredictable context compared to the neutral context, while the latter two did not differ from each other ($t[41] = 0.95$, $p = .348$). Similarly, a main effect of condition was found for arousal ($F[2, 82] = 37.67$, $p = < .001$, $\eta_p^2 = .479$) and anxiety ($F[2, 82] = 36.47$, $p = < .001$, $\eta_p^2 = .47$, $GG\text{-}\varepsilon = .87$), revealing increased arousal ratings for the predictable ($t[41] = 7.37$, $p < .001$) and unpredictable context ($t[41] = 6.96$, $p < .001$) compared to neutral context as well as increased anxiety ratings for both predictable ($t[41] = 8.13$, $p < .001$) and unpredictable context ($t[41] = 6.65$, $p < .001$). Similarly to the valence ratings, the predictable and unpredictable context did not elicit differences in arousal ($t[41] = 1.06$, $p = .297$) or anxiety ratings ($t[41] = 0.90$, $p = .372$).

Regarding the cue ratings, a similar pattern evolved. In the post-rating analyses, ratings of the cues superimposed on the equivalent context were used, as those were the stimuli seen during the experiment. A main effect of condition was found for the valence ratings ($F[2, 82] = 45.90$, $p < .001$, $\eta_p^2 = .53$) with decreased valence ratings for the predictable ($t[41] = 7.95$, $p < .001$) and unpredictable cue ($t[41] = 7.88$, $p < .001$) compared to the neutral cue but no difference between predictable and unpredictable cue ($t[41] = 0.81$, $p = .936$). Similarly, a main effect of condition for the arousal ratings ($F[2, 82] = 38.87$, $p < .001$, $\eta_p^2 = .49$) was found, showing that the predictable ($t[41] = 7.33$, $p < .001$) and unpredictable cue ($t[41] = 7.05$, $p < .001$) elicited increased arousal compared to the neutral cue, but again there was no difference between the predictable and the unpredictable cue ($t[41] = 0\ 00$, $p = 1.00$). For the anxiety ratings a main effect of condition ($F[2, 82] = 38.40$, $p < .001$, $\eta_p^2 = .48$) showed increased anxiety ratings for the predictable ($t[41] = 7.66$, $p < .001$) and the unpredictable cue ($t[41] = 6.51$, $p < .001$) compared to the neutral cue, while these two stimuli were similarly anxiogenic ($t[41] = 0.88$, $p = .387$). Regarding the perceived sympathy for the persons serving as cues, significant differences were found between the three conditions ($F[2, 82] = 33.32$, $p = < .001$, , $\eta_p^2 = .45$) with lower sympathy ratings for the predictable ($t[41] = 7.58$, $p < .001$) and unpredictable cue ($t[41] = 6.06$, $p < .001$) compared to the neutral cue, but similar sympathy ratings in the latter two conditions ($t[41] = 1.33$, $p = .190$). The mean valence, arousal and anxiety ratings for both contexts and cues are depicted in Figure 18.

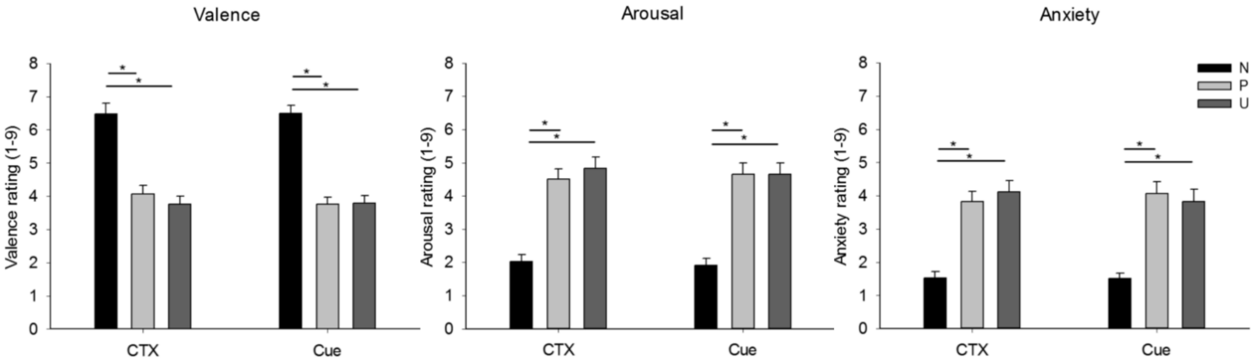

Figure 18: Mean subjective ratings of context and cue stimuli after the experimental phase.
Mean ratings and standard errors of the mean are displayed. All stimuli were rated on valence (left), arousal (middle), and anxiety (right) for the context and cue stimuli.

4.3.3 EEG data

Cortical response to contexts

A repeated measures ANOVA including the within-factors condition (N, P, U), laterality (left, right) and time (5 time windows a 12,000 ms) was performed for the ssVEP signal elicited by the context during the whole 60 seconds presentation. A main effect of condition ($F[2, 82] = 9.64$, $p < .001$, $\eta_p^2 = .42$) revealed increased ssVEP amplitudes during the predictable context ($t[41] = 7.02$,

$p < .001$) and the unpredictable context ($t[41] = 5.65$, $p < .001$) compared to the neutral condition for the whole 60 seconds, while there was no significant difference found between predictable and unpredictable context ($t[41] = 0.48$, $p = .633$). A main effect of time ($F[4, 164] = 53.51$, $p < .001$, $\eta_p^2 = .566$, $GG\text{-}\varepsilon = .30$) showed a significant decrease of ssVEP signal from the first (0-12 seconds) to the second time window (12-24 seconds) across all three conditions ($t[41] = 7.49$, $p < .001$) and to all other following time windows ($t[41] = 6.62$, $p < .001$; $t[41] = 7.87$, $p < .001$; $t[41] = 7.32$, $p < .001$ respectively). As laterality did not return any significant effect ($F[1, 41] = 0.42$, $p = .520$, $\eta_p^2 = .01$), this factor was disregarded in any further analyses and the two sensor clusters were combined.

In order to include only epochs without any cue presentation, four time phases were cut out before, between and after the cue presentations within each trial (0-7,000; 17,000-24,000; 34,000-41,000; 51,000-58,000 ms after context onset). Figure 19 shows the mean scalp topography of the four phases for each condition. A repeated measures ANOVA including the factors condition and four time phases excluding the cue presentations within each trial showed a main effect of condition ($F[2, 82] = 23.04$, $p < .001$, $\eta_p^2 = .36$) and phase ($F[3, 123] = 54.93$, $p < .001$, $\eta_p{}^2 = .57$, $GG\text{-}\varepsilon = .43$). Furthermore a significant interaction of condition and phase was found ($F[6, 246] = 2.26$, $p = .039$, $\eta_p^2 = .05$, $GG\text{-}\varepsilon = .62$). The conditions did not show any differential cortical activation in the first phase ($F[2, 82] = 2.18$, $p = .119$, $\eta_p^2 = .051$). In the second phase, a significant difference between conditions was found ($F[2, 82] = 29.53$, $p < .001$, $\eta_p^2 = .42$) with increased cortical activation for the predictable ($t[41] = 6.00$, $p < .001$) and the unpredictable condition ($t[41] = 7.41$, $p < .001$) compared to the neutral context. The predictable and unpredictable context did not differ from each other ($t[41] = 1.15$, $p = .259$). A similar pattern was found in the third phase ($F[2, 82] = 22.25$, $p < .001$, $\eta_p^2 = .35$), in which the predictable ($t[41] = 5.88$, $p < .001$) as well as the unpredictable context ($t[41] = 5.49$, $p < .001$) elicited increased cortical activation relative to the neutral context but did not differ from each other ($t[41] = 1.07$, $p = .290$). In the fourth phase, the main effect of condition ($F[2, 82] = 28.17$, $p < .001$, $\eta_p^2 = .407$) revealed a significant increase in cortical activity during both the predictable ($t[41] = 5.26$, $p < .001$) and the unpredictable context ($t[41] = 6.88$, $p < .001$) compared to the neutral context. Additionally, the unpredictable context elicited a heightened ssVEP amplitude compared to the predictable context ($t[41] = 2.73$, $p = .009$).

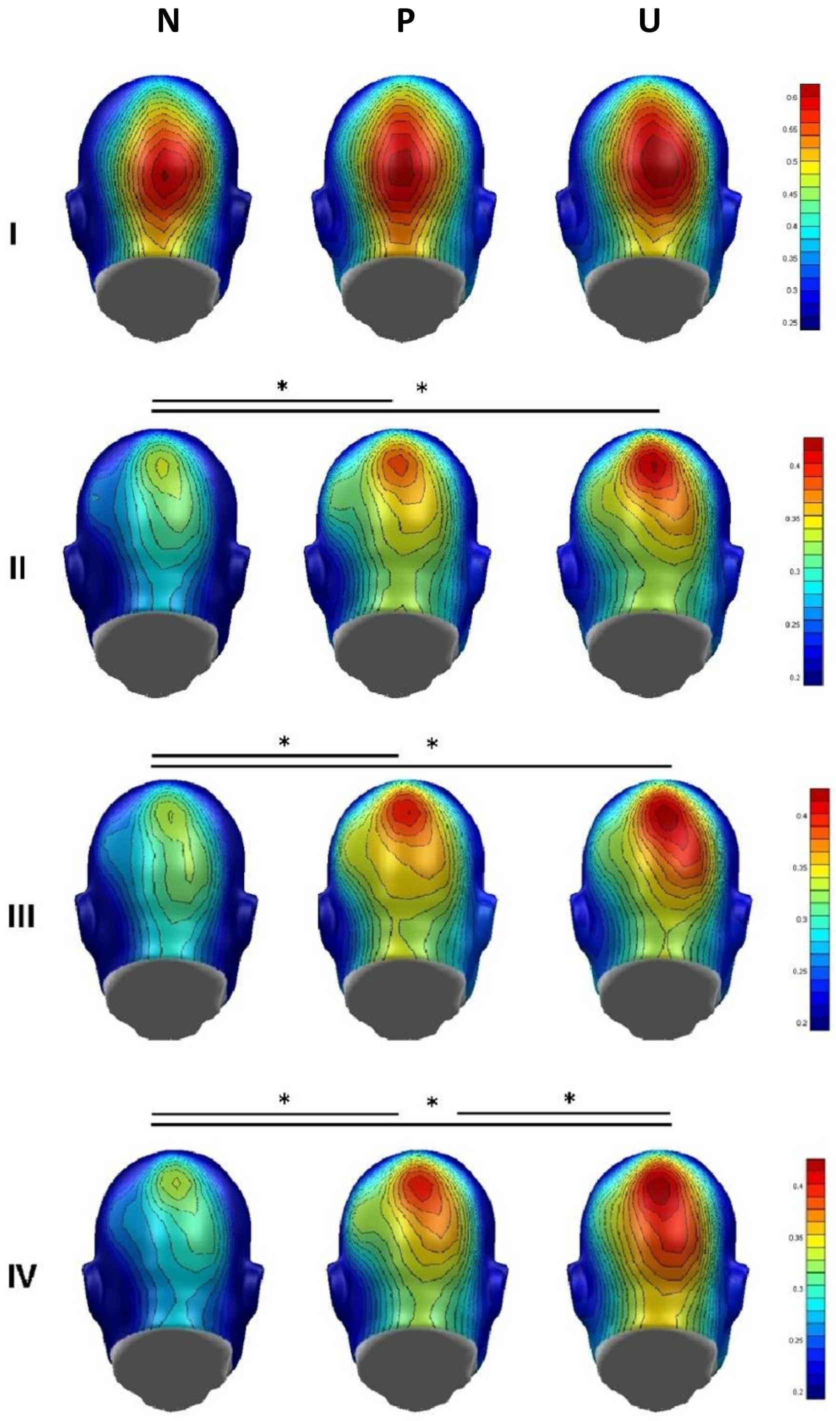

Figure 19: Scalp topographies of ssVEP amplitudes in response to the neutral, predicable, and unpredictable context.
The ssVEP signal was averaged and analyzed separately for four selected phases (7 seconds) of the trial during which no cues were presented. The first phase was from 0-7,000ms the second from 17,000-24,000ms, the third phase from 34,000-41,000 and the fourth phase lasted from 51,000-58,000 ms after context onset.

Cortical response to cues

In order to assess the effects of cue processing depending on predictability, a separate analysis for the cue trials was performed. A grand mean scalp topography averaged over the presentation time of the cues is depicted in Figure 20. The ssVEP signal elicited by the cue was analyzed in 3 x 4 repeated measures ANOVA including the within-factors condition (N, P, U) and 4 time windows a 1,000 ms. The three cues elicited marginally significantly different cortical responses (F[2, 82] = 2.86, p = .063, η_p^2 = .065). As this main effect was of major interest and included in the hypotheses, post-hoc paired sampled t-tests were performed. These showed an increased ssVEP signal elicited by the predictable compared to the neutral cue (t[41] = 2.17, p = .036), while neither the comparison between neutral and unpredictable cue (t[41] = 1.31, p = .196) nor between predictable and unpredictable cue returned significant (t[41] = 1.21, p = .234). A similar analysis was performed using the ssVEP signal elicited by the context during cue presentation, as one advantage of the frequency tagging. The repeated measures ANOVA with the within-factors of condition and time revealed a main effect of condition (F[2, 82] = 3.68, p = .029, η_p^2 = .08) and a main effect of time (F[3, 123] = 4.47, p = .009, η_p^2 = .10, GG-ε = .83). In the predictable condition, the context during cue presentation elicited increased ssVEP amplitudes compared to the neutral context (t[41] = 3.21, p = .003), while in the unpredictable condition, the context did not show any differential cortical activation compared to the neutral context (t[41] = 1.46, p = .153) and compared to the predictable context (t[41] = 1.07, p = .291). Regarding the temporal change of the ssVEP signal, a significant increase of the ssVEP signal across all conditions from the 1,000 to 2,000 ms (t[41] = 3.22, p = .002) was then followed by a marginal decrease from the second to the third time window (t[41] = 1.75, p = .089).

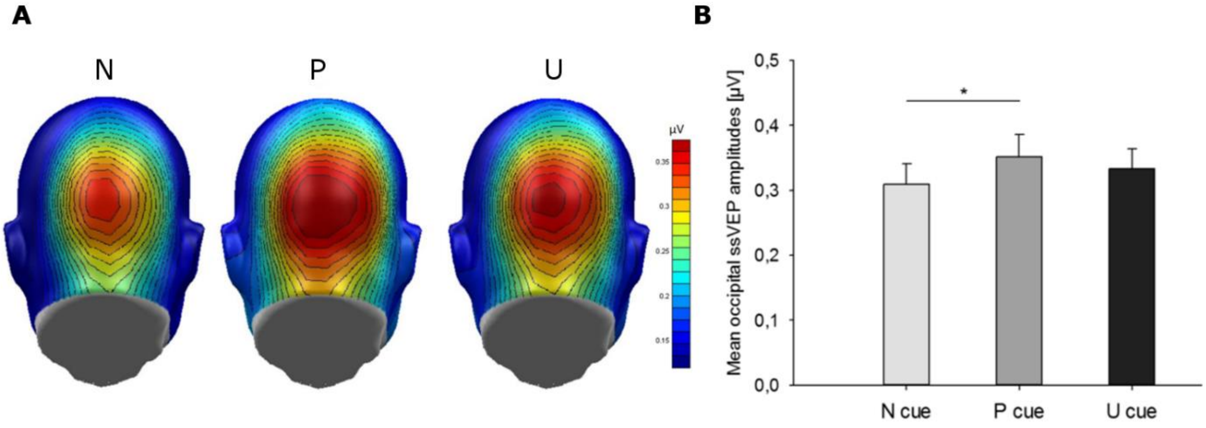

Figure 20: Grand mean ssVEP amplitudes in response the cues.
(A) Grand mean scalp topographies of the ssVEP amplitudes elicited by the frequency of the cues within each condition, averaged over 4,000 ms. (B) Mean ssVEP amplitudes averaged over the occipital sensor cluster and the whole presentation time of 4,000 ms

Competition analysis

In order to assess relative activation elicited by the cue compared to the context during the cue presentation the competition indices computed for each condition was included in a repeated measures ANOVA including the factor condition. There was no significant difference between conditions regarding competition effects (F[2, 82] = 0.47, p = .627, η_p^2 = .01) (M = 0.50, SD = 0.07 for the neutral, M = 0.49, SD = 0.07 for the predictable and M = 0.49, SD = 0.07 for the unpredictable condition).

4.3.4 Heart rate

Response to context

The repeated measures ANOVA returned a significant main effect of condition (F[2, 76] = 5.62, p = .005, η_p^2 = .13) and time (F[9, 342] = 14.37, p < .001, η_p^2 = .27, GG-ε = .35), as well as a significant interaction between these factors (F[18, 684] = 2.80, p = .010, η_p^2 = .07, GG-ε = .36). After the first two seconds, for which conditions did not differ between each other (ts < 1.23, ps < .228), both predictable and unpredictable contexts elicited a significantly decreased heart rate compared to the neutral context between 2,000 and 8,000 ms (ts > 2.20, ps < .034). More approximate to the cue appearance between 8,000 to 9,000 ms and 9,000 to 10,000 ms, the unpredictable condition did not differ anymore from the neutral condition (*t*[38] = 1.89, *p* = .066; *t*[38] = 1.02, *p* = .313), while this was still true (at least marginally) for the predictable condition (*t*[38] = 1.89, *p* = .066; *t*[38] = 2.00, *p* = .052). The predictable and unpredictable context did not elicit any differential heart rate in all time windows (*t*s > 1.66, *p*s > .104). The heart rate deceleration for the predictable and unpredictable context compared to the neutral context is depicted in Figure 21A. While there was no difference found between the first second and the last second within the neutral context (*t*[38] = 1.12, *p* = .270), both predictable (*t*[38] = 3.45, *p* = .001) and unpredictable context (*t*[41] = 2.26, *p* = .03).elicited a significant decrease from the first to the last time window. Within the neutral condition, a significant increase from the first to the second (*t*[38] = 2.80, *p* = .008), and from the second to the third time window (*t*[38] = 2.74, *p* = .009) was found, followed by a decrease from 3,000 to 4,000 ms (*t*[38] = 2.20, *p* = 034) and from 6,000 to 7,000 ms (*t*[38] = 2.21, *p* = .033). Within the predictable condition, the heart rate decreased significantly between 3,000 and 5,000 ms (*t*[38] = 3.47, *p* = .001; *t*[38] = 2.36, *p* = .023), while it significantly increased again more proximate to the cue presentation from 8,000 - 9.000 ms (*t*[38] = 2.36, *p* = .024). Within the unpredictable condition, a short heart rate increase in the 1,000 ms (*t*[38] = 2.48, *p* = .018) was followed by a significant heart rate decrease similar to the predictable condition between 2,000 - 5000 ms (*t*[38] = 2.12, *p* = .041; *t*[38] = 3.11, *p* = .004, *t*[38] = 1.71, *p* = .095).

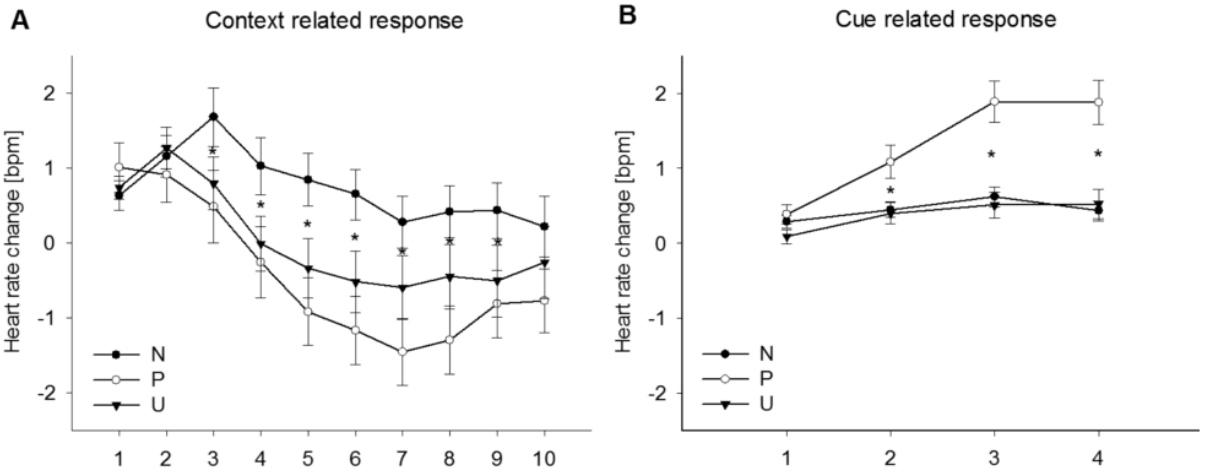

Figure 21: Heart rate changes for the neutral, predictable, and unpredictable condition.
(A) Averaged heart rate changes for the first 10 seconds of each trial during context presentation
(B) Heart rate changes during 4 seconds of cue presentations. Each time window is averaged over 1,000 ms.

Response to cues

The repeated measures ANOVA concerning the heart changes during cue presentation returned a significant main effect of condition (F[2, 70] = 15.01, p < .001, η_p^2 = .30, GG-ε = .78) and time (F[3, 105] = 26.07, p < .001, η_p^2 = .427, GG-ε = .52), as well as a significant interaction between those factors (F[6, 210] = 10.44, p < .001, η_p^2 = .23, GG-ε = .49). As seen in Figure 21B, the predictable cue elicited a significant heart rate acceleration in the three time intervals between 1,000 and 4,000 ms compared to the neutral (t[35] = 2.51, p = .017; t[38] = 4.83, p < .001;, t[38] = 4.39, p < .001) and unpredictable condition (t[38] = 2.98, p = .005; t[38] = 5.17, p < .001; t[38] = 4.38, p < .001). Within the first time window, the unpredictable condition showed a marginally decreased heart rate compared to the neutral (t[38] = 1.75, p = .088) and the predictable condition (t[38] = 1.75, p = .088). Within the neutral condition there was a marginal increased heart rate from the first to the second time window (t[35] = 1.96, p = .058), and similarly within the unpredictable condition (t[35] = 2,42, p = .021) while the following successive time windows did not show any change any more (ts < 1.64, ps > .109 and ts < 1.19, ps > .241). In contrast, the predictable condition elicited an accelerative heart rate for all successive time windows between 0 - 3,000 ms (t[35] = 4.29, p < .001; t[35] = 6.65, p < .001; t[35] = 1.96, p = .058).

4.3.1 Influence of anxiety

Ratings

Correlations between explicit ratings for both the cue and the contextual stimuli were correlated with state and trait anxiety taken from the STAI as well as social anxiety from the SPAI.

These revealed that the differences in reported anxiety between predictable and neutral cue as well as between unpredictable and neutral cue correlated significantly with both state (r = .34, p = .027 and r = .44, p = .004) and trait anxiety (r = .28, p = .071 and r = .31, p = .045). These suggest increased differential self-reported anxiety to both cues somehow associated with the aversive event (predictable and unpredictable cue) compared to the neutral cue with increasing state and trait anxiety. No correlations were found regarding the contextual stimuli (*r*s < .25, *p*s > .109).

EEG data

A marginally significant negative correlation was found between social anxiety and the differential ssVEP amplitudes in response to the predictable cue compared to neutral cue (*r* = -.29, *p* = .060). No relationship was found between the averaged differential ssVEP amplitudes in response to the context stimuli and the participants' anxiety level (*r*s < .17, *p*s > .262).

Heart rate

State or trait anxiety did not show any influence on differential heart rate within the predictable and unpredictable condition compared to the neutral condition during presentation of the cues (*r*s < .25, *p*s > .141). However, with increased social anxiety the difference between the unpredictable and neutral (*r* = .33, *p* = .038) and between the unpredictable and predictable context (*r* = .37, *p* = .021) increased.

4.4 Discussion

Previous studies already aimed at a differentiation of the affective states of fear and anxiety by comparing manifestations of psychophysiological activation such as the startle reflex during predictable and unpredictable threat (Grillon, Baas, Cornwell et al., 2006; Grillon et al., 2004). The current study investigated the differential influence of the predictability of threat on cortical activation as well as autonomic changes, in order to demarcate the state of fear as a phasic response during imminent threat and the state of sustained anxiety, where threat is more distant and undetermined. Within a long lasting context stimuli, the cue in the predictable condition signaled the occurrence of an aversive sound and therefore was expected to elicit a phasic fear response. Within the unpredictable condition, the aversive sound could not be predicted and therefore a state of chronic apprehension was expected during the context stimulus. A neutral condition served as a control condition. The assessment of continuous activation during predictable and unpredictable threat in the present study (instead of the assessed “snap-shots” measured with startle responses) could be realized with the help of ssVEPs and continuous heart rate assessment.

As expected, the context in the absence of cue elicited subjective arousal and anxiety for the unpredictable compared to the neutral context, as well as decreased valence ratings. This was also true for the predictable context. It seems that although the context in this condition was never associated with the US directly, it still carried a high significance. Regarding the ratings for the cues embedded in the respective context, a similar pattern for the subjective experience evolved. Both cues of the unpredictable and predictable condition were experienced as more unpleasant, more arousing and more anxiogenic compared to the neutral cue. From these subjective measures, it may be assumed, that the all stimuli associated with the US, even when not in an explicit manner, elicited explicit affective responses.

During both the predictable and unpredictable condition, increased cortical activation in visual areas was visible for the whole presentation time of 60 seconds. As both the context and cue stimuli were presented within this presentation time, this result was expected. Interestingly, when only considering time windows without cue presentation, the ssVEP amplitudes showed a differential activation in response to the unpredictable compared to the predictable threat. This facilitated sensory processing of the unpredictable context only evolved in the last window of the trial. While during the predictable context, sustained attentional resources were reduced as the threat of an US occurrence dissolved with the last cue presentation, the threat was still present in the unpredictable context and therefore increased attentional resources were still maintained within the unpredictable condition. Regarding the key characteristics of anxiety as a state of a sustained, long-lasting response due to the unpredictable threat, this could be – at least partly – be seconded by these findings.

It was expected that the electrocortical activation elicited by the cues should differentiate between phasic fear and sustained anxiety to this effect, that increased ssVEP amplitudes should be observable during the predictable cue, as representing the most imminent threat. In fact, this increased cortical activation was found in response to the predictable cue compared to the neutral cue. Interestingly, the context surrounding the predictable cue during the 4 seconds presentation elicited similarly increased ssVEP amplitudes. Therefore, not only the specific cue itself, but also the external surrounding context, which is present during the aversive event gains motivational significance and threat value. Similar results were observed for visual contexts, which contained a fearful face. Here, the aversive cue led to an amplification of the contexts, indicated by enhanced ssVEP amplitudes (Wieser & Keil, 2014).

It seems that unpredictable and predictable threat similarly draw attentional resources and lead to increased electrocortical activation when no crucial cue is present. One explanation could be that the time between cues, when only the context is present, serves for both conditions as a time of uncertainty about the occurrence of the aversive event. While in the unpredictable condition, the occurrence is not predictable in any way, even in the predicable condition, the context signaled some uncertainty about when the cue occurs and consequently, the aversive noise would appear.

The context and cue received similarly facilitated cortical processing and did not compete for limited attentional resources, contrary to expectations (Hindi Attar et al., 2010; Müller et al., 2008; Wieser et al., 2012). The learned motivational significance of cues and contexts seem to be equivalent between conditions, as both are somehow associated with the aversive sound and therefore neither context nor cue can be disregarded in each condition and both are drawing similarly enhanced processing resources. That simultaneously presented stimuli can be processed without competition effect could be shown also for stimuli with high emotional content and spatial attention (Keil et al., 2005).

The current study first revealed a decreased heart rate response in the predictable and unpredictable condition for the first ten seconds of the trial, in which only the context was present. As soon as the cue was present, heart rate significantly increased in the predictable condition compared to the neutral and unpredictable condition. The fear bradycardia, as the heart rate deceleration is known in the animal literature, reflects an orienting response during the post-encounter phase of the defense cascade model, showing a hyperalertness (Fanselow, 1994; Löw et al., 2008). In the circa-strike phase, in which the threat becomes proximal, physiological arousal increases, such as a heart rate acceleration and increased skin conductance (Fanselow, 1994; Lang et al., 2000). In the present study, subjects showed an orienting response with a heart rate deceleration when no predictions can be made regarding the aversive event, that is during presentation of the contextual stimuli. As soon as the feared event – that is the cue in the predictable condition – is presented, heart rate significantly increases compared to the two other conditions, as now the source of threat is imminent and the likelihood of the aversive event exponentially increases. These results support previous findings of differential heart rate response depending on the proximity and imminence of threatening stimuli (Löw et al., 2008).

The defense cascade model could explain the similarities and difference between predictable and unpredictable threat in the electrocortical and physiological activation in this study. During the post-encounter phase of the model, a facilitated processing of all perceptual processes is reflected by increased electrocortical activation in occipital areas. In this phase, where the anticipated threat is not as imminent yet, the organism needs to evaluate the situation in order to prepare any suitable responses. The increased cortical responses during the unpredictable condition, reflecting increased mobilization of attentional resources are in line with this explanation. Even during the predictable condition, a preparation for the approaching cue and subsequently also for the occurrence of the aversive event takes place, as seen with increased ssVEP amplitudes in response to the context in the predictable condition. However, a differentiation between these two conditions is found during the cue presentation. The occurrence of the predictable cue can be classified into the circa-strike phase during defense. The proximity of the unpleasant event leads to increased physiological activations in order to prepare immediate responses. These are increased ssVEP amplitudes, as well as an increased heart rate in response to the cue in the predictable condition in the present study.

An influence of both state and trait anxiety could be shown on the self-reported anxiety in response to predictable and unpredictable cue compared to the neutral cue. The anxiogenic effect of both cues associated with the aversive event (independent of the predictability) increased with higher anxiety. These results follow findings of increased conditionability in cue conditioning studies for high anxious individuals (Orr et al., 2000; Peri et al., 2000). Similarly, context learning also showed a stronger reactivity for the anxiety context compared to the safety context for high anxious individuals (Glotzbach-Schoon et al., 2013). An increased amygdala activity during the threat context in the extinction phase was related to higher trait anxiety (Barrett & Armony, 2009).

The present study aimed at differentiating the two states of fear and anxiety, as it could been shown before in various studies using startle response and fMRI studies (Alvarez et al., 2011; Davis et al., 2010; Grillon et al., 2004; Hasler et al., 2007). The present study allowed tracking phasic fear and sustained anxiety continuously and therefore dissociating these affective states quantitatively and qualitatively. Increased activation in occipital areas reflecting facilitated processing of perceptual processes were found for both predictable and unpredictable threat. Without the availability of the cue, which gives more information about the occurrence of the aversive event, unpredictable and predictable threat similarly received sensory facilitation, whereby during unpredictable threat a longer lasting activation was found.

5 General discussion

Context conditioning serves as a paradigm to model sustained anxiety. Especially for the etiology of anxiety disorders like GAD or PD the exposure to unpredictable and uncontrollable events is discussed as important factors (Mineka & Zinbarg, 2006). As such, it is important to understand the processes during contextual learning, during which unpredictable aversive consequences lead to sustained anxiety. Differential context conditioning is one possibility to examine the processing during the acquisition and maintenance of sustained anxiety and compare the resulting anxiety responses to responses within a safety condition. While it is important to understand the neural and physiological correlates of sustained anxiety itself, it is additionally advantageous to extend the focus of research on how novel cues, especially faces, are perceived and processed when individuals experience states of anxiety.

This thesis presents a differential context conditioning paradigm, which was implemented with the intention to obtain a continuous electrocortical correlate of sustained anxiety in a threat compared to a safety context. The attention mechanisms during the processing of contextual anxiety on implicit and explicit response levels were assessed in three studies. Conditioning effects with increased ssVEP amplitudes showing facilitated sensory processing and heart rate decelerations indicating an orienting response were expected in response to the anxiety context. An additional focus was set on the processing of social cues and the influence of contextual information on these cues. In the first two studies, social cues were presented in the anxiety and the safety contexts in order to compare the differential processing of these cues within periods of threat and safety. It was the question, whether all cues received increased attentional resources due to general hypervigilance induced by anxiety or if some cues of heightened significance, (e.g. persons compared to objects and angry faces compared to neutral faces), are preferentially processed in the anxiety context. Finally, the thesis aimed at delineating the correlates of sustained anxiety found in the first two studies from responses evoked by phasic fear in a paradigm combining cue and context conditioning.

5.1 Context conditioning effects

One main hypothesis of this study comprised that a previously neutral context elicits sustained anxiety responses after it was associated with an unpredictable aversive event, which are reflected in electrocortical activations. Anxiety responses in a threat compared to responses in a safety context were expected to manifest themselves on explicit and implicit levels. Indeed, successful contextual learning occurred on the implicit as well as the explicit level.

Regarding the emotional-cognitive level, in all three studies the anxiety context was rated as more unpleasant, more arousing and more anxiogenic, thereby confirming the hypothesis of successful context conditioning reflected on the explicit level. The perception of contextual stimuli did not change after novel distractor stimuli were presented, but was persistent over time.

As expected a priori, these conditioning effects should also manifest themselves in a sensory amplification of the threat context, as indicated by increased ssVEP amplitudes while being in the anxiety context compared to responses in the safety context. Indeed, these augmented ssVEP amplitudes in response to the anxiety context could be confirmed in all three studies. These enhanced electrocortical responses reflect increased attentional resources towards a stimulus of significance, both mediated by stimulus properties, (Keil et al., 2003; Moratti et al., 2006) and top-down attention mechanisms (Müller et al., 2006). As previous cue conditioning studies showed, a cue known to predict an electric shock elicited increased occipital ssVEP amplitudes (Miskovic & Keil, 2013a; Moratti & Keil, 2005). Sources this oscillatory response are located in the extended visual cortex together with contributions from the striate cortex and higher order cortices (Di Russo et al., 2007; Müller et al., 1997; Wieser & Keil, 2011). In this thesis, for the first time sustained activations could be shown in context conditioning as an indicator of increased attention facilitating sensory processing of the anxiety context.

However, awareness had an impact on the conditioning effect in the second study. The two level account of fear conditioning discusses conditioning in terms of two dissociable routes (Hamm & Weike, 2005). Implicit learning occurs through a direct thalamic-amygdaloid route and is reflected in fear potentiated startle responses. Explicit learning depends on the conscious representation of the US-CS contingency and is quantified in ratings as well as skin conductance responses reflecting physiological arousal (Hamm & Weike, 2005). In Study 2, unaware participants did not show the expected correlates of contextual anxiety on cortical, physiological, and emotional-cognitive levels, as I found in the first study, while participants being aware of the context-US contingency showed differential response patterns. It might be that in more complex conditioning paradigms, especially when adding contextual information which needs to be considered in the learning process, awareness plays a different role than during simple cue conditioning. In a combined context and cue conditioning paradigm, where the context served as occasion setter for a CS to be signaled as an actual CS+, Ewald (2014) reported in her dissertation that both fear potentiated startle and explicit ratings were affected by contingency awareness. Only aware participants showed enhanced startle response to the CS+ in the fear context, while unaware subjects did not differentiate between cues or contexts. The psychophysiological measures used in this thesis, i.e., ssVEPs and heart rate change, cover different aspects of affective stimulus processing. While heart rate seems to be dependent on the expectancy of an aversive event (Epstein & Roupenian, 1970), cortical activity in occipital areas is independent on declarative cognitive processes as CS-US expectancy, but is linearly related to actual associative strength (Moratti & Keil, 2009). In addition, different aspects of associative learning processes might have been assessed here compared to the typical conditioning studies examining contingency awareness, which mainly obtained fear potentiated startle or SCR. Therefore, any assumptions derived from the results related to the impact of awareness in context conditioning need to be interpreted with care. Firstly, only in one of the two studies, which applied this new context conditioning paradigm, found any effects of awareness. Secondly, the sample size in the second study of the unaware and especially the aware group was small; as such the results are only restrictedly interpretable.

As a third hypothesis regarding contextual anxiety responses, I expected sustained anxiety to be indicated by a heart rate deceleration as an orienting response in the anxiety context. In all experiments, the heart rate response reliably reflected contextual anxiety (when only considering aware subjects in Study 2). Moreover, a differentiation between imminent threat and diffuse threat, was reflected in the heart rate response. In the acquisition phase of Study 1 and 2, when the US occurrence was frequent, the threat context elicited a heart rate acceleration. This was also seen for the cued fear response in the NPU-threat test. In the test phases and during unpredictable threat in the NPU paradigm, sustained anxiety was related to a heart rate deceleration, as I expected it a priori as a marker of contextual anxiety. The differential course in the defense response between acquisition and test phase might be accounted to the characteristics of unpredictability. While temporal unpredictability relates to the uncertainty about *when* to expect negative consequences, cue unpredictability refers to *whether* to expect the aversive event at this time point at all. There is evidence that both cue and temporal unpredictability are important to consider in models of anxiety (Davies & Craske, 2015). In the acquisition phase, a temporal unpredictability was realized, while a cue unpredictability was not attained due to the 100 % reinforcement rate.

Although heart rate response is discussed as a typical marker of defensive engagement during threat, there is surprisingly only a small amount of recent studies, which applied heart rate responses to measure fear or anxiety conditioning effects. Citing the defense cascade model again, heart rate is one indicator of threat imminence (Bradley et al., 2001; Fanselow, 1994). When the aversive event is perceived as a diffuse threat without the immediate need to act yet – potentially also due to the unpredictability of the threat – a heart rate deceleration is observed. This post-encounter phase is associated with sustained anxiety in the literature (Bradley et al., 2001; Davis et al., 2010). In the circa-strike phase the increasing imminence of the threat - when the threat is immediately expected – leads to a heart rate acceleration typically seen in fear responses. Especially for phobic patients, an encounter with the phobic event leads to this increased heart rate (Globisch, Hamm, Esteves, & Öhman, 1999), while a recent conditioning study (Costa, Bradley, & Lang, 2015) but also studies using simple picture viewing (Bradley et al., 2001; Lang & Bradley, 2010; Moratti et al., 2004) reported defensive heart rate deceleration for fear stimuli. Taken together, cued fear responses in the NPU-threat test, as well as responses during the acquisition phase in context conditioning elicited a heart rate acceleration. In both cases, threat was highly imminent. In contrast to this acceleration, heart rate response consistently showed a deceleration after the association between contexts and aversive event was established and the reinforcement was less obvious to the subjects, which corresponds to a situation with a highly unpredictable and diffuse threat. The unpredictable threat in the NPU-Study similarly elicited a heart rate deceleration.

Importantly, augmented ssVEP amplitudes and decreased cardiovascular responses should show a temporal specificity and be reflected during the whole time the anxiety context is present in order to validate the concept of anxiety as a sustained, tonic response. In all three studies, I found evidence for a sensory amplification of contextual anxiety. One important feature of anxiety is the characterization as a tonic defensive response in comparison to phasic fear (Davis et al., 2010). As such, the studies aimed at mapping the long-lasting anxiety on a neural and physiological level. Both heart rate and ssVEPs are methods that allow tracking response patterns continuously. The facilitated sensory processing of the anxiety context found in the first study was maintained as long as the context was present. The results support the categorization of anxiety as a sustained, long-lasting response, as both elevated heart rate and cortical responses were maintained for the whole context presentation. A similar sustained response was found in the third study, which used another paradigm but still showed this long-lasting sensory facilitation of the threat context. Previously, brain responses during the anticipation of unpredictable threat were found in the visual areas in concert with subcortical structures (Hasler et al., 2007). These support the idea of an increased vigilance with a sensory amplification of the threat information, as it was found in the first study. That this defensive activation was actually maintained in the anxiety context underscores the nature of anxiety as a sustained response. However, the open question remains why this tonic response was scarcely observable in the second study, and mainly as an increased initial response. In a recent fMRI study, Andreatta et al. (2015) showed a differentiation between initial and sustained brain activation in a context conditioning paradigm in virtual reality. The hippocampus and the amygdala as two structures which are repeatedly found to be active during fear and anxiety conditioning (Alvarez et al., 2008; Maren et al., 2013; Marschner et al., 2008), were part of the sustained anxiety network. Entering a threatening context activated the orbitofrontal, dorsolateral, and dorsomedial prefrontal cortex. As such, they are discussed to be part of a network involved in threat expectancy and threat appraisal (Andreatta et al., 2015) and possibly mediate the cognitive-emotional ratings of the contexts. The initial threat appraisal led in all three studies to more negative ratings for the threat context, thereby revealing a successful conditioning process. In the second study, all participants showed an initial anxiety response on a cognitive-emotional level and an initial cortical activation to the anxiety context. Surprisingly however, no sustained activation in response to the anxiety context was found in this study. In the test phase, when the reinforcement rate was reduced and novel distractor stimuli appeared in both contexts, only subjects who were aware of the context-US contingency maintained an amplification of the threat context compared to the safety context, reflected in a differential heart rate response and ssVEP amplitudes. Taken together, the results of Study 1 and 3 combined give the strongest evidence for the continuous context amplification, as I expected it, as a marker of sustained anxiety. As in all three studies the identical EEG sensors clustering was used, the results are ideal to compare.

Additionally, the sensory amplification indicated by heightened electrocortical responses to the CTX+ were expected to not attenuated by simultaneously presented distractor stimuli. The anxiety response reflected in cortical and physiological activation seemed to be robust enough to survive the simultaneous presentation of novel stimuli. Although facial stimuli represent an important and evolutionary significant category for humans (Öhman & Dimberg, 1978) and receive preferentially processing resources, the motivational significance of the anxiety context was not undermined.

As both facilitation of sensory information and physiological activation during threat are part of the response pattern during contextual threat, I also intended to explore how the increased ssVEP amplitudes and increase in attention mechanisms are reflected on physiological defensive activation measured in heart rate. Moratti and Keil (2005) analyzed both cardiovascular activity and steady-state cortical activation during a cue conditioning paradigm and reported a cortical amplification of the CS+ solely for subjects with a heart rate acceleration during acquisition. The same sample group showed an orienting response, i.e. heart rate deceleration, in the extinction phase. Results of Study 2 could partly support this positive relationship between conditioning effects reflected in heart rate and ssVEP amplitudes. That is, the conditioning strength, i.e. the differential activation pattern between anxiety and safety context of the heart rate response was linearly related to the differential cortical activity of the two contexts. In general, the indices of contextual anxiety that were collected in the three studies partly correlated with each other. That is, differential cortical activation in response to the contexts was linearly related to anxiety ratings of the contexts and in the second study with heart rate change to the threat context. This suggests that the facilitated sensory processing, i.e., enhanced ssVEP amplitudes of the threat context is also reflected in, and connected with declarative cognitive processes of explicit evaluation of this context stimulus. Moreover, the increased ssVEP amplitudes also relate to heart rate change induced by anxiety. The physiological and neural change in contextual anxiety therefore represents a widespread response pattern, culminating to the idea that motivational relevance of a stimulus changes and facilitates sensory perception of this stimulus (Lang et al., 1990). In other studies, ratings were found to determine avoidance behavior in the threat context (Glotzbach et al., 2012) and thereby supporting the findings of interrelated physiological threat related changes.

The first and second study applied the same paradigm with long-lasting pictures as contextual stimuli. The only difference was for the semantic category of distractor stimuli during the test phase: object vs. social agent in the first study and different emotional expressions in the second study. The test phase in both studies focused on the additional research question of the contextual influence on cue (face) processing, whose results are discussed in the succeeding chapter.

5.2 Processing of facial cues within threat

While a main focus of the thesis was the perception and processing of sustained anxiety within a context conditioning paradigm, a second research question arose regarding the perception and processing of faces within this state of sustained anxiety. While it is important to understand the learning mechanisms contributing to the etiology of anxiety, we also need to understand how anxiety changes the perception and processing of people. Initially, the idea was to clarify whether social cues, as inherently more relevant to humans, and non-social cues are differentially affected by anxiety contexts (Study 1). In a second step, persons conveying different emotional expressions were presented in both threatening and safe contexts in order to explore the reciprocal influences of contexts and facial cues (Study 2). As a nondirectional hypothesis I expected either enhanced responses to all stimuli within the anxiety contexts, irrespective of semantic category of the stimuli or emotional facial expression (general hypervigilance). On the other hand, a preferential enhancement of faces compared to objects and of angry faces compared to neutral faces might be expected in accordance with a selective hypervigilance.

With the data from three studies, I showed that context conditioning led to sensory amplification of the threat context. With this threat-induced hypervigilance, sensory processing of cues perceived within the contextual anxiety was expected to be altered as well (e.g. Righart & de Gelder, 2005). In the first study, at first glance no evidence was found that the contextual information changed the processing of the novel cues in general or specifically of the social cues. However, when only looking at the part, where the learning experience was still proximate, the objects compared to the social cues received a sensory facilitation in the anxiety compared to the safety context. This was a surprising finding, as instead of the social cue as a highly relevant stimulus compared to a random object, this object elicited increased cortical activity in occipital areas. As discussed earlier, this might be due to the nature of the objects as an oddball (Mercado et al., 2006). An unexpected novel stimulus in the context of an office room, might receive increased attentional resources due to the hypervigilance elicited by the threatening contextual information, as every new (and maybe especially unexpected) cue could give more information about when to expect the next aversive event.

More evidence for the impact of threatening information on the processing of facial cues comes from the second study. Initially, the angry face received increased attentional resources, irrespective of the context information surrounding the face and thereby supporting findings of studies of preferential processing of threatening faces (Dimberg, 1987; Öhman & Mineka, 2001). This preferential processing of threatening faces seems to occur fast and automatically. Time-wise, it seems that emotion-modulated processing happens before the integration with the contextual information takes place (Diéguez-Risco, Aguado, Albert, & Hinojosa, 2013). Participants, who learned the contingency of the contexts and the aversive event and actually expressed sustained anxiety responses on an implicit and explicit level, also showed a context-dependent effect on the processing of faces. All faces irrespective of facial expression, elicited augmented ssVEP amplitudes in the threat context compared to the safety context. This gives evidence for a general hypervigilance induced by contextual threat, leading to a preferential processing of all novel stimuli within a state of anxiety. This hypothesis of a general hypervigilance promotes the enhanced processing of all facial stimuli when perceived in a threatening environment, irrespective of the emotional load. The present findings go in line with previous work revealing that anticipatory anxiety compared to a neutral context leads to an enhanced reactivity to cues (Bublatzky et al., 2013; Bublatzky & Schupp, 2012; Dunning et al., 2013; Righart & de Gelder, 2005). In a threatening context consisting of inhalation of stress sweat, both neutral and ambiguous faces elicited enhanced neural responses (Rubin et al., 2012).

Taken together, in Study 1, I found a differential processing of cues in the anxiety and safety context, depending on the stimulus category. However, when only using faces, without any non-social control stimulus, a preferential processing of all faces irrespective of their emotional content was visible. It seems to be of some relevance, which different stimulus categories are used when investigating anxiety-dependent processing of cues. The NPU-threat test of the third study, although its primary purpose was to examine the differences and similarities of predictable and unpredictable threat, might add further hints concerning the impact of contextual information on cues. When the predictable and unpredictable cues appeared in the respective context, they had a different meaning for the participants. In the predictable threat, the person itself was associated with the aversive event and therefore elicited a fear response. In the unpredictable condition, the person did not have any relevance for the participants to predict the occurrence of the US. As such, the person in the predictable condition elicited a sensory amplification, reflected by increased heart rate and ssVEP amplitudes. On the other hand, the face in the unpredictable condition, where the context signaled the occurrence of an unpredictable threat and the cue did not carry any additional information, did not elicit increased responses. Consequently, the person appearing in a context of unpredictable threat did not benefit from the hypervigilance found in sustained anxiety. However, these results only give limited information regarding context-dependent cue processing, as in the NPU paradigm, the motivational significance of the social cues changes drastically for each condition. As such, the cue in the unpredictable condition was regarded as highly irrelevant compared to the predictable condition and as such not comparable to the findings of the other two studies. A meta-analysis examining the processing of emotional faces vs. emotional scenes revealed that an emotional network elicited by a facial expression is distinct from a network elicited by an emotional scene, even when controlling for the basic features of each stimulus class (Sabatinelli et al., 2011). The interaction between these two networks, as an influence of contextual information on the processing of threatening facial expression, might therefore be not visible in the observed brain areas in the current studies, as these networks show overlaps only in distinct areas.

In contrast to previous studies, I used pictures of persons instead of disembodied faces as stimuli to examine the influence of threatening contexts on their perception and processing. As more and more studies underscore the importance of contextual information, it is important to meet the concerns raised by researchers about the use of context-less faces as stimulus material in emotion processing for years (Barrett et al., 2011). In other words, we have to move from the disembodied, context-less faces conveying emotional expressions to an incorporation of more contextual information when using faces as stimuli.

As environmental stress influences the processing of social cues and social anxiety has an influence on face processing, social anxiety was taken into account for the context depending processing of faces. Previous studies highlighted the importance of the context, in which the perceiver is situated, i.e. a stressful situation, for the processing of facial cues regarding social anxiety (Garner et al., 2006; Grillon & Charney, 2011). In the first study, for both implicit psychophysiological measures an influence of trait anxiety on the processing of contextual anxiety was found. That is, the difference between the threat and safety context diminished with increasing participants' anxiety. Prominently, this was also true for social anxiety but mainly when the social distractor was present. Obviously, a fear relevant cue in social anxiety, i.e. a person, leads to an impaired ability to differentiate between external threat and safety. The impaired safety learning in anxiety patients was described before (Duits et al., 2015; Lissek et al., 2005; Peri et al., 2000). Interestingly, in the present study it was only present when a fear relevant cue – compared to a completely irrelevant object, - was visible within both contexts. In the second study, only a marginal correlation between social anxiety and ssVEP amplitudes in response to the contexts were reported, depending on the presence of an angry face. This correlation suggests that with increasing social anxiety, the anxiety context received more processing resources only when an angry face was present. So high socially anxious individuals direct heightened resources to a threat context when an additional threat cue, that is the angry face, is present. Wieser and Keil (2014) observed a context amplification in the presence of a fearful face, showing that the context, which surrounds a threat cue, is as relevant as the cue itself and therefore additionally receives heightened sensory processing. Likewise, in the NPU-threat test of the third study, the context during the predictable threat drew increased attentional resources, similar to the cue itself, and elicited heightened ssVEP amplitudes. The second study could show a similar finding, while at the same time restricting it to social anxiety as a mediating factor.

Here it needs to be mentioned, that the type of facial expression, i.e. angry vs. fearful face, might be essential for any cue-context interactions. While both expressions transfer threat information, a fearful and an angry face have different effects on attention processes. Angry faces attract and hold attention (Williams et al., 2005). Perceived within an anxiety context, they serve as an additional threat cues themselves, and receive preferential processing (Mogg & Bradley, 1998). Fearful faces, on the other hand, might signal a warning of a potential environmental threat, and therefore broaden the attentional focus (Palermo & Rhodes, 2007), as seen in the study from Wieser and Keil (2014). Therefore, a follow-up study should include both angry and fearful facial expressions when examining the influence of contextual threat information on them.

5.3 Differentiation of fear and anxiety

In two of the three studies, I explicitly focused on processing of anxiety induced by a context conditioning paradigm. In these, sustained sensory facilitation and hypervigilance reflected this contextual anxiety on an electrocortical and physiological level, i.e. heart rate and ssVEP amplitudes, as well as respectively increased evaluations of the threat context. In a third study, I aimed at demarcating the concepts of fear and anxiety as results of cue and context conditioning, by modulating the predictability of the aversive event. In previous studies, potentiated startle responses were used as an index of fear and anxiety (e.g. Grillon, Baas, Cornwell et al., 2006; Grillon et al., 2004). Although a reliable measure, it fails to account for the temporal characteristics employed by the differentiation of fear and anxiety, as a phasic and a sustained response, respectively. It was expected, that the differences between anxiety and fear are reflected in time varying cortical responses. Specifically, I expected that the context associated with the unpredictable threat elicits long-lasting enhanced ssVEP amplitudes compared to the other contexts. Responses to the predictable cue were expected to elicit heightened short-term electrocortical responses, indicating cued fear. As in the first two studies, sustained enhanced ssVEP amplitudes for the context were found when the US was presented unpredictable. However, the context in the predictable condition, where subjects knew when to expect the loud noise, similarly received increased attentional resources. This sensory amplification of both contexts compared to the neutral context was maintained for the whole trial length. Supporting to the definition of anxiety as a long-lasting, tonic response compared to phasic fear (Davis et al., 2010), the augmented oscillatory potentials evoked by the unpredictable threat were actually maintained for a longer period of time than to the predictable context.

Next to an electrocortical representation of cued fear and contextual anxiety, differential heart rate patterns of fear and anxiety were expected in line with the defense cascade model. That is, a state of anxiety, i.e. unpredictable threat, should induce a deceleration of heart rate. Phasic fear, as the state of ultimate defensive reactions in the circa-strike phase, on the other hand, should elicit a heart rate acceleration. Indeed, the heart rate response showed distinct response patterns for predictable and unpredictable threat. In the unpredictable condition, the context elicited a heart rate deceleration, similarly to the response in the test phase of the two previous studies. The presence of diffuse threat, due to the inability to predict its occurrence, led to an orienting response. This hypervigilance in a state of anxiety helps to orient to the sensory input. Thereby, the processing of further contextual details is fostered to receive more information from the environment regarding the threat in order to prepare for possible action (Bradley et al., 2001). Interestingly, this orienting response was also observed for the context of the predictable condition. In this phase, participants waited for the presentation of the cue in order to find out when the loud noise will appear. During this time, the threat was as unpredictable as in the actual unpredictable condition, as participants did not know, when the cue as predictor of the US will occur. When keeping this in mind, the orienting response during the context in both predictable and unpredictable condition is an allegeable finding. This heart rate deceleration was recently shown to conditioned cues (Costa et al., 2015), as well as to inherently aversive stimuli (Bradley et al., 2001). As a marker of sustained anxiety, heart rate deceleration could be confirmed in all three studies of this thesis. With increasing imminence of the threat, the defensive physiological response changes as the innervation of the sympathetic nervous system dominates, leading to a heart rate acceleration. A study, which examined the influence of threat proximity on defensive response patterns described that with increasing proximity a threatening stimulus led to a heart rate change from a deceleration to an acceleration compared to a safety condition (Löw et al., 2008). These findings are translatable to the results of the NPU paradigm (as well as of the other two studies).

In previous studies, mainly the potentiated startle response as measure of fear and anxiety was applied (Grillon, Baas, Pine et al., 2006; Grillon, Baas, Cornwell et al., 2006; Grillon et al., 2004). More recently, fMRI-studies aimed at comparing brain response patterns of predictable and unpredictable threat (Alvarez et al., 2011; Haaker et al., 2013; Lonsdorf et al., 2014). Here, I focused at completing the overall picture with applying measures that allow continuous recording during fear and anxiety and reflect involvement of attention networks, modulated by emotional arousal (Moratti et al., 2004). While previous studies focused on describing two distinct networks of fear and anxiety (Davis et al., 2010), it is also important to keep in mind that these two affective states also have a common circuit of emotional processing around the amygdala (Alvarez et al., 2011). While receiving sensory information, the amygdala also projects in a feedback loop to all levels of the visual pathway and exerts modulatory control over sensory processing (Amaral et al., 2003). As the most prominent finding regarding the differentiation of fear and anxiety, attentional resources are similarly drawn by predictable and unpredictable threat, as both require and receive facilitated sensory processing. As such, the facilitated sensory processing during phasic fear and anxiety can be seen as the common interface of both.

5.4 Influence of trait anxiety on contextual learning

Trait anxiety is a potential influence on associative learning processes. However, scientific studies about the influence of participants' anxiety on context conditioning are still scare. Therefore, trait and state anxiety were assessed in order to examine the influence on contextual anxiety and phasic fear in all three studies. This influence was expected to be represented either by increased anxiety responses to the conditioned context during conditioning or by an impaired safety learning for high anxious individuals.

A decreased ability to differentiate between threat and safety cues leads to increased sustained anxiety indicated by physiological and explicit measures (Grillon, 2002a). An impaired safety learning is discussed as one marker for anxiety disorders (Lissek et al., 2005). The deficient safety learning in high anxious individuals as well anxiety patients leads to an increased chronic expectation of the US and therefore generates and maintains sustained anxiety (Baas, van Ooijen, Goudriaan, & Kenemans, 2008; Grillon, 2002a). As such, increased anxiety responses to unpredictable threat were found as a psychophysiological marker for PD and PTSD (Grillon et al., 2008; Grillon et al., 2009). Therefore, I expected to find differences in the physiological or cortical responses to anxiety depending on trait anxiety, as one risk marker for the development of anxiety disorders. Indeed, a hint towards impaired safety learning for participants with higher trait anxiety was found in the first study. More precisely, for cortical activation and heart rate an influence of trait anxiety on the processing of contextual anxiety was found. That is, the difference between the threat and safety context diminished with increasing participants' anxiety. These results are similar to what was reported previously in differential cue conditioning studies (Duits et al., 2015; Lissek et al., 2005; Mineka & Oehlberg, 2008). Recently, a fMRI study from our lab transferred these findings to sustained contextual anxiety, and reported a decreasing amygdala response with increasing participants' trait anxiety (Andreatta et al., 2015). On the other hand, results of the third study showed that both predictable and unpredictable threat elicited increased subjective anxiety in subjects with higher trait anxiety, thereby supporting previous reports of a faster acquisition of enhanced startle responses for anxiety conditioned contexts (Glotzbach-Schoon et al., 2013).

In total, a consistent sign for the influence of trait anxiety on the associative learning processes was not found. As the findings in this thesis are results of different context conditioning paradigms and only of correlational nature further studies are needed to investigate the relationship of trait anxiety on associative contextual learning processes.

5.5 Limitations

While I could successfully introduce a context conditioning paradigm and adapt a previously established one for the present methodological requirements, there are some limitations that need to be discussed.

Unpredictability of an aversive event is considered a key characteristic of anxiety. Thereby, both cue unpredictability and temporal unpredictability need to be considered in models of anxiety in order to elicit strongest responses (Davies & Craske, 2015). The context conditioning paradigm used in two of the studies yielded a temporal unpredictability, as subjects did not know *when* to expect an US in the threat context. The cue unpredictability, however, was maybe not as well implemented, as a 100 % reinforcement rate was realized and participants learned that they definitely will experience the loud noise in every trial. In future studies, both unpredictability characteristics should be considered in order to maximize sustained anxiety responses and subsequently increase any possibly impact on further face processing.

In two of the three studies, the study sample consisted only of female participants. The basis of this decision was a study by Grillon (2008), which reported an enhanced reactivity for sustained anxiety for women compared to men. As the main focus in my studies was to primarily obtain a continuous cortical representation of contextual anxiety, female participants were selected for the study samples in order to maximize conditioning results. While this restricted sample might be beneficial for initially establishing a new paradigm, further studies should try to include a broader sample in order to generalize the present results.

While I was interested in the influence of trait anxiety on learning processes in context conditioning, the associations were based only on correlation analyses. As now the paradigm is established, it would be helpful to recruit explicitly high and low anxious individuals. Additionally, it would be informative to collect other personality traits such as self-reported measures of attention of control (Baas, 2013).

The concept of ‘‘belongingness’’ between the stimuli and a US, sometimes referred to as “preparedness” (Seligman, 1971), describes that a better fitting between a CS, i.e. a face, and an US, i.e., a scream, leads to stronger conditioning responses and resistance to extinction (Hamm, Vaitl, & Lang, 1989). This associability of stimuli based on semantic parameters, however, varies within the used paradigms in this thesis. The use of nonliving objects, such as the office rooms and the clock as cues in comparison to faces, leads to a not easily solvable conflict of the semantic belongingness between conditioned cues, conditioned contexts, and the US. The loud noise as an aversive stimulus is easier semantically associated to the clock than the faces, and probably easier to the rooms than the persons. This could, at least partially explain the higher reactivity for the object than the person cue in the anxiety context in the first study. It might also explain the fact that an explicit contingency learning was necessary for a preferential processing of the faces in the threat context, as the association was not build as automatically in the second study. A rash answer to this problem would be the suggestion to use a scream as unconditioned stimulus in the future, as it is already established in conditioning paradigms with facial stimuli (Britton, Lissek, Grillon, Norcross, & Pine, 2011; Lau et al., 2008). However, then we would create the same problem with the deficient associability of a scream with an empty room. This was already described in a recent study applying the NPU-threat test with social stimuli and a scream-US, which failed to consistently produce an anxiety response to unpredictable threat in the absence of the social cue (Cohen Kadosh et al., 2015).

5.6 Summary and outlook

In sum, this thesis aimed at modeling sustained anxiety with the help of a context conditioning paradigm. Attention mechanisms were examined during the acquisition and maintenance of contextual anxiety, reflected in ssVEP activation and heart rate response as implicit measures, as well as explicit evaluations of the contexts. In three studies, I could endorse anxiety as a sustained defensive response, with augmented ssVEP amplitudes and heart rate change in the threat context. Due to the unpredictability of the aversive event, the individuals responded with a hypervigilance in the threat context, reflected in a facilitated processing of sensory information and an orienting response. This hypervigilance had an impact of processing of novel cues, which appeared in the anxiety context. As a second focus of this thesis, I could show that – depending on the contrasting semantic categories – the stimuli perceived in a state of anxiety received increased attentional resources. Against expectations, no modulation of the contextual influence was found by the emotional arousal conveyed by the facial expression, but all faces received increased attentional resources, as increased ssVEP amplitudes indicate (Keil et al., 2010; Moratti et al., 2004). A third research question examined the differentiation of predictable and unpredictable threat as an operationalization of fear and anxiety on a cortical and physiological level. Confirming previous studies, a fear response to the predictable cue was found, indicated by increased oscillatory response and accelerated heart rate. Both predictable and unpredictable threat yielded sensory amplification of the contexts, when no cue gave information regarding the occurrence of the next aversive event. According to expectations, the response in the unpredictable context showed longer-term sensory facilitation of the threat context. The hypervigilance during the absence of any predicting cue was supported by a heart rate deceleration, usually classified as one index of an anxiety responses (Blanchard et al., 1993; Davis et al., 2010).

As an etiological model of anxiety, associative learning processes play a crucial role (Mineka & Oehlberg, 2008; Mineka & Zinbarg, 2006). While early approaches were criticized as too simplistic, taking more factors into account, such as individual differences and contextual factors, helps to further develop these models and therefore to gain more understanding in the development and maintenance of anxiety disorders and their complexity on an individual level. The findings of my thesis help to add pieces to the big picture. In general, studying emotions are approached on different levels, that include verbal reports, physiological activation and behavioral response (Lang, 1995). Here, I covered two of these three equally important components, which are partly interrelated with each other. In order to develop a more coherent picture of the impact of contextual information on the development of sustained anxiety, the processing of stimuli within a state of anxiety, the assessment of conditioning, and its effects on multiple response levels are important. Notably, we need to understand not only the acquisition of sustained anxiety, but also how anxiety patients, once suffering from phases of sustained anxiety and excessive worrying, such as in PD, perceive and process their environment and the stimuli within.

As a marker for anxiety disorders, individuals with higher trait anxiety are more susceptible to threat, and fear and anxiety learning than others. Based on correlational analysis, trait anxiety showed to have a differential influence on contextual learning. One aspect that could not be regarded in this thesis is the influence of a genetic predisposition for the development of an anxiety disorder. In a next step, both conditioning paradigms should be used with participants carrying risk alleles for anxiety disorders to examine whether their contextual learning and the cortical processing of predictable and unpredictable threat differs.

The question is how concepts of fear and anxiety contribute to the development and anxiety disorders and how a deficiency in safety learning and/or increased reactivity to learned threat cues exerts influence. To obtain this goal, it is important to combine context and cue stimuli in associative learning models, but also consider the implications such as changed perception and processing of novel cues, i.e. persons, in states of anxiety. This view offers additional topics, which should be discussed in research and therapy.

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7 Annex

A Written informed consent Study 1

B Written informed consent Study 2

C Written informed consent Study 3

D Demographic data questionnaire

E Trial order and pseudo-randomization Study 1

F Trial order and pseudo-randomization Study 2

G Trial order and pseudo-randomization Study 3

H Written instructions Study 3

A Written informed consent Study 1

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Aufklärungstext zur Studie

„Lernen und Aufmerksamkeit“

Sehr geehrte Versuchsteilnehmerin, sehr geehrter Versuchsteilnehmer,

Sie nehmen an der Studie „Lernen und Aufmerksamkeit II“ teil, bei der wir untersuchen möchten, wie aversive oder unangenehme Reize Ihre Gehirnaktivität verändern. Sie werden aus der Teilnahme keinen unmittelbaren Nutzen für sich ziehen können. Wir hoffen jedoch, durch unsere Arbeit mehr darüber erfahren zu können, welche unmittelbaren physiologischen Reaktionen und welche gefühlsmäßigen und motivationalen Veränderungen unangenehme Reize hervorrufen können. Wenn Sie möchten, werden wir Ihnen nach der Untersuchung gerne die Hintergründe und Ziele dieser Untersuchung ausführlich schildern.

Vor der Untersuchung werden Sie einige Fragebögen ausfüllen, in denen wichtige Daten bezüglich Ihrer Person festgehalten werden. Dann wird der Versuchsleiter zur Messung Ihrer Herzrate zwei Messelektroden auf Ihrer Brust anbringen. Dazu wird Ihre Haut mit etwas Alkohol gereinigt, damit der Widerstand zwischen Haut und Messelektrode so gering wie möglich ist. Aufgrund dieser Hautreinigung kann es zu Hautrötungen und leichten Hautirritationen kommen, die aber normalerweise innerhalb kurzer Zeit abklingen. Außerdem wird auf Ihren Kopf ein angefeuchtetes Netz mit 128 Elektroden gelegt, um Ihrer Gehirnaktivität zu messen. Diese Flüssigkeit, in dem das Netz liegt besteht nur aus Wasser, Baby-Shampoo und Kalium und ermöglicht eine optimale Erfassung ihrer Gehirnaktivität.

In der Untersuchung werden Sie auf einem Computerbildschirm Bilder sehen. Die Bilder werden flickern und dabei werden Sie manchmal lautes weißes Rauschen hören. Die Geräusche sind ziemlich unangenehm, aber vollkommen unschädlich. Ihre Aufgabe ist es, nur die Bildern anzusehen

Die Teilnahme an der Untersuchung ist völlig freiwillig. Sie können jederzeit - ohne Angabe von Gründen - die Teilnahme abbrechen. Dadurch entstehen Ihnen keinerlei persönliche Nachteile. Für Ihre Teilnahme an der Untersuchung erhalten Sie 2 Versuchspersonenstunden.

Alle Daten dienen ausschließlich Forschungszwecken, werden vertraulich behandelt und ohne Namensgebung unter einer Codenummer abgespeichert. Der Codierungsschlüssel wird nach Abschluss der Studie vernichtet. Bis dahin können Sie auch noch nach der Untersuchung die Löschung ihrer Daten verlangen.
Falls Sie noch weitere Frage haben, fragen Sie bitte jetzt.

Einverständniserklärung

Ich bin einverstanden, an dem Experiment „Lernen und Aufmerksamkeit“ teilzunehmen und dass die erhobenen Daten in anonymisierter Form wissenschaftlich ausgewertet werden.

Ich bin darüber informiert worden, dass ich jederzeit aus der Untersuchung ausscheiden kann, ohne dass mir persönliche Nachteile entstehen.

Mit meiner Unterschrift erkläre ich, dass ich das Vorhaben und diese Information verstanden habe, meine Fragen zufrieden stellend beantwortet wurden und ich freiwillig und aus eigenem Entschluss an der Untersuchung teilnehme.

Würzburg, den ____________________ Unterschrift _________________________

Name und Anschrift ____

____

Unterschrift des Versuchsleiters ______________________ Code_______________

B Written informed consent Study 2

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Aufklärungstext zur Studie

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Würzburg, den ____________________ Unterschrift _________________________

Name und Anschrift ____

____

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C Written informed consent Study 3

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Aufklärungstext der Studie
„Alles vorhersagbar?“

Sehr geehrte Versuchsteilnehmerin, sehr geehrter Versuchsteilnehmer,

Sie nehmen an der Studie „Alles vorhersagbar?“ teil, bei der wir untersuchen möchten, wie aversive Reize Ihre Gehirnaktivität verändern. Sie werden aus der Teilnahme keinen unmittelbaren Nutzen für sich ziehen können. Wir hoffen jedoch, durch unsere Arbeit mehr darüber erfahren zu können, welche unmittelbaren physiologischen Reaktionen und welche gefühlsmäßigen und motivationalen Veränderungen unangenehme Reize hervorrufen können. Wenn Sie möchten, werden wir Ihnen nach der Untersuchung gerne die Hintergründe und Ziele dieser Untersuchung ausführlich schildern.

Vor der Untersuchung werden Sie einige Fragebögen ausfüllen, in denen wichtige Daten bezüglich Ihrer Person festgehalten werden. Dann wird der Versuchsleiter zur Messung Ihrer Herzrate zwei Messelektroden auf Ihrer Brust anbringen. Dazu wird Ihre Haut mit etwas Alkohol gereinigt, damit der Widerstand zwischen Haut und Messelektrode so gering wie möglich ist. Aufgrund dieser Hautreinigung kann es zu Hautrötungen und leichten Hautirritationen kommen, die aber normalerweise innerhalb kurzer Zeit abklingen. Außerdem wird auf Ihren Kopf ein angefeuchtetes Netz mit 128 Elektroden gelegt, um Ihrer Gehirnaktivität zu messen. Diese Flüssigkeit, in dem das Netz liegt, besteht nur aus Wasser, Baby-Shampoo und Kalium und ermöglicht eine optimale Erfassung ihrer Gehirnaktivität.

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Die Teilnahme an der Untersuchung ist völlig freiwillig. Sie können jederzeit - ohne Angabe von Gründen - die Teilnahme abbrechen. Dadurch entstehen Ihnen keinerlei persönliche Nachteile. Für Ihre Teilnahme an der Untersuchung erhalten Sie 9€.

Alle Daten dienen ausschließlich Forschungszwecken, werden vertraulich behandelt und ohne Namensgebung unter einer Codenummer abgespeichert. Der Codierungsschlüssel wird nach Abschluss der Studie vernichtet. Bis dahin können Sie auch noch nach der Untersuchung die Löschung ihrer Daten verlangen.
Falls Sie noch weitere Frage haben, fragen Sie bitte jetzt.

2

Einverständniserklärung

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Ich bin darüber informiert worden, dass ich jederzeit aus der Untersuchung ausscheiden kann, ohne dass mir persönliche Nachteile entstehen.

Mit meiner Unterschrift erkläre ich, dass ich das Vorhaben und diese Information verstanden habe, meine Fragen zufrieden stellend beantwortet wurden und ich freiwillig und aus eigenem Entschluss an der Untersuchung teilnehme.

Würzburg, den ____________________ Unterschrift _________________________

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D Demographic data questionnaire

VORBEFRAGUNG

Wir möchten Sie bitten, einige Angaben zu ihrer Person zu machen. Diese sind notwendig, da individuelle Faktoren (wie z.B. Ihr Alter) einen Einfluss auf die Testergebnisse haben könnten

Sie können sich darauf verlassen, dass diese streng vertraulich bleiben.

1. Alter: : ……………………..

2. Geschlecht: männlich …………………………… ☐
weiblich …………………………… ☐

3. Familienstand: ledig ………………………………………… ☐
verheiratet ………………………………… ☐
in Lebensgemeinschaft lebend ………. ☐
geschieden ………………………………… ☐
getrennt lebend …………………………… ☐
verwitwet …………………………………. ☐

4. Studienfach: ………………………………………… Semesterzahl: …………..

5. Händigkeit : ☐ rechts ☐ links ☐ beidhändig

6. Haben Sie eine Sehschwäche? ☐ ja ☐ nein

Wird Ihre Sehfähigkeit ausreichend korrigiert? ☐ ja ☐ nein

7. Leiden Sie an einer psychiatrischen oder neurologischen Erkrankung (wenn ja, welche)?

____

8. Hatten Sie schon einmal einen epileptischen Anfall oder ist bei Ihnen in der Familie eine Epilepsieerkrankung bekannt? ☐ ja ☐ nein

9. Nehmen Sie regelmäßig oder zurzeit Medikamente (welche, wie oft)?

____

E Trial order and pseudo-randomization Study 1

	Order 1	**Order 2**	**Order 3**	**Order 4**
Frequencies	CTX = 15 Hz	CTX = 12 Hz	CTX = 15 Hz	CTX = 12 Hz
	Cues = 12 Hz	Cues = 15 Hz	Cues = 12 Hz	Cues = 15 Hz
Stimuli	CTX+ = brown	CTX+ = brown	CTX+ = red	CTX+ = red
	CTX- = red	CTX- = red	CTX- = brown	CTX- = brown
Conditioning	CTX+	CTX+	CTX-	CTX+
	CTX+	CTX+	CTX-	CTX-
	CTX-	CTX-	CTX+	CTX+
	CTX-	CTX-	CTX+	CTX-
	CTX-	CTX+	CTX-	CTX-
	CTX-	CTX-	CTX+	CTX-
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	CTX-	CTX+	CTX+	CTX-
	CTX+	CTX-	CTX-	CTX-
	CTX+	CTX+	CTX-	CTX+
	CTX-	CTX-	CTX-	CTX+
	CTX+	CTX+	CTX+	CTX-
	CTX+	CTX+	CTX-	CTX-
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	CTX-	CTX+	CTX+	CTX+
Test phase	CTX-(soc)	CTX+(obj)	CTX-(soc)	CTX-(soc)
	CTX+(soc)	CTX-(obj)	CTX+(soc)	CTX+(obj)
	CTX-(obj)	CTX+(soc)	CTX+(obj)	CTX+(soc)
	CTX+(obj)	CTX-(soc)	CTX-(obj)	CTX-(obj)
	CTX+(obj)	CTX-(obj)	CTX-(soc)	CTX+(soc)
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	CTX-(soc)	CTX+(soc)	CTX-(obj)	CTX+(obj)
	CTX-(obj)	CTX+(obj)	CTX+(obj)	CTX-(obj)
	CTX+(soc)_US	CTX-(obj)	CTX-(soc)	CTX-(obj)
	CTX-(soc)	CTX+(obj)_US	CTX-(obj)	CTX+(obj)_US
	CTX-(obj)	CTX-(soc)	CTX+(soc)_US	CTX-(soc)
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	CTX-(obj)	CTX+(soc)	CTX+(obj)	CTX+(soc)
	CTX-(soc)	CTX-(obj)	CTX+(soc)	CTX+(obj)
	CTX+(obj)	CTX+(soc)	CTX+(obj)_US	CTX-(obj)
	CTX-(soc)	CTX-(obj)	CTX+(soc)	CTX+(soc)
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F Trial order and pseudo-randomization Study 2

	Order 1	Order 2	Order 3	Order 4
Frequencies	CTX = 15 Hz	CTX = 12 Hz	CTX = 15 Hz	CTX = 12 Hz
	Cues = 12 Hz	Cues = 15 Hz	Cues = 12 Hz	Cues = 15 Hz
Stimuli	CTX+ = brown	CTX+ = brown	CTX+ = red	CTX+ = red
	CTX- = red	CTX- = red	CTX- = brown	CTX- = brown
Conditioning	CTX+	CTX+	CTX-	CTX+
	CTX+	CTX+	CTX-	CTX-
	CTX-	CTX-	CTX+	CTX+
	CTX-	CTX-	CTX+	CTX-
	CTX-	CTX+	CTX-	CTX-
	CTX-	CTX-	CTX+	CTX-
	CTX+	CTX+	CTX+	CTX+
	CTX+	CTX-	CTX-	CTX+
	CTX-	CTX-	CTX+	CTX+
	CTX-	CTX+	CTX+	CTX-
	CTX+	CTX-	CTX-	CTX-
	CTX+	CTX+	CTX-	CTX+
	CTX-	CTX-	CTX-	CTX+
	CTX+	CTX+	CTX+	CTX-
	CTX+	CTX+	CTX-	CTX-
	CTX-	CTX-	CTX+	CTX+
	CTX+	CTX+	CTX-	CTX+
	CTX-	CTX-	CTX+	CTX-
	CTX-	CTX+	CTX+	CTX+
	CTX+	CTX-	CTX-	CTX-
	CTX+	CTX-	CTX-	CTX+
	CTX+	CTX+	CTX+	CTX-
	CTX-	CTX-	CTX-	CTX-
	CTX-	CTX+	CTX+	CTX+
Test phase	CTX+(happy)	CTX-(happy)	CTX+(happy)	CTX-(neutral)
	CTX+(neutral)	CTX+(neutral)	CTX+(angry)	CTX+(angry)
	CTX-(neutral)	CTX-(neutral)	CTX+(neutral)	CTX-(happy)
	CTX+(happy)	CTX+(angry)_US	CTX-(happy)	CTX+(happy)
	CTX-(neutral)	CTX-(neutral)	CTX-(neutral)	CTX+(angry)_US
	CTX+(happy)_US	CTX+(neutral)	CTX+(neutral)_US	CTX-(happy)
	CTX+(happy)	CTX-(happy)	CTX+(angry)	CTX+(neutral)
	CTX-(happy)	CTX-(angry)	CTX-(neutral)	CTX-(neutral)
	CTX-(angry)	CTX+(angry)	CTX+(neutral)	CTX-(angry)
	CTX-(angry)	CTX+(neutral)	CTX-(angry)	CTX+(angry)
	CTX+(happy)	CTX-(angry)	CTX-(angry)	CTX-(happy)
	CTX-(angry)	CTX-(neutral)	CTX-(neutral)	CTX+(neutral)
	CTX+(neutral)	CTX+(happy)	CTX+(angry)	CTX-(happy)
	CTX+(angry)	CTX-(neutral)	CTX-(angry)	CTX+(neutral)
	CTX-(neutral)	CTX+(happy)	CTX+(angry)	CTX+(neutral)
	CTX-(happy)	CTX+(angry)	CTX+(happy)	CTX+(happy)_US
	CTX-(neutral)	CTX+(happy)_US	CTX-(happy)	CTX-(neutral)
	CTX+(angry)_US	CTX-(happy)	CTX-(neutral)	CTX-(angry)
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G Trial order and pseudo-randomization Study 3

	Order 1	**Order 2**	**Order 3**	**Order 4**	**Order 5**	**Order 6**
Frequencies	CTX= 15 Hz Cues= 12 Hz	CTX= 12 Hz Cues= 15 Hz	CTX= 15 Hz Cues= 12 Hz	CTX= 12 Hz Cues= 15 Hz	CTX= 15 Hz Cues= 12 Hz	CTX= 12 Hz Cues= 15 Hz
Context Stimuli	N = red P = brown U = gray	N = brown P = gray U = red	N = gray P = red U = brown	N = red P = brown U = gray	N = brown P = gray U = red	N = gray P = red U = brown
Cue Stimuli	N = 3 P =1 U = 2	N = 2 P = 3 U = 1	N = 1 P = 2 U = 3	N = 3 P =1 U = 2	N = 2 P = 3 U = 1	N = 3 P =1 U = 2
Sequence	P	P	U	U	U	P
	N	N	N	N	N	N
	U	U	P	P	P	U
	N	N	N	N	N	N
	U	U	P	P	P	U
	N	N	N	N	N	N
	P	P	U	U	U	P
	U	U	P	P	P	U
	N	N	N	N	N	N
	P	P	U	U	U	P
	N	N	N	N	N	N
	P	P	U	U	U	P
	N	N	N	N	N	N
	U	U	P	P	P	U
	‘break‘	‘break‘	‘break‘	‘break‘	‘break‘	‘break‘
	P	P	U	U	U	P
	N	N	N	N	N	N
	U	U	P	P	P	U
	N	N	N	N	N	N
	U	U	P	P	P	U
	N	N	N	N	N	N
	P	P	U	U	U	P
	U	U	P	P	P	U
	N	N	N	N	N	N
	P	P	U	U	U	P
	N	N	N	N	N	N
	P	P	U	U	U	P
	N	N	N	N	N	N
	U	U	P	P	P	U

H Written instructions Study 3

The following instructions were presented on the screen before the start of the experiment, always followed by the appropriate context and cue stimuli.

„In der "kein Geräusch-Bedingung" werden Sie kein unangenehmes Geräusch hören.

Vor jedem dieser Durchgänge steht „KEIN GERÄUSCH“.

In dieser Bedingung sehen Sie immer folgenden Raum und folgende Person:

In der vorhersagbaren Bedingung werden Sie selbst vorhersagen können, wann das unangenehme Geräusch kommt.

Das unangenehme Geräusch wird nur präsentiert, wenn die Person im Raum sichtbar ist.

Vor jedem dieser Durchgänge steht „VORHERSAGBAR“.

In dieser Bedingung sehen Sie immer folgenden Raum und folgende Person:

In der unvorhersagbaren Bedingung werden Sie nicht vorhersagen können, wann das unangenehme Geräusch kommt.

Es kann sowohl präsentiert werden, wenn die jeweilige Person sichtbar ist oder auch wenn nur der Raum zu sehen ist.

Vor jedem dieser Durchgänge steht „UNVORHERSAGBAR“.

In dieser Bedingung sehen Sie immer folgenden Raum und folgende Person:“

Publication list

Research articles in peer-reviewed journals

Kastner, A. K., Pauli, P., & Wieser, M. J. (2015). Sustained attention in context conditioning: Evidence from steady-state VEPs. International journal of psychophysiology. doi:10.1016/j.ijpsycho.2015.03.005

Wangelin, B. C., Bradley, M. M., **Kastner, A.**, & Lang, P. J. (2012). Affective engagement for facial expressions and emotional scenes: The influence of social anxiety. *Biological Psychology, 91*(1), 103–110. doi:10.1016/j.biopsycho.2012.05.002

Published abstracts

Flohr, E. L. R., **Kastner, A. K**., Rimkus, S., Pauli, P., & Wieser, M. J. (2014). A scent of anxiety: Processing of faces in a conditioned olfactory context. *Psychophysiology*, *51,* S63. doi: 10.1111/psyp.12280.

Kastner, A. K., Pauli, P., & Wieser, M. J. (2013). Anxiety reduces electro-cortical differentiation between safe and threat context: evidence from steady-state VEPs. *Psychophysiology*, *50,* S63. doi:10.1111/psyp.12120

Kastner, A. K., Pauli, P., & Wieser, M. J. (2013). Context Matters: How a Threatening Context Modulates Processing of Social versus non-Social Cues. *Psychologie und Gehirn,* Würzburg, Germany.

Kastner, A. K., Pauli, P., & Wieser, M. J. (2013). Faces alter context: steady-state visually evoked potentials during context conditioning for high and low socially anxious individuals. *Cognitive Neuroscience (CNS),* Boston, MA, USA.

Kastner, A. K., Pauli, P., & Wieser, M. J. (2014). Cortical activation during phasic fear and sustained anxiety – Evidence from steady-state VEPs. *Cognitive Neuroscience (CNS),* San Francisco, CA, USA.

Kastner, A. K., Pauli, P., & Wieser, M. J. (2014). Influences of social anxiety on processing of conditioned contexts: Evidence from steady-state VEPs. *Psychologie und Gehirn,* Würzburg, Germany.

Kastner, A. K., Flohr, E. L. R. Rimkus S., Pauli, P., & Wieser, M. J. (2014). Modeling anxiety using olfactory context conditioning. *Psychophysiology*, *51,* S63. doi:10.1111/psyp.12280

Curriculum Vitae

Affidavit

I hereby confirm that my thesis entitled “Attention mechanisms in contextual anxiety and cued fear and its influence on processing of social cues” 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